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Doctoral dissertation in medical science

Application of metabolomics to understand the metabolic response evoked by the intake of high-carbohydrate or low-carbohydrate meal with cinnamon and capsicum by people at risk of type 2 diabetes development

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1. List of articles included in the dissertation

− Original article:

-Hameed, Ahsan, Edyta Adamska-Patruno, Joanna Godzien, Przemyslaw Czajkowski, Urszula Miksza, Karolina Pietrowska, Joanna Fiedorczuk, Monika Moroz, Witold Bauer, Julia Sieminska, Maria Górska, Adam Jacek Krętowski, and Michal Ciborowski. 2022. "The Beneficial Effect of Cinnamon and Red Capsicum Intake on Postprandial Changes in Plasma Metabolites Evoked by a High-Carbohydrate Meal in Men with Overweight/Obesity" *Nutrients* 14, no. 20: 4305. https://doi.org/10.3390/nu14204305

− **Review articles:**

-Hameed, Ahsan, Patrycja Mojsak, Angelika Buczynska, Hafiz Ansar Rasul Suleria, Adam Kretowski, and Michal Ciborowski. 2020. "Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM" *Journal of Clinical Medicine* 9, no. 7: 2257. https://doi.org/10.3390/jcm9072257

-Hameed, Ahsan, Mauro Galli, Edyta Adamska-Patruno, Adam Krętowski, and Michal Ciborowski. 2020. "Select Polyphenol-Rich Berry Consumption to Defer or Deter Diabetes and Diabetes-Related Complications" *Nutrients* 12, no. 9: 2538. https://doi.org/10.3390/nu12092538

2. List of a candidate's publications: (Number of publications, Impact Factor, points of Ministry of Science and Higher Education (MNiSW)):

- Articles included in the dissertation
- Articles not included in the dissertation
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Article type	Number	Impact Factor	MNiSW point
Articles included in the dissertation	3	18.1	420
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Summary	25	82.7	1588

3. INTRODUCTION

Obesity has become a significant problem. The global population with obesity has increased by 3-folds since 1975, and there are 1.9 billion adults with overweight, and among them, 650 million with obesity [1]. Obesity is a multifactorial disease; nevertheless, the major reason for weight gain is considered an energy imbalance between energy intake and energy expended. Exceed body fat content is no longer a cosmetic issue since it is a serious risk factor for type 2 diabetes mellitus (T2DM), cardiovascular disease, as well as some types of cancer development, which influences significantly the quality of life [2]. Because dietary factors play one of the most important roles, therefore a dietary intervention is considered the main approach for circumventing weight gain.

The most attention is paid to dietary fats and carbohydrates intake, and although a lot of researches have been conducted, their role in obesity prevention and treatment is very controversial. Therefore, also dietary recommendations of macronutrient intake for patients with overweight/obesity and/or T2DM are being discussed and modified continously. From a low-calorie diet (LCD) comprising mainly of fats with a limited carbohydrate restriction to promoting a high-carbohydrate diet (HCD) and low-in-fat-diet when the role of diabetes in developing cardiovascular disease has been established [3]. The rationale for advocating HCD with low-fat content was the decreasing plasma cholesterol concentration with a negligible increase in the fasting plasma glucose (FPG), however, these kind of diets failed to prevent obesity, insulin resistance, metabolic syndrome, and T2DM development [4]. Therefore, a high-fat diet (HFD) with low carbohydrates content is again regaining the grounds for the general notion of reduction/control over body weight and its metabolic consequences such as blood glucose concentrations [3]. Numerous human studies have reported the effects of HCD/LCD consumption with comparable results in case of body weight, fasting plasma glucose, insulin concentration, glycated haemoglobin (HbA1c), low-density lipoprotein cholesterol (LDL) or high-density lipoprotein cholesterol (HDL) reduction [5, 6].

Except different macronutrients composition in a diet, there is also an increased interest in use of plant-based foods to maintain health and well-being [7]. One of the promising "functional fruits" are berries, which contain a vast collection of polyphenols beneficial for health, e.g. anthocyanins, phenolic acids, flavonoids, flavanols, alkaloids, etc. There is an increasing number of evidences that inclusion of berries in human diet is associated with a reduced risk of development or even reversal of several health issues, including diabetes, obesity or hypertension. Clinical and non-clinical findings reporting positive health effects of the consumption of berries-based products in the prevention of T2DM development and reduction of T2DM-related complications together with the metabolic composition of berries and their potential mechanisms of action are presented in the review paper which is a part of this dissertation (Hameed A. et al. Nutrients 12, no. 9: 2538) [2]. Among functional plants with anti-obesity and/or antidiabetic activity cinnamon and capsicum recently gained the attention of the scientific community [8-10]. Both spices not only improve the physical cues of food but also grant the favourable healthy effects to its consumers. Cinnamon has been already associated with improved the glycemic control, insulin resistance, and hyperlipidemia, mostly by altering the expression of key regulatory genes involved in glucose-lipid metabolism pathways, and by reducing proinflammatory interleukins, prostaglandins, and nitric oxides production [11]. Similarly, capsicum or chili pepper compounds possess anti-obesity/anti-diabetic properties and could be used for the prevention and treatment of above-mentioned conditions [12]. Capsicum intake was noted to promote the satiety, fat oxidation, energy expenditure, plasma markers of glucose/lipid metabolism, obesity related systematic inflammation, and gut epithelial barrier function [10, 13].

Every dietary intervention should be long enough to observe its clinical effects, however, analyses of postprandial changes in plasma metabolites can show which metabolic

pathways are modulated shortly after thefood intake, and observation of these short-term changes can be useful to understand the possible long-term consequences of particular diets . Therefore, in the present study we used untargeted metabolomics to evaluate a short-term postprandial changes in plasma metabolites evoked by a single meal intake.

Metabolomics allows a systematic analysis of small molecules in a biological system. It can be used to study inter-individual variability in metabolizing same foods in health and disease states, therefore it is a key research tool in the studies aiming to develop a personalized nutrition. Among others, changes in metabolome can reflect a genetic background or be evoked by environmental factors, drugs or diet [14]. Blood metabolome is vulnerable to rapid changes in response to meal challenge. It has already been shown that, depending on the genetic background, specific meal types may evoke similar or different postprandial changes in plasma metabolome [15, 16]. As it has been presented in the review paper included in this dissertation, metabolomics studies provided a wide spectrum of potential biomarkers of diabetes (Hameed A. et al. Journal of Clinical Medicine 9, no. 7: 2257) [17].

In the first part of this study we evaluated a short-term postprandial metabolic response of participants with normal body weight (NW) or with overweight/obesity (OW/OB) to HC (high carbohydrate) and NC (normo carbohydrate) meals intake. We have observed differences in postprandial metabolites‘ levels between subjects with OW/OB and NW after HC, but not after NC meal intake. Therefore, in the second part of the study we evaluated potential benefits of cinnamon/capsicum, in individuals with OW/OB after HC meal intake with cinnamon and capsicum capsule compared to placebo.

4. Study Aims:

1. Application of global metabolomics apparocah to evaluate and compare the postprandial metabolic response to high-carboyderate and normal-carbohyderate meals in lean-to-obese subjects prone to T2DM.

2. Application of untargeted metabolomics to unveil the effect of capsicum and cinnamon intake on high carbohyderate meals in people prone to diabetes

5. MATERIALS AND METHODS

Ethics

This first substudy was a part of bigger project, which has been registered at www.clinicaltrials.gov as NCT03792685. The study protocol and procedures of both substudies were approved by the local Ethics Committee of Medical University of Bialystok, Poland (R-I-002/35/2009, R-I-002/314/2018, and R-I-002/95/2019). All of the participants signed informed consent before enrollment to the study. The study procedures were conducted following the ethical standards of human experimentation and with the Declaration of Helsinki.

Participants and study design

The participants enrolled to these studies were volunteers of Polish-Caucasian origin. Only males were included to the meal-challenge tests because of the possible sexual dimorphism of investigated factors [18]. Exclusion criteria included T2DM or prediabetes, as well as other disorders or any treatments that might affect obtained results. Also, subjects who followed any special diet or dietary patterns were excluded from the study. Participants were asked for and instructed to maintain their regular lifestyle throughout the study and to avoid coffee, alcohol, and excessive physical activity for at least one day before each test day. During the meal challenge test men stayed in bed, in a quiet room at thermoneutral conditions (22–25ºC).

Participants of the first study (n=24), based on the BMI criteria, were classified to OW/OB study group (BMI>25, n=12) or to NW study group (BMI<25, n=12). In these subjects, two meal-challenge-tests visits were performed in a crossover design, with an interval of 2–3 weeks. Some of the participants refused to take part in both meal-challenge tests visits; therefore, all of them underwent a meal-challenge-test with HC-meal intake and 18 of them with NC-meal intake. After fasting blood collection (at least 8h of fasting state),

subjects received a standardized HC-meal (300 mL, Nutridrink Juice Style, Fat Free, Nutricia, Poland, which provided 450 kcal: 89% of energy from carbohydrate, 11% from protein, and 0% from fat) or NC-meal (360 mL, Cubitan, Nutricia, Poland, which provided 450 kcal: 45% of energy from carbohydrate, 30% from protein, and 25% from fat).

In the second intervention study 20 male individuals with OW/OB were enrolled into double-blind, placebo-controlled trial consisted of two study visits with HC meal intake with the capsule containing 2 g of cinnamon (Cinnamomum verum, Dary Natury Pvt., Ltd., Koryciny, Poland) and 200 mg of capsicum (Capsicum annum, Organic Cayenne Pepper ground, Lebensbaum, Diepholz, Germany) or placebo capsule (composed of maltodextrin only), in randomized order. A wash-over period between study visits was around 1-3 weeks. A study meal was composed of wheat roll (100g), fruit jam (50g) and fruit juice (200ml). The clinical and anthropometric characteristics of study participants are shown in Table 1.

Table 1. Baseline characteristics of studied individuals.

Anthropometric Parameters	HC/NC Meal Study			Cinnamon/ Capsicum Study	*p*-value * for Comparison with OW/OB Group
	OW/OB	**NW**	***p*-value***		
Age [years]	37.8 ± 6.3	35.3 ± 8.6	0.4	46 ± 8.4	0.02
BMI [kg/m^2]	30.8 ± 5.4	23.8 ± 1.6	0.0009	32.0 ± 4.3	0.2
Body Fat contents [%]	28.0 ± 6.3	17.0 ± 5.3	0.0001	31.4 ± 4.1	0.2
Fat free mass [%]	69.8 ± 12.2	66.3 ± 6.6	0.4	67.9 ± 7.5	0.6
WHR	0.998 ± 0.06	0.91 ± 0.06	0.003	1.03 ± 0.05	0.1
Fasting glucose concentration [mg/dL]	87.9 ± 5.8	84.2 ± 8.1	0.2	101.05 ± 8.98	0.0002
Fasting Insulin concentration [IU/mL]	12.7 ± 9.3	6.5 ± 1.7	0.06	13.8 ± 4.5	0.2
HOMA–IR	2.8 ± 2.1	1.3 ± 0.3	0.05	3.46 ± 1.2	0.05
HOMA–β	186.3 ± 121.1	157.5 ± 150.4	0.6	136.32 ± 56.3	0.4
HbA1c	5.3 ± 0.3	5.2 ± 0.3	0.3	5.4 ± 0.3	0.9

This Table is from the original article being a part of this dissertation. * - For quantitative variables with normal distribution, the parametric *t*-test was used; for the other variables, the non-parametric Mann–Whitney test was applied. The data are represented as the mean ± STD, and *p*-values < 0.05 were considered significant. HC: high-carbohydrate, NC: normo-carbohydrate, OW/OB: individuals with overweight/obesity, NW: individuals with normal weight BMI: body mass index, WHR: waist-hip ratio, HOMA–IR: Homeostatic Model Assessment of Insulin Resistance, HOMA–β: Homeostatic Model Assessment of β–cell function, HbA1c: glycated haemoglobin.

Study Procedures

At the screening visit, the demographic data and anthropometric measurements, as well as body weight, body composition analysis, and blood collections, were performed. In subjects who met inclusion criteria, the meal-challenge test visits were conducted as described in details previously [15, 19]. The metabolomics analyses were performed on plasma samples from the blood collected at fasting (0‘) and 30‘, 60‘, 120‘, and 180‘ minutes after a meal intake.

Metabolomics measurements

The metabolomics analyses were performed using an HPLC system (1290 Infinity, Agilent Technologies, Santa Clara, CA, USA) with mass spectrometry detection (6550 iFunnel Q-TOF, Agilent Technologies, Santa Clara, CA, USA). Samples were prepared by mixing 1 volume of plasma with 4 volumes of freezing cold (–20°C) methanol/ethanol (1:1) to perform simultaneous precipitation of proteins and extraction of metabolites. Obtained samples were analyzed in the randomized order. Applied methodology has been described previously [16, 20].

Metabolomics data pre-treatment

Data treatment included cleaning of background noise and unrelated ions through molecular feature extraction (MFE) tool in Mass Hunter Qualitative Analysis Software (B.06.00, Agilent, Santa Clara, CA, USA). Mass Profiler Professional (B.12.6.1, Agilent Technologies, Santa Clara, CA, USA) software was used to perform quality assurance (QA) procedure and data filtration. QA procedure covered a selection of metabolic features with good repeatability. The features detected in >80% of quality control (QC) samples and with RSD <30% were kept for further data treatment. Additionally data was filtered to keep metabolic features present in 80% of the samples in at least one of the time points.

Calculations and statistical analyses

Based on the relation between time points and the signal intensity of each metabolite, the areas under the curve (AUCs) were calculated using a trapezoid rule in R software environment (version 3.4.3, https://www.R-project.org/). The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and the Homeostatic Model Assessment of β-cell function (HOMA-B) were calculated using the standard formulas: HOMA-IR = fasting plasma glucose concentration (mmol/L)] fasting insulin concentration (U/mL)]/22.5, HOMA-B = 20 fasting insulin (U/mL)/fasting glucose (mmol/L) - 3.5.

Statistical analysis of metabolomics data was performed on each metabolite's AUCs or intensities of metabolic features (baseline comparison between individuals from OW/OB and NW groups). The following comparisons were performed: AUCs after HC meal between individuals from OW/OB and NW groups, AUCs after NC meal between individuals from OW/OB and NW groups, AUCs after HC meal for cinnamon/capsicum and placebo group. Selection of statistically significant metabolites was performed implementing multivariate statistics. Partial least squares discriminant analysis (PLS-DA) models were computed for each comparison using the SIMCA-P+ 13.0.3 software (Umetrics, now Sartorius Stedim, Sweden). Volcano plots were created using variable importance in the projection (VIP), and p(corr) values obtained from the PLS-DA models. Variables with VIP>1.0 and absolute p(corr) >0.4 were considered significant.

Parametric (t-test) or non–parametric (Mann–Whitney test) was applied for clinical and anthropometric data, depending on data normality.

Metabolites' Identification

The identity of significant metabolites was confirmed by matching the experimental MS/MS spectra to MS/MS spectra from such databases as METLIN, KEGG, LIPIDMAPS,

and HMDB or by matching experimental m/z, fragmentation spectra and retention time with these obtained for metabolite's standards (if available). Fragmentation experiments were repeated with identical chromatographic conditions to the primary analysis. Phospholipids were identified based on a previously described characteristic fragmentation pattern [21]. A detailed fragmentation data of identified metabolites (except phospholipids) is presented in Table 2.

Table 2. A detailed fragmentation data of identified metabolites.

Metabolite	Monoisotopic mass [Da]	RT [min]	Fragments [m/z]	Ion
Piperidine	85.0892	0.3	86.0963, 69.0696, 58.0652, 44.0498, 43.0544, 41.0385	[M+H]+
Lactic acid	90.03185	0.3	87.0072, 71.0128, 44.9970, 43.0178, 41.0022	[M-H]-
Indoxylsulfuric acid	213.0097	0.7	212.0021, 132.0448, 80.9653, 79.9573	[M-H]-
Uric acid	168.0282	0.2	167.0214, 124.0150, 96.0201, 69.0093, 41.9987	[M-H]-
Bilirubin	584.2621	8.0	585.271, 299.138 583.254, 285.124	[M+H]+ [M-H]-
Androsterone sulfate	370.1814	3.9	369.1741, 96.9603	[M-H]-
Arachidonic acid	304.2397	7.1	303.2322, 285.2223, 259.2420, 231.2104, 205.1968, 59.0138	[M-H]-
Hydroxy stearic acid	300.2659	7.4	255.107, 138.911, 116.929, 100.934, 84.938	[M-H]-
LTA4	318.21915	6.2	299.198, 273.219, 255.212, 201.160, 127.075, 59.012	[M-H]-
HETE	320.2348	5.8	319.2272, 301.2168, 257.2270, 221.1542, 179.1079, 155.0709, 135.1175, 107.0866, 69.0342, 59.0134	[M-H]-
Docosenamide	337.3343	7.4	338.3416, 321.3144, 303.3055, 163.1477, 149.1326, 114.0912, 97.1009, 83.0857, 69.0702, 57.0704	[M+H]+
Linoleamide	279.2558	5.5	280.2636, 263.2353, 245.2225, 175.147, 109.101, 97.107, 95.0853,	[M+H]+

			83.0849, 81.068, 69.0705, 67.0549, 57.0696	
Palmitoylethanolamide	297.30246	7.8	298.3096, 256.2636, 116.1090, 102.0914, 88.0760, 57.0696, 43.0539	[M+H]+
Stearoylethanolamide	327.31308	7.3	328.3209, 311.2942, 310.3147, 109.1025, 95.0861, 71.0854, 67.0520, 62.0601, 57.0708, 44.0498	[M+H]+
Lauroyldiethanolamide	287.2456	5	288.2538, 227.1997, 106.0861, 88.0753, 70.0645, 57.0685	[M+H]+
Ketosphingosine	297.2661	6.0	298.274, 281.243, 263.234, 121.101, 97.101, 95.086, 93.068, 87.045, 83.086, 71.086, 69.07, 67.053, 57.07, 55.055	[M+H]+
Hexadecasphinganine	273.2662	4.1	274.274, 256.258, 106.083, 88.074, 70.065, 57.07	[M+H]+
Sphingosine 18:3	295.25055	5.7	296.2564, 279.2305, 261.2197, 233.2253, 184.8892, 153.1273, 121.0999, 107.0862, 95.0845, 83.0851, 81.0703, 69.0697	[M+H]+
Sphingosine 16:0	271.2509	4.5	272.258, 254.247, 106.086, 88.076, 57.069	[M+H]+
Sphingosine-1-phosphate	379.2489	5	380.2562, 362.2412, 264.2685, 247.2434, 82.0646, 57.0682	[M+H]+
Sphinganine C17:0	287.282	4.2	288.290, 270.279	[M+H]+
Arachidonic Acid methyl ester	318.2559	8	319.2632, 287.2362, 269.2253	[M+H]+
SM d34:2	846.4822	8.2	845.4749, 785.6480, 168.0416, 144.9231, 116.9279, 100.9334	[M-H+144.92]-
SM d32:1	820.4666	8	819.4593, 759.6315, 168.0407, 144.9231, 116.9281, 100.9335	[M-H+144.92]-

Metabolic pathway (enrichment) analysis and biological significance

Pathway analysis was performed using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/) [22]. Two analyses were performed. The first pathway analysis was performed for metabolites significantly discriminating OW/OB group from NW group after HC meal, while the second for metabolites significantly changing in the

cinnamon/capsicum intervention study. The Kyoto Encyclopedia of Genes and Genomes (KEGG) based Homo sapiens library was selected for analysis with a hypergeometric test in over-representation analysis and relative-betweenness centrality in pathway typology analysis (to estimate node importance). Pathway significance was determined from pathway enrichment analysis and based upon the values for each compound in the dataset.

6. Results and discussion of articles included in dissertation

Comprehensive untargeted metabolomics analysis of fasting and postprandial plasma samples was carried out to investigate and compare the effects of HC/NC meals intake on the plasma metabolome of participants with overweight/obesity (OW/OB group) and individuals with normal body weight (NW group). Metabolites discriminating individuals from OW/OB and NW groups at the fasting state are presented in Table 3. clearly indicating differences in metabolic profiles between two populations with different BMIs.

Table 3. Significant serum metabolites discriminating individuals with overweight/obesity from lean individuals at baseline.

Metabolites	OW/OB vs. NW		
	Change * [%]	**VIP**	**Absolute *p*(corr)**
Bilirubin	–30	1.82	0.52
Leucine (S)	33	1.45	0.65
Valine	–28	1.88	0.51
Piperidine	41	1.80	0.73
Linoleamide (S)	66	1.65	0.53
Dodecanamide (S)	32	1.51	0.57
Palmitoleamide (S)	79	2.17	0.68
HETE	43	1.78	0.49
LPC 18:1	21	1.29	0.43
LPA 16:0	20	1.39	0.51
LPI 16:0	51	1.48	0.53
LPI 18:0	31	1.15	0.43
LPI 18:1	37	1.82	0.61
PC 36:5	–45	1.98	0.64
PC 38:6	–26	1.60	0.47
PC O–36:5 or P–36:4	–30	1.83	0.52
PC 38:5	–44	2.67	0.67

This is a modified Table 2 from the original article being a part of this dissertation. * - Positive/negative value of percent of change means higher/lower intensity of metabolite in people with overweight/obesity in comparison to lean individuals. The *p*(corr) and VIP values were calculated

based on respective PLS-DA models. Variables with VIP > 1.0 and absolute *p*(corr) > 0.4 were considered significant. RT: retention time, VIP: variable importance into the projection, *p*(corr): predictive loading value, HETE: hydroxyeicosatetraenoic acid, LPC: lysophosphatidylcholine, LPA: lysophosphatidic acid, LPI: lysophosphatidylinositol, PC: phosphatidylcholine.

Discriminating metabolites mainly belong to different classes of lipids, i.e. phosphatidylcholine (PC), lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), lysophosphatidic acid (LPA), fatty acid amides (FAA) as well as hydroxyeicosatetraenoic acid. Changes in BCAA, bilirubin and piperidine were also noted. Baseline metabolic profiles discriminating people with overweight/obesity from those with normal weight indicate mainly for dysregulation of cellular lipid and amino acids metabolism. It is known that people with obesity have two-fold higher lipolytic fluxes what may result in the alteration of fasting metabolic profile [23]. Presented research showed a decreased level of several PCs when individuals from OW/OB group were compared with those from the NW group. Similar findings were also reported by Bagheri et al. [24], who observed reduced fasting plasma levels of long-chain PCs in individuals with overweight/obesity. Also Boulet et al. [25], observed a positive association between various LPC and different anthropometric variables, i.e. BMI, body-fat mass as well as subcutaneous and visceral adipose tissue content, what was widely discussed in the original article included to this dissertation. Other lipid entities increased in participants with overweight/obesity include FAA (i.e. dodecanamide, linoleamide, and palmitoleamide). FAA are linked to the the endocannabinoid system which correct functionality is significant to maintain and recover energy homeostasis [26].

We also noted higher fasting plasma levels of two BCAA (leucine and valine) in subjects with OW/OB. The impaired catabolism of BCAA may lead to reduced expression of branched-chain-keto acid dehydrogenase and branched-chain aminotransferase which prompt obesity. Observed in our study disrupted fasting plasma metabolic profile of participants with overweight/obesity can be an early predictor of insulin resistance, impaired glucose tolerance, prediabetes, as well as T2DM [17].

As it can be seen in Table 4, postprandial metabolic profile of subjects with OW/OB was substantially perturbed, especially after HC meal intake.

Table 4. A list of meal–dependent metabolites that changed postprandially, discriminating people with overweight/obesity from lean individuals.

Metabolite	NC meal OW/OB vs. NW			HC meal OW/OB vs. NW			Change in Cinnamon/ Capsicum Study*
	Change [%]	**VIP**	**Absolute *p*(corr)**	**Change [%]**	**VIP**	**Absolute *p*(corr)**	
Androsterone sulfate	–8	NA	NA	114	**1.49**	**0.51**	↓
Indoxyl sulfate	–25	NA	NA	155	**2.71**	**0.67**	↓
Lactic acid	10	NA	NA	73	**2.05**	**0.56**	Not changing
Uric acid	−16	NA	NA	145	**1.55**	**0.63**	↓
Hydroxy stearic acid	–17	NA	NA	54	**1.74**	**0.51**	↑
Hexanoylcarnitine	−16	0.42	0.12	84	**1.88**	**0.42**	ND
HETE	–11	NA	NA	129	**1.93**	**0.64**	ND
Sphinganine C17:0	11	0.54	0.10	−49	**1.89**	**0.50**	↑
Sphinganine C16:0	−8	0.15	0.07	−60	**1.79**	**0.56**	↑
Sphingosine C16:0	12	0.88	0.27	−40	**1.51**	**0.53**	Not changing
Sphingosine C18:3	550	**2.63**	**0.55**	57	0.42	0.27	↓
Lauroyldiethanolamide	−4	0.26	0.08	−71	**2.87**	**0.78**	↑
Linoleamide	682	**3.75**	**0.77**	38	0.74	0.40	Not changing
Palmitoyl N-Isopropylamide	69	**1.75**	**0.50**	36	0.67	0.39	↑
LPC 14:0	2	0.22	0.14	185	**2.22**	**0.65**	↓
LPC O-15:0	–19	NA	NA	125	**1.02**	**0.65**	↑
LPC 16:0	−23	0.22	0.14	161	**1.79**	**0.56**	↓
LPC 17:0 sn-1	–15	NA	NA	91	**1.96**	**0.51**	Not changing
LPC 17:0 sn-2	–14	NA	NA	94	**2.08**	**0.54**	Not changing
LPC 19:0	–22	NA	NA	92	**1.64**	**0.69**	↓
LPC 20:1	−36	0.28	0.02	274	**2.17**	**0.55**	↓
LPE 16:0	23	NA	NA	255	**1.91**	**0.65**	Not changing
LPE O-16:0	2	NA	NA	211	**1.96**	**0.66**	↑
LPE P-16:0 or LPE O-16:1	–30	NA	NA	195	**1.91**	**0.70**	↓
LPE P-19:1	−23	0.18	0.02	133	**1.67**	**0.67**	↓
LPE P-18:0 or LPE O-18:1	–25	NA	NA	113	**1.17**	**0.63**	↑
LPE P-20:0 or LPE O-20:1	10	NA	NA	64	**1.39**	**0.53**	↓
LPE 20:3	15	0.45	0.12	−71	**2.49**	**0.53**	↑
LPA 22:4	30	1.19	0.29	101	**1.53**	**0.52**	Not changing
LPI 16:0	41	NA	NA	224	**1.40**	**0.68**	ND
LPI 18:0	22	NA	NA	98	**1.59**	**0.58**	ND
LPI 18:1	26	NA	NA	76	**1.44**	**0.67**	↑
LPI 20:4	−22	NA	NA	41	**1.50**	**0.42**	↓

PC 32:1	73	1.52	0.34	116	**1.48**	**0.49**	↓
PC 38:5	−8	0.54	0.23	78	**1.06**	**0.49**	↓
SM 32:1	19	0.97	0.26	48	**1.09**	**0.46**	↓

This is a modified Table 3 from the original article being a part of this dissertation. * - the direction of arrow shows a direction of AUC Change after Cinnamon/Capsicum Intake. Positive/negative value of percent of change means higher/lower AUC of postprandial change of metabolite level in people with overweight/obesity in comparison to lean individuals. The *p*(corr) and VIP values were calculated based on respective PLS-DA models. Variables with VIP > 1.0 and absolute *p*(corr) > 0.4 were considered significant and are bolded. NA: values not available as it was not possible to build PLS-DA model based on this data set. ND: not enough quality data for this metabolite were recorded in the cinnamon/capsicum study. Not changing means that a difference between the AUC for placebo and cinnamon/capsicum was below 5%. HC: high-carbohydrate, NC: normo-carbohydrate, OW/OB: individuals with overweight/obesity, NW: individuals with normal weight, AUC: area under the curve, RT: retention time, *p*(corr): predictive loading value, VIP: variable importance into the projection, HETE: hydroxyeicosatetraenoic acid, LPA: lysophosphatidic acid, LPC: lysophosphatidylcholine, LPE: lysophosphoethanolamine, LPI: lysophosphatidylinositol, PC: phosphatidylcholine, SM: sphingomyelin.

Most of affected metabolites were phospholipids, sphingolipids, and FAA. The AUC of several phospholipids (LPEs, LPCs, and LPIs), indoxylsulfuric acid, lactic acid or uric acid was significantly increased in individuals with OW/OB when compared to subjects with normal body weight, but only after HC meal intake. For some metabolites (sphingosine 18:3, lauroyldiethanolamide, and palmitoyl N-isopropylamide) significantly increased AUC was found after NC, but not HC meal, while for others significant differences in AUC between the studied groups were observed after both meals. For most of the metabolites we noted an increased postprandial AUC in OW/OB group in comparison to the NW study participants.

Other authors have reported similar results. Gonzalez-Granda et al. [27] studied the effect of consuming high-fructose meals by indivuiduals with different body weight and obserwed a marked increase of phospholipids and diacyl-LPCs levels in obese individuals in response to fructose-rich diet. Most of the meal-type discriminatory metabolites were lipids. Their postprandial AUC was found accelerated in OW/OB subjects in response to HC-meal. Taking together all of these results we may conclude thatpopulation with BMI above normal range is more susceptible to dysregulation of lipid metabolism due to HC-meals intake than individuals with BMI within the normal ranges. These findings are in line with the result of

the study in which lipidome of individuals with obesity and prediabetes was investigated, and perturbations in the level of several PL, LPC, LPE, LPI, SM, and ceramides in people with obesity have been reported [28]. Another study indicated three phospholipids, i.e. LPC 18:2, PC 32:1, and PC 34:2, as the early predictors of diabetes in the susceptible population [29]. These studies underline the positive association of HC-meal intake and plasma/serum levels of phospholipids, fatty liver index, and weight gain. Tulipani et al. [30] noted an inversed relationship of LPCs 17:0, 18:1, and 18:2 with the BMI, body weight, hip and waist circumferences. This contradiction in findings might be arising since OW/OB individuals in this study group were largely normoglycemic. Additionally, carbohydrate-type, glycemic index, and quantity of intake were considered important for determining the overall blood glucose, lipid metabolism, and metabolic response as well [31].

After HC-meal intake we noted also significantly exacerbated the postprandial AUC of hydroxy stearic acid and hydroxyeicosatetraenoic acid (HETE) in subjects with OW/OB when compared to NW study group. HETE and its various isoforms have been found to activate the inflammatory markers and reduce the secretion of insulin [32]. Moreover, we noted a higher postprandial AUC of hydroxy stearic acid and lower postprandial AUC of sphingolipids (with the exception for sphingosine C18:3) in OW/OB subjects in comparison to NW individuals in response to HC-meal intake. Sphingosine is the key precursor of *de novo* biosynthesis pathways of ceramides, and higher plasma concentrations of sphingosine and ceramides were noted in obese and insulin-resistant Zucker rats [33] and diabetic individuals [34]. Another metabolite with meal-dependent postprandial profile is androsterone sulphate. We noted significantly higher AUC for this metabolite after HC-meal intake in OW/OB group participants. The relationship between the steroid hormones, obesity and T2DM has been already discussed [35], and it was found independently associated with a decreased risk of T2DM development in postmenopausal women and healthy men [36]. We founf that

postprandial AUC of indoxyl sulfate was higher in individuals with OW/OB in comparison to normal body weight individuals after an HC-meal intake. Indoxyl sulfate is may also be involved in the pathogenesis of metabolic syndrome, cardiovascular disease and chronic kidney disease [37]. All of these results show that HC-meal intake can make individuals with OW/OB more vulnerable to above-mentioned diseases.

Different response of participants with OW/OB in comparison to NW participants is mostly observed after an HC meal intake and discriminating metabolites are mostly lipids. It has been already observed that ingested capsaicinoids can prevent diet-induced obesity in rats [38]. Moreover, it was noted that also cinnamon extract may regulate plasma levels of adipose-derived factors and expression of multiple genes related to carbohydrate metabolism and lipogenesis [39]. A beneficial effects of capsicum annum and cinnamon supplementation on metabolic syndrome and diabetes management have been reviewed recently. Literature data indicate different anti-diabetic/obesity mechanisms of cinnamon and capsicum action [10, 40], therefore, we decided to combine them into one capsule and evaluate their ability to diminish changes in plasma metabolome evoked by an HC meal intake in men with OW/OB. As it has been presented in the original article included to this dissertation, we noted different AUCs for postprandial changes in metabolites level when cinnamon/capsicum capsule was taken with an HC meal in comparison to placebo capsule intake. Metabolites significantly affected by the cinnamon/capsicum intake are presented in details in the Table 5.

For the most of metabolites significant after HC-meal intake, the opposite direction of change was observed after the cinnamon/capsicum intervention, what indicate that these spices may diminish the effect induced by an HC meal intake. Although considering exactly the same metabolites, only six significant after HC-meal intake were also significantly changing after cinnamon/capsicum intervention. Performed pathway analyses indicate that the most of metabolic pathways affected by an HC meal intake are the same that are affected by the cinnamon/capsicum ingestion with an HC meal. Although participants of both substudies

were males with overweight/obesity, those from cinnamon/capsicum substudy were older and more insulin resistant. It may explain why the lists of significant metabolites are not more consistent. However, high similarities of affected pathways, together with the opposite direction of noted changes in postprandial metabolites affected by an HC meal intake in comparison to HC-meal intake with cinnamon/capsicum, indicate that these natural spices may regulate metabolic pathways, which are perturbed by this kind of meal in men with OW/OB.

Table 5. A list of metabolites significantly changing in the cinnamon/capsicum intervention study.

Metabolite	Change [%]	VIP	Absolute *p*(corr)
Sphingosine-1-phosphate	170	1.26	0.49
Sphinganine C17:0	1,758,409	2.11	0.88
Arachidonic Acid methyl ester	688,141	1.32	0.58
Docosenamide	159	2.43	0.99
LPC 14:0	**−27**	**1.13**	**0.48**
LPC 16:0	**−100**	**1.52**	**0.64**
LPC 18:2	−8	1.79	0.79
LPC 20:1	**−34**	**1.50**	**0.67**
LPE P-16:0	**−17**	**1.22**	**0.54**
LPE P-19:1	**−29**	**2.01**	**0.87**
LPE 20:3	**1,134,744**	**1.36**	**0.56**
LPE 20:4	−52	1.35	0.38
PC 32:4	−59	1.05	0.44
PC36:2	−99	1.38	0.54
PC 38:4	−99	1.32	0.53
PC 40:6	−90	1.62	0.66
PC 40:7	2884	1.20	0.46
PC 16:0/20:4	−88	1.50	0.42
PC O-36:2 or PC P-36:1	17,503	2.12	0.86
PC O-40:5 or PC P-40:5	−100	1.72	0.69
SM d34:2	−27	2.43	0.86
SM d32:1	−30	2.35	0.78

This is a modified Table 4 from the original article being a part of this dissertation. Positive/negative value of percent of change means higher/lower AUC of postprandial change of metabolite level after cinnamon/capsicum capsule intake in comparison to placebo capsule intake. Metabolites significant after HC meal in OW/OB vs. NW comparisons are bolded. The *p*(corr) and VIP values were calculated based on the PLS-DA models. Variables with VIP > 1.0 and absolute *p*(corr) > 0.4 were considered significant. RT: retention time, *p*(corr): predictive loading value, VIP: variable importance into the projection, LPC: lysophosphatidylcholine, LPE: lysophosphoethanolamine, PC: phosphatidylcholine, PE: phosphoethanolamine, SM: sphingomyelin.

7. Conclusions

This first substudy has shown the differences in postprandial metabolic response to HC-meal in men with overweight/obesity in comparison to normal weight men. Several metabolites and metabolic pathways were affected after an HC-meal intake in participants with owerweight/obesity, but not in those with normal body weight. A substantial increase of postprandial AUCs was mainly noted for different classes of lipids. The second substudy showed ingestion of the cinnamon/capsicum together with an HC-meal by men with overweight/obesity can diminish observed unfavourable postprandial metabolic response evoked by such a meal. Further work aiming to confirm observed relationships and to explore the mechanisms related to the perturbed metabolic pathways should be performed. Both biochemical and animal experiments would be needed to validate obtained results.

8. Articles included in dissertation

1) **Hameed, Ahsan**, Patrycja Mojsak, Angelika Buczynska, Hafiz Ansar Rasul Suleria, Adam Kretowski, and Michal Ciborowski. 2020. "Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM" *Journal of Clinical Medicine* 9, no. 7: 2257. https://doi.org/10.3390/jcm9072257

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Review

Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM

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Abstract: Diabetes mellitus, a disease of modern civilization, is considered the major mainstay of mortalities around the globe. A great number of biochemical changes have been proposed to occur at metabolic levels between perturbed glucose, amino acid, and lipid metabolism to finally diagnoe diabetes mellitus. This window period, which varies from person to person, provides us with a unique opportunity for early detection, delaying, deferral and even prevention of diabetes. The early detection of hyperglycemia and dyslipidemia is based upon the detection and identification of biomarkers originating from perturbed glucose, amino acid, and lipid metabolism. The emerging "OMICS" technologies, such as metabolomics coupled with statistical and bioinformatics tools, proved to be quite useful to study changes in physiological and biochemical processes at the metabolic level prior to an eventual diagnosis of DM. Approximately 300–400 such metabolites have been reported in the literature and are considered as predicting or risk factor-reporting metabolic biomarkers for this metabolic disorder. Most of these metabolites belong to major classes of lipids, amino acids and glucose. Therefore, this review represents a snapshot of these perturbed plasma/serum/urinary metabolic biomarkers showing a significant correlation with the future onset of diabetes and providing a foundation for novel early diagnosis and monitoring the progress of metabolic syndrome at early symptomatic stages. As most metabolites also find their origin from gut microflora, metabolism and composition of gut microflora also vary between healthy and diabetic persons, so we also summarize the early changes in the gut microbiome which can be used for the early diagnosis of diabetes.

Keywords: metabolomics; validated biomarkers; early biomarkers; diabetes mellitus; pre-diabetes; glucose intolerance; insulin resistance; obesity; gut microbiota

1. Introduction

Diabetes mellitus (DM) is the most prevalent modern civilization disease and 8th major mainstay of mortalities globally. This worldwide pandemic of DM is associated with large financial strain on the healthcare systems of many countries, especially developing ones. The current projections from the International Diabetic Federations (IDF) predict that the number of diabetic patients will hit 629 million by 2045 and 34 million more people are at risk of developing DM than in 2015. Approximately 19 million more adult people are undiagnosed than in 2015 and 1 in 6 births are influenced by hyperglycemia in pregnancies [1]. Governments have allocated and spent almost USD 792 billion worldwide for the treatments of DM, out which almost 377 and 166 billion dollars were allocated and spent only by

the US and European Union (EU) countries. In the developing countries the financial burden of DM treatment is mostly beard by patients themselves and it accounts for 25–40% of their monthly income. However, to make matters worse, 4 out of 5 people with diabetes live in low- and middle-income countries [1,2]. Therefore, new approaches are needed to lessen this financial pressure on both patients and healthcare systems which is achievable only by reducing the epidemic of DM.

To circumvent the onset and/or delay in the progress of DM, it is most effective to recognize the early stages of DM before major systematic damage (i.e., retinopathy, microvascular complications, nephropathy etc.) occurs. Currently, a number of laboratory-based-diagnostic-tools are available for the early detection of metabolic syndrome and related diseases which include impaired glucose tolerance (IGT), impaired fasting glucose (IFG), combined glucose tolerance (CGT) tests, anthropometric measurements and insulin sensitivity indexes [3]. These laboratory-based-tests are related to the (pre)diabetic state which may take years to proceed to chronic DM. The aforementioned diagnostic tests are directly related to glucose and insulin homeostasis. However, non-glucose/insulin related reliable and validated biomarkers are needed to complement our knowledge on diabetes development and indicate prognostic biomarkers. Regarding this, it has also been reported in the literature that metabolic syndrome (pre-diabetic/diabetic state) is likely to alter the metabolic pathways related to sugars, lipids, amino acids and their resulting metabolites resulting in the perturbed respective metabolites level in predisposed individuals compared to healthy ones [4]. These altered metabolite levels may serve as non-glucose/non-insulin related reliable and validated diagnostic biomarkers to recognize the pre-diabetic stage. Furthermore, many beneficial metabolites are produced as a result of intestinal microflora's metabolism on sugars, lipids and amino acids. Therefore, metagenomic studies of gut microbiota have also been considered complementary to metabolomics studies to summarize the reported changes in the gut microbiota ecosystem.

A high throughput analytical technique such as metabolomics is immensely popular in epidemiological studies to provide the mining of these new reported biomarkers of disease risks and severity. This approach has the capability to detect the perturbation of one's body metabolic pathways affected by disease and hence permit new insights into the physiological and pathophysiological development of disease [5]. The metabolomics technique has been used for screening potential diabetic patients for early diagnosis, prevention or delayed onset of type 2 diabetes mellitus (T2DM). Literature is full of many prospective, randomized, blinded, nested and case-controlled cohort studies where the populations of different geographical localities have been recruited and tested for decades to comprehensively understand the prognosis of DM in both vulnerable and non-vulnerable subjects. These studies came up with a wide range of early T2DM biomarkers, especially of sugars, lipids and amino acids origin. Most of these (pre)diabetes reporting biomarkers originating from sugars, lipids and amino acids metabolism are considered validated biomarkers. However, a few conflicting studies are also presented in literature and discussed in this review too. Most of these conflicts in findings may arise due to not considering varying covariates in research design and personal or communal genetic variations.

The data regarding the early biomarkers of DM (T2DM) in human beings is really dispersed and exhaustive. Therefore, the objectives of this comprehensive review were, for the first time in literature, to collect, compile and update as much reliable and validated early DM biomarkers data in one manuscript from published literature, which will be convenient for both physicians and potential DM sufferers to score their risk factors.

2. Metabolomics and Early Biomarker of Type 2 Diabetes Mellitus (T2DM)

The global prevalence of the T2DM pandemic has attracted wide attention due to its financial burden on health care systems. The failure in diagnosing prediabetes by conservative laboratory-based diagnosis tools at their latest stages could also be partially blamed for this pandemic. The laboratory-based diabetes diagnostic tools which are currently available include fasting blood glucose levels, insulin sensitivity indexes, oral glucose tolerance tests (OGTT) and glycated hemoglobin (HbA1c). It is estimated that up to 60% of cases of T2DM have never been diagnosed and/or misdiagnosed due to the sensitivity limitations of these assays at prediabetic and diabetic threshold levels [6].

Additionally, these trivial assays are not involved in staging the progress of diabetes as T2DM is considered a non-static condition and it keeps evolving from acute prediabetic (hypoglycemia, hyperosmolar hyperglycemic syndrome, ketoacidosis, and lactic acidosis) to chronic diabetic (diabetic coronary artery disease (CAD), cerebral vascular disease, diabetic retinopathy (DR), diabetic peripheral neuropathy, lower extremity vascular disease, diabetic nephropathy (DN) and diabetic foot disease) states [7]. The misdiagnosis and mistreatment of prediabetes and prediabetic complications has not only exposed people to non-essential medications with possible side effects but also to a source of economic loss. Preventing the onset of T2DM and/or diagnosing the early stages of diabetes followed by respective targeted treatment is the most economical way to treat T2DM before the occurrence of systematic damage and chronic complications. Apparently, the failure of the diabetic diagnostic tools currently available to diagnose prediabetes makes the search inevitable for new biomarkers/predictors to complement the current diagnostic measures. Recently, the inclusion of low plasma adiponectin concentration as a strong predictor for future T2DM development [8] further suggests the significant scope of setting the complementary biomarkers of T2DM risk. Moreover, highly sensitive and specific biomarkers are urgently needed in order to early diagnose T2DM. Metabolomics provides a great opportunity to indicate these novel biomarkers.

Metabolomics has been increasingly used in epidemiological studies for unveiling the novel association between metabolic pathways and disease. It is referred to a systematic study of identification and quantification of low molecular-weight metabolites in a given biological sample. The pool of these metabolites plays an important physiological role in the biological systems and is considered a promising candidate for studying disease phenotype as disrupted levels of metabolites were found in prediabetic individuals [9]. Two major platforms are used in metabolomics research, i.e., mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. MS, the most frequently employed approach, is high-throughput and sensitive but destructive in nature. It is often used in combination with separation techniques such as (high/ultra-pressure) liquid chromatography, gas chromatography and capillary electrophoresis. NMR, on the other hand, is non-destructive, robust and reproducible with minimal sample preparation, separation and ionization steps. As no single platform exists to capture the global profile of the whole metabolome, a multiplatform approach is mostly applied to get an all-inclusive understanding of metabolic variations and widen the “window” of significant metabolic discrepancies [10]. The application of metabolomics in T2DM studies has been in progress for the last two decades with the successful provision of novel insights into the pathophysiological mechanism and metabolic profiling. These studies have come up with the identification of novel biomarkers related to insulin resistance and T2DM biomarkers [3,4,10]. It is highly probable that a growing number of these novel T2DM biomarkers can be translated into clinical applications that will upgrade the current medical routine in regards to personalized medicine.

3. Biomarkers of Disturbed Protein Metabolism

Protein and glucose metabolism are tightly linked and accordingly regulated at metabolic and molecular levels. Dietary and endogenous amino acids (AA) relate to glucose metabolism via gluconeogenesis. The catabolic breakdown of AA provides the fuel for gluconeogenesis. The deamination of AA forms ketoacids such as oxaloacetate and pyruvate which feed the gluconeogenesis [11,12]. On the other hand, AA are also de novo biosynthesized from the Krebs cycle-derived carboxylic acid-intermediates by transamination. These free AA modulate the AA-type-dependent glucagon and insulin secretion and hence glucose metabolism [13]. In short, AA are not only an energy reservoir for gluconeogenesis, but their de novo biosynthesiz influences the glucagon and insulin release. In prediabetes, the glucagon not only over-activates the gluconeogenesis in the liver and kidneys but also disrupts the de novo biosynthesis of AA, which makes the AA good candidate biomarkers. In the meantime, many studies have claimed a positive association of branched-chained AA (BCAA), aromatic AA and other AA with the risk of developing T2DM as follows.

AA: Elevated (serum/plasma) AA level is an important early biomarker of glucose intolerance, insulin insensitivity and, subsequently, diabetes. Many epidemiological cohort studies have witnessed

an increased level of AA in newly diagnosed diabetic patients in the follow up investigations hinting towards an impaired glucose/hexoses metabolism [14]. An increased serum AA level reduced the insulin sensitivity and uptake of hexoses by offering competition at the substrate level to glucose oxidation and also by interfering with insulin signaling [11,13]. Higher serum AA levels were found to diminish the peripheral uptake of hexoses along with the suppression of endogenous glucose production (EGP) and body glucose disposal by 25% [15]. Similarly, the rate of glycogen synthesis was recorded to reduce up to 64% with a rise in serum AA levels under insulin stimulated conditions which accounted for AA modulated reduced glucose absorption in the body. This decline in glucose absorption is accompanied by the down-regulation of glycogen synthase, glucose transporter, phosphorylations and G6P [11,16]. Many studies have recorded this increased AA level as a future risk factor of developing T2DM. Most of these AA not only belong to BCAA but also to aromatic and aliphatic AA. In a closely matched case-control study, three aromatic (phenylalanine, tyrosine, and tryptophan) and one aliphatic AA (lysine) were found to be associated with the future risk of T2DM. The predictive power of lysine was also viable after the OGTT while comparing cases vs. controls. The additional stratified analysis in follow-up duration recorded the retainability of the predictive power of these AA up to 12 years from the baseline. For each increase in the SD of these AA, the odds of future T2DM development increased by 57–102%, further signaling towards a 2- to 3.5-fold higher probability of developing T2DM in top quartile individuals. The conditional regression analysis with isoleucine, phenylalanine and tyrosine also reported a five- to seven-fold higher probability of developing T2DM in top quartile individuals [17]. In the Insulin Resistance Atherosclerosis (IRAS) cohort, the individuals of four ethnicities (European-American, Hispanic, and African-American) who converted to T2DM in their follow-up of five years recorded similar results. A distinctive metabolome profile was noted in T2DM-converted subjects compared to healthy controls with significantly higher concentrations of phenylalanine, tyrosine, combined glutamine and glutamate, and valine associated with insulin resistance (Table 1). An 11–15% increase in these AA was seen whereas a 22% lower level of glycine was noted in highly insulin resistant individuals. A nominal difference was also noted between high/low insulin-resistant persons to T2DM-convertees. Alanine and aspartate/asparagine levels increased in the T2DM-converters only compared to high/low insulin resistant persons [18]. The SABRE (Southall and Brent Revisited) cohort study comprised of non-diabetic South Asian migrants in Europe/UK pinpointed nine AA in relation with the biomarker of obesity and insulin resistance in a follow up of 19 years. This study also described a significant positive correlation of phenylalanine, tyrosine and alanine, a weak positive relationship of histidine, and a significant negative correlation of glutamine and glycine with insulin resistance and glycemia [19]. In another Asian-Japanese cohort study, the authors measured the level of plasma-free AA (BCAA and aromatic AA) in correlation with obesity and diabetes and was able to predict the future diabetes risk in a minimal time of four years. This study further cited the negative correlation of clusters of glycine, serine, glutamine, and asparagine with obesity, body mass index (BMI), insulin resistance and 120 min insulin resistance assay. The increment of 1 SD of the odds ratios of the plasma-free AA increased the future risk of T2DM, metabolic syndrome, dyslipidemia or hypertension by 2.06%, 3.04%, 1.98%, and 1.42% respectively [20]. The longitudinal, nested and cross-sectional studies from two Chinese cohorts, Shanghai Obesity Study (SHOS) and Shanghai Diabetes Study (SHDS), also noted increased serum levels of aromatic AA at baseline in those individuals who develop T2DM in a follow up of 10 years [21]. The Finnish cohort study of 9369 nondiabetic or newly diagnosed T2DM Finnish men, namely population-based Metabolic Syndrome in Men (METSIM) also cited increased concentrations of tyrosine, glutamine and alanine in a 4.7-year follow-up [22]. An aliphatic AA called 2-aminoadipic acid was also found to be an early biomarker for T2DM risk. The degradation of lysine usually results in the 2-aminoadipic acid that may serve as a substrate for tryptophan catabolism. A strong association of 2-aminoadipic acid with T2DM risk was for the first time cited by Wang et al. [23]. The fasting plasma levels of prediabetic patients were found to be high in this amino acid. Following adjustments for age, sex, BMI and fasting conditions, the conditional logistics regression models noted 60% odds of future T2DM risk after each standard deviation (SD)

increment of a logged biomarker. The twelve years-follow up-period showed 4-fold higher odds of developing T2DM in the top quartiles of plasma 2-aminoadipic acid concentration. The adjustment of data with respect to parental history, dietary habits, lifestyle, fat/protein/carbohydrate intake and total caloric intake did not bring about any variations in this risk factor [24,25]. The independent work on Malmo diets and cancer studies also served as a replication of these results, which also indicated a 57% rise in the T2DM risk for each increment of SD of 2-aminoadipic acid concentration. Aromatic and branched-chain amino acids are also biomarkers of incidence of T2DM, but recent studies have not found any correlation between 2-aminoadipic acid and aromatic or branched-chain amino acids. However, a modest association of 2-aminoadipic acid with lysine, kynurenic acid and anthranilic acid was observed [16,23]. The Dongfeng–Tongji (DFTJ) and Jiangsu non-communicable disease (JSNCD) independent nested case control cohort models were also used to predict the identified metabolites using the traditional risk factors [26]. Qiu et al. [26] identified 52 metabolites, among which 20 AA were found to have associated positively with DM risk in both models. The dietary variables also did not change the four AA biomarkers out of 20 (i.e., alanine, phenylalanine, tyrosine and palmitoylcarnitine) which had a false discovery rate correction (FDR) < 0.01. The exploratory analysis of the pooling of other metabolites also identified an additional 12 metabolites, including such AA as glutamate, betaine, ornithine, leucine/isoleucine, valine and proline which achieved FDR < 0.01. The four metabolites i.e., phenylalanine, alanine, palmitoylcarnitine and tyrosine were found consistently associated with the risk of T2DM. Another aliphatic AA called alanine is a hepatic substrate and stimulator for gluconeogenesis and glucagon secretion and its circulating amount was also found to be a predictive metabolite of T2DM risk in many cross-sectional and prospective studies [4,27]. Elevated levels of alanine have already been found to have a positive association with T2DM in Finland, UK, and South Asia populations, as stated above. Moreover, the evident relationship of phenylalanine and tyrosine with the risk of T2DM was also significant due to an increase in insulin resistance through blocking the transport/phosphorylation of glucose. In the case of tyrosine, it is a far more powerful indicator of T2DM risk in South Asians [27]. The population-based KORA (Cooperative Health Research in the Region of Augsburg) and European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort study identified five baseline metabolites out of 131 (using pairwise comparison, multivariate (logistic) regression analyses followed by non-parametric random forest and the stepwise parametric regression) specifically associated with the pre-diabetic conditions to examine their ability to forecast the pre-diabetic conditions earlier [4]. The non-parametric random forest and the stepwise parametric regression recorded glycine, (lysophosphatidylcholines (LysoPC) (18:2), LysoPC (17:0), LysoPC (18:1) and C2) as novel candidate biomarkers of T2DM. In pursuit of establishing the predictive values of these metabolites, the baseline concentration of these metabolites in the KORA cohort (118 incident and 471 healthy controls) were compared, which revealed significant differences only for glycine and LysoPC (18:2). Each increment in the standard deviation of these two metabolites associated with a 33% less risk of future diabetes. The individuals in the fourth quartile were at three times less risk of diabetes than people whose serum glycine and LysoPC (18:2) levels were at first quartile. Therefore, the baseline decreases in the serum levels of glycine and LysoPC (18:2) were cited as powerful indicators of the future onset of diabetes [4]. Later on, the replicative prospective study with EPIC-Potsdam cohort also reported similar results using targeted metabolomics citing an increased phenylalanine concentration and reduced glycine concentration as biomarkers for future T2DM occurrence [28]. Earlier, the work of Pontiroli et al. [29] also confirmed a low level of glycine as a result of progressive insulin resistance which, in turn represses, the expression of ALAS-H catalyzing the conversion of glycine and succinyl-CoA into 5-aminolevulinic acid. In short, most of the early diabetes biomarker mining studies find AA as early predictors of T2DM. In summary, with the exceptions of glycine, serine, asparagine and histidine, most of studies stated increased concentrations of AA as a risk predictor of the future onset of T2DM. The predicting power of different AA may vary depending on the early diabetic stage, ethnicities and genetic background.

Table 1. A comprehensive list of altered metabolites of lipids and amino acid (AA) origins found in cohort studies mentioned in the respective sections.

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
1	Branched-chain-amino acids/Amino acids (BCAA/AA)	*N*-Acetylaspartate	↓	plasma/urine	Alanine and aspartate metabolism
2		Phosphocreatine	↑	plasma/urine	Creatine biosynthesis and amino acid
3		Creatinine	↑	plasma/urine	Metabolism, glycine, serine and threonine
4		Glycine	↓	plasma/urine	Metabolism
5		Guanidinoacetate	↑	plasma/urine	AA metabolism
6		Butyrylglycine	↓	plasma/urine	FA metabolism
7		Caproylglycine	↓	plasma/urine	Fatty acid metabolism
8		*N*-Acetylglutamate	↑	plasma/urine	Glutamate pathway (link with urea cycle)
9		Choline	↑	plasma/urine	Glycine, serine and threonine metabolism
10		Threonine	↑	plasma/urine	AA metabolism
11		Valerylglycine	↓	plasma/urine	AA metabolism
12		Alanine	↑	plasma/urine	Glycolysis, alanine and aspartate metabolism
13		2-Oxoadipate	↑	plasma/urine	Lysine degradation
14		Lysine	↑	plasma/urine	biosynthesis
15		Glutaric acid	↓	plasma/urine	Lysine degradation, fatty acid metabolism
16		Methionine	↑	plasma/urine	Methionine metabolism
17		Taurine	↑	plasma/urine	Taurine and hypotaurine metabolism
18		Tyrosine	↑	plasma/urine	Tryptophan metabolism
19		Indoxyl sulfate	↓	plasma/serum	Tyrosine metabolism
20		Citrulline	↑	plasma/serum	Urea cycle
21		L-Argininosuccinic acid	↑	plasma/serum	
22		*N*-Acetyl citrulline	↑	plasma/serum	
23		Ornithine	↑	plasma/serum	
24		Isobutyrlglycine	↓	plasma/serum	val, leu and ileu degradation
25		Isovalerate	↓	plasma/serum	
26		Isovalerylglycine	↓	plasma/serum	
27		Methylmalonate	↓	plasma/serum	
28		Valine	↑	plasma/serum	

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
29		Glutamylvaline	↑	plasma/serum	dipeptide metabolism
30		Gamma-glutamylisoleucine	↑	plasma/serum	*g*-glutamyl metabolism
31		3-hydroxybutyrate (BHB)	↑	plasma/serum	ketone bodies degradation
32		Phenylacetylglutamine	↑	plasma/serum	Phenylalanine and tyrosine degradation
33		Phenylalanine	↑	plasma/serum	Phenylalanine and tyrosine degradation
34		Homocitrulline	↑	plasma/serum	Urea cycle
35		Phenylacetylglutamine	↑	plasma/serum	Dipeptide
36		Glutamylvaline	↑	plasma/serum	Saturated fatty acids
37		Gamma-glutamylisoleucine	↑	plasma/serum	*g*-glutamyl metabolism
38		*N*-acetylalanine	↑	plasma/serum	BCAA metabolism
39		Cysteine	↓	plasma/serum	Amino-sugars metabolism
40		Leucine	↑	plasma/serum	AA metabolism
41		2-ketoisocaproic acid and 2-hydroxybutanoic	↑	plasma/serum	Leucine and methionine metabolism
42		cystine	↑	plasma/serum	AA metabolism
43		Histidine	↑	plasma/serum	AA metabolism
44		Lysine/serine/aspergine	↓	plasma/serum	AA metabolism
45		5-L-Glutamyl-taurine	↑	Urine	AA metabolism
46		4-Oxoproline	↑	Urine	AA metabolism
47		L-Valine	↑	Urine	AA metabolism
48		*N*-formylproline	↑	Urine	AA metabolism
49		*N*-(3-hydroxybenzoyl)glycine	↑	Urine	AA metabolism
50		3-Hydroxyphenylacetic acid	↑	Urine	AA metabolism
51		Glucuronide compound	↑	Urine	AAmetabolism
52		d-Glutamicacid	↑	Urine	Amino acids metabolism
53		Glutamine	↓	plasma/serum	Amino acids metabolism
54		2-aminoadipic acid	↑	plasma/serum	Tryptophan metabolism

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
55	(Acyl)carnitines	Total carnitine	↑	plasma/serum	Mitochondrial fatty acids metabolism
56		Free Carnitine	↑	plasma/serum	
57		Acetylcarnitine (C2)	↓	plasma	
58		Propionylcarnitine (C3), C14:2 and C18 acylcarnitines	↓	plasma	
59		Hexanoylcarnitine (C6), Octanoylcarnitine (C8), Decanoylcarnitine (C10), Myristoylcarnitine (C14)	↑	plasma	
60		Malonylcarnitine, Oleoylcarnitine (C18:1)			
61		Suberoylcarnitine (C8-dicarb)	↑	plasma	
62		Summed C10-C14 acylcarnitines	↑	plasma	
63		2-methylbutyroylcarnitine	↑	plasma	
64		3-dehydroxycarnitine	↑	plasma	
65		Butyrylcarnitine (C4)	↓	plasma	
66		Isobutyrylcarnitine	↑	plasma	
67		Valerylcarnitine	↑	plasma	
68		Isovalerylcarnitine	↑	plasma	
69		3-Hydroxy-isovalerylcarnitine	↑	plasma	
70		3-Methyl-crotonylcarnitine	↑	plasma	
71		Hexanoylcarnitine (C6)	↑	plasma	
72		Phenylacetylcarnitine	↑	plasma	
73		Phenylpropionylcarnitine	↑	plasma	
74		4-Phenyl-butyrylcarnitine	↓	plasma	
75		4-Methyl-hexanoylcarnitine	↑	plasma	
76		Octanoylcarnitine (C8)	↑	plasma	
77		cis-3,4-Methylene-heptanoylcarnitine	↑	plasma	
78		Decanoylcarnitine (C10)	↑	plasma	
79		cis-4-Decenoylcarnitine	↑	plasma	
80		cis-3,4-Methylene-nonanoylcarnitine	↑	plasma	

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
81		Lauroylcarnitine (C12)	↑	plasma	
82		Myristoylcarnitine (C14)	↑	plasma	
83		Linoleoylcarnitine (C18:2)	↑	plasma	
84		Adipoylcarnitine (C6-dicarb)	↑	plasma	
85		Suberoylcarnitine (C8-dicarb)	↑	plasma	
86		C18:2-carnitine	↑	plasma	
87		C20-carnitine	↑	plasma	
88		C20:4-carnitine	↑	plasma	
89		C26-carnitine	↑	plasma	
90	Organic acids	Malonate	↑	plasma	Fatty acids metabolism
91		Lactate	↑	plasma	Glycolysis
92		Acetate	↑	plasma	Glycolysis, ala and asp metabolism
93		Valeric acid	↑	plasma	Glycolysis, fatty acid b-oxidation
94		Formate	↑	plasma	Glyoxylate and dicarboxylate
95		N1-Methylnicotinamide	↑	plasma	Nicotinate, nicotinamide metabolism
96		N1-Methylnicotinic acid	↑	plasma	
97		Nicotinamide-n-oxide	↑	plasma	
98		*N*-Methyl-2-pyridone-5-carboxamide	↑	plasma	
99		*N*-Methyl-4-pyridone-3-carboxamide	↑	plasma	
100		3-Ureidopropanoate	↑	plasma	Purine metabolism
101		Orotate	↑	plasma	Pyrimidine metabolism
102		Isocaproyl	↓	plasma	Steroid and hormone production
103		(s)-Malate	↓	plasma/serum	TCA cycle metabolism
104		2-Oxoglutarate	↑	plasma/serum	
105		cis-Aconitate	↓	plasma/serum	
106		Citrate	↑	plasma/serum	
107		Fumarate	↑	plasma/serum	

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
108		Succinate	↑	plasma/serum	
109		m-Hydroxyphenyl propionic acid	↑	plasma/serum	Phenyl alanine metabolism (bacterial)
110		m-Hydroxyphenyl propionic acid sulfate	↑	plasma/serum	
111		Phenyl sulfate	↓	plasma/serum	
112		Hippurate	↑	plasma/serum	
113		5-Hydroxykynurenine	↑	plasma	Amino acids metabolism
114		3-deoxyarabinohexonic acid	↑	serum	Fatty acid metabolism
115		Uronic acid	↑	plasma/serum	Glucose metabolism
116		Erythronate	↑	plasma	Amino-sugars metabolism
117		Gluconic acid	↑	plasma	Carbohydrate metabolism
118		Benzoic acid	↓	plasma/urine	Phenolic metabolite
119		Acetic acid	↓	plasma/urine	Carbohydrate metabolism
120		Propionic acid	↓	plasma/urine	Carbohydrate metabolism
121		Butyric acid	↓	plasma/urine	Carbohydrate metabolism
122		Isovaleric acid	↓	plasma/urine	Carbohydrate metabolism
123		Valeric acid	↑	plasma/urine	Carbohydrate metabolism
124		Succinic acid	↑	plasma/urine	Carbohydrate metabolism
125		Formic acid	↑	plasma/urine	Carbohydrate metabolism
126		Lactic acid	↑	plasma/urine	Carbohydrate metabolism
127		Capric acid	↑	plasma/urine	Carbohydrate metabolism
128		Caprylic acid	↑	plasma/urine	Carbohydrate metabolism
129		Citrate	↑	plasma/urine	Carbohydrate metabolism
130		Ethylmalonic acid	↑	plasma/urine	Carbohydrate metabolism
131		Fumarate	↑	plasma/urine	Carbohydrate metabolism
132		Glutaric acid	↑	plasma/urine	Carbohydrate metabolism
133		Glycolic acid	↑	plasma/urine	Carbohydrate metabolism
134		β-Hydroxybutyrate	↑	plasma/urine	Carbohydrate metabolism
135		α-Hydroxybutyrate	↑	plasma/urine	Carbohydrate metabolism

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
136		2-Hydroxyisocaproic acid	↑	plasma/urine	Carbohydrate metabolism
137		α-Ketoglutarate	↑	plasma/urine	Carbohydrate metabolism
138		Lactate	↑	plasma/urine	Carbohydrate metabolism
139		Methylmalonic acid	↑	plasma/urine	Carbohydrate metabolism
140		Orotic acid	↑	plasma/urine	Carbohydrate metabolism
141		Oxalic acid	↑	plasma/urine	Carbohydrate metabolism
142		Oxaloacetate	↑	plasma/urine	Carbohydrate metabolism
143		Pyroglutamic acid	↓	plasma/urine	Carbohydrate metabolism
144		Pyruvate	↑	plasma/urine	Carbohydrate metabolism
145		Sebacic acid	↑	plasma/urine	Carbohydrate metabolism
146		Suberic acid	↓	plasma/urine	Carbohydrate metabolism
147		Succinate	↑	plasma/urine	Carbohydrate metabolism
148		Lactate	↓	plasma/urine	Carbohydrate metabolism
149		Hippuric acid	↑	plasma/urine	Carbohydrate metabolism
150		Indole-3-carboxylic acid	↑	plasma/urine	Carbohydrate metabolism
151		Phenyllactic acid	↑	urine	Carbohydrate metabolism
152		Glyoxylate	↑	urine	Energy metabolism
153		2-Hydroxybutyrate	↑	plasma/urine	Energy metabolism
154		3-Hydroxybutyrate	↑	plasma/urine	Energy metabolism
155		3-Hydroxy-3-(3-hydroxyphenyl) propanoic acid	↑	plasma/urine	Energy metabolism
156		5-Hydroxymethyl-2-furancarboxylic acid	↑	plasma/urine	Energy metabolism
157		Benzoic acid	↑	plasma/urine	Energy metabolism
158		2-Hydroxy-*N*-valerate	↓	plasma/serum	Fatty acids metabolism
159		Docosanoic acid	↑	plasma	Free fatty acid synthesis
160		2-Hydroxyvaleric acid	↑	plasma	Free fatty acid synthesis
161	Free fatty acids	C12:0	↑	plasma	
162		C14:0	↑	plasma	
163		C15:0	↑	plasma	
164		C16:0	↑	plasma	

Lipid metabolism

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
165		C16:1n-9	↑	plasma	
166		C16:1n-7	↑	plasma	
167		C18:0	↑	plasma	
168		C18:1n-9	↑	plasma	
169		C18:1n-7	↑	plasma	
170		C18:2n-6	↑	plasma	
171		C18:3n-3	↑	plasma	
172		C18:3n-6	↑	plasma	
173		C20:0	↑	plasma	
174		C20:1n-9	↑	plasma	
175		C20:2n-7	↑	plasma	
176		C20:3n-6	↑	plasma	
177		C20:4n-6	↑	plasma	
178		C20:5n-3	↓	plasma	
179		C22:1n-9	↑	plasma	
180		C22:4n-6	↑	plasma	
181		C22:5n-6	↓	plasma	
182		C22:5n-3	↑	plasma	
183		C22:6n-3	↑	plasma	
184		LysoPC 16:0, 18:0	↑	plasma	Phospholipid metabolism
185		PE C34:2, PE C36:2, PE C38:4,	↑	plasma	
186		DG 16:0/22:5, DG 16:0/22:6, DG 16:1/18:0, DG 16:1/18:1, DG 16:0/16:0, DG 18:0/18:1, DG 16:0/18:0, DG 16:0/20:4, DG 14:0/18:1, DG 16:0/20:3, and DG 18:0/18:2	↑	plasma	Phospholipid metabolism
187		LysoPC C17:0, lysoPC C18:1, LysoPC (18:2), LysoPC C20:4, Lyso C 22:6, LysoPC C18:3, LysoPC C20:5, Lyso-PC Lyso-PC C20:C36:3, Lyso-PC C38:5, Lyso-PC 40:1, Lyso-PC C18:2, Lyso-PC C34:3, Lyso-PC C42:5, Lyso-PC C40:6, Lyso-PC C44:5, Lyso-PC C44:4	↓	plasma	Phospholipid metabolism
188		phosphatidylinositol (PI) (PI 38:4, 36:2, 36:3, 34:2)	↓	plasma	Phospholipid metabolism

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
189		phosphatidylethanolamine (PE) (PE 38:6, PE 38:5, PE 38:4 and PE 36:3	↓	plasma	Phospholipid metabolism
190		Cholesteryl-β-d-glucoside	↑	plasma	Phospholipid metabolism
191		Cholesteryl-β-d-glucoside fragment	↑	plasma	Cholesterol metabolism
192		1,2 Distearyole phosphatidyle serine	↑	plasma	Cholesterol metabolism
193		Lyso PE 18:2, LysoPE (20:0/0:0), LysoPE (20:2/0:0), LysoPE (20:1/0:0)	↑	plasma	Phospholipid metabolism
194		TAG 52:1, TAG 50:0, TAG 48:1, TAG 46:1, TAG 44:1 TAG 48:0	↑	plasma	Lipids metabolism
195		PC 34:2, PC 40:1, PC 36:3, and PC 38:5	↑	plasma	
196		SM 22:0	↑	plasma	Phospholipid metabolism
197		TAG 58:10, TAG 56:9, TAG 60:12	↓	plasma	Phospholipid metabolism
198		PC 38:6, 18:2, C34:4	↓	plasma	Phospholipid metabolism
199		TAG 50:0 + TAG 58:10	↑	plasma	Lipids metabolism
200		PC 22:4/dm18:0, PCO-20:0/O-20:0, PCO-18:0/22:5,LysoPCdm16:0	↑	plasma	Phospholipid metabolism
201		LysoPCdm16:0	↑	plasma	Phospholipid metabolism
202		GlcCer (d18:0/18:0) PC (16:0/O-16:0) PC (O-14:0/18:0)	↓	plasma	Phospholipid metabolism
203		diacyl-PC36:1, PC32:1, PC40:5, and PC38:3	↑	plasma	Phospholipid metabolism
204		PC (18:2/dm16:0) PC (O-16:0/18:3) PC (O-16:0/18:3)	↑	plasma	Phospholipid metabolism
205		PC (P-16:0/18:2)	↑	plasma	Phospholipid metabolism
206		glycerophosphorylcholine [M]	↓	plasma	Glycerolipids metabolism
207		PC a C20:4 (alt) [B]	↓	plasma	Glycerolipids metabolism
208		PC aa (OH, COOH) C28:4	↓	plasma	Glycerolipids metabolism
209		PC aa C34:4	↓	plasma	Glycerolipids metabolism
210		SM C14:0, C16:1, SM C22:2, SM C18:1, dihydroceramides d18:0/C18:0, d18:0/C22:0, ceramide d18:1/C18:0	↓	plasma	Glycerolipids metabolism
211		PE aa C34:2, PE aa C36:2, PE aa C38:4	↑	plasma	Glycerolipids metabolism

Table 1. *Cont.*

Sr. no.	(Parent)-Class of Compound	Metabolites	Nature of Variation	Source	Associated Pathway
212		Gangliosides C16:0 and C18:0 and glucosylceramides (C16:0, C22:0, C24:0 and C24:1)	↑	plasma	Lipid/fatty acid metabolism
213		PC aa C34:4, PC 34:4	↓	plasma	Glycerolipids metabolism
214		arachidonate	↓	plasma	Polyene metabolism
215		myristate (14:0), palmitate (16:0), oleic acid, heptadecanoic acid, margarate (17:0), stearate (18:0), 10-heptadecenoate (17:1n7), oleate (18:1n9), linoleate (18:2n6), linoleamide (18:2n6), linolenate (18:3n3 or 6), eicosenoate (20:1n9 or 11), dihomo-alpha-linolenate (20:3n3), adrenate (22:4n6), TG 14:1/16:1/18:0, TG 16:1/16:1/16:1	↑	plasma	FA metabolism
216		cholesterol esters (CE) (CE 24:1, and CE 22:0)	↑	plasma	FA metabolism
217		2-hydroxypalmitate	↑	plasma	Medium-chain FA metabolism
218		2-hydroxystearate	↑	plasma	SFA metabolism
219		caproate (6:0), heptanoate (7:0), pelargonate (9:0)	↓	plasma	
220		10-undecenoate (11:1n1)	↓	plasma	
221		arachidonate (20:4n6)	↓	plasma	
222		3-hydroxybutanoic acid (b-hydroxybutryrate)	↑	plasma	Lipid/fatty acid metabolism
223		20-Hydroxy-leukotriene E4, 5-methoxytryptamine, Endomorphin-1	↑	plasma	Lipid/fatty acid metabolism
224		2-ketoisocaproic acid	↑	serum	Lipid/fatty acid metabolism
225		α-hydroxyisobutyric acid	↑	serum	Lipid/fatty acid metabolism
226		β-hydroxybutyric acid	↑	serum	Lipid/fatty acid metabolism
227		1-monopalmitin	↑	serum	Lipid/fatty acid metabolism
228		1-monostearin	↑	serum	Lipid/fatty acid metabolism
229		Cholic acid	↑	urine	Lipid/fatty acid metabolism
230		Suberic acid	↓	urine	Lipid/fatty acid metabolism
231		Glycocholic acid	↑	urine	Bile acid metabolism
232		3,4,5-Trihydroxypentanoic acid	↑	plasma/serum	Lipid/fatty acid metabolism
233		Galactonic acid	↑	plasma/serum	Lipid/fatty acid metabolism
234		2-Hydroxyglutaric acid	↑	plasma/serum	Lipid/fatty acid metabolism

BCAA: the first report of BCAA (i.e., valine, isoleucine, and leucine) correlation with insulin resistance, impaired insulin signaling and diabetes appeared in 1970 [30]. Since then, an overwhelming number of published data have advocated the predictive and pathogenic relationship of increasing plasma BCAA concentration with obesity, insulin insensitivity and diabetes. The literature assertions of higher plasma BCAA levels are mainly due to the concept of insulin resistance resulting from the activation of the rapamycin molecular target (mTOR). It is currently poorly understood which mechanisms are involved in increasing the BCAA level. However, most of the mechanistic work on this topic cited the downregulation of mitochondrial branched-chain keto acid dehydrogenase (BCKDH) and branched-chain aminotransferase (BCATm) expression followed by under-transamination and thereafter under-decarboxylation and under-dehydration of BCAA [31]. Many in vitro and in vivo studies stated that increasing BCAA (especially leucine) encouraged the insulin resistance by the activation of mTORC1 and S6 kinase followed by the phosphorylation of insulin receptor substrates S1 and S2. The deprivation of individual BCAA can promote the activity of mTORC1/S6K and adenosine monophosphate-activated protein kinase (AMPK) signaling pathways resulting in improved insulin sensitivity [32,33]. The prospective roles of reporting biomarker BCAA + aromatic AA in the pathogenesis of T2DM were further investigated in a Finnish cohort study stating the BCCA + aromatic AA as an early predictor of insulin resistance in young Finnish adults after 6 years of follow-up study [34]. A recent cohort study on 3000 volunteers found BCAA to be a valid indicator of the future risk of DM [20]. Various prospective, case-controlled and nested studies on the subjects of different ethnicities found elevated levels of BCAA in the pre-diabetic, insulin-resistant and T2DM subjects [35,36]. The longitudinal nested control-case study conducted on UK Caucasians found three BCAA as early predictors of T2DM risk [17]. A 12-year follow-up study found 2.5- to 3.5-fold higher odds of T2DM risk in the top quartiles of individuals as compared to those individuals whose plasma amino acids were in the lowest quartiles. The adjustments of predictive models for parental history, dietary variations and serum triglycerides further elevated the odds ratios of metabolites, especially amino acids. The replication analysis with Malmo and cancer diets also established a substantial relationship of four amino acids (leucine, tyrosine, valine, and phenylalanine) with incidence of diabetes. In Malmo diets, the three amino acid combination (isoleucine, tyrosine, phenylalanine) quadrupled the incident of diabetes compared to lowest quartiles [17]. Tillin et al. [19] conducted a multi-ethnic cohort study for a 19 year period of follow-up and witnessed that 14–35% of the population who had shown higher BCAA level at baseline developed T2DM. The logistic regression results of this study clearly bespoke about the obvious involvement of BCAA (odds ratio (OR) = 3.14 to 3.36) in the development of diabetes. Chen and his colleagues also reported that after 10 years of follow-up, a higher positive correlation was detected between the baseline five BCAA and incidence of DM in the understudied Chinese population [37]. The seven years of follow up in the EPIC-Potsdam cohort study found a positive correlation between future DM risk and valine, isoleucine and leucine [28]. A similar study was also performed on the Chinese Han ethnic folks who are among the highest diabetes vulnerable group [38]. This study verified the prediction-ability of a chosen model and proposed higher levels of alanine, lactate, β-hydroxybutyric acid, phosphate, leucine, α-hydroxyisobutyric acid, serine, isoleucine, palmitic acid, pyroglutamic acid, stearic acid, oleic acid, 1-monopalmitin, arachidonic acid and 1-monostearin while substantial lower levels of 2-ketoisocaproic acid as early biomarkers of T2DM. Similarly, Fiehn et al. [35] noted a 50% higher concentration of plasma leucine with a 27% higher amount of its catabolic secondary metabolite called 2-ketoisocaproic acid (α-ketoisocaproate). The mean concentration of plasma valine was 20% higher in pre-diabetic weight/age matched African-American ethnical subjects. The enrichment of the plasma AA pool with valine and leucine is also strongly correlated with the plasma acetyl-carnitine concentration. The AA score (sum of BCAA + aromatic AA) was found to be in association with baseline insulin resistance/HOMA-IR even after the adjustment for metabolic factors. The magnitude of this association was found to be more pronounced for men than for women. The authors stated that both the BCAA + aromatic AA were strong predictors of insulin insensitivity in men, whereas only valine, leucine and phenylalanine showed a positive

correlation for HOMA-IR in women [22,35,39]. Stancakova et al. [22] observed that among all BCAA, isoleucine was found to be strongest and most reliable predictor of insulin resistance. Some studies concluded that fasting plasma BCAA levels are a reliable predictor of T2DM, whereas circulating BCAA correlated positively with the indices of uncontrolled blood glucose and insulin resistance in overweight individuals [40]. Some studies also documented the correlation of plasma BCAA is only significant in prediabetic obese subjects who started losing significance upon losing weight and improving insulin sensitivity [41]. Other work has also stated the BCAA is solely responsible for insulin resistance, at least under the circumstances of high tissue FA availability and in high-fat feeding conditions [33]. With respect to age, no obvious difference was noted in the correlation of BCAA with insulin resistance between youth and adults' subjects [42]. Although an ethnicity based studies came up with some mixed conclusions. The cohort studies on the Asian population experienced and supported BCAA as a validated biomarker for the prognosis and development of T2DM [19,43,44], whilst, at the same time a predictive model study in American-Indians failed to developed a reporter BCAA notion [45]. The difference in the predictability of BCCA for the future risk of DM in different ethnicities (Caucasian Hispanic and Africans Americans) was further investigated by Lee et al. [46] and dictated that diabetic risk was more prevalent among the Hispanic Caucasians compared to African Americans. Chen et al. [21] recently conducted plasma metabolomics on the subjects recruited from the SHDS cohort study in which they identified increased (2-folds) BCAA even at baseline between the future diabetic patients and the healthy controls. The fitting of basic and advanced regression models to these BCAA metabolites with 3–14 confounding factors verified the correlation of BCAA with the future risk of diabetes without any dependence on physical activity factors. The discrimination between the healthy and diabetic groups was also evident in the area-under-curve (AUC) interpretations of these BCAA. As expected, the regrouping of diabetic and healthy controls also exhibited a strong relationship of BCAA with diabetes in diabetic people with fold change (FC) >2 and odds ratio (OR) >1.5 [21]. Subsequently, the correlation of the worsening of metabolic control of glucose with the BCAA was investigated, which was important to confirm further the BCAA status of a validated early biomarker of diabetes. The authors used the UCD-T2DM rat models, which were homozygous for β-cell defects with diabetes-prone obese ancestors [41,47]. Piccolo and his colleagues asserted that metabolites of BCAA are more robust markers of insulin resistance than BCAA themselves. 2-ketoisocaproate and 2-hydroxybutanoic, the metabolites of leucine and methionine/cysteine respectively, have been implicated as the most reliable markers of diabetes risk as these metabolites were lower before the onset of insulin resistance and their level increased just after 3 weeks of detected metabolic insulin resistance. Besides BCAA, all other gluconeogenic and ketogenic amino acids (i.e., alanine, glycine, methionine, serine, thrionine, trypsine and ornithine) were found to have been reduced by 16–36% in the pre-diabetic UCD-T2DM rat model. The plasma BCAA correlation with total fasting plasma glucose, adiposity and insulin become more significant with the worsening of the metabolic control of glucose [41,47]. In addition to the work of Piccolo et al. [41,47], many metabolomics studies also established the fact that catabolic products of BCAA also have equal predictive qualities to BCAA. The BCAA-derived short-branched fatty acid and branched-chain keto acids are new to this list. The odd carbon number acylcarnitines, another BCAA breakdown products, are also considered as the latest reported biomarker of insulin resistance development [48,49]. However, the only disadvantage with BCAA-derived metabolites is having a lower plasma/tissue concentration and stability than BCAA, resulting in higher analytical variations. The intake of BCAA with a high-fat diet for 9–16 weeks increased (2.3- to 3.1-fold) subcutaneous/visceral fat mass and respiratory quotient (RQ), HOMA-IR in obese Wister rats followed by up-regulation of 14 energy metabolism-related hormones (glucagon-like peptide-1 (GLP-1), amylin, pancreatic polypeptide, resistin and insulin-like growth factor binding protein-3-IGFBP-3). The evaluation of BCAA related metabolites and HOMA showed a linear correlation, which signified the BCAA responsible for obesity-related morbidities. Like HFD, BCAA diets contributed equally towards the development of insulin resistance as HFD, HFD + BCAAA and BCAA diets impaired the phosphorylation of AKT/protein kinase B in individual experiments.

A BCAA rich diet equally raised insulin resistance and weight gain like HF diets, but the addition of BCAA in HF diets reduced food intake [32,50]. Please note that diabetic and obesity-promoting effects of BCAA are only possible when all three BCAA were used in combination and supplementation of single BCAA usually does not increase the insulin resistance [51]. Many studies documented a reduced level of other AA after the increase of BCAA and aromatic AA. In this context, the most favorable idea is that higher BCAA plasma levels (in insulin-resistant cases) markedly reduce the catabolism in main tissues which, in turn, limits the obligatory AA concentrations in tissues considered responsible for normal metabolism. There are also many reports in the literature supporting this school of thought and indicating the reduced expression and activities of two initial catabolic BCAA enzymes (branched-chain amino acid aminotransferase-BCATm branched-chain a-ketoacid dehydrogenase-BCKD) in the liver and adipose tissue. T2DM patients also showed a 20% less whole-body clearance of BCAA [52,53]. Literature has also shown the declining outcome of the impairment of BCAA oxidation or turnover in T2DM or obese people [54]. In fact, additional work is needed to find the exact reasons for elevated levels of BCAA (if any) and to track down the fate of proteins and BCAA metabolism in diabetic and obese subjects. The plasma biotin status is also crucial for catabolic carboxylation enzymes of cysteine, TCA cycle anaplerosis and BCAA. Low biotin levels were found in insulin resistant patients with higher 2-hydroxybutyric acid (2-HB) metabolite. The higher 2-HB concentration induced the dysfunctional bioactivity of biotin tissues which in turn affects the cysteine/BCAA/TCA cycle anaplerosis metabolism [35]. In summary, the plasma concentration of BCAA has a positive relation with the future risk of T2DM. The comparative predictive-power of BCAA may vary with leucine and isoleucine cited as relatively the most powerful reporter BCAA biomarker of T2DM. The level of BCAA enabling this group of metabolites to be considered as a reliable biomarkers needs to be defined with respect to sex and ethnicities. The intake of BCAA could pose serious health problems in individuals with unknown insulinemia, dyslipidemia and glycemia.

4. Biomarkers of Disturbed Lipid Metabolism

Homeostasis of lipid metabolism is a tightly regulated act at various molecular and cellular levels in healthy subjects whereas obesity is the central risk factor for disturbances in homeostasis of lipid metabolism (hence T2DM pathogenesis) leading to the accumulation of excess fat, dysregulated glucose and lipid metabolism, impaired insulin and adipocyte signaling, and various other pathologies related to cardiovascular disease, arthrosclerosis and inflammation. This alternation in the lipid metabolism at the cellular level usually occurs years before the diagnosis of diabetes. Many studies in the literature cited this varied window-period from altered lipid metabolism to a final diagnosis of T2DM in many cohort studies related to biomarkers. These biomarkers belong to dynamic classes of lipids but we, for the convenience of readers, will state only those signature early biomarkers of T2DM risk which belong to the three main classes of lipids.

Glycerolipids and phospholipids (PL): high-density-lipoprotein-cholesterol (HDL-C), low-density-lipoprotein-cholesterol (LDL-C), triglycerides (TG), total cholesterol (TC) and BMI are the typical dyslipidemic risk factors/biomarkers for T2DM. However, recently, several cohort studies focused on the lipidomics of the subjects for finding novel biomarkers of T2DM. All these lipidomics studies found varying quantities of diacyl phosphatidylcholines (PC), glycerophospholipids (GPL), phosphatidylethanolamines (PE), alkylacyl phosphatidylcholines (PC), lysophosphatidylcholines (LysoPC), alkylacyl phosphatidylethanolamines (PE), triacylglycerols (TG), lysophosphatidylethanolamines (LysoPE), sphingomyelins (SM), cholesterol esters (ChoE) and ceramides (Cer) in the (pre)diabetic compared to non-diabetic patients [3,4,55–59]. These lipid metabolites are diabetic risk predictors in human beings. To investigate the mechanism by which these lipids contribute to the prediction of diabetic risk, many studies compared the lipidome of (pre)-diabetic persons with healthy controls in several cohort studies with a follow up of ≥5 years. A prospective case-control cohort study on European Caucasians identified 34 metabolites significantly associated with the early risk of T2DM. The risk of T2DM was positively associated

with phenylalanine, hexose, and diacyl-phosphatidylcholines (36:1, 32:1, 40:5 and 38:3), while an inverse relation was detected with sphingomyelin (16:1), glycine, acyl-alkyl-phosphatidylcholines, lysoPC (18:2) as well as PC (34:3, 42:5, 40:6, 44:5 and 44:4) (Table 1) [28]. The unsupervised PCA divided the metabolites into two metabolic factors. Metabolite factor 1 (i.e., sphingomyelins, acyl-alkyl-phosphatidylcholines and lysophosphatidylcholines) reduced the incidence of T2DM by 69% and metabolite factor 2 (i.e., BCAA, diacyl-phosphatidylcholines, propionyl carnitine, aromatic amino acids, and hexose) increased the risk of T2DM almost 4 times. [28]. Some metabolites of phospholipid metabolism (lysophosphatidylcholine C18:2, acyl-alkyl-phosphatidylcholines, and glycine) were found in hyper-insulin-sensitivity cases whereas some phospholipid metabolism metabolites (e.g., diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, sphingomyelin C16:1) were associated with insulin resistance and less insulin secretion. It is worthy to note that choline derived phospholipids were significantly associated with the risk of T2DM [60,61]. These kinds of phospholipids also acted as a potent antioxidant to prevent the oxidation of lipoproteins and also required for the secretions of VLDL and VHDL from hepatic tissues [62]. These choline derived phospholipids are in a positive relationship with the serum HDL and any dietary deficiency of choline can lead to blood scarcity of phospholipids and hence HDL. Higher levels of acyl-alkyl-phosphatidyl choline (except diacyl-alkyl-phosphatidyl cholines) also correlated with reduced TG blood level and with improved insulin sensitivity. However, the shorter chain and saturated phosphatidylcholines are positively associated with the risk of T2DM and longer chain unsaturated phosphatidylcholines are protective against it (Table 1) [63]. Wang-Settler et al. [4] also identified low levels of LysoPC 18:2 and glycine as an early indicators of the onset of T2DM in a prospective crested case-controlled study. Suhre et al. [64,65] also identified numerous glycerophospholipids associated with the T2DM risk in the KORA F3 case-controlled cohort study. The PCs (34:4, 36:3, 38:5, and 40:1) and LysoPC (20:4) are negatively associated with diabetes. On the other hand, Pes (34:2, 36:2 and 38:4) of the same carbon chain lengths increased in diabetic subjects. The METSIM (Metabolic Syndrome in Men), a prospective population cohort study, adopted a global lipidomic profiling approach and found elevated levels of one PL cluster (LC8), 4 TG clusters (LC9 to LC12) and a decreased concentration of ether alkylacyl phospholipids (PL) cluster at baseline in undiagnosed prediabetic-progressors [58]. The dyslipidemia biomarkers (LDL-C, HDL-C, ALT and total TG) have also shown a positive correlation with the TG clusters (LC9 to LC12), whereas a negative correlation with PL cluster (LC5) and arachidonic acid containing PCs. The lipid profile of normal glucose tolerance (NGT)-non-progressors was similar to the prediabetic non-progressors both at baseline and at the end of a five-year follow-up. LysoPC, SM, highly-unsaturated LCTGs and ceramide-containing lipid clusters increased in prediabetic non-progressors compared to healthy-non-progressors [58]. Rhee and his team correlated dyslipidemia and risk assessment of diabetes incidence. A strong association of TG with a single double bond and low carbon number with the risk of T2DM has been witnessed by Rhee and his colleagues [66]. The short-chained monounsaturated TG were linked with the high prevalence rate of diabetes, whereas large carbon number monounsaturated TG were related to a reduced risk of diabetes. The multivariate adjustments of a regression model with LysoPE, PC, SM and diacylglycerols (DAG) retained the same results. A total of 9 analytes were screened after the regression analysis adjustments with respect to age, sex, BMI, fasting insulin, cholesterol and parental history. With each increment in the SD of the odds ratios of these nine metabolites, the prospects of diabetes incidence increased by 1.35–1.94-fold. The acute studies with exercise and even with administration of glipizide, have also shown that short chain unsaturated TG decreased with OGTT and long chain unsaturated TG increased (Table 1) [59,67]. The plasma levels of these TG were further corroborated with the insulin resistance. The TG levels were found to differ abruptly and differently over the course of studies consisting of 12 years. The integration of negative and positive risk factors of TG with relatively higher carbon numbers and unsaturation index improved the prediction ability of the used model [68]. However, it is still the subject of investigation whether these lipids served only as diabetes predictors or also contributed towards the pathogenesis of DM. In addition to TG, the logistic regression-based

predicting models were also proposed for identifying and predicting PL-based-biomarkers in the discovery and validation of cohort sets. These models proposed LysoPC (18:2), LysoPC (32:1), LysoPC (34:2e), TG (17:1),TG (50:5) TG (50:1), TG (18:1), TG (54:5), TG (18:2), TG (56:4) and ether lipid PC (42:6e), as validated biomarkers for early diagnosis/prediction of T2DM [58,59]. Similar kinds of outcomes have been reported by the RISC and Botnia cohort studies which described a reciprocal relationship of LysoPC (18:2) with the risk of T2DM. The fasting plasma level of LysoPC (18:2) measured at baseline independently predicted the risk of T2DM with the same power as of 2 h plasma glucose level [69]. The AusDiab cross-sectional cohort study of undiagnosed T2DM patients also showed an increased AUC for 17 lipid risk factors belong to five classes of lipids i.e., diacylglycerols (DG) (DG 16:0/22:5, 16:0/22:6, 16:1/18:0, 16:1/18:1, 16:0/16:0, 18:0/18:1, 16:0/18:0, 16:0/20:4, 14:0/18:1, 16:0/20:3, and 18:0/18:2), TG (14:1/16:1/18:0, 16:1/16:1/16:1), cholesterol esters (CE) (CE 24:1 and CE 22:0), PE 40:6 and dihydroceramide (DHC) (dhCer 18:0). The inclusion of DAG, in addition to TG and Hb1Ac, in the predicting models significantly improved the independent stratification of patients of impaired glucose tolerance (IGT) from the whole population of NGT. The incorporation of DHC, PEe and CE not only represented elevated levels of TG in potential IGT patients but also reflected separated biological processes in prediabetic patients compared to healthy ones [70]. Stahlman et al. [71] also unveiled the increased composition of DG (16:0/22:5, 16:0/16:0 and 16:0/22:6) and triacylglycerol species in the VLDL-C and LDL-C diabetic dyslipidemic women. Another cross-sectional explorative cohort study on age and health matched lean and obese (prone to T2DM) human subjects, disclosed that abundance of six metabolites varied considerably between the lean and obese persons and considered the predictors of body fat mass. The lipid metabolite (PC 42:0) was found to be abundant in the obese subjects whereas PC (32:1), PC 32:0, and PC (40:5) were higher in the lean subjects [57]. The rest of the lipid-based body fat mass predictor metabolites belong to carnitines and have been described in their respective section. The relationship of obesity with T2DM was further dissected by the metabolomics done by the team of Tulipani et al. [72]. This study unveiled the relationship of glycemic impairment with obesity based on the three lysoPC. These three lysoPC i.e., lysoPC (17:0, 18:1, and 18:2), showed a strong inverse correlation with BMI, body weight, hip circumference and waist. The levels of these lysoPC decreased in those obese subjects who were in the highest quartile i.e., more prone to develop diabetes. The serum phospholipids also showed the same nature of relationships with the dyslipidemic biomarkers however, this relationship was less significant than that of lysoPC [72]. Tulipani and his colleagues also described the elevated levels of nonpolar sphingolipids (dihydro)ceramides (d18:0/18:0 and d18:0/22:0), ceramide (d18:1/18:0) and sphingomyelin (18:0) in those human subjects which later developed T2DM. Suhre et al. [64] conducted a fully comprehensive metabolomics study on a subgroup of T2DM diabetic males (55 years old) of the KORA F3 cohort. This study described phosphatidylcholines PC (34:4) and the lysoPC (20:4) in reciprocal relationship with the risk of T2DM whereas PC with PUFA side chains i.e., PC (40:1, 36:3 and 38:5) were found to be in a positive relationship with T2DM. At the same time, PE with the same side chain length i.e., PE (34:2, 36:2 and 38:4) were found increased in the T2DM patients [64]. The individuals with single nucleotide polymorphism (SNP), another greater risk factor for T2DM, also showed elevated levels of non-esterified fatty acids (NEFAs), acylcarnitines (C2 and C3), several SM, lysoPC and PC in rs7903146 risk allele carriers. The list of metabolites which were observed down-regulated includes SM-OH (24:1), lysoPC (16:0, 16:1 and 17:0) [73]. The difference in PC level between different genotype groups was not significant; however, the unsaturated PC were down-regulated significantly in the SNP-transcription factor 7-like 2 (TCF7L2) group [73]. In an attempt to discriminate the human subjects with NGT, pre-diabetes and T2DM, Zeng et al. [74] found five classifiers metabolites, i.e. 20-hydroxy-leukotriene E4, LysoPC 20:4, 5-methoxytryptamine, Endomorphin-1 and LysoPC 20:3 between NGT and pre-diabetic groups. Similarly five other metabolites i.e., iso-valeraldehyde, linoleic acid, LysoPC (18:1), 2-pyrroloylglycine and dityrosine were found to be strong discriminators between the pre-diabetic and diabetic subjects. The plasma level of PC (18:3/20:3) was found to be increased in pre-diabetes in comparison to NGT subjects whereas PC (18:0/18:2 and 16:0/14:0) reduced in pre-diabetes

in comparison to T2DM. Various lysoPC species i.e., lysoPC (20:4, 18:3, 20:5 and 20:3) were also found to be decreased in T2DM patients in comparison to pre-diabetes [74]. Another, recently published work found altered classes of glycerophopsholipids, nucleotide and (deoxy) sugars in the large prospective nested case-controlled study in diabetic and non-diabetic patients. Out of >1300 detected metabolites only 34 were found higher in diabetic patients throughout the study period. Among lipid-based classes, only six metabolites (i.e., PC (22:4/dm18:0, O-20:0/O-20:0 and, O-18:0/22:5) as well as LysoPC (16:0)) showed strong association with T2DM risk. The individuals within the highest tertiles of these metabolites were found to be 4-fold more prone to T2DM [75]. Zhao and his colleagues conducted a cohort study on 3665 American Indians (sixty-five 3-generation and 29 two-generation families), which lasted for 5.5 years [45]. Approximately 9.3% of people became diabetic, whereas 7.5% of the population developed impaired fasting glucose during the study duration. The study found new metabolic lipid-based biomarkers which significantly can predict the risk of T2DM. A total of seven metabolites (PC (22:6/20:4), 3S-7-hydroxy-29, 39, 49, 59, 8-pentamethoxyisoflavan (HPMF), MEIR, LDYR, X-490, 2-hydroxybiphenyl (2HBP) and X-1178) were found in significant association with T2DM risk. The 2HBP and m/z ratio 1178.804 (X-1178, unknown) were found in significant positive association with T2DM risk. Whereas the other 5 detected biomarkers (HPMF, PC 22:6/20:4, two peptides Met-Glu-Ile-Arg and Leu-Asp-Tyr-Arg, and metabolite m/z ratio 490.816 X-490) were found in persons with a decreased risk of T2DM [45]. The study conducted by Conor and his colleagues [76] reported similar results as Zhao et al. [45]. This work disclosed 80–89% higher odds ratios with each rise in the SD of 2HBP and X-1178 (unknown metabolite). On the other hand, 32–42% less risk of T2DM incidence was noticed with each SD increase in HPMF, peptides and PC PC22:6/20:4. García-Fontana et al. [77] also segregated the T2DM with CVD, T2DM without CVD, and control healthy human subjects based on four phospholipids metabolites. These four metabolites were PC (16:1(9Z)/2:0), O-12:0/2:0), LysoPC (O-16:0/0:0), and LPE (18:2(9Z,12Z)). These four metabolites, belonging to three different phospholipids classes, were found decreased in concentration in the T2DM patients. While two metabolites, namely LysoPE (18:2(9Z,12Z)) and LysoPC (O-16:0) discriminated diabetic and diabetic with CVD patients. The levels of these metabolites were further decreased in the diabetic with CVD patients compared to only diabetic patients [77]. The consumption of HFD also induced the specific validated biomarker of pre-diabetes which can be used to predict future risk of T2DM. Wigger et al. [78] fed the six diabetic and obese mice models (DBA/2J, C57BL/6J, Balb/cJ, AJ, 129S2/SvPas, and AKR/J) for a period of three months to check the response of varying genetic background to lipid consumptions. In PL, 3 ceramides, 2 lactosylceramides, and 1 dihydroceramide showed a constructive relation with HOMA-IR and fasting insulin levels whilst six-lipid species showed a negative correlation with the insulin sensitivity suggesting these metabolites as early biomarkers of (pre)-diabetes owing to HFD consumption. This targeted ceramide metabolomic intervention disclosed the elevated levels of Cer (d18:1/18:0, d18:1/20:0 and d18:1/22:0) three years before the diagnosis of T2DM. The plasma concentration of dihydroceramide Cer (d18:0) was found increased 9 years ahead of T2DM occurrence. These findings were further validated by the targeted ceramide profiling in plasma of another CoLaus cohort study which also confirmed the elevated plasma Cer (d18:0) levels from baseline in the diabetic group compared to healthy ones [78]. Moreover, the oversupply of saturated fats in sedentary subjects is known to induce the accumulation of ceramides as a result of up-regulation of sphingolipids biosynthetic pathways (Figure 1) [79]. The accumulation of ceramides promoted insulin resistance by down-regulating the activity of glucose uptake facilitator Akt/PKB [80]. Among many ceramides' species, two recent and independent studies pinpointed the 16:0 ceramide as main component inducing insulin resistance [81]. The plasma lipidomic of HFD-fed diabetic C57BL/6J mice showed a significantly higher 16:0 ceramide level in the study group than in the control group mice [82]. The 16:0 ceramide expression levels were up-regulated in the liver, white adipose subcutaneous tissue, and brown adipose tissue of obese mice compared to lean mice, both on HFD. At the same time, the knockout of 16:0 ceramide in HFD fed C57BL/6J mice significantly improved insulin sensitivity, energy expenditure and glucose homeostasis [83]. The suggested mechanism of

ceramide (over)-biosynthesis involves the dysfunctionality of adipose tissues resulting in excess production of fatty acid precursors of ceramides and DAG which in turn activate the protein kinase C (PKC) notorious for halting insulin signaling in muscles and liver. The inhibition of ceramide biosynthesis also promoted the conversion of white adipose tissue to brown adipose tissue and hence improved lipid and glucose metabolism [84]. Of note, ceramides serve as building blocks of complex sphingolipids like glycosphingolipids and sphingomyelins and this involves a complex set of biochemical reactions catalyzed by serine palmitoyltransferase (SPT). The AA serine can be replaced with L-alanine to carry out this reaction which results in the formation of neurotoxic 1-deoxysphingolipids. The levels of 1-deoxysphingolipids were also found elevated in the plasma of pre-diabetic and diabetic patients. On the basis of these findings, Othman et al. [85] found that the 1-deoxysphingolipids (1-deoxysphingosine (1-deoxySO) and 1-deoxysphinganine (1-deoxySA)) can be used as early biomarkers/risk factors of T2DM. The plasma metabolomics of adult Rhesus monkeys fed on high-fat/fructose-diet for a period of 8–66 months also exhibited the same elevated levels of dihydroceramides and ceramides as obese pre-diabetic and diabetic mice [86]. The noted ceramides species which increased enormously in the pre-diabetic/diabetic include 14:0, 16:0, 22:0 and 24:0 compared to controls. Ceramides are believed to be further metabolized into sphingosine (Sph), sphingomyelin (SM), sphingosine-1-phosphate (S-1-P), sphinganine (Sa) and sphinganine-1-phosphate (Sa-1-P) (Figure 1). The plasma level of Sa and Sph were severely elevated in the diabetic monkeys whereas the levels of these metabolites remained unchanged in the prediabetic monkeys compared to healthy control monkeys. S-1-P was also found to be increased in both diabetic/prediabetic groups whereas Sa-1-P remained unchanged [86]. Gangliosides are a downstream products of ceramides and also belong to sphingolipids. The plasma levels of two gangliosides (16:0 and 18:0) and four glucosylceramides (16:0, 22:0, 24:0 and 24:1) were also found to be elevated in both the prediabetic and diabetic groups compared to controls. The Spearman's correlation analysis showed a negative relationship between the HOMA-IR and total ceramides, deoxy-sphinganine and 14:0, 16:0, 22:0, 24:0 ceramides [86]. Similar results have been reported previously by the Huas et al. [87] who reported the elevated levels of 18:0, 20:0, 24:1 and total ceramide in the type 2 diabetic human subjects. Elevated levels of myristic, palmitic, stearic, linoleic, oleic and arachidonic acids were also pinpointed by the Xu et al. [88], however, Xu and his colleagues mentioned a decreased level of glycerophspholipids in persons in transition from NGT to IFG. This discrepancy in these results might arise due to the inclusion or exclusion of certain covariates which ultimately affected the final results. The plasma lipidome of (diabetic/non-diabetic) cynomolgus monkey also proposed different plasma polar lipids biomarkers for the prediction of T2DM. This study found elevated levels of phosphatidylglycerol (PG) and PC accompanying lowered plasma concentrations of phosphatidylinositol (PI) (PI 38:4, 36:2, 36:3, 34:2), and PE (38:6, 38:5, 38:4 and 36:3) [89].

Gestational diabetes (GDM) is a condition of high-blood-sugar in healthy pregnant women. GDM affects 3–9% of global pregnancies and women with GDM are considered at an increased risk of developing T2DM. The TG, PL and TC are found to increase in the last trimester of pregnancy. In pregnant GDM subjects, the relative levels of TG were higher than the normo-glycemic pregnant women [90]. Lu and his colleagues [91] identified five positively correlated predictor lipid species i.e., TG (48:1), TG (51:1), and PC (32:1) and two negatively correlated i.e., choline ether phospholipid (PCae) (40:4) and PCae (40:3) with post-load glucose levels. After the adjustment of maternal BMI, age and correction of multiple testing, only the PCae (40:4) were found to be significantly associated with GDM [92]. The correlation of TG (48:1), TG (51:1), and PC (32:1) with T2DM has also been reported previously in the Framingham cohort study which described the elevated levels of these three lipid species in diabetic AusDiab subjects [92]. The presence of these three lipid species which possess a single double bond also implies the existence of monounsaturated fatty acids (MUFA) (i.e., palmitoleate and oleate) in the LCMS spectra. In another cohort study, the hepatic formation of palmitoleate and oleate and circulating plasma palmitoleate and oleate levels have been linked to T2DM risk [93]. Recently, Petry et al. [94] also explored the paternally transmitted genotype and maternal

lipid metabolomics revealing considerable associations of TG (44:1) with maternally transmitted fetal imprinted alleles affecting the maternal glucose metabolism during pregnancy starting from the end of the first trimester. This lipid was found associated with insulin resistance in the Framingham Offspring Study too [66,68]. The abundance of TG (44:1) around the 15th week of pregnancy was seen as a risk factor for GDM. Furthermore, a strong correlation was found between the HOMA-IR and TG (44:1) in the DISCOVERY cohort study too. Increased serum levels of total fatty acids, TG, linoleic, arachidonic, esterified cholesterol, glycolytic and Krebs cycle metabolites, 1,5-anhydroglucitol, glucose, palmitoleic, FA derivatives, lysophospholipids, taurine-bile acids and docohexaenoic acids were also witnessed in GDM females compared to NGT females [95,96]. The data-driven approach also identifies distinguishing phospholipids i.e., (LysoPC (16:2), PC (36:3), PG (40:5), PC (48:1), LysoPC (18:0), PC (19:0), PC (32:3), LysoPC (16:0), PC (14:1/dm16:0), PE (15:0/dm18:1) PC (34:6), PC (36:1) and LysoPC (17:0)), long-chain/short-chain fatty acids (LCFA/SCFA) among pregnant women with GDM history, women with NGT (control) and women with NGT but in the upper quartile of glycemic distribution. The metabolites of phospholipids and LCFA/SCFA were higher in the control group compared to the group at the upper quartile. Comparing the GDM group with the upper quartile group, 72 unique metabolic features were identified in which 2-oxoglutaramate metabolite was twice as abundant in GDM than those in the upper quartile group [97]. A nested, pair-matched, case-control study on the GDM women of the Study of Women, Infant Feeding and Type 2 Diabetes after GDM Pregnancy (SWIFT) cohort participants successfully developed a prediction model of GDM-to-T2DM transition, with 83% discrimination power (AUC), comprising of a four-structure metabolic signature (a) hexoses, (b) PC (40:5), (c) BCAA, and (d) SM (14:1) (OH) [98]. The GDM pregnant women of the SWIFT cohort also exhibited lowered levels of PC and sphingolipids than normal healthy controls [99]. Additionally, smaller nested case-controlled study also proposed another validated prediction model consisting of six general dyslipidemic risk factors and three polar lipid metabolites i.e., phosphatidylserine (PS) 38:4, PE (P-36:2) and cholesteryl ester (CE) 20:4 [100]. The univariate receiver operating characteristic (ROC) analysis in a nested, case-controlled, pair-matched study of Asian and Hispanic origin women discovered the elevated levels of fasting triacyglycerlas (TAG) at baseline in those subjects who developed T2DM in a follow up of two years [101]. The multivariate-ROC analysis deliberately exhibited 12 lipid metabolites belong to TAG, ceramide, NEFA, lactosylceramide (LCer), LPC, LPE, PE and SM classes of lipids. The TAG (myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), α-linolenic acid, linoleic acid, dihomo-γ-linoleic acid (20:3), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:5)) levels were increased in the newly diagnosed T2DM females whom progressed from GDM while the rest of lipid metabolites decreased compared to controls. The lactosylceramides (LCer), ceramides and SM which were found decreased in the newly diagnosed T2DM females were LCer (16:0), LCer (24:1), Cer (16:0), Cer (20:0), Cer (22:0), Cer (24:1) and SM (20:1) [101]. These findings of Khan et al. [102] are in contradiction to the previous cohort findings (as described above) in respect to levels of ceramides and other sphingolipids. The reason for this conflict may be due to the inclusion of obesity as a covariate in their statistical analysis while Khan and his colleagues controlled the obesity covariates by pair-matching of BMI in the population. In short, most of PL are considered validated and reliable future risk biomarkers of T2DM. The inclusion and consideration of a positive relationship of short-chained-monounsaturated TG with T2DM also improved the predicting-power of models and came up as unswerving biomarkers. LysoPC and LysoPE were found in a reverse relationship with the occurrence of T2DM except in SNP-diabetes. TG, LCFA, SM, ChoE, and Cer also increased in concentration and showed a direct correlation with the onset of T2DM. In the case of PC, it is difficult to generalize the results for this highly diverse class of PL. Most PC are also increased in the prediabetic state, however, depending on the nature and function of individual PC metabolite, few PC compounds are also found in reciprocal relationship with the risk of PC. The risk factor biomarkers of GDM are different than the prediabetes biomarkers.

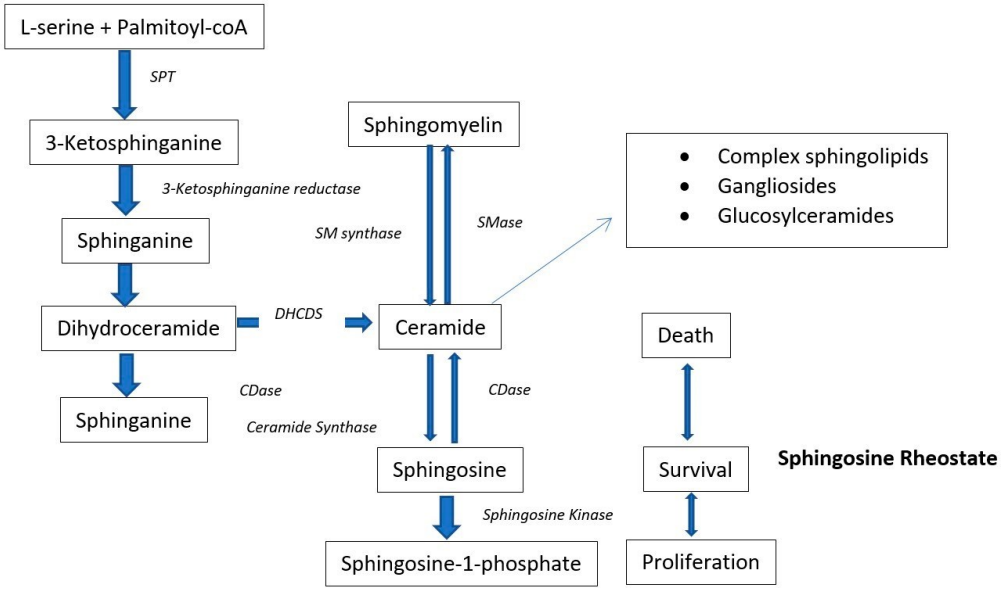

Figure 1. Schematic presentation of the de novo biosynthesis of ceramide and ceramide metabolism. SMase, sphingomyelinase; CDase, ceramidase; SM synthase, sphingomyelin synthase. (Modified from: Brozinick, Hawkins [87]).

Acylcarnitines: acylcarnitines were also found to be potent early reporters of impaired glucose tolerance or otherwise T2DM risk. They are usually generated during the esterification of fatty acids (FA) as per requirement of transporting the FA into mitochondria. The disturbed mitochondrial bioenergetics or mitochondrial stress leads to mitochondrial dysfunctioning which is another phenotype of pre-diabetes leading to a build up of these FA and FA-derived metabolites resulting in incomplete FA oxidation [103,104]. Based on these findings, it was proposed that incomplete oxidation of FA prior to the IR may provide an opportunity to explore novel biomarkers of diabetes. Additionally, carnitines are considered solely responsible for the transportation of LCFA across the inner membranes of mitochondria for β-oxidation and incomplete oxidation of these carnitines produces an intermediated carnitines oxidation called acylcarnitines [103]. Malonyl-CoA, a precursor of malonylcarnitine, plays a significant role in the completion of β-oxidation of fatty acids by inhibiting the expression of carnitine palmitoyltransferase I. The expression level of Malonyl-CoA was found to decrease in the obese and diabetic subjects leading to incomplete β-oxidation of FA and generating acylcarnitines [105]. Furthermore, cellular lipotoxicity also happened due to excessive accumulations of these partially oxidized FA in livers, muscles, adipocytes andpancreatic β-cells paving the way to IR and loss of pancreatic β-cells function. This recognized pre-diabetic state is followed by the less disintegration of LCFA and blunted oxidation of carbohydrates at a cellular level. This event is further accompanied by the imbalance of accumulating LCFA and cellular oxidative capacity leading towards the pile up of lipid-derived moieties (especially carnitines, ceramides, diacylglycerol) which attenuate the insulin signaling by activating the protein kinase C enzymes, and inhibiting Vakt/PKB murine thymoma viral oncogene homolog/protein kinase B respectively. The perturbation of LCFA catabolism in mitochondria leads to the build up of SCFA which aids in the pro-inflammatory cascades and insulin resistance [35,106].

Elevated plasma/serum circulating levels of several SC acylcarnitines (C2, C3, C4, C5, C6, C8), medium chained acylcarnitines (C10, C10:1, C12, C12:1, C14, C14:1) and LC acylcarnitines (C16, C18, C18:1, C20) have already been reported in the pre-diabetic, glucose intolerant and established diabetic cases [55,104]. Many studies focused on identifying these incomplete oxidized LCFA products to set up the signature biomarkers for IR or T2DM [35,106]. The plasma metabolic fingerprinting of weight/age-matched diabetes/non-diabetic African-American women with/without uncoupling protein 3 (UCP3) (missense polymorphism, g/g or g/a), that reduced the oxidation of LCFA, revealed two

acylcarnitines namely glutamate and 2-oxoglutarate (α-ketoglutarate) were found to be substantially increased in diabetic women with a g/g allele. The already perturbed BCAA and cysteine catabolism also contributed to the stressful anaplerotic process related to IR as isoleucine, leucine, valine and cysteine are the precursors of succinyl-CoA and succinate. Concurrent with this concept, enrichment of plasma valine occurred with the reduction of propionylcarnitine accompanying worsening of blood glucose control and plasma accumulation of acetylcarnitines [35]. Adams and his team [106] discretely found 42 acylcarnitines and free carnitine in overweight, obese diabetic and non-diabetic subjects with or without UCP3 g/g or g/a polymorphism. Two carnitines were found in different amounts with respect to genotype in g/g and g/a polymorphic subjects. In diabetic polymorphic subjects, C12 carnitines were higher in g/g subjects compared to g/g diabetic patients. Non-diabetic polymorphic patients showed more variation in butyrylcarnitine with 57% reduced content in g/a participants. The concentrations of lactate carnitines were lowered in both polymorphic non-diabetic and diabetic subjects. Irrespective of genotype factors, total carnitines:carnitine ratios rose up to 150–170% in diabetic patients. Acetylcarnitine was the most abundant carnitine moiety which also rose to 157% in diabetic patients (Table 1). Among the medium-chain (MC) carnitines, the level of C6–C10 carnitines rose to 300% in T2DM patients accompanied by a 36% reduction in propionylcarnitine concentration. The blood level of only one carnitine called propionylcarnitine was found in inverse relation with the glucose level in blood [106]. A cross-sectional prospective cohort study with subjects from Nutrition and Health of Aging Population in China (NHAPC) identified that individuals who developed T2DM in the follow-up of 6 years showed higher baseline plasma concentrations of SC, MC and LC acylcarnitines [107]. A strong correlation has also been found to exist between these (especially LC) acylcarnitines and (baseline) fasting blood sugar, metabolic traits, HbA1c and insulin resistance. Upon classification of acylcarnitines, only LC acylcarnitines have shown a strong association with the future risk of T2DM. The per unit increase in SD of the odds ratios of these acylcarnitines showed a 2.48- to 9.41-fold increase in risk ratio (RR) for the individuals in upper quartile. The results presented by Sun et al. [107] only presented the correlation of baseline acylcarnitines and T2DM in Asian populations so recently these findings were retested and confirmed by the another nested, case-controlled cohort study of the PREDIMED (Mediterranean Diet in the Primary Pprevention of Cardiovascular Disease) framework [108]. This study demonstrated that SC acylcarnitines (C2, C3, C4OH, C5, and C6) and MC acylcarnitines (C16, C12) more firmly predict the future risk of T2DM compared to LC acylcarnitines (C18, C18:1, and C20). Additionally, the correction of *p* and the adjustment of baseline plasma glucose resulted in the strongest positive association of C5 acylcarnitines with the future risk of diabetes. The authors also measured the per annum changes in the acylcarnitines level which were in line with the baseline results. The per unit increase in the SD of C3, C4OH and C5 carnitines increased the risk ratio of future T2DM up to 44% [108]. Another case control study was conducted on the American population (sedentary lean, obese with glucose intolerance and obese with T2DM) targeting only 46 compounds of acylcarnitines. The authors noted an obvious increase of saturated and unsaturated LC acylcarnitines, C14–OH– and C16–OH–CN, and plasma free acylcarnitines both in obese + T2DM and obese + glucose intolerant subjects relative to sedentary lean non-diabetic people. In relation to pre-diabetic and lean non-diabetic individuals, the T2DM people with obesity were found to have increased plasma levels of SC and MC acylcarnitines [109]. The lean diabetic patients were found to have significantly elevated plasma levels of C4– and C6–CN acylcarnitines whereas obese diabetic subjects were found to have higher plasma levels of C4-dicarboxylcarnitine (C4 DC–CN). Regarding gender, two notable exceptions were C3 and C5 acylcarnitines which were found to be higher only in obese diabetic men compared to obese diabetic women [108]. Another two human clinical trials also described the acylcarnitine pattern in obese and lean persons with/without diabetes suggesting short chained acylcarnitines as a reliable biomarker of future risk of T2DM in both sexes [32,106]. But C3 acylcarnitines were increased in obese diabetic subjects compared to C3 acylcarnitines levels in obese but yet non-diabetic subjects [32,106]. The untargeted metabolomics study on the plasma samples of 578 Swedish men recruited under the framework of the Uppsala Longitudinal Study of Adult Men

(ULSAM) [60] detected four acylcarnitines raised in the pre-diabetic subjects and among these four acylcarnitines, two MC acylcarnitines (C10, and C12 carnitines) have been found to be involved in the early prediction and pathogenesis of insulin resistance [110]. The findings of another published work found that MC acylcarnitines began to increase before the LC acylcarnitines, therefore, MC acylcarnitines' altered levels are more potent early signs of mitochondrial dysfunctions and disease progression. Recently, Libert et al. [111] also explored the possible plasma acylcarnitines difference in metabolic-well-but-lean, overall-metabolic-well-but-obese, metabolic-unwell-and-prediabetic and diabetic-obese human subjects. The data of this study clearly mentioned the increased plasma level of SC acylcarnitines and ratio of SC acylcarnitines: total acylcarnitines in overall-metabolic-well-but-obese, metabolic-unwell-and-prediabetic and diabetic-obese human subjects compared to metabolic-well-but-lean. Libert and his colleagues [111] found elevated levels of 3-OH-butyrylcarnitine and 3-hydroxybutyrate in metabolic-unwell-and-prediabetic and diabetic-obese human subjects compared to metabolic-well-but-lean control subjects. A few previous studies also mentioned the increased plasma level of malonylcarnitine or sum of 3-OHbutyrylcarnitine and malonylcarnitine in prediabetic and diabetic subjects [55,107]. It is worth adding here that Zhang and his colleagues also added contradictory results which showed that human subjects with NGT, pre-diabetes and newly diagnosed T2DM could not be differentiated merely on the basis of short-chain and medium-chain acylcarnitines [56]. The authors noted higher concentrations of LC carnitine esters (i.e., carnitine C22, palmitoylcarnitine C16, carnitine C20, 3-OH-hexadecanoylcarnitine C16-OH and carnitine C24) in the newly diagnosed pre-diabetes group and T2DM groups. The concentration of free acylcarnitines was significantly higher in the pre-diabetic (25.33 mmol/L) and newly diagnosed diabetic subjects (25.33 mmol/L) compared to subjects with normal glucose tolerance (20.28 mmol/L) (Table 1). The correlation of serum acylcarnitines with prediabetic states such as impaired glucose tolerance (IGT) and isolated impaired fasting glycaemia (IFG) were differentiated depending on serum concentrations of acetylcarnitine. The serum concentrations of acetylcarnitine (C2), tetradecenoylcarnitine (C14:1) and octadecenoylcarnitine (C18:1), were found to have a positive correlation with the IGT whilst C2, hexanoylcarnitine (C6), octenoylcarnitine (C8:1), decenoylcarnitine (C10:1), malonylcarnitine/hydroxybutyrylcarnitine (C3DC + C4OH), hydroxyhexadecanoylcarnitine (C16OH) and tetradecenoylcarnitine (C14:1), were found in significantly higher levels in the T2DM patients. The two groups IGT and IFG were distinguished by the serum levels of tetradecadienylcarnitine (C14:2), tetradecenoylcarnitine (C14:1) and octadecenoylcarnitine (C18:1) [56]. The authors also correlated body fat with the serum (free) carnitines levels and found that a significant positive correlation exists between body fat and free carnitine and the acylcarnitines (C16:1, C8:1, C6, C5, C4, C3, C2) but negatively with C14:2 and C18 acylcarnitines [55]. Previously, a metabolomics approach was also used to reveal the correlation of body mass fat with various metabolites (especially acylcarnitines) abundance between the healthy lean and healthy obese subjects who also underwent the dietary (hypocaloric diet), bariatric surgery and physical exercise interventions. The targeted serum metabolomics showed glutamine and the C18:1, C18:2, C14:1-OH, and C2 carnitines as varyingly abundant in the serum of the two groups. C3 carnitines (with 6 isoforms) were found to be the most powerful body fat mass related markers which were found to be up-regulated in obese subjects and remained uninfluenced by the exercise intervention. However, the low carbohydrate diet intake following bariatric surgery reduced the expression of C3 carnitines which bespeaks higher expression of C3 in body fat mass. The physical activity resulted in an increase of carnitines in both the lean and obese groups, however, this increase was found to be diminished after 24 h [57].

Urinary metabolomics using MS also revealed that diabetic and obese subjects excrete more urinary LC acylcarnitines in comparison to healthy controls [104]. Van der Kloet et al. [99] also employed a urinary metabolomics approach to differentiate progressive and non-progressive forms of albuminuria. The diabetic patients with progressive forms of albuminuria were found to have higher urinary metabolites from acidic/carboxylic acidic (i.e., benzoic acid, 5-hydroxymethyl-2-furancarboxylic acid, galactonic acid, and hippuric acid), acyl-glycines (i.e., 2-phenylacetoxy-propionyl, glycine, salicyluric

acid, and 3-methylcrotonylglycine), acylcarnitines, and tryptophan metabolism metabolite compared to non-progressive forms of albuminuria. Dellow et al. [112] also added that persons with lose glycemic control excrete more urinary acylcarnitines which might be the result of reduced renal absorption or carnitines acylation. Tamamogˇ ullari et al. [102] reaffirmed that serum levels of total and free carnitines were higher in T2DM patients having no complications rather than diabetic patients with diabetic retinopathy.

The role of acylcarnitines in progression from gestational diabetes mellitus (GDM) to T2DM has also been recently studied [103,113]. These studies noted significantly higher plasma levels of SC acylcarnitines in newly diagnosed GDM women. These works also emphasizes on MC acylcarnitines due to their unlearned role in the pathogenesis of GDM to T2DM. These MC acylcarnitines (i.e., octanoylcarnitine (C8-acylC), hexanoylcarnitine (C6-acylC), laurylcarnitine (C12-acylC), decanoylcarnitine (C10-acylC)) were also observed in the newly diagnosed GDM patients compared to NGT subjects [103,113]. Gall et al. [114] registered the decrease in MC acylcarnitines especially decanoylcarnitine in the case of insulin resistance. The population-based KORA cohort study also reported a decreased level of three metabolites namely glycine, acetylcarnitine, LPC (18:2) in GDM women [4]. The transition of GDM to T2DM was also studied in a SWIFT sub-cohort study which further added the increased level of C6 and C8 acylcarnitines in those GDM patients which transit from GDM to T2DM in a follow-up of two years [115]. Anderson et al. [97] used the data-driven approach (free of any hypothesis) and reported a decrease in the LC acylcarnitines (i.e., dodecanoyl-, octanoyl-, decanoyl-, and tetradecanoyl-) in both pregnant women groups with previous GDM history and those found in the upper quartiles of the glycemic index compared to the control group. In short, acylcarnitines are not only reliable early reporting biomarkers of DM, but can also be used to differentiate different states of metabolic syndrome such as pre-diabetic, IR, obese, SNP-diabetic, GDM and IPD. Some of acylcarnitines such as glutamate, propionylcarnitine malonylcarnitine, sum of 3-OH-butyrylcarnitine and malonylcarnitine, 2-oxoglutarate, C3, C5, C4–OH, C12, C14, C14:1, C16, C18:1, C20 carnitines, butyrylcarnitine, 3-OH-hexadecanoylcarnitine, dodecanoylcarnitine, octanoylcarnitine, decanoylcarnitine and tetradecanoyl carnitine can be readily currently employed to diagnose subjects with IFG, T2DM, IR, GDM, IPD, and IGT.

Free fatty acids (FFA): two common origins of circulating FFA are de novo lipogenesis from excessive carbohydrates and cleavage of TG in chylomicrons. The FFA metabolism dysregulation is a key event in the emergence of insulin resistance and Randle et al. [116] proposed the preferential oxidation of FFA over glucose as a major episode leading to metabolic syndrome. The significant correlation of plasma concentration of FFA with dyslipidemia and T2DM was seen for the first time and confirmed in the studies of Jones et al. [117] and Taylor et al. [118]. These studies reported significant alterations in the plasma levels of LCFA between the diabetic and control groups. Later on, some studies also suggested the involvement of altered plasma FFA levels in influencing insulin sensitivity and impaired glucose metabolism. The saturated FA (SFA) including palmitic and stearic acid were found in positive relation with glucose intolerance, impaired insulin sensitivity, impaired insulin secretion and HbA1c. The unsaturated oleic acid was a biomarker of inadequate diabetes control [119]. The lipidomics studies of erythrocytes membranes showed a greater palmitate content (31.1 ± 2.4% in T2DM, 25.4 ± 3.1% in controls, $p < 0.005$) in isolated erythrocyte membranes with a higher SFA/unsaturated FA ratio affecting the fluidity of cells [120]. Grapov and his colleagues [121] investigated the relationship between NEFA and signaling lipids (oxylipins and endocannabinoids) influencing the insulin signaling, inflammation and adipose function. The authors noted a 114% increase in the circulating FFA at baseline which additionally positively correlated with the glucose intolerance in diabetic patients. The net concentrations of SFA (14:0, 16:0, 18:0, 19:0, 20:0), MUFA (16:1n7, 18:1n7, 18:1n9, 20:1n9), PUFA (18:2n6, 18:3n3, 22:4n6, and 22:5n3), and trans-FA (trans 16:1n7, trans 18:2n6) were found to be elevated in T2DM patients. The linoleic acid and α-linolenic acid-derived epoxides also increased by 47–127% and showed a positive correlation with SFA and MUFA in diabetic patients. The arachidonate-derived 14,15- and 11,12-dihydroxyeicosatrieneoates were also increased by

86% in diabetic patients. The OPLS-DA based predictive model also described a shift in the metabolic profile. Four FFA i.e., 18:1n9, 18:0, docosahexaenoyl-ethanolamide (DoHex-EA) and 22:5n6 were the main discriminating FFA between healthy and diabetic individuals. The 18:0 and 18:1n9, and 20:4n6 and 22:5n6 were the early reporters of diabetes related changes in the activity of stearoyl-CoA desaturase and LCPUFA biosynthesis [121]. The Uppsala cross-sectional cohort study found higher serum concentrations of SFA namely palmitic acid (16:0), myristic acid (14:0), and palmitoleic acid (16:1w-7) and reduced serum concentration of omega3/omega6 fatty acid in individuals who develop diabetes over the course of 10 years. The logistic regression based predicting model used in this study identified oleic acid, palmitoleic acid, dihomo-7-linolenic acid and stearic acid as contributors towards hyperglycemia and dyslipidemia [122]. The relationship of individual FFA with T2DM was further elaborated in literature by Wang et al. [123]; Lapolla et al. [124] and Yang et al. [125]. The work of Wang et al. [123] clearly elaborated that the percentage of SFA and MUFA was higher at the baseline in those people who developed T2DM over the course of 9 years in the Atherosclerosis Risk in Communities (ARIC) Study. With respect to individual FA, the findings of Wang et al. [123] are in complete agreement with the previous findings of Vessby et al. [122]. Among the cholestrolemic esterified FA, (16:0), palmitoleic (16:1n-7), and dihomo-α-linolenic (20:3n-6) were found in positive association with incidence of T2DM, whereas in PL, the C16:0 and C18:0 were in positive relationship with the T2DM occurrence [123]. Yang et al. [125] used the PCA non-linear mapping (PCA–NLM) approach and also found FFA i.e., 18:1n9, 12:0, 18:3n3, 18:1n7, 20:5n3, 20:2, 22:5, and 22:6 as early discriminators between potential T2DM patients and healthy controls. Conflicting results have also been reported in the literature stating only even-chained SFA (14:0 to 18:0) in a constructive association with development of diabetes whereas LC SFAs (20:0 to 24:0) and odd chain SFA showed opposite trends [126]. The most comprehensive work in this respect and to also quantitatively relate the FFA with T2DM was published by Tan et al. [127] who used the competitive adaptive reweighted sampling (CARS) method with PLS-DA to seek the most probable biomarkers of diabetes. This study found three FFA i.e., α-linolenic acid (18:3n-3), oleic acid (18:1n-9), and eicosapentaenoic acid (20:5n-3) as the predictive biomarkers of future T2DM risk. The elongation of FFA was also correlated with the insulin resistance and LCFA-elongase (Elov16)-led conversion of palmitate to stearate was found to play a major role in the emergence of pathogenesis of T2DM [128]. The prospective cohort study on healthy/diabetic Uyghur and Kazak Chinese distinguished T2DM individuals from healthy controls were having high 22:6 and 20:4n6, and lower 22:0, 14:1, 18:3n6, and 20:3n6 fatty acids plasma concentrations from baseline [129]. The FFA are also believed to attach with peroxisome proliferator-activated receptors (PPAR) and modulate the transcription factors contributing towards metabolic syndrome. Attaching with the PPAR prompted the proinflammatory responses and impaired endothelial function in non-pregnant healthy subjects [130]. Pankow et al. [131] stated that the correlation of high fasting FFA with the incident of T2DM is independent of sex, waist/thigh ratio, percent body fat, fasting TG concentration, and insulin-mediated glucose uptake. A more recent cohort study reported 3–4 times higher serum FFA levels amongst the newly diagnosed and long time-monitored diabetic patients whereas a moderate correlation was found in the pre-diabetic patients. The authors further recommend the measurement of serum FFA concentration in combination with existing diabetic diagonostic tools to improve the diagnostic accuracy. This study ranked the earlier diagnostic accuracy from the newly diagnosed diabetic patients to those monitored for a long time [132]. Yi et al. [133] demonstrated both esterified and non-esterified FA to screen the DM patients from healthy ones. The esterified FA were mostly storage lipids (cholesterol, TG, PL esters) which are also considered the precursors of non-esterified fatty acid. Taking into consideration other variables (body weight, age and sex), the authors identified nine potential biomarkers (total NEFAs, 24:0, 20:3, 14:0, 18:1n7, 16:1n9, 16:0, 18:1n9, 18:0) to separate the T2DM and healthy individuals. Among these FFA, the coefficients of 16:0, 18:1n9, and 18:0 were quite high in T2DM patients compared to controls [133]. The MCFA, beta-hydroxyisovalerate and arachidonates was lowered in T2DM while LCFA including essential FA linolenate and linolate was elevated in comparison to controls. This study demonstrated decreased PC (hence HDL and total

cholesterol) and increased PE (TG) contents in diabetic patients [65]. In line with these results, Gall et al. [114] also found lower levels of multiples glycerophosphocholine species which are highly associated with insulin resistance.

Furthermore, the existing evidence in literature on this topic is still inconclusive and not many studies unequivocally consider the dietary intake of carbohydrates, fats and alcohol influencing de novo lipogenesis of FA. Addressing these issues, some nutritional interventional cohort studies focused on these shortcomings and started to correlate the dietary fat intake with the elevated level of FFA and incidence of diabetes. In a large US prospective Health Professionals Follow-Up Study (HPFS), plasma metabolomics of the participants with the validated self-reported HFD consumptions found 31–52% less risk of incidence of diabetes with the increased plasma concentrations of marker dairy fat FA namely 15:0, 17:0, and *trans*-16:1n-7 [134]. The correlation of dairy fat consumption derived trans-palmitoleate concentrations with dyslipidemic biomarker LDL-C was also investigated in a multi-ethnic cohort study "Multi-Ethnic Study of Atherosclerosis". The consumption of dairy fat was found to be positively associated with an increased plasma concentration of trans-palmitoleate and this trans-palmitoleate concentration in turn positively correlated with LDL-C and negatively with fasting insulin levels and TG [135]. The increased FFA concentration interferes with the insulin signaling causing peripheral insulin resistance or reduces the accessibility of insulin to skeletal muscles ultimately reducing the glucose transport towards muscles. This lipotoxcity also harms the β-pancreatic cells resulting in the impairment of insulin secretion.

Previously, many studies have also established the relationship of FFA level with the GDM in pregnant women. The experimentally created acute elevation in the FFA caused an increase in insulin resistance in both non-pregnant/pregnant women with/without GDM/T2DM. Elevated blood levels of FFA also resulted in the up to 47% diminution of insulin-stimulated glucose uptake and glycogen synthesis [136]. More recently, the relationship of maternal individual FFA level with GDM and inflammatory response has also been studied [137]. This study indicated a positive relationship of palmitic, stearic, dihomo-γ-linolenic (DGLA), and arachidonic acids with the C-peptide level, cytokines/adipokines, and GDM. In addition to GDM, the other most undiagnosed form of prediabetes is isolated post-challenge diabetes (IPD). The interpretation of T2DM based only on fasting blood sugar fails due to the fact that IPD is often overlooked and misdiagnosed due to its normal fasting glucose levels during the screening process of T2DM, and many authors devised some metabolomics strategies to cover up this gap [138,139]. These studies focused on the FFA profile of such patients and cited concentrations of most of FFA were substantial enough to discriminate between the healthy controls, T2DM and IPD. The concentrations of FFA were lower in healthy controls than T2DM and IPD. Moreover, the three FFA (i.e., 18:1, 18:2 and 18:3) can be used as validated biomarkers for diagnosing the T2DM/IPD subjects from the healthy ones. However, to use 18:2 for screening purposes of T2DM, it is necessary to determine the concentrations of 16:0 and 18:0 too, to differentiate the healthy patients from T2DM. 16:0 can be used to distinguish T2DM and IPD individuals [138,139]. In another study, the authors stated the 15 most significantly varying metabolites among healthy, T2DM, and IPD individuals. The concentration of oleic acid, cholesteryl-β-d-glucoside, linoleic acid, 1,2-distearoyl phosphatidyl serine increased in the T2DM and IPD groups whereas other metabolites, such as lysoPE, DHEA-S, lysoPC, and 5-hydroxykynurenine were considerably lower in both of these groups as compared to healthy individuals. The concentration of three metabolites namely DHEA-S, linoleic acid and oleic acid were found to discriminate IPD and T2DM patients [28].

5. Biomarkers of Disturbed Microbiome and Microbiome-Related Metabolites

Organic acids (OA) are produced by intestinal microfloral action on sugars and proteins in addition to glycolysis, lipolysis, gluconeogenesis and AA metabolism. Therefore, it is quite important to also discuss the OA as early biomarkers of diabetes.

OA: human OA metabolites are the compounds with carbon chain length varying from C2 to C6 and majorly produced by the fermentation of carbohydrates and AA. Straight-chain OA are usually

produced by the colon-inhibited-microfloral fermentation of starchy and fibrous dietary material whereas branched-chain-OA (BCOA) are produced by bacterial metabolism of AA. The other ways of OA production are the various metabolic pathways i.e., glycolysis, lipolysis, gluconeogenesis and Krebs cycle. Any variations in intestinal microfloras' compositions usually bring about the changes in the concentrations of plasma/urine/serum/fecal OA which made these short-chain entities tremendous biomarkers of various metabolic syndromes [140]. It is estimated that human beings fulfill their 10% daily energy requirements from these OA [141] and crucial involvement of OA in metabolic procedures (e.g., mitochondrial energy production, nutrient deficiencies, free radical overload, intestinal dysbiosis, and so on) importantly relate them to various biological processes. These gut microflora-produced OA are usually absorbed in the colon to maintain the necessary redox balance and in exchange of bicarbonates. OA are also transported from the lumen to different organs where these compounds act as a substrate and are involved in energy homeostasis signaling including lipids, glucose, and cholesterol metabolism in tissues [142]. There is accumulated evidence in literature proposing the (short-chained)-OA the biomarker for DM and other health conditions [143]. Being the central metabolic regime of energy molecules, Krebs cycle is the first to be perturbed in the case of diabetes-induced enzymatic variation which after all can cause an insufficient recycling of OA. Therefore, many studies also declared OA as a marker of DM, central nervous system diseases, organic acidurias and other metabolic disorders etc. [143–146].

Three OA namely acetic acid, propionic acid and butyrate are known for their health-promoting activities. The plasma levels of these OA were found to decrease in persons prone to IR, IGT, IFG IPD, obesity and T2DM [147]. Butyrate is a more important reporter of early obesity induced metabolic syndrome in this regard as its decreased concentration was found in obese and pre-diabetic individuals. On the other hand, the increased concentration of butyrate is a symbol of well-being as it suppresses the insulin resistance and weight gain. The increased production of butyrate, by modulation of intestinal micoflora diversity, led to less increased IR and weight gain in HFD-diabetic mice [148]. The inoculation of butyrate producing gut microflora in germ-free-lean mice with metabolic syndrome also showed improved insulin sensitivity [149]. Recently, metagenomic data also supported the fact of a substantial decrease in the butyrate producing gut microbiota in obese mice [150]. The butyrate supplementation in HFD also reduced weight gain and improved insulin sensitivity in obese C57BL/6 mice [142]. Beside butyrate, propionic and acetic acids, patients prone to metabolic syndrome or diabetes were found to have up to a 14-fold higher OA (adipic acid, suberic acid, 3-hydroxyisovaleric acid, 2-hydroxybutyric acid, aceto-acidic acid, 5-hydroxyhexanoic acid, lactic acid, 3-hydroxybutyric acid, fumaric acid, 5-hydroxic hexanoic acid, 2, 3-dideoxypentonic acid) content in their urine due to the phenomenon of generous liberation of FFA from adipose tissue followed by β-oxidation of FA [143,144,146]. Interestingly, an increased concentration of most of these OA suppressed the insulin signaling by activating the free fatty acid receptor 2 (FFAR2) while simultaneously promoting the glucose/lipids metabolism in tissues and preventing the lipid accumulation in adipocyte tissues (i.e., weight loss) [149]. Moreover, immune and adipose tissues have two G-protein-coupled receptors (GPCR) (i.e., GPCR 41 and GPCR 43) for (short chained)-OA which are further linked to G-protein for further downstream targeting. Among the major four classes of G-proteins (i.e., Gi/o, Gs, G12/13, and Gq/11), each class is specified for certain GPCRs which proposed the involvement of SCOA in modulating the response of adipose/immunity tissues [151]. The existence of OA receptors on the surface of immune and adipose tissues suggested the modulatory roles of these metabolites in biological processes. The GPCR41/GPCR43 knockout mice studies showed a huge inflammatory response in various metabolic conditions due to GPCR41/GPCR43-/-immune/adipose cells [152] Whilst the supplementation of butyrate or propionate decreased the mRNA expression levels of inflammatory cytokines GPCR41/GPCR43 knockout mice. The acetate, propionate and butyrate reduced the TNF-α, cytokine and chemokine release from the monocytes and neutrophils while promoting prostaglandin E2 production. These studies concluded that SCOA has a modulatory role in the inflammatory status of obese tissues and thus in IR [153,154].

Han and his team [144] also stated a 0.1–66% increase in plasma contents of 10 OA (acetate, propionate, isobutyrate, 2-methylbutyrate, 3-methylevalerate, isovalerate, valerate, caporate, isocaporate), including three C5 and C6 isomers and two C4 isomers, in T2DM diagnosed patients. The gas chromatography–mass spectrometry (GC-MS)-based metabolomics analysis of serum OA unveiled a higher concentration of glucose intermediates i.e., 3-OH-butyric, lactic acid, adipic, succinic, citric, palmitic, and phenyl acetic acids) in diabetic subjects. The higher excretions of these OA were also cited as an indicator of underlying undiagnosed conditions belonging to metabolic syndrome. The higher excretion of adipic acid, 3-OH-butyric acid and suberic acid were synonymous to the occurrence of ketogenesis and the formation of C6–C8 fatty acids due to beta-oxidation in pre-diabetic patients [155]. The pre-diabetic patients were found to excrete higher levels of 2-OH-butyric, hydroxy-isobutyric acid and 3-OH-butyric acid and low levels of sebacic acid, whereas, obese people only excrete higher amount of suberic acid relative to non-obese and non-diabetic subjects. Higher excretion of hydroxy-isobutyric acid in diabetic patients also indicated a higher fat metabolism resulting in higher serum levels of C4 moieties [155]. Chou et al. [156] carried out the serum metabolomics using the GC-MS platform to discriminate normal, diabetic and IPD patients. This study stated sixteen early biomarkers of diabetes clearly distinguished the individuals at T2DM risk from healthy controls and nine metabolites successfully differentiating the people at risk of diabetes or IPD. Two OA biomarkers namely pyroglutamic acid and α-hydroxybutyrate (α-HB) clearly distinguished the IPD and non-IPD (healthy and T2DM), and healthy vs. IPD individuals, respectively [146,157,158]. β-HB, ethylmalonic acid, and α-HB proved to be signature biomarkers to distinguish between healthy controls and T2DM patients. Moreover, lactate and ethylmalonic acid categorized individuals at IPD and T2DM risk [156,159]. The levels of β-HB and α-HB increased in ascending order from healthy controls to IPD and diabetic patients. The α-HB has also been proposed as a biomarker of the IR and IGT [88]. Most of the detected OA metabolites were identified as TCA cycle intermediates which level was found to fluctuate mostly in diabetes, CKD and CHD. Some studies also cited altered levels of these metabolites in the cardiac and nerve tissues of diabetic persons [160]. Citrate and pyruvate were already declared in perturbed amounts in the urine of diabetic patients. The TCA intermediate succinate was found to be increased in the plasma of patients who developed T2DM [161]. The work of Yuan et al. [146] also defined SCOA (e.g., 4-aminobenzoic acid and oxyl acetic acid) as a predicting biomarker for T2DM risk. Chou et al. [156] also stated pyroglutamic acid as a promising biomarker for distinguishing IPD and healthy non-IPD patients whereas pyroglutamic acid was found decreased in diabetic patients [88]. Sato et al. [162] also observed higher concentrations of isovaleric acids in T2DM patients in a blind, randomized and case-control cohort study. Many studies have documented the higher excretion of other OA (especially uric acid) in diabetic patients compared to healthy controls [140,163,164]. These published results also claimed a higher urine OA being the main causative of uric acid stones and nephrolithiasis in diabetic subjects. The uric acid and sodium retention was also observed in insulin resistance and HOMA-IR model. It also proved a strong relation between high uric acid and the low urine pH and insulin resistance [165]. A low urinary pH can also be a validated marker of future insulin resistance as Abate et al. [166] selected his subjects merely on the basis of the presence of uric acid kidney stones. But later these subjects unveiled less insulin sensitivity and lower urinary pH. Therefore, the patients with low urinary pH are at a stage of increased risk of T2DM development. These studies successfully proposed and defended the so-called obvious correlation between low urinary pH and T2DM/obesity, however, these studies also came up with some major limitations such as ignoring the major dietary, environmental and lifestyle factors in these cross-sectional cohort studies. These factors were partially addressed by Maalouf et al. [167] who provided the fixed metabolic diet to the diabetic patients for the whole study duration. The T2DM patients showed higher net acid excretion (NAE) (NH_4^+ + TA) value which symbolized the higher net acid production in diabetic patients. However, this data not only lacks the focus of mechanisms responsible for higher acid production in diabetes but also states urinary anionic measurements being non-significantly different between diabetic and non-diabetic subjects which helped out to formulate the idea of GI alkali loss in diabetic individuals.

The accumulation of pancreatic fats which affects the bicarbonates secretion and exocrine activities may result in GI alkali loss in T2DM. Alternatively, some other studies proposed greater intestinal micoflora fermentation due to a delayed transient time in the colon in diabetic patients [168]. A low NH_4^+/NAE ratio was also noticed in diabetic patients in some studies [169] with higher TA values in diabetic patients. The impaired NH_4^+ secretion might be due to increased FA supply to renal tubules resulting in compromised NH_4^+ excretion [167]. The work of Sato et al. [162] also published amazing data about the effects of the administration of different diets and the consequent effects on organic acids in diabetic and non-diabetic patients. The diets rich in saturated fatty acid/total fat negatively interlinked with the organic acid concentration in both groups. Carbohydrates exhibited a positive association with all fecal/plasma organic acids of diabetic patients. A further negative linkage was found between the duration of diabetes and two organic acids named acetic acid and propionic acid. Isovaleric acid was the organic acid least influenced by the dietary habits of diabetic subjects [162]. Conclusively, it is also possible to distinguish the normal, pre-diabetic, IPD, obese and T2DM individuals based on the targeted serum/plasma/urine metabolomics focusing OA as early biomarkers of diabetes and or various stages of diabetes.

Human gut microbiota signature biomarkers: the microbial lives of the whole gastro-intestinal tract are collectively known as gut microflora or gut microbiota. The total number of gastro-intestinal microbiota exceeds 100 trillion which is 3-fold more than the total number of human body cells, which is why the gut environment is considered as a whole functional organ [170]. The five important and predominant phyla of gut microbiome are *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Verrucomicrobia*. The composition of these phyla keeps changing throughout the gastro-intestinal tract depending on various confounding factors such as individual lifestyle, dietary habits, health and physiological factors determining which phenotype would be developed. Regardless of this confounding drawback, gut microbial studies identified some gut microbial signatures as early biomarkers for metabolic syndromes. Moreover, the gut microflora controls many metabolic reactions by the production of many beneficial secondary metabolites (e.g., choline, phenols, bile acids, and SCFAs etc.) involved in the various metabolic signaling pathways. Recent findings have shown that gut microbiota is not only involved in maintaining optimal human health but it is also culpably involved in the pathogenesis of metabolic diseases [150,171–173].

Recent studies have focused on unveiling the effect of changed dietary habits on the composition and ecosystem of gut microflora. The consumption of fiber-deficient diets resulted in a lesser extent of fermentation in gut and hence less production of systematic anti-inflammatory short-chain fatty acids (SCFA). These SCFA are important for the synthesis and production of immunoglobulin A and immune-supportive cytokines and failure to produce these health-supporting SCFA results in dysbiosis which is in turn implicated in the increased incidence of diabetes and inflammatory diseases [174]. There have also been reports that the population of health-promoting SCFA-producing gut-bacteria found to have decreased in individuals at risk of developing diabetes [175]. SCFA also promotes the production of glucgone-like-peptide 1 (GLP1) and coheres with G-protein-coupled receptors. The production of GLP1 impedes hepatic gluconeogenesis and glucagon secretion; promotes insulin sensitivity and satiety and hence encourages weight loss. SCOA are another class of secondary metabolites generally produced by the gut microflora. It has been noted that butyrate producing gut-bacteria became less abundant followed by an increase in the population of *Lactobacillus* spp. and *Betaproteobacteria* in obesity and T2DM compared to non-obese control healthy subjects [150,168,171]. The gut microflora population also becomes deficient in the Firmicutes and Clostridia in future T2DM patients as noted by Laresn et al. [168]. The lowering of *Clostridium* in diabetic patients worsens the glucose metabolism since this species is primarily involved in the conversion of primary bile acid into secondary bile acid entities (i.e., cholic and chenodeoxycholic acids) in the large intestine [176,177]. The secondary bile acid entities actually activate the farnesoid X receptor (FXR) and G-protein-coupled receptor 1. The activation of G-protein-coupled receptor 1 ensures the release of GLP-1 which is important for proper pancreatic and hepatic functioning. FXR also controls the glucose metabolism

and weight loss maintenance by down-regulating the expression of 6-biphosphatase-1, fructose-1, glucose-6-phosphatase and gluconeogenic phosphoenolpyruvate carboxykinase [177,178]. So a lowered population of gut *Clostridium* is another signal of obesity and perturbed glucose metabolism in glucose intolerant persons. Firmicutes (Gram-positive) and Bacteroidetes (Gram-negative) bacteria comprised almost 90% of gut microflora and some studies proclaimed the ratio of Firmicutes-to-Bacteroidetes as a predictor of dietary habits and hence metabolic disorders linked to these dietary life styles. The obese mice model studies registered a high Bacteroidetes-to-Firmicutes ratio with an increased proportion of *Bacteroidetes* [179]. A positive correlation was also noted between the blood glucose level and the ratios of *Clostridium coccoides/Eubacterium rectale*, Bacteroides/Prevotella, and Bacteroidetes/Firmicutes groups. So the lowering of SCFA, SCOA and butyrate producing bacteria, *Firmicutes*, *Clostridia* population are the early biomarkers of T2DM in glucose intolerant patients. Additionally, an increased population of Gram-negative Bacteroidetes and Proteobacteria hinted their role in the pathogenesis onset of T2DM via an endotoxin-led-inflammatory response as lipopolysaccharides and endotoxins were found in higher concentration in their cell membranes [168]. Similarly, the transplantation of fecal microflora from healthy and lean individuals containing the specific gut bacteria into diabetic persons ameliorated the insulin sensitivity in the recipients. This transplantation improved the population of SCFA and SCOA producing strains of bacteria in the insulin-resistant persons [180].

In recent years, human gut microbiome data has been used in conjunction with the metabolome data to comprehensively assess the nutritional and metabolic health status of human beings. Qin et al. [150] did a metagenomic study of gut microflora exploring the metagenome association with T2DM in 345 diabetic + non-diabetic subjects. This metagenome-wide-study showed the enrichment of genes with opportunistic pathogens such as *Clostridium hathewayi*, *Bacteroides caccae*, *Clostridium symbiosum*, *Clostridium ramosum*, *E. coli*, *Eggerthella lenta* in T2DM individuals whilst SCFA, SCOA-producing bacteria, including SS3/4, *Clostridiales* sp., *Faecalibacterium prausnitzii*, *E. rectale*, *Roseburia inulinivorans*, and *Roseburia intestinalis* were found to be enriched in non-diabetic persons. Le Chatelier et al. [181] documented that low bacterial richness/low bacterial gene count favors obesity, insulin resistance and low-grade inflammation and fatty liver. The authors further added that based on the metagenomic analysis of 46 genera of low bacterial gene count and high bacterial gene count, one can distinguish the persons who are more prone to obesity/T2DM compared to those whom are less. Bacterial species such as *Ruminococcus* and *Bacteroides* species which were more dominant in the low bacterial gene count could be early predictors of metabolic disorders such obesity and T2DM whereas dominant species (*Bifidobacterium*, *Faecalibacterium prausnitzii*, *Alistipes*, *Akkermansia* and *Lactobacillus*) of high bacterial gene count are mostly associated with resistance to T2DM. This study supported the idea of a lack of SCFA and SCOA producing bacteria, mucus degrading bacteria and sulfate reducing species (*Desulfovibrio*) in the low bacterial gene count group [181]. Conflicting data were also registered by the European cohort study describing the upregulated abundances of only *A. muciniphila* in individuals with less severe metabolic syndrome, however, this upregulation in the abundance of *A. muciniphila* was concurrent with an increased microbial diversity exhibiting the population-dependent relationship of *A. muciniphila* and T2DM [171]. However, all three of the aforementioned cohort studies unanimously declared the *Lactobacillus* species, *Clostridiales* and SCFA/SCOA-producing bacteria as main early discriminants among healthy, glucose intolerant and diabetic human subjects [150,171,181]. The supplementation of *A. muciniphila* in high-fat diet-induced obese mice also increased high-glucose-tolerance and lowered inflammation by lessening the lipopolysaccharides and lipid oxidation [182]. The skewing of gut microbial population, during the early, glucose intolerant and undiagnosed stages of diabetes, affects the cell-to-cell integrity in the gut lining resulting in a leaky gut with increased permeability leading to perturbed immune response and intestinal inflammation. All these variables attributed to influence the T-cell mediated autoimmunity and related autoimmune disorders including diabetes [183,184]. Zhang et al. [185] also performed the metagenomic study using the 16S RNA and short-gun sequencing method to find the gut microflora signature markers among the healthy, pre-diabetic, and newly diagnosed T2DM patients. The healthy controls were rich in *Haemophilus parainfluenzae* T3T1 and *F. prausnitzii* but

less abundant in *A. muciniphila* and *Clostridiales* spp. SS3/4. The butyrate producing *Roseburia intestinalis* and *Faecalibacterium prausnitzii* were found to be deficient in T2DM subjects with the abundance of *Streptococcus mutans*, *Lactobacillus gasseri* and certain *Clostridiales*. A fall in the populations of anti-inflammatory strains of *Faecalibacterium prausnitzii* and *Roseburia* (butyrate-producing *Clostridialis*) were seen as an early sign of T2DM compared to healthy controls whereas enrichment of *Akkermansia muciniphila* strains come along with signs of improved metabolic control in the obese mice [186].

The composition of gut microflora cannot always be synonymous to a function and that is why, recently, some studies extended their investigation by correlating the gut microbiota composition with their gut-microbe-derived metabolomics. These studies found increased BCAA levels in the glucose intolerant patients prone to T2DM and this increased production of BCAA was correlated with the increased prevalence of two bacterial strains i.e., *P. copri* and *B. vulgatus* [187]. Moreover, diet is considered a key player in shaping the relationship of gut microbiota composition and metabolism with the risk factors of T2DM, so many studies have devoted their objective on the effect of specific diet consumption and their consequent effects on gut microbial communities and their metabolites. The effect of macronutrient constituents of diet has been particularly discussed in the literature. The prevalent intake of animal protein diets increased the T2DM related gut microbial signatures (i.e., *Bacteroides* and *Clostridia*) with the decrease of SCFA- and SCOA-producing bacteria e.g., *Bifidobacterium adolescentis*. The comparison of plant-based-protein rich diets and animal-origin-protein rich diets was also studied in detail which pointed to the dominance of *Bacteroides*, *Alistipes* and *Bilophila* (bile-tolerant anaerobes) in the latter group of diet recipients [188]. The consumption of a high protein and low carbohydrate diet also diminished the population of SCOA producing bacteria such as *Roseburia* and *Eubacterium rectale* [189]. The consumption of a high-fat diet also caused an increased in the population of *Clostridia* and *Bacteroides* whereas the intake of low-fat diet resulted in the abundance of SCOA-producing bacteria. The consumption of saturated fat-rich-diet encouraged the setup of *Faecalibacterium prausnitzii* and monounsaturated fat-rich-diet intake reduced the total gut bacterial load [190]. Fish oil-fed mice harbored an elevated population of SCOA (especially butyrate and lactic acid) producing bacteria i.e., *Lactobacillus*, *Streptococcus*, *Verrucomicrobia*, *Bifidobacterium* and *Adlercreutzia* whilst lard-feeding showed an increased population of *Bilophila* and *Bacteroides* [191]. The consumption of sugars (fructose, sucrose, glucose) increased the ratio of *Bifidobacteria*:*Bacteroides*. The addition of lactose with other sugars replicated the same results but with lower population of *Clostridia* species [192]. The consumption of artificial sweeteners produced inverted results with an elevated community of *Bacteroides* [193]. The intake of non-digestible carbohydrate-rich diet (fiber/probiotic) stimulated the community growth of beneficial anti-obesity fermentative commensal microbiota i.e., *Bifidobacteria* and *Lactobacilli* [194] whereas, on the other hand, prebiotics reduced the signature gut microbial biomarkers of obesity and T2DM, i.e., *Clostridium* and *Enterococcus* [195]. The intake of probiotics and prebiotics was found to decrease the risk factors and signature biomarkers of T2DM by stimulating a decrease in blood glucose levels. The supplementation of probiotic strains (*Escherichia coli Nissle 1917*, *Lactobacillus plantarum* 2142, *Lactobacillus rhamnosus* GG) or the supernatant of their spent cultures or their metabolites increased the blood concentration of insulin, insulin sensitivity, improved glucose tolerance and relieved the oxidative stress and oxidative stress-related proinflammatory cytokines [196–198]. The administration of probiotics/prebiotics in addition with diet in different mouse models (KK-Ay, NOD, and alloxan-induced-diabetic mice) witnessed the fading of signature T2DM biomarkers i.e., lowered HbA1c, FFA, LDL-H, TG, FBG, and HOMA-IR with subsequent increase in the *Bifidobacterium* and butyrate producing gut microbes [199]. The supplementation of diet with polyphenols of varying origins increased the community size of *Prevotella*, *Bifidobacterium*, *Bacteroides*, *Bacteroides uniformis*, *Enterococcus*, *Blautia coccoides-E. rectale* and *Eggerthella lenta* groups [200,201]. In summary, diminishing intestinal bacterial richness is the first biomarker of undiagnosed metabolic syndrome. Considering the dietary, lifestyle and physical activity covariates, the persistent gut prevalence of certain (pathogenic) microflora (i.e., *Bacteriodetes*, *Clostridium coccoides*, *Proteobacteria*, *Clostridiales*, *Lactobacillus* spp., *Betaproteobacteria*, *Streptococcus mutans*, *P. copri*, *B. vulgatus*, *Lactobacillus*

gasseri, *Ruminococcus* spp. *E. coli*, *Eggerthella lenta*) are also early risk predictors for metabolic syndrome and pre-diabetic conditions. Likewise, a decrease in the intestinal population of SCFA and beneficial SCOA are also reporters of a future risk of metabolic syndrome and diabetes.

6. Conclusions

The metabolic syndrome and diabetes mellitus are becoming more prevalent in both developed and developing countries. The onset of diabetes can be deferred or even prevented if intervention is accurate at early stages. The existing diagnostic clinical tools are not considered sufficient for early prediction of these conditions. However, several perturbed metabolic biomarkers have been proposed for early prediction of metabolic syndrome. These predictive and risk-factoring metabolic biomarkers have been discovered using high-throughput technologies used in many cohort and predictive modeling (metabolomics) studies. Reported metabolic biomarkers mainly belong to AA, BCAA, SCOA, acylcarnitines, phopsholipids and FFA. These metabolites are considered the intermediate metabolites of carbohydrates, lipids and amino acid-altered metabolism which ultimately distorted the gluconeogenesis, glycolysis, lipolysis, the tricarboxylic acid cycle and proteolysis pathways. These disturbed biomarkers showed significant correlations with elevated blood/plasma/serum glucose level, fasting plasma glucose, insulin resistance, glucose intolerance, HOMA-IR, OGTT and obesity. Moreover, most of these early diabetic biomarkers have been validated and are being used in newly established phenome centers for the purpose of population-based screening. Gut microbes have also been found to be influenced by (the early stages of) T1DM, T2DM and obesity. With some exceptions, the abundance of Firmicutes, Actinobacteria, SCOA-producing-bacteria, Bifidobacteriaceae (Actinobacteria) and *Clostridium* phyla was found to be lower in metabolic syndrome, obese and diabetic persons whereas the population of Bacteroidetes, *Lactobacillus* spp., Rikenellaceae, Proteobacteria, *A. muciniphila* and *Desulfovibrio* spp. was found to be richer in metabolic syndrome, obese, and diabetic individuals compared to healthy subjects.

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Abbreviations

1-deoxysphinganine	(1-deoxySA)
2-hydroxybiphenyl	(2HBP)
8-pentamethoxyisoflavan	(HPMF)
adenosine monophosphate-activated protein kinase	(AMPK)
alkylacyl phosphatidylcholines	(PCe)
alkylacyl phosphatidylethanolamines	(PEe)
amino acids	(AA)
area-under-curve	(AUC)
Atherosclerosis Risk in Communities	(ARIC)
branched-chain a-ketoacid dehydrogenase	(BCKD)
branched-chain amino acid aminotransferase	(BCATm)
branched-chain aminotransferase	(BCATm)
branched-chain keto acid dehydrogenase	(BCKDH)
branched-chained AA	(BCAA)

branched-chain-OA	(BCOA)
Ceramides	(Cer)
cholesterol esters	(CE)
cholesterol esters	(ChoE)
cholesteryl ester	(CE)
choline ether phospholipid	(PCae)
combined glucose tolerance	(CGT)
Cooperative Health Research in the Region of Augsburg	(KORA)
coronary artery disease	(CAD)
deoxysphingosine	(1-deoxySO)
diabetes mellitus	(DM)
diabetic nephropathy	(DN)
diabetic retinopathy	(DR)
diacylglycerols	(DAG)
dihydroceramides	(DHC)
Dongfeng-Tongji	(DFTJ)
endogenous glucose production	(EGP)
European Prospective Investigation into Cancer and Nutrition	(EPIC)
False Discovery rate	(FDR)
farnesoid X receptor	(FXR)
fold change	(FC)
Free Fatty Acid Receptor 2	(FFAR2)
Free fatty acids	(FFA)
Gestational diabetes	(GDM)
glucagon-like peptide-1	(GLP-1)
glucose-6-phosphotase	(G6P)
glycated hemoglobin	(HbA1c)
glycerophospholipids	(GPL)
G-protein-coupled receptors	(GPCR)
Health Professionals Follow-Up Study	(HPFS)
High-density-lipoprotein-cholesterol	(HDL-C)

References

1. The International Diabetes Federation (IDF). *Can. J. Diabetes* **2009**, *33*, 13. [CrossRef]
2. World Health Organization. *Organisation for Economic Co-Operation and Development (OECD)*; WHO: Geneva, Switzerland, 2016.
3. Savolainen, O.; Fagerberg, B.; Lind, M.V.; Sandberg, A.-S.; Ross, A.B.; Bergström, G. Biomarkers for predicting type 2 diabetes development—Can metabolomics improve on existing biomarkers? *PLoS ONE* **2017**, *12*, e0177738. [CrossRef] [PubMed]
4. Wang-Sattler, R.; Yu, Z.; Herder, C.; Messias, A.C.; Floegel, A.; He, Y.; Heim, K.; Campillos, M.; Holzapfel, C.; Thorand, B.; et al. Novel biomarkers for pre-diabetes identified by metabolomics. *Mol. Syst. Biol.* **2012**, *8*, 615. [CrossRef] [PubMed]
5. Liu, R.; Hong, J.; Xu, X.; Feng, Q.; Zhang, D.; Gu, Y.; Shi, J.; Zhao, S.; Liu, W.; Wang, X.; et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat. Med.* **2017**, *23*, 859–868. [CrossRef]
6. Klein, M.; Shearer, J. Metabolomics and Type 2 Diabetes: Translating Basic Research into Clinical Application. *J. Diabetes Res.* **2015**, *2016*, 3898502. [CrossRef] [PubMed]
7. Santaguida, P.L.; Balion, C.; Hunt, D.; Morrison, K.; Gerstein, H.; Raina, P.; Booker, L.; Yazdi, H. Diagnosis, prognosis, and treatment of impaired glucose tolerance and impaired fasting glucose. In *AHRQ Evidence Report Summaries*; Agency for Healthcare Research and Quality: Rockville, MD, USA, 2005.
8. Fagerberg, B.; Kellis, D.; Bergström, G.; Behre, C.J. Adiponectin in relation to insulin sensitivity and insulin secretion in the development of type 2 diabetes: A prospective study in 64-year-old women. *J. Intern. Med.* **2010**, *269*, 636–643. [CrossRef] [PubMed]

9. Yuan, M.; Breitkopf, S.B.; Yang, X.; Asara, J.M. A positive/negative ion–switching, targeted mass spectrometry–based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. *Nat. Protoc.* **2012**, *7*, 872–881. [CrossRef]
10. Huang, Y.; Tian, Y.; Li, G.; Li, Y.; Yin, X.; Peng, C.; Xu, F.; Zhang, Z. Discovery of safety biomarkers for realgar in rat urine using UFLC-IT-TOF/MS and 1H NMR based metabolomics. *Anal. Bioanal. Chem.* **2013**, *405*, 4811–4822. [CrossRef]
11. Bender, D.A. The metabolism of "surplus" amino acids. *Br. J. Nutr.* **2012**, *108*, S113–S121. [CrossRef]
12. Schutz, Y. Protein Turnover, Ureagenesis and Gluconeogenesis. *Int. J. Vitam. Nutr. Res.* **2011**, *81*, 101–107. [CrossRef]
13. Wester, T.; Kraft, G.; Dardevet, D.; Polakof, S.; Ortigues-Marty, I.; Rémond, D.; Savary-Auzeloux, I. Nutritional regulation of the anabolic fate of amino acids within the liver in mammals: Concepts arising fromin vivostudies. *Nutr. Res. Rev.* **2015**, *28*, 22–41. [CrossRef]
14. Chen, S.; Akter, S.; Kuwahara, K.; Matsushita, Y.; Nakagawa, T.; Konishi, M.; Honda, T.; Yamamoto, S.; Hayashi, T.; Noda, M.; et al. Serum amino acid profiles and risk of type 2 diabetes among Japanese adults in the Hitachi Health Study. *Sci. Rep.* **2019**, *9*, 7010. [CrossRef] [PubMed]
15. Krebs, M.; Krssak, M.; Bernroider, E.; Anderwald, C.H.; Brehm, A.; Meyerspeer, M.; Nowotny, P.; Roth, E.; Waldhäusl, W.; Roden, M. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. *Diabetes* **2002**, *51*, 599–605. [CrossRef] [PubMed]
16. Patti, M.E.; Brambilla, E.; Luzi, L.; Landaker, E.J.; Kahn, C.R. Bidirectional modulation of insulin action by amino acids. *J. Clin. Investig.* **1998**, *101*, 1519–1529. [CrossRef]
17. Wang, T.J.; Larson, M.G.; Vasan, R.S.; Cheng, S.; Rhee, E.P.; McCabe, E.; Lewis, G.D.; Fox, C.S.; Jacques, P.F.; Fernandez, C.; et al. Metabolite profiles and the risk of developing diabetes. *Nat. Med.* **2011**, *17*, 448–453. [CrossRef] [PubMed]
18. Palmer, N.D.; Stevens, R.D.; Antinozzi, P.A.; Anderson, A.; Bergman, R.N.; Wagenknecht, L.E.; Newgard, C.B.; Bowden, N.W. Metabolomic profile associated with insulin resistance and conversion to diabetes in the Insulin Resistance Atherosclerosis Study. *J. Clin. Endocrinol. Metab.* **2014**, *100*, E463–E468. [CrossRef] [PubMed]
19. Tillin, T.; Hughes, A.; Wang, Q.; Würtz, P.; Ala-Korpela, M.; Sattar, N.; Forouhi, N.G.; Godsland, I.F.; Eastwood, S.V.; McKeigue, P.M.; et al. Diabetes risk and amino acid profiles: Cross-sectional and prospective analyses of ethnicity, amino acids and diabetes in a South Asian and European cohort from the SABRE (Southall And Brent REvisited) Study. *Diabetologia* **2015**, *58*, 968–979. [CrossRef] [PubMed]
20. Yamakado, M.; Nagao, K.; Imaizumi, A.; Tani, M.; Toda, A.; Tanaka, T.; Jinzu, H.; Miyano, H.; Yamamoto, H.; Daimon, T.; et al. Plasma Free Amino Acid Profiles Predict Four-Year Risk of Developing Diabetes, Metabolic Syndrome, Dyslipidemia and Hypertension in Japanese Population. *Sci. Rep.* **2015**, *5*, 11918. [CrossRef] [PubMed]
21. Chen, T.; Ni, Y.; Ma, X.; Bao, Y.; Liu, J.; Huang, F.; Hu, C.; Xie, G.; Zhao, A.; Jia, W.; et al. Branched-chain and aromatic amino acid profiles and diabetes risk in Chinese populations. *Sci. Rep.* **2016**, *6*, 20594. [CrossRef]
22. Stancáková, A.; Civelek, M.; Saleem, N.K.; Soininen, P.; Kangas, A.; Cederberg, H.; Paananen, J.; Pihlajamäki, J.; Bonnycastle, L.L.; Morken, M.A.; et al. Hyperglycemia and a Common Variant of GCKR Are Associated with the Levels of Eight Amino Acids in 9,369 Finnish Men. *Diabetes* **2012**, *61*, 1895–1902. [CrossRef] [PubMed]
23. Wang, T.J.; Ngo, D.; Psychogios, N.; Dejam, A.; Larson, M.G.; Vasan, R.S.; Ghorbani, A.; O'Sullivan, J.; Cheng, S.; Rhee, E.P.; et al. 2-Aminoadipic acid is a biomarker for diabetes risk. *J. Clin. Investig.* **2013**, *123*, 4309–4317. [CrossRef] [PubMed]
24. Tsutsui, H.; Maeda, T.; Toyo'Oka, T.; Min, J.Z.; Inagaki, S.; Higashi, T.; Kagawa, Y. Practical Analytical Approach for the Identification of Biomarker Candidates in Prediabetic State Based upon Metabonomic Study by Ultraperformance Liquid Chromatography Coupled to Electrospray Ionization Time-of-Flight Mass Spectrometry. *J. Proteome Res.* **2010**, *9*, 3912–3922. [CrossRef]
25. Arora, S.; Henderson, S.O.; Long, T.; Menchine, M. Diagnostic Accuracy of Point-of-Care Testing for Diabetic Ketoacidosis at Emergency-Department Triage. *Diabetes Care* **2011**, *34*, 852–854. [CrossRef]
26. Qiu, G.; Zheng, Y.; Wang, H.; Sun, J.; Ma, H.; Xiao, Y.; Li, Y.; Yuan, Y.; Yang, H.; Li, X.; et al. Plasma metabolomics identified novel metabolites associated with risk of type 2 diabetes in two prospective cohorts of Chinese adults. *Int. J. Epidemiol.* **2016**, *45*, 1507–1516. [CrossRef] [PubMed]

27. Vangipurapu, J.; Stancˇáková, A.; Smith, U.; Kuusisto, J.; Laakso, M. Nine Amino Acids Are Associated with Decreased Insulin Secretion and Elevated Glucose Levels in a 7.4-Year Follow-up Study of 5,181 Finnish Men. *Diabetes* **2019**, *68*, 1353–1358. [CrossRef] [PubMed]
28. Floegel, A.; Stefan, N.; Yu, Z.; Mühlenbruch, K.; Drogan, D.; Joost, H.-G.; Fritsche, A.; Häring, H.-U.; De Angelis, M.H.; Peters, A.; et al. Identification of Serum Metabolites Associated with Risk of Type 2 Diabetes Using a Targeted Metabolomic Approach. *Diabetes* **2013**, *62*, 639–648. [CrossRef] [PubMed]
29. Pontiroli, A.E.; Pizzocri, P.; Caumo, A.; Perseghin, G.; Luzi, L. Evaluation of insulin release and insulin sensitivity through oral glucose tolerance test: Differences between NGT, IFG, IGT, and type 2 diabetes mellitus. A cross-sectional and follow-up study. *Acta Diabetol.* **2004**, *41*, 70–76. [CrossRef] [PubMed]
30. Felig, P.; Marliss, E.; Ohman, J.L.; Cahill, G.F. Plasma Amino Acid Levels in Diabetic Ketoacidosis. *Diabetes* **1970**, *19*, 727–729. [CrossRef]
31. Lackey, D.E.; Lynch, C.J.; Olson, K.C.; Mostaedi, R.; Ali, M.; Smith, W.H.; Karpe, F.; Humphreys, S.; Bedinger, D.; Dunn, T.N.; et al. Regulation of adipose branched-chain amino acid catabolism enzyme expression and cross-adipose amino acid flux in human obesity. *Am. J. Physiol. Metab.* **2013**, *304*, E1175–E1187. [CrossRef]
32. Newgard, C.B.; An, J.; Bain, J.R.; Muehlbauer, M.J.; Stevens, R.D.; Lien, L.F.; Haqq, A.M.; Shah, S.H.; Arlotto, M.; Slentz, C.A.; et al. A Branched-Chain Amino Acid-Related Metabolic Signature that Differentiates Obese and Lean Humans and Contributes to Insulin Resistance. *Cell Metab.* **2009**, *9*, 565–566. [CrossRef]
33. Mihalik, S.J.; Michaliszyn, S.F.; Heras, J.D.L.; Bacha, F.; Lee, S.; Chace, D.H.; DeJesus, V.R.; Vockley, J.; Arslanian, S. Metabolomic Profiling of Fatty Acid and Amino Acid Metabolism in Youth with Obesity and Type 2 Diabetes. *Diabetes Care* **2012**, *35*, 605–611. [CrossRef]
34. Würtz, P.; Soininen, P.; Kangas, A.; Rönnemaa, T.; Lehtimäki, T.; Kahonen, M.; Viikari, J.S.; Raitakari, O.T.; Ala-Korpela, M. Branched-Chain and Aromatic Amino Acids Are Predictors of Insulin Resistance in Young Adults. *Diabetes Care* **2013**, *36*, 648–655. [CrossRef] [PubMed]
35. Fiehn, O.; Garvey, W.T.; Newman, J.W.; Lok, K.H.; Hoppel, C.L.; Adams, S.H. Plasma Metabolomic Profiles Reflective of Glucose Homeostasis in Non-Diabetic and Type 2 Diabetic Obese African-American Women. *PLoS ONE* **2010**, *5*, e15234. [CrossRef] [PubMed]
36. Zhang, X.; Saaddine, J.B.; Chou, C.-F.; Cotch, M.F.; Cheng, Y.J.; Geiss, L.S.; Gregg, E.; Albright, A.L.; Klein, B.E.K.; Klein, R. Prevalence of Diabetic Retinopathy in the United States, 2005–2008. *JAMA* **2010**, *304*, 649–656. [CrossRef]
37. Chen, C.; Xu, Y.; Guo, Z.; Yang, J.; Wu, M.; Hu, X.-S. The application of visceral adiposity index in identifying type 2 diabetes risks based on a prospective cohort in China. *Lipids Health Dis.* **2014**, *13*, 108. [CrossRef] [PubMed]
38. Zeng, M.; Che, Z.; Liang, Y.; Wang, B.; Chen, X.; Li, H.; Deng, J.; Zhou, Z. GC–MS Based Plasma Metabolic Profiling of Type 2 Diabetes Mellitus. *Chromatographia* **2009**, *69*, 941–948. [CrossRef]
39. Stancˇáková, A.; Javorský, M.; Kuulasmaa, T.; Haffner, S.M.; Kuusisto, J.; Laakso, M. Changes in Insulin Sensitivity and Insulin Release in Relation to Glycemia and Glucose Tolerance in 6,414 Finnish Men. *Diabetes* **2009**, *58*, 1212–1221. [CrossRef]
40. Wilson, P.W.F.; Meigs, J.B.; Sullivan, L.; Fox, C.S.; Nathan, D.M.; D'Agostino, R.B. Prediction of Incident Diabetes Mellitus in Middle-aged Adults. *Arch. Intern. Med.* **2007**, *167*, 1068–1074. [CrossRef]
41. Piccolo, B.D.; Graham, J.L.; Stanhope, K.L.; Fiehn, O.; Havel, P.; Adams, S.H. Plasma amino acid and metabolite signatures tracking diabetes progression in the UCD-T2DM rat model. *Am. J. Physiol. Metab.* **2016**, *310*, E958–E969. [CrossRef]
42. Michaliszyn, S.F.; Sjaarda, L.A.; Mihalik, S.J.; Lee, S.; Bacha, F.; Chace, D.H.; De Jesús, V.R.; Vockley, J.; Arslanian, S. Metabolomic profiling of amino acids and β-cell function relative to insulin sensitivity in youth. *J. Clin. Endocrinol. Metab.* **2012**, *97*, E2119–E2124. [CrossRef]
43. Tai, E.S.; Tan, M.L.S.; Stevens, R.D.; Low, Y.L.; Muehlbauer, M.J.; Goh, D.L.M.; Ilkayeva, O.R.; Wenner, B.R.; Bain, J.R.; Lee, J.J.M.; et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia* **2010**, *53*, 757–767. [CrossRef] [PubMed]
44. Yamada, C.; Kondo, M.; Kishimoto, N.; Shibata, T.; Nagai, Y.; Imanishi, T.; Oroguchi, T.; Ishii, N.; Nishizaki, Y. Association between insulin resistance and plasma amino acid profile in non-diabetic Japanese subjects. *J. Diabetes Investig.* **2015**, *6*, 408–415. [CrossRef] [PubMed]

45. Zhao, J.; Zhu, Y.; Hyun, N.; Zeng, N.; Uppal, K.; Tran, V.T.; Yu, T.; Jones, D.; He, J.; Lee, E.T.; et al. Novel Metabolic Markers for the Risk of Diabetes Development in American Indians. *Diabetes Care* **2014**, *38*, 220–227. [CrossRef] [PubMed]
46. Lee, C.C.; Watkins, S.M.; Lorenzo, C.; Wagenknecht, L.E.; Il'Yasova, R.; Chen, Y.-D.I.; Haffner, S.M.; Hanley, A.J. Branched-Chain Amino Acids and Insulin Metabolism: The Insulin Resistance Atherosclerosis Study (IRAS). *Diabetes Care* **2016**, *39*, 582–588. [CrossRef]
47. Piccolo, B.D.; Comerford, K.B.; Karakas, S.E.; Knotts, T.A.; Fiehn, O.; Adams, S.H. Whey Protein Supplementation Does Not Alter Plasma Branched-Chained Amino Acid Profiles but Results in Unique Metabolomics Patterns in Obese Women Enrolled in an 8-Week Weight Loss Trial. *J. Nutr.* **2015**, *145*, 691–700. [CrossRef]
48. Menni, C.; Fauman, E.; Erte, I.; Perry, J.R.; Kastenmuller, G.; Shin, S.-Y.; Petersen, A.-K.; Hyde, C.; Psatha, M.; Ward, K.J.; et al. Biomarkers for Type 2 Diabetes and Impaired Fasting Glucose Using a Nontargeted Metabolomics Approach. *Diabetes* **2013**, *62*, 4270–4276. [CrossRef]
49. She, P.; Olson, K.C.; Kadota, Y.; Inukai, A.; Shimomura, Y.; Hoppel, C.L.; Adams, S.H.; Kawamata, Y.; Matsumoto, H.; Sakai, R.; et al. Leucine and Protein Metabolism in Obese Zucker Rats. *PLoS ONE* **2013**, *8*, e59443. [CrossRef]
50. Pearson, E.R.; Adamski, J. The search for predictive metabolic biomarkers for incident T2DM. *Nat. Rev. Endocrinol.* **2018**, *14*, 444–446. [CrossRef]
51. Zhang, X.; Cui, Y.; Fang, L.; Li, F. Chronic High-Fat Diets Induce Oxide Injuries and Fibrogenesis of Pancreatic Cells in Rats. *Pancreas* **2008**, *37*, e31–e38. [CrossRef]
52. Devkota, S.; Layman, D. Protein metabolic roles in treatment of obesity. *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13*, 403–407. [CrossRef]
53. Nairizi, A.; She, P.; Vary, T.C.; Lynch, C.J. Leucine supplementation of drinking water does not alter susceptibility to diet-induced obesity in mice. *J. Nutr.* **2009**, *139*, 715–719. [CrossRef] [PubMed]
54. Gougeon, R.; Morais, J.A.; Chevalier, S.; Pereira, S.; Lamarche, M.; Marliss, E.B. Determinants of Whole-Body Protein Metabolism in Subjects with and Without Type 2 Diabetes. *Diabetes Care* **2007**, *31*, 128–133. [CrossRef] [PubMed]
55. Mai, M.; Tonjes, A.; Kovács, P.; Stumvoll, M.; Fiedler, G.M.; Leichtle, A.B. Serum Levels of Acylcarnitines Are Altered in Prediabetic Conditions. *PLoS ONE* **2013**, *8*, e82459. [CrossRef] [PubMed]
56. Zhang, X.; Zhang, C.; Chen, L.; Han, X.; Ji, L. Human serum acylcarnitine profiles in different glucose tolerance states. *Diabetes Res. Clin. Pract.* **2014**, *104*, 376–382. [CrossRef]
57. Oberbach, A.; Blüher, M.; Wirth, H.; Till, H.; Kovács, P.; Kullnick, Y.; Schlichting, N.; Tomm, J.M.; Rolle-Kampczyk, U.; Murugaiyan, J.; et al. Combined Proteomic and Metabolomic Profiling of Serum Reveals Association of the Complement System with Obesity and Identifies Novel Markers of Body Fat Mass Changes. *J. Proteome Res.* **2011**, *10*, 4769–4788. [CrossRef]
58. Suvitaival, T.; Bondia-Pons, I.; Yetukuri, L.; Pöhö, P.; Nolan, J.J.; Hyotylainen, T.; Kuusisto, J.; Oresic, M. Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men. *Metabolism* **2018**, *78*, 1–12. [CrossRef]
59. Henriksen, E.J. Invited Review: Effects of acute exercise and exercise training on insulin resistance. *J. Appl. Physiol.* **2002**, *93*, 788–796. [CrossRef]
60. Cole, L.K.; Vance, J.E.; Vance, D.E. Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim. Biophys. Acta (BBA) Mol. Cell Biol. Lipids* **2012**, *1821*, 754–761. [CrossRef]
61. Magnusson, C.D.; Haraldsson, G.G. Ether lipids. *Chem. Phys. Lipids* **2011**, *164*, 315–340. [CrossRef] [PubMed]
62. Wallner, S.; Schmitz, G. Plasmalogens the neglected regulatory and scavenging lipid species. *Chem. Phys. Lipids* **2011**, *164*, 573–589. [CrossRef]
63. Pietiläinen, K.H.; Sysi-Aho, M.; Rissanen, A.; Seppänen-Laakso, T.; Yki-Jarvinen, H.; Kaprio, J.; Oresic, M. Acquired Obesity Is Associated with Changes in the Serum Lipidomic Profile Independent of Genetic Effects—A Monozygotic Twin Study. *PLoS ONE* **2007**, *2*, e218. [CrossRef] [PubMed]
64. Suhre, K.; Meisinger, C.; Döring, A.; Altmaier, E.; Belcredi, P.; Gieger, C.; Chang, D.; Milburn, M.V.; Gall, W.E.; Weinberger, K.M.; et al. Metabolic Footprint of Diabetes: A Multiplatform Metabolomics Study in an Epidemiological Setting. *PLoS ONE* **2010**, *5*, e13953. [CrossRef] [PubMed]

65. Suhre, K.; Schwartz, J.E.; Sharma, V.K.; Chen, Q.; Lee, J.R.; Muthukumar, T.; Dadhania, D.M.; Ding, R.; Ikle, D.N.; Bridges, N.D.; et al. Urine Metabolite Profiles Predictive of Human Kidney Allograft Status. *J. Am. Soc. Nephrol.* **2015**, *27*, 626–636. [CrossRef]
66. Rhee, E.P.; Cheng, S.; Larson, M.G.; Walford, G.A.; Lewis, G.D.; McCabe, E.; Yang, E.; Farrell, L.; Fox, C.S.; O'Donnell, C.J.; et al. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. *J. Clin. Investig.* **2011**, *121*, 1402–1411. [CrossRef] [PubMed]
67. Holloszy, J.O. Exercise-induced increase in muscle insulin sensitivity. *J. Appl. Physiol.* **2005**, *99*, 338–343. [CrossRef] [PubMed]
68. Rhee, M.; Ho, Y.-L.; Raghavan, S.; Vassy, J.L.; Cho, K.; Gagnon, D.; Staimez, L.R.; Ford, C.N.; Wilson, P.W.F.; Phillips, L.S. Random plasma glucose predicts the diagnosis of diabetes. *PLoS ONE* **2019**, *14*, e0219964. [CrossRef] [PubMed]
69. Ferrannini, E.; Natali, A.; Camastra, S.; Nannipieri, M.; Mari, A.; Adam, K.-P.; Milburn, M.V.; Kastenmuller, G.; Adamski, J.; Tuomi, T.; et al. Early Metabolic Markers of the Development of Dysglycemia and Type 2 Diabetes and Their Physiological Significance. *Diabetes* **2013**, *62*, 1730–1737. [CrossRef]
70. Wong, G.; Barlow, C.K.; Weir, J.M.; Jowett, J.B.M.; Magliano, D.J.; Zimmet, P.Z.; Shaw, J.; Meikle, P.J. Inclusion of Plasma Lipid Species Improves Classification of Individuals at Risk of Type 2 Diabetes. *PLoS ONE* **2013**, *8*, e76577. [CrossRef]
71. Stahlman, M.; Pham, H.T.; Adiels, M.; Mitchell, T.W.; Blanksby, S.J.; Fagerberg, B.; Ekroos, K.; Borén, J. Clinical dyslipidaemia is associated with changes in the lipid composition and inflammatory properties of apolipoprotein-B-containing lipoproteins from women with type 2 diabetes. *Diabetologia* **2012**, *55*, 1156–1166. [CrossRef]
72. Tulipani, S.; Palau-Rodriguez, M.; Alonso, A.M.; Cardona, F.; Marco-Ramell, A.; Zonja, B.; De Alda, M.L.; Muñoz-Garach, A.; Sánchez-Pla, A.; Tinahones, F.J.; et al. Biomarkers of Morbid Obesity and Prediabetes by Metabolomic Profiling of Human Discordant Phenotypes. *Clin. Chim. Acta* **2016**, *463*, 53–61. [CrossRef]
73. Then, C.; Wahl, S.; Kirchhofer, A.; Grallert, H.; Krug, S.; Kastenmuller, G.; Römisch-Margl, W.; Claussnitzer, M.; Illig, T.; Heier, M.; et al. Plasma Metabolomics Reveal Alterations of Sphingo- and Glycerophospholipid Levels in Non-Diabetic Carriers of the Transcription Factor 7-Like 2 Polymorphism rs7903146. *PLoS ONE* **2013**, *8*, e78430. [CrossRef] [PubMed]
74. Zeng, H.; Tong, R.; Tong, W.; Yang, Q.; Qiu, M.; Xiong, A.; Sun, S.; Ding, L.; Zhang, H.; Yang, L.; et al. Author Correction: Metabolic Biomarkers for Prognostic Prediction of Pre-diabetes: Results from a longitudinal cohort study. *Sci. Rep.* **2017**, *7*, 16165. [CrossRef] [PubMed]
75. Drogan, D.; Dunn, W.; Lin, W.; Buijsse, B.; Schulze, M.B.; Langenberg, C.; Brown, M.; Floegel, A.; Dietrich, S.; Rolandsson, O.; et al. Untargeted Metabolic Profiling Identifies Altered Serum Metabolites of Type 2 Diabetes Mellitus in a Prospective, Nested Case Control Study. *Clin. Chem.* **2015**, *61*, 487–497. [CrossRef] [PubMed]
76. Connor, S.C.; Hansen, M.K.; Corner, A.; Smith, R.F.; Ryan, T.E. Integration of metabolomics and transcriptomics data to aid biomarker discovery in type 2 diabetes. *Mol. BioSyst.* **2010**, *6*, 909–921. [CrossRef]
77. Fontana, B.G.; Morales-Santana, S.; Díaz, C.; Rozas-Moreno, P.; Genilloud, O.; Pérez, F.V.; Del Palacio, J.P.; Muñoz-Torres, M.; Muñoz-Torres, M. Metabolomic profile related to cardiovascular disease in patients with type 2 diabetes mellitus: A pilot study. *Talanta* **2016**, *148*, 135–143. [CrossRef]
78. Wigger, L.; Cruciani-Guglielmacci, C.; Nicolas, A.; Denom, J.; Fernandez, N.; Fumeron, F.; Marques-Vidal, P.; Ktorza, A.; Kramer, W.; Schulte, A.; et al. Plasma Dihydroceramides Are Diabetes Susceptibility Biomarker Candidates in Mice and Humans. *Cell Rep.* **2017**, *18*, 2269–2279. [CrossRef]
79. Mittermayer, F.; Caveney, E.; De Oliveira, C.; Gourgiotis, L.; Puri, M.; Tai, L.-J.; Turner, J.R. Addressing Unmet Medical Needs in Type 2 Diabetes: A Narrative Review of Drugs under Development. *Curr. Diabetes Rev.* **2015**, *11*, 17–31. [CrossRef]
80. Stratford, S.; Hoehn, K.L.; Liu, F.; Summers, S.A. Regulation of Insulin Action by Ceramide. *J. Biol. Chem.* **2004**, *279*, 36608–36615. [CrossRef] [PubMed]
81. Raichur, S.; Brunner, B.; Bielohuby, M.; Hansen, G.; Pfenninger, A.; Wang, B.; Bruning, J.C.; Larsen, P.J.; Tennagels, N. The role of C16:0 ceramide in the development of obesity and type 2 diabetes: CerS6 inhibition as a novel therapeutic approach. *Mol. Metab.* **2019**, *21*, 36–50. [CrossRef]
82. Raichur, S.; Wang, S.T.; Chan, P.W.; Li, Y.; Ching, J.; Chaurasia, B.; Dogra, S.; Öhman, M.K.; Takeda, K.; Sugii, S.; et al. CerS2 Haploinsufficiency Inhibits β-Oxidation and Confers Susceptibility to Diet-Induced Steatohepatitis and Insulin Resistance. *Cell Metab.* **2014**, *20*, 687–695. [CrossRef] [PubMed]

83. Turpin-Nolan, S.; Nicholls, H.T.; Willmes, D.M.; Mourier, A.; Brodesser, S.; Wunderlich, C.M.; Mauer, J.; Xu, E.; Hammerschmidt, P.; Brönneke, H.S.; et al. Obesity-Induced CerS6-Dependent C16:0 Ceramide Production Promotes Weight Gain and Glucose Intolerance. *Cell Metab.* **2014**, *20*, 678–686. [CrossRef]
84. Holland, W.L.; Brozinick, J.T.; Wang, L.-P.; Hawkins, E.D.; Sargent, K.M.; Liu, Y.; Narra, K.; Hoehn, K.L.; Knotts, T.A.; Siesky, A.; et al. Inhibition of Ceramide Synthesis Ameliorates Glucocorticoid-, Saturated-Fat-, and Obesity-Induced Insulin Resistance. *Cell Metab.* **2007**, *5*, 167–179. [CrossRef]
85. Othman, A.; Saely, C.H.; Muendlein, A.; Vonbank, A.; Drexel, H.; Von Eckardstein, A.; Hornemann, T. Plasma 1-deoxysphingolipids are predictive biomarkers for type 2 diabetes mellitus. *BMJ Open Diabetes Res. Care* **2015**, *3*, e000073. [CrossRef] [PubMed]
86. Brozinick, J.T.; Hawkins, E.; Bui, H.H.; Kuo, M.-S.; Tan, B.; Kievit, P.; Grove, K. Plasma sphingolipids are biomarkers of metabolic syndrome in non-human primates maintained on a Western-style diet. *Int. J. Obes.* **2012**, *37*, 1064–1070. [CrossRef] [PubMed]
87. Haus, J.M.; Kashyap, S.R.; Kasumov, T.; Zhang, R.; Kelly, K.R.; DeFronzo, R.A.; Kirwan, J. Plasma Ceramides Are Elevated in Obese Subjects with Type 2 Diabetes and Correlate with the Severity of Insulin Resistance. *Diabetes* **2008**, *58*, 337–343. [CrossRef]
88. Xu, F.; Tavintharan, S.; Sum, C.F.; Woon, K.; Lim, S.C.; Ong, C.N. Metabolic Signature Shift in Type 2 Diabetes Mellitus Revealed by Mass Spectrometry-based Metabolomics. *J. Clin. Endocrinol. Metab.* **2013**, *98*, E1060–E1065. [CrossRef]
89. Shui, G.; Stebbins, J.W.; Lam, B.D.; Cheong, W.F.; Lam, S.M.; Grégoire, F.; Kusonoki, J.; Wenk, M.R. Comparative Plasma Lipidome between Human and Cynomolgus Monkey: Are Plasma Polar Lipids Good Biomarkers for Diabetic Monkeys? *PLoS ONE* **2011**, *6*, e19731. [CrossRef] [PubMed]
90. Di Cianni, G.; Miccoli, R.; Volpe, L.; Lencioni, C.; Del Prato, S. Intermediate metabolism in normal pregnancy and in gestational diabetes. *Diabetes/Metab. Res. Rev.* **2003**, *19*, 259–270. [CrossRef] [PubMed]
91. Meikle, P.J.; Wong, G.; Barlow, C.K.; Weir, J.M.; Greeve, M.A.; MacIntosh, G.L.; Almasy, L.; Comuzzie, A.G.; Mahaney, M.C.; Kowalczyk, A.; et al. Plasma Lipid Profiling Shows Similar Associations with Prediabetes and Type 2 Diabetes. *PLoS ONE* **2013**, *8*, e74341. [CrossRef] [PubMed]
92. Lu, L.; Koulman, A.; Petry, C.J.; Jenkins, B.; Matthews, L.; Hughes, I.A.; Acerini, C.L.; Ong, K.K.; Dunger, D.B. An Unbiased Lipidomics Approach Identifies Early Second Trimester Lipids Predictive of Maternal Glycemic Traits and Gestational Diabetes Mellitus. *Diabetes Care* **2016**, *39*, 2232–2239. [CrossRef] [PubMed]
93. Jacobs, S.; Schiller, K.; Jansen, E.H.J.M.; Boeing, H.; Schulze, M.B.; Kröger, J. Evaluation of various biomarkers as potential mediators of the association between Δ5 desaturase, Δ6 desaturase, and stearoyl-CoA desaturase activity and incident type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition–Potsdam Study. *Am. J. Clin. Nutr.* **2015**, *102*, 155–164. [CrossRef] [PubMed]
94. Petry, C.J.; Koulman, A.; Lu, L.; Jenkins, B.; Furse, S.; Prentice, P.; Matthews, L.; Hughes, I.A.; Acerini, C.L.; Ong, K.K.; et al. Associations between the maternal circulating lipid profile in pregnancy and fetal imprinted gene alleles: A cohort study. *Reprod. Biol. Endocrinol.* **2018**, *16*, 82. [CrossRef]
95. Chen, X.; Scholl, T.O.; Leskiw, M.; Savaille, J.; Stein, T.P. Differences in Maternal Circulating Fatty Acid Composition and Dietary Fat Intake in Women with Gestational Diabetes Mellitus or Mild Gestational Hyperglycemia. *Diabetes Care* **2010**, *33*, 2049–2054. [CrossRef] [PubMed]
96. Dudzik, D.; Ż órawski, M.; Skotnicki, M.; Zarzycki, W.; Kozlowska, G.; Bibik-Malinowska, K.; Vallejo, M.; Garcia, A.; Barbas, C.; Ramos, M.P.; et al. Metabolic fingerprint of Gestational Diabetes Mellitus. *J. Proteom.* **2014**, *103*, 57–71. [CrossRef] [PubMed]
97. Anderson, S.G.; Dunn, W.B.; Banerjee, M.; Brown, M.; Broadhurst, D.I.; Goodacre, R.; Cooper, G.J.S.; Kell, D.B.; Cruickshank, J.K. Evidence That Multiple Defects in Lipid Regulation Occur before Hyperglycemia during the Prodrome of Type-2 Diabetes. *PLoS ONE* **2014**, *9*, e103217. [CrossRef]
98. Allalou, A.; Nalla, A.; Prentice, K.J.; Liu, Y.; Zhang, M.; Dai, F.F.; Ning, X.; Osborne, L.R.; Cox, B.J.; Gunderson, E.P.; et al. A Predictive Metabolic Signature for the Transition from Gestational Diabetes Mellitus to Type 2 Diabetes. *Diabetes* **2016**, *65*, 2529–2539. [CrossRef] [PubMed]
99. Van Der Kloet, F.M.; Tempels, F.W.A.; Ismail, N.; Van Der Heijden, R.; Kasper, P.T.; Rojas-Cherto, M.; Van Doorn, R.; Spijksma, G.; Koek, M.; Van Der Greef, J.; et al. Discovery of early-stage biomarkers for diabetic kidney disease using ms-based metabolomics (FinnDiane study). *Metabolomics* **2011**, *8*, 109–119. [CrossRef]

100. Lappas, M.; Mundra, P.A.; Wong, G.; Huynh, K.; Jinks, D.; Georgiou, H.M.; Permezel, M.; Meikle, P.J. The prediction of type 2 diabetes in women with previous gestational diabetes mellitus using lipidomics. *Diabetologia* **2015**, *58*, 1436–1442. [CrossRef]
101. Khan, S.R.; Mohan, H.; Liu, Y.; Batchuluun, B.; Gohil, H.; Al Rijjal, D.; Manialawy, Y.; Cox, B.J.; Gunderson, E.P.; Wheeler, M.B. The discovery of novel predictive biomarkers and early-stage pathophysiology for the transition from gestational diabetes to type 2 diabetes. *Diabetologia* **2019**, *62*, 687–703. [CrossRef]
102. Tamamogˇullari, N.; Siligˇ, Y.; Içagˇasiogˇlu, S.; Atalay, A. Carnitine Deficiency in Diabetes Mellitus Complications. *J. Diabetes Complicat.* **1999**, *13*, 251–253. [CrossRef]
103. Batchuluun, B.; Al Rijjal, D.; Prentice, K.J.; Eversley, J.A.; Burdett, E.; Mohan, H.; Bhattacharjee, A.; Gunderson, E.P.; Liu, Y.; Wheeler, M.B. Elevated Medium-Chain Acylcarnitines Are Associated with Gestational Diabetes Mellitus and Early Progression to Type 2 Diabetes and Induce Pancreatic β-Cell Dysfunction. *Diabetes* **2018**, *67*, 885–897. [CrossRef]
104. Möder, M.; Kießling, A.; Löster, H.; Brüggemann, L. The pattern of urinary acylcarnitines determined by electrospray mass spectrometry: A new tool in the diagnosis of diabetes mellitus. *Anal. Bioanal. Chem.* **2003**, *375*, 200–210. [CrossRef]
105. Saggerson, E.D. Malonyl-CoA, a Key Signaling Molecule in Mammalian Cells. *Annu. Rev. Nutr.* **2008**, *28*, 253–272. [CrossRef] [PubMed]
106. Adams, S.H.; Hoppel, C.L.; Lok, K.H.; Zhao, L.; Wong, S.W.; Minkler, P.E.; Hwang, D.H.; Newman, J.W.; Garvey, W.T. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. *J. Nutr.* **2009**, *139*, 1073–1081. [CrossRef] [PubMed]
107. Sun, L.; Liang, L.; Gao, X.; Zhang, H.; Yao, P.; Hu, Y.; Ma, Y.; Wang, F.; Jin, Q.; Li, H.; et al. Early Prediction of Developing Type 2 Diabetes by Plasma Acylcarnitines: A Population-Based Study. *Diabetes Care* **2016**, *39*, 1563–1570. [CrossRef] [PubMed]
108. Guasch-Ferré, M.; Ruiz-Canela, M.; Li, J.; Zheng, Y.; Bulló, M.; Wang, D.D.; Toledo, E.; Clish, C.; Corella, D.; Estruch, R.; et al. Plasma Acylcarnitines and Risk of Type 2 Diabetes in a Mediterranean Population at High Cardiovascular Risk. *J. Clin. Endocrinol. Metab.* **2018**, *104*, 1508–1519. [CrossRef] [PubMed]
109. Mihalik, S.J.; Goodpaster, B.H.; Kelley, D.E.; Chace, D.H.; Vockley, J.; Toledo, F.G.; Delany, J.P. Increased Levels of Plasma Acylcarnitines in Obesity and Type 2 Diabetes and Identification of a Marker of Glucolipotoxicity. *Obesity* **2010**, *18*, 1695–1700. [CrossRef]
110. Nowak, C.; Hetty, S.; Salihovic, S.; Castillejo-López, C.; Ganna, A.; Cook, N.; Broeckling, C.D.; Prenni, J.E.; Shen, X.; Giedraitis, V.; et al. Glucose challenge metabolomics implicates medium-chain acylcarnitines in insulin resistance. *Sci. Rep.* **2018**, *8*, 8691. [CrossRef]
111. Libert, D.M.; Nowacki, A.S.; Natowicz, M.R. Metabolomic analysis of obesity, metabolic syndrome, and type 2 diabetes: Amino acid and acylcarnitine levels change along a spectrum of metabolic wellness. *PeerJ* **2018**, *6*, e5410. [CrossRef]
112. Dellow, W.J.; Chambers, S.T.; Lever, M.; Lunt, H.; Robson, R.A. Elevated glycine betaine excretion in diabetes mellitus patients is associated with proximal tubular dysfunction and hyperglycemia. *Diabetes Res. Clin. Pract.* **1999**, *43*, 91–99. [CrossRef]
113. Gunderson, E.P.; Hurston, S.R.; Ning, X.; Lo, J.C.; Crites, Y.; Walton, D.; Dewey, K.G.; Azevedo, R.A.; Young, S.; Fox, G.; et al. Lactation and Progression to Type 2 Diabetes Mellitus After Gestational Diabetes Mellitus. *Ann. Intern. Med.* **2015**, *163*, 889–898. [CrossRef] [PubMed]
114. Gall, W.E.; Beebe, K.; Lawton, K.A.; Adam, K.-P.; Mitchell, M.W.; Nakhle, P.J.; Ryals, J.A.; Milburn, M.V.; Nannipieri, M.; Camastra, S.; et al. α-Hydroxybutyrate Is an Early Biomarker of Insulin Resistance and Glucose Intolerance in a Nondiabetic Population. *PLoS ONE* **2010**, *5*, e10883. [CrossRef] [PubMed]
115. Gunderson, E.P.; Matias, S.; Hurston, S.R.; Dewey, K.G.; Ferrara, A.; Quesenberry, C.P.; Lo, J.; Sternfeld, B.; Selby, J.V. Study of Women, Infant feeding, and Type 2 diabetes mellitus after GDM pregnancy (SWIFT), a prospective cohort study: Methodology and design. *BMC Public Health* **2011**, *11*, 952. [CrossRef]
116. Randle, P.; Garland, P.; Hales, C.; Newsholme, E. The glucose fatty-acid cycle its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1963**, *281*, 785–789. [CrossRef]
117. Jones, D.; Carter, R.; Mann, J. Indirect Evidence of Impairment of Platelet Desaturase Enzymes in Diabetes Mellitus. *Horm. Metab. Res.* **1986**, *18*, 341–344. [CrossRef] [PubMed]

118. Taylor, A.J.; Jennings, P.E.; Barnett, A.H.; Pandov, H.I.; Lawson, N. An alternative explanation for the changes in erythrocyte fatty acids observed in diabetes mellitus. *Clin. Chem.* **1987**, *33*, 2083–2085. [CrossRef]
119. Mandal, S.; Causevic, A.; Dzudzevic-Cancar, H.; Semiz, S. Free fatty acid profile in Type 2 diabetic subjects with different control of glycemia. In *CMBEBIH 2017*; Springer: Singapore, 2017.
120. Leekumjorn, S.; Cho, H.J.; Wu, Y.; Wright, N.T.; Sum, A.K.; Chan, C. The role of fatty acid unsaturation in minimizing biophysical changes on the structure and local effects of bilayer membranes. *Biochim. Biophys. Acta (BBA) Biomembr.* **2009**, *1788*, 1508–1516. [CrossRef]
121. Grapov, D.; Adams, S.H.; Pedersen, T.L.; Garvey, W.T.; Newman, J.W. Type 2 Diabetes Associated Changes in the Plasma Non-Esterified Fatty Acids, Oxylipins and Endocannabinoids. *PLoS ONE* **2012**, *7*, e48852. [CrossRef]
122. Vessby, B.; Aro, A.; Skarfors, E.; Berglund, L.; Salminen, I.; Lithell, H. The Risk to Develop NIDDM Is Related to the Fatty Acid Composition of the Serum Cholesterol Esters. *Diabetes* **1994**, *43*, 1353–1357. [CrossRef]
123. ARIC Study Investigators; Wang, L.; Folsom, A.R.; Zheng, Z.-J.; Pankow, J.S.; Eckfeldt, J.H. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: The Atherosclerosis Risk in Communities (ARIC) Study. *Am. J. Clin. Nutr.* **2003**, *78*, 91–98. [CrossRef]
124. Lapolla, A.; Sartore, G.; Della Rovere, G.R.; Romanato, G.; Zambon, S.; Marin, R.; Manzato, E.; Fedele, D. Plasma fatty acids and lipoproteins in type 2 diabetic patients. *Diabetes/Metab. Res. Rev.* **2006**, *22*, 226–231. [CrossRef] [PubMed]
125. Yang, J.; Xu, G.; Hong, Q.; Liebich, H.M.; Lutz, K.; Schmülling, R.-M.; Wahl, H.G. Discrimination of Type 2 diabetic patients from healthy controls by using metabonomics method based on their serum fatty acid profiles. *J. Chromatogr. B* **2004**, *813*, 53–58. [CrossRef]
126. Forouhi, N.G.; Koulman, A.; Sharp, S.J.; Imamura, F.; Kröger, J.; Schulze, M.B.; Crowe, F.; Sánchez, M.-J.; Guevara, M.; Beulens, J.W.J.; et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: The EPIC-InterAct case-cohort study. *Lancet Diabetes Endocrinol.* **2014**, *2*, 810–818. [CrossRef]
127. Tan, B.; Liang, Y.; Yi, L.; Li, H.; Zhou, Z.; Ji, X.; Deng, J. Identification of free fatty acids profiling of type 2 diabetes mellitus and exploring possible biomarkers by GC–MS coupled with chemometrics. *Metabolomics* **2009**, *6*, 219–228. [CrossRef]
128. Matsuzaka, T.; Shimano, H.; Yahagi, N.; Kato, T.; Atsumi, A.; Yamamoto, T.; Inoue, N.; Ishikawa, M.; Okada, S.; Ishigaki, N.; et al. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. *Nat. Med.* **2007**, *13*, 1193–1202. [CrossRef] [PubMed]
129. Ma, X.; Meng, L.; Li, L.; Ma, L.-N.; Mao, X. Plasma Free Fatty Acids Metabolic Profile Among Uyghurs and Kazaks with or Without Type 2 Diabetes Based on GC-MS. *Exp. Clin. Endocrinol. Diabetes* **2017**, *126*, 604–611. [CrossRef] [PubMed]
130. Tripathy, D.; Mohanty, P.; Dhindsa, S.; Syed, T.; Ghanim, H.; Aljada, A.; Dandona, P. Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes* **2003**, *52*, 2882–2887. [CrossRef] [PubMed]
131. Pankow, J.S.; Duncan, B.B.; Schmidt, M.I.; Ballantyne, C.M.; Couper, D.; Hoogeveen, R.; Golden, S.H. Fasting plasma free fatty acids and risk of type 2 diabetes: The atherosclerosis risk in communities study. *Diabetes Care* **2004**, *27*, 77–82. [CrossRef]
132. Spiller, S.; Blüher, M.; Hoffmann, R. Plasma levels of free fatty acids correlate with type 2 diabetes mellitus. *Diabetes Obes. Metab.* **2018**, *20*, 2661–2669. [CrossRef]
133. Yi, L.; He, J.; Liang, Y.-Z.; Yuan, D.-L.; Chau, F.-T. Plasma fatty acid metabolic profiling and biomarkers of type 2 diabetes mellitus based on GC/MS and PLS-LDA. *FEBS Lett.* **2006**, *580*, 6837–6845. [CrossRef]
134. Yakoob, M.Y.; Shi, P.; Willett, W.C.; Rexrode, K.M.; Campos, H.; Orav, E.J.; Hu, F.B.; Mozaffarian, D. Circulating Biomarkers of Dairy Fat and Risk of Incident Diabetes Mellitus Among Men and Women in the United States in Two Large Prospective Cohorts. *Circulation* **2016**, *133*, 1645–1654. [CrossRef] [PubMed]
135. Mozaffarian, D.; de Oliveira Otto, M.C.; Lemaitre, R.N.; Fretts, A.M.; Hotamisligil, G.; Tsai, M.Y.; Siscovick, D.S.; Nettleton, J.A. Trans-Palmitoleic acid, other dairy fat biomarkers, and incident diabetes: The Multi-Ethnic Study of Atherosclerosis (MESA). *Am. J. Clin. Nutr.* **2013**, *97*, 854–861. [CrossRef] [PubMed]
136. Boden, G.; Chen, X.; Capulong, E.; Mozzoli, M. Effects of free fatty acids on gluconeogenesis and autoregulation of glucose production in type 2 diabetes. *Diabetes* **2001**, *50*, 810–816. [CrossRef] [PubMed]

137. Chen, X.; Stein, T.P.; Steer, R.A.; Scholl, T.O. Individual free fatty acids have unique associations with inflammatory biomarkers, insulin resistance and insulin secretion in healthy and gestational diabetic pregnant women. *BMJ Open Diabetes Res. Care* **2019**, *7*, e000632. [CrossRef]
138. Liu, L.; Wang, M.; Yang, X.; Bi, M.; Na, L.; Niu, Y.; Li, Y.; Sun, C. Fasting Serum Lipid and Dehydroepiandrosterone Sulfate as Important Metabolites for Detecting Isolated Postchallenge Diabetes: Serum Metabolomics via Ultra-High-Performance LC-MS. *Clin. Chem.* **2013**, *59*, 1338–1348. [CrossRef]
139. Louet, J.-F.; Chopra, A.R.; Sagen, J.V.; An, J.; York, B.; Tannour-Louet, M.; Saha, P.K.; Stevens, R.D.; Wenner, B.R.; Ilkayeva, O.R.; et al. The Coactivator SRC-1 Is an Essential Coordinator of Hepatic Glucose Production. *Cell Metab.* **2010**, *12*, 606–618. [CrossRef]
140. Turnbaugh, P.J.; Ley, R.E.; Mahowald, M.A.; Magrini, V.; Mardis, E.R.; Gordon, J.I. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **2006**, *444*, 1027–1031. [CrossRef]
141. Macfarlane, S.; Macfarlane, G.T. Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* **2003**, *62*, 67–72. [CrossRef]
142. Gao, Z.; Yin, J.; Zhang, J.; Ward, R.E.; Martin, R.J.; Lefevre, M.; Cefalu, W.T.; Ye, J. Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes* **2009**, *58*, 1509–1517. [CrossRef] [PubMed]
143. Niwa, T. The protein metabolite theory as a mechanism for the progression of renal failure. *J. Ren. Nutr.* **2001**, *11*, 181–182. [CrossRef]
144. Han, J.; Lin, K.; Sequeira, C.; Borchers, C.H. An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography–Tandem mass spectrometry. *Anal. Chim. Acta* **2015**, *854*, 86–94. [CrossRef] [PubMed]
145. Niwa, T.; Maeda, K.; Ohki, T.; Saito, A.; Tsuchida, I. Gas chromatographic—Mass spectrometric profile of organic acids in urine and serum of diabetic ketotic patients. *J. Chromatogr. B Biomed. Sci. Appl.* **1981**, *225*, 1–8. [CrossRef]
146. Yuan, K.; Kong, H.; Guan, Y.; Yang, J.; Xu, G. A GC-based metabonomics investigation of type 2 diabetes by organic acids metabolic profile. *J. Chromatogr. B* **2007**, *850*, 236–240. [CrossRef]
147. Lin, H.V.; Frassetto, A.; Kowalik, E.J., Jr.; Nawrocki, A.R.; Lu, M.M.; Kosinski, J.R.; Hubert, J.A.; Szeto, D.; Yao, X.; Forrest, G.; et al. Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. *PLoS ONE* **2012**, *7*, e35240. [CrossRef] [PubMed]
148. Yadav, H.; Lee, J.-H.; Lloyd, J.; Walter, P.; Rane, S.G. Beneficial Metabolic Effects of a Probiotic via Butyrate-induced GLP-1 Hormone Secretion. *J. Biol. Chem.* **2013**, *288*, 25088–25097. [CrossRef] [PubMed]
149. Kimura, I.; Ozawa, K.; Inoue, D.; Imamura, T.; Kimura, K.; Maeda, T.; Terasawa, K.; Kashihara, D.; Hirano, K.; Tani, T.; et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* **2013**, *4*, 1829. [CrossRef]
150. Qin, J.; Li, Y.; Cai, Z.; Li, S.; Zhu, J.; Zhang, F.; Liang, S.; Zhang, W.; Guan, Y.; Shen, D.; et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **2012**, *490*, 55–60. [CrossRef] [PubMed]
151. Al-Lahham, S.H.; Roelofsen, H.; Priebe, M.; Weening, D.; Dijkstra, M.; Hoek, A.; Rezaee, F.; Venema, K.; Vonk, R.J. Regulation of adipokine production in human adipose tissue by propionic acid. *Eur. J. Clin. Investig.* **2010**, *40*, 401–407. [CrossRef]
152. Maslowski, K.M.; Vieira, A.T.; Ng, A.; Kranich, J.; Sierro, F.; Yu, D.; Schilter, H.C.; Rolph, M.S.; Mackay, F.; Artis, D.; et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* **2009**, *461*, 1282–1286. [CrossRef]
153. Puddu, A.; Sanguineti, R.; Montecucco, F.; Viviani, G.L. Evidence for the Gut Microbiota Short-Chain Fatty Acids as Key Pathophysiological Molecules Improving Diabetes. *Mediat. Inflamm.* **2014**, *2014*, 162021. [CrossRef]
154. Roelofsen, H.; Priebe, M.G.; Vonk, R.J. The interaction of short-chain fatty acids with adipose tissue: Relevance for prevention of type 2 diabetes. *Benef. Microbes* **2010**, *1*, 433–437. [CrossRef]
155. Villarreal-Peréz, J.Z.; Villarreal-Martínez, J.Z.; Lavalle-González, F.; Torres-Sepúlveda, M.D.R.; Ruiz-Herrera, M.D.C.; Cerda-Flores, R.M.; Castillo-García, E.R.; Rodríguez-Sánchez, I.P.; Martínez-De-Villarreal, L.E. Plasma and urine metabolic profiles are reflective of altered beta-oxidation in non-diabetic obese subjects and patients with type 2 diabetes mellitus. *Diabetol. Metab. Syndr.* **2014**, *6*, 129. [CrossRef] [PubMed]

156. Chou, J.; Liu, R.; Yu, J.; Liu, X.; Zhao, X.; Li, Y.; Liu, L.; Sun, C. Fasting serum α-hydroxybutyrate and pyroglutamic acid as important metabolites for detecting isolated post-challenge diabetes based on organic acid profiles. *J. Chromatogr. B* **2018**, *1100*, 6–16. [CrossRef] [PubMed]
157. Gaster, M.; Nehlin, J.O.; Minet, A.D. Impaired TCA cycle flux in mitochondria in skeletal muscle from type 2 diabetic subjects: Marker or maker of the diabetic phenotype? *Arch. Physiol. Biochem.* **2012**, *118*, 156–189. [CrossRef] [PubMed]
158. Zhang, X.; Wang, Y.; Hao, F.; Zhou, X.; Han, X.; Tang, H.; Ji, L. Human Serum Metabonomic Analysis Reveals Progression Axes for Glucose Intolerance and Insulin Resistance Statuses. *J. Proteome Res.* **2009**, *8*, 5188–5195. [CrossRef]
159. Landaas, S. The formation of 2-hydroxybutyric acid in experimental animals. *Clin. Chim. Acta* **1975**, *58*, 23–32. [CrossRef]
160. Hinder, L.M.; Vivekanandan-Giri, A.; McLean, L.L.; Pennathur, S.; Feldman, E.L. Decreased glycolytic and tricarboxylic acid cycle intermediates coincide with peripheral nervous system oxidative stress in a murine model of type 2 diabetes. *J. Endocrinol.* **2013**, *216*, 1–11. [CrossRef]
161. Van Diepen, J.A.; Robben, J.H.; Hooiveld, G.J.; Carmone, C.; Alsady, M.; Boutens, L.; Bekkenkamp-Grovenstein, M.; Hijmans, A.; Engelke, U.F.H.; Wevers, R.A.; et al. SUCNR1-mediated chemotaxis of macrophages aggravates obesity-induced inflammation and diabetes. *Diabetologia* **2017**, *60*, 1304–1313. [CrossRef]
162. Sato, J.; Kanazawa, A.; Ikeda, F.; Yoshihara, T.; Goto, H.; Abe, H.; Komiya, K.; Kawaguchi, M.; Shimizu, T.; Ogihara, T.; et al. Gut Dysbiosis and Detection of "Live Gut Bacteria" in Blood of Japanese Patients with Type 2 Diabetes. *Diabetes Care* **2014**, *37*, 2343–2350. [CrossRef]
163. Kootte, R.S.; Vrieze, A.; Holleman, F.; Dallinga-Thie, G.M.; Zoetendal, E.G.; De Vos, W.M.; Groen, A.K.; Hoekstra, J.B.L.; Stroes, E.S.; Nieuwdorp, M. The therapeutic potential of manipulating gut microbiota in obesity and type 2 diabetes mellitus. *Diabetes Obes. Metab.* **2011**, *14*, 112–120. [CrossRef]
164. Cani, P.D.; Bibiloni, R.; Knauf, C.; Waget, A.; Neyrinck, A.M.; Delzenne, N.; Burcelin, R. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet-Induced Obesity and Diabetes in Mice. *Diabetes* **2008**, *57*, 1470–1481. [CrossRef] [PubMed]
165. Maalouf, N.M.; Cameron, M.A.; Moe, O.W.; Adams-Huet, B.; Sakhaee, K. Low Urine pH: A Novel Feature of the Metabolic Syndrome. *Clin. J. Am. Soc. Nephrol.* **2007**, *2*, 883–888. [CrossRef]
166. Abate, N.; Chandalia, M.; Cabo-Chan, A.V.; Moe, O.W.; Sakhaee, K.; Abate, M.C.N. The metabolic syndrome and uric acid nephrolithiasis: Novel features of renal manifestation of insulin resistance. *Kidney Int.* **2004**, *65*, 386–392. [CrossRef] [PubMed]
167. Maalouf, N.M.; Cameron, M.A.; Moe, O.W.; Sakhaee, K. Metabolic basis for low urine pH in type 2 diabetes. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*, 1277–1281. [CrossRef] [PubMed]
168. Larsen, N.; Vogensen, F.K.; Berg, F.V.D.; Nielsen, D.S.; Andreasen, A.S.; Pedersen, B.K.; Abu Al-Soud, W.; Sørensen, S.J.; Hansen, L.H.; Jakobsen, M. Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. *PLoS ONE* **2010**, *5*, e9085. [CrossRef]
169. Bobulescu, I.A.; Dubree, M.; Zhang, J.; McLeroy, P.; Moe, O.W. Effect of renal lipid accumulation on proximal tubule Na+/H+ exchange and ammonium secretion. American journal of physiology. *Ren. Physiol.* **2008**, *294*, F1315–F1322. [CrossRef]
170. Fukuda, S.; Ohno, H. Gut microbiome and metabolic diseases. *Semin. Immunopathol.* **2013**, *36*, 103–114. [CrossRef]
171. Karlsson, F.; Tremaroli, V.; Nookaew, I.; Bergström, G.; Behre, C.J.; Fagerberg, B.; Nielsen, J.; Bäckhed, F. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* **2013**, *498*, 99–103. [CrossRef]
172. Aw, W.; Fukuda, S. Toward the comprehensive understanding of the gut ecosystem via metabolomics-based integrated omics approach. *Semin. Immunopathol.* **2014**, *37*, 5–16. [CrossRef]
173. Wen, L.; Ley, R.E.; Volchkov, P.Y.; Stranges, P.B.; Avanesyan, L.; Stonebraker, A.C.; Hu, C.; Wong, F.S.; Szot, G.L.; Bluestone, J.A.; et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* **2008**, *455*, 1109–1113. [CrossRef]
174. Jiang, W.; Wu, N.; Wang, X.; Chi, Y.; Zhang, Y.; Qiu, X.; Hu, Y.; Li, J.; Liu, Y.-L. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci. Rep.* **2015**, *5*, 8096. [CrossRef] [PubMed]

175. Mikkelsen, K.H.; Knop, F.K.; Frost, M.; Hallas, J.; Pottegård, A. Use of Antibiotics and Risk of Type 2 Diabetes: A Population-Based Case-Control Study. *J. Clin. Endocrinol. Metab.* **2015**, *100*, 3633–3640. [CrossRef] [PubMed]
176. Hylemon, P.B.; Zhou, H.; Pandak, W.M.; Ren, S.; Gil, G.; Dent, P. Bile acids as regulatory molecules. *J. Lipid Res.* **2009**, *50*, 1509–1520. [CrossRef] [PubMed]
177. Yamagata, K.; Daitoku, H.; Shimamoto, Y.; Matsuzaki, H.; Hirota, K.; Ishida, J.; Fukamizu, A. Bile Acids Regulate Gluconeogenic Gene Expression via Small Heterodimer Partner-mediated Repression of Hepatocyte Nuclear Factor 4 and Foxo1. *J. Biol. Chem.* **2004**, *279*, 23158–23165. [CrossRef]
178. Ryan, K.; Tremaroli, V.; Clemmensen, C.; Kovatcheva-Datchary, P.; Myronovych, A.; Karns, R.; Wilson-Pérez, H.E.; Sandoval, D.A.; Kohli, R.; Bäckhed, F.; et al. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* **2014**, *509*, 183–188. [CrossRef]
179. Schwiertz, A.; Taras, D.; Schafer, K.; Beijer, S.; Bos, N.A.; Donus, C.; Hardt, P.D. Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity* **2010**, *18*, 190–195. [CrossRef]
180. Vrieze, A.; Van Nood, E.; Holleman, F.; Salojärvi, J.; Kootte, R.S.; Bartelsman, J.F.; Dallinga–Thie, G.M.; Ackermans, M.T.; Serlie, M.J.; Oozeer, R.; et al. Transfer of Intestinal Microbiota from Lean Donors Increases Insulin Sensitivity in Individuals with Metabolic Syndrome. *Gastroenterology* **2012**, *143*, 913–916.e7. [CrossRef]
181. Le Chatelier, E.; Nielsen, T.; Qin, J.; Prifti, E.; Hildebrand, F.; Falony, G.; Almeida, M.; Arumugam, M.; Batto, J.M.; Kennedy, S.; et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* **2013**, *500*, 541–546. [CrossRef]
182. Forslund, K.; Hildebrand, F.; Nielsen, T.; Falony, G.; Le Chatelier, E.; Sunagawa, S.; Prifti, E.; Vieira-Silva, S.; Gudmundsdottir, V.; Pedersen, H.K.; et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **2015**, *528*, 262–266. [CrossRef]
183. De Vadder, F.; Kovatcheva-Datchary, P.; Goncalves, D.; Vinera, J.; Zitoun, C.; Duchampt, A.; Bäckhed, F.; Mithieux, G. Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. *Cell* **2014**, *156*, 84–96. [CrossRef]
184. De Vadder, F.; Kovatcheva-Datchary, P.; Zitoun, C.; Duchampt, A.; Bäckhed, F.; Mithieux, G. Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis. *Cell Metab.* **2016**, *24*, 151–157. [CrossRef] [PubMed]
185. Zhang, X.; Shen, D.; Fang, Z.; Jie, Z.; Qiu, X.; Zhang, C.; Chen, Y.; Ji, L. Human Gut Microbiota Changes Reveal the Progression of Glucose Intolerance. *PLoS ONE* **2013**, *8*, e71108. [CrossRef] [PubMed]
186. Plovier, H.; Everard, A.; Druart, C.; Depommier, C.; Van Hul, M.; Geurts, L.; Chilloux, J.; Ottman, N.; Duparc, T.; Lichtenstein, L.; et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat. Med.* **2016**, *23*, 107–113. [CrossRef] [PubMed]
187. Pedersen, H.K.; Gudmundsdottir, V.; Nielsen, H.B.; Hyotylainen, T.; Nielsen, T.; Jensen, B.A.; Forslund, S.K.; Hildebrand, F.; Prifti, E.; Falony, G.; et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* **2016**, *535*, 376–381. [CrossRef] [PubMed]
188. David, L.A.; Maurice, C.F.; Carmody, R.N.; Gootenberg, D.; Button, J.E.; Wolfe, B.E.; Ling, A.V.; Devlin, A.S.; Varma, Y.; Fischbach, M.A.; et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **2013**, *505*, 559–563. [CrossRef] [PubMed]
189. Russell, W.R.; Gratz, S.W.; Duncan, S.H.; Holtrop, G.; Ince, J.; Scobbie, L.; Duncan, G.; Johnstone, A.M.; Lobley, G.E.; Wallace, R.J.; et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am. J. Clin. Nutr.* **2011**, *93*, 1062–1072. [CrossRef]
190. Fava, F.; Gitau, R.; Griffin, B.A.; Gibson, G.R.; Tuohy, K.; Lovegrove, J.A. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. *Int. J. Obes.* **2012**, *37*, 216–223. [CrossRef]
191. Caesar, R.; Tremaroli, V.; Kovatcheva-Datchary, P.; Cani, P.D.; Bäckhed, F. Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. *Cell Metab.* **2015**, *22*, 658–668. [CrossRef]
192. Francavilla, R.; Calasso, M.; Calace, L.; Siragusa, S.; Ndagijimana, M.; Vernocchi, P.; Brunetti, L.; Mancino, G.; Tedeschi, G.; Guerzoni, E.; et al. Effect of lactose on gut microbiota and metabolome of infants with cow's milk allergy. *Pediatr. Allergy Immunol.* **2012**, *23*, 420–427. [CrossRef] [PubMed]

193. Suez, J.; Korem, T.; Zeevi, D.; Zilberman-Schapira, G.; Thaiss, C.A.; Maza, O.; Israeli, D.; Zmora, N.; Gilad, S.; Weinberger, A.; et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **2014**, *514*, 181–186. [CrossRef] [PubMed]
194. Machiels, K.; Joossens, M.; Sabino, J.; De Preter, V.; Arijs, I.; Eeckhaut, V.; Ballet, V.; Claes, K.; Van Immerseel, F.; Verbeke, K.; et al. A decrease of the butyrate-producing speciesRoseburia hominisandFaecalibacterium prausnitziidefines dysbiosis in patients with ulcerative colitis. *Gut* **2013**, *63*, 1275–1283. [CrossRef]
195. Singh, R.K.; Chang, H.-W.; Yan, D.; Lee, K.M.; Ucmak, D.; Wong, K.; Abrouk, M.; Farahnik, B.; Nakamura, M.; Zhu, T.H.; et al. Influence of diet on the gut microbiome and implications for human health. *J. Transl. Med.* **2017**, *15*, 73. [CrossRef] [PubMed]
196. Pászti-Gere, E.; Szeker, K.; Csibrik-Nemeth, E.; Csizinszky, R.; Marosi, A.; Palocz, O.; Farkas, O.; Galfi, P. Metabolites of Lactobacillus plantarum 2142 Prevent Oxidative Stress-Induced Overexpression of Proinflammatory Cytokines in IPEC-J2 Cell Line. *Inflammation* **2012**, *35*, 1487–1499. [CrossRef] [PubMed]
197. Matsuzaki, T.; Takagi, A.; Ikemura, H.; Matsuguchi, T.; Yokokura, T. Intestinal microflora: Probiotics and autoimmunity. *J. Nutr.* **2007**, *137*, 798S–802S. [CrossRef] [PubMed]
198. Hald, S.; Schioldan, A.G.; Moore, M.E.; Dige, A.; Lærke, H.N.; Agnholt, J.; Knudsen, K.E.B.; Hermansen, K.; Marco, M.L.; Gregersen, S.; et al. Effects of Arabinoxylan and Resistant Starch on Intestinal Microbiota and Short-Chain Fatty Acids in Subjects with Metabolic Syndrome: A Randomised Crossover Study. *PLoS ONE* **2016**, *11*, e0159223. [CrossRef] [PubMed]
199. Manirarora, J.N.; Parnell, S.A.; Hu, Y.-H.; Kosiewicz, M.M.; Alard, P. NOD Dendritic Cells Stimulated with Lactobacilli Preferentially Produce IL-10 versus IL-12 and Decrease Diabetes Incidence. *Clin. Dev. Immunol.* **2011**, *2011*, 630187. [CrossRef]
200. Kim, Y.; Keogh, J.; Clifton, P.M. Polyphenols and Glycemic Control. *Nutrients* **2016**, *8*, 17. [CrossRef]
201. Hooper, L.; Kay, C.D.; Abdelhamid, A.; Kroon, P.A.; Cohn, J.S.; Rimm, E.B.; Cassidy, A. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: A systematic review and meta-analysis of randomized trials. *Am. J. Clin. Nutr.* **2012**, *95*, 740–751. [CrossRef]

2) **Hameed, Ahsan,** Mauro Galli, Edyta Adamska-Patruno, Adam Krętowski, and Michal Ciborowski. 2020. "Select Polyphenol-Rich Berry Consumption to Defer or Deter Diabetes and Diabetes-Related Complications" *Nutrients* 12, no. 9: 2538. https://doi.org/10.3390/nu12092538

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Review

Select Polyphenol-Rich Berry Consumption to Defer or Deter Diabetes and Diabetes-Related Complications

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Abstract: Berries are considered "promising functional fruits" due to their distinct and ubiquitous therapeutic contents of anthocyanins, proanthocyanidins, phenolic acids, flavonoids, flavanols, alkaloids, polysaccharides, hydroxycinnamic, ellagic acid derivatives, and organic acids. These polyphenols are part of berries and the human diet, and evidence suggests that their intake is associated with a reduced risk or the reversal of metabolic pathophysiologies related to diabetes, obesity, oxidative stress, inflammation, and hypertension. This work reviewed and summarized both clinical and non-clinical findings that the consumption of berries, berry extracts, purified compounds, juices, jams, jellies, and other berry byproducts aided in the prevention and or otherwise management of type 2 diabetes mellitus (T2DM) and related complications. The integration of berries and berries-derived byproducts into high-carbohydrate (HCD) and high-fat (HFD) diets, also reversed/reduced the HCD/HFD-induced alterations in glucose metabolism-related pathways, and markers of oxidative stress, inflammation, and lipid oxidation in healthy/obese/diabetic subjects. The berry polyphenols also modulate the intestinal microflora ecology by opposing the diabetic and obesity rendered symbolic reduction of Bacteroidetes/Firmicutes ratio, intestinal mucosal barrier dysfunction-restoring bacteria, short-chain fatty acids, and organic acid producing microflora. All studies proposed a number of potential mechanisms of action of respective berry bioactive compounds, although further mechanistic and molecular studies are warranted. The metabolic profiling of each berry is also included to provide up-to-date information regarding the potential anti-oxidative/antidiabetic constituents of each berry.

Keywords: berries; metabolic syndrome; precision nutrition; hyperglycemia; hyperlipidemia; diabetes; omics; metabolomics; genomics

1. Introduction

Diabetes mellitus (DM) is a multifactorial disease with high mortality worldwide. Chronic DM is the eighth-leading cause of deaths globally, responsible for 1.5 million deaths each year [1]. According to the World Health Organization (WHO), in 2013, 381 million adults were diagnosed with DM, which increased to 422 million in 2016 and is expected to double by 2030. Type 1 (T1DM) represents 15% of cases, and the remaining cases are type 2 (T2DM) [1]. T2DM is primarily treated with pharmacotherapeutic drugs, evidence-based alternative approaches, and functional food-based approaches [2]. Pharmacotherapeutic approaches generally consist of monotherapy

or binary/poly-therapy, depending on severity. Most physicians use the binary approach and prescribe insulin-secretogenic sulfonylurea drugs and the insulin sensitivity enhancer metformin. Additional drugs address diabetes-induced vascular complications, with the average number of prescribed daily drugs being as high as four [3]. Combined drug therapy is associated with long-term side-effects and other costs, resulting in non-adherence [4]. Moreover, evidence-based alternative approaches may have safety and toxicity issues due to which precision nutrition-based approaches have recently been proposed as alternatives to defer or deter T2DM and its complications.

The provision of individualized dietary and nutritional recommendations is referred to as precision nutrition. Polyphenol-rich fruits (including berries) are the primary components of precision nutrition, and consumption of these fruits, like berries, represent a potential "frontline strategy" for combating T2DM in obese or overweight patients. Substantial evidence suggests that T2DM onset can be prevented or managed by berries and/or berries-derived-tailored dietary intake, exercise, and the maintenance of healthy body weights (BWs) [5]. Therefore, targeted berries-nutrition is considered analogous to an individualized medicinal approach, providing effective and safe nutritional interventions for DM prevention and management. Furthermore, the American Diabetes Association and Dietary Guidelines for Americans also strongly recommend diets rich in anthocyanin and polyphenols to protect against and manage DM [6]. Increasing evidence shows that berry consumption also reduces DM risk, including a recent study showing that Finnish men who regularly consumed berries reduced their T2DM risks by up to 35% [7]. Due to the significance of berry consumption and the lack of comprehensive studies examining berry consumption effects specifically on DM, this study aimed to collect and summarize all studies examining the relationship between berry consumption and DM.

DM is a metabolic syndrome with concordance changes in insulin sensitivity and/or availability. This insulin insensitivity and/or deficiency induces derangements in metabolic pathways related to glucose, lipids, and protein metabolism. Berry, or its byproduct, intake not only opposes these derangements by normalizing the metabolic homeostasis of glucose, lipids, and protein metabolism, but also improves insulin sensitivity and secretary indexes. Therefore, all available in vitro and in vivo studies involving whole berries or berry bioproduct consumption and citing the normalization of insulin signaling, secretion, and sensitivity, restoring the altered glucose, lipid, and protein metabolism, and reduction of oxidative stress and inflammatory cytokines were included. In order to determine the hypoglycemic and hypolipidemic potential of berries, studies that added berries to high-fat (HFD) and high-carbohydrate (HCD) diets, defined as diets with >45% fat and >60% carbohydrates, respectively, were also included. In addition to HFD and/or HCD, disruption of intestinal endothelium and homeostasis resulting in epithelial inflammation, increased permeability (i.e., dysbiosis), and alteration in gut microbial taxonomic composition and diversity (increase in Firmicutes:Bacteroidetes ratio, and reduction in intestinal mucosal barrier dysfunction (IMBD) restoring bacterial families, proteolytic and glycolytic microflora, short-chain fatty acids (SCFA), and organic acids (SCOA) producing microflora) are also considered risk factors to obesity and DM. IMBD associated bacterial families protect the epithelial layer of the intestine whereas SCFA and SCOA played important role in the synthesis and production of immunoglobulins and immune-supportive cytokines to protect against dysbiosis and metabolic disorders. In this context, the impact of berry or berry product intake on the attenuation of obesity-associated disorders and dysbiosis was also reviewed. Studies involving the metabolic fingerprinting of berries were also described to represent the possible number of compounds considered responsible for their antioxidative and antidiabetic actions.

Consequently, this review aimed to discuss scientific evidence regarding a positive role of berry consumption on the prevention or delay of diabetes development and reduction or avoidance of diabetes-related complications. Moreover, a detailed composition of different berries is also presented.

2. Methods

Studies examining berry consumption and T2DM were searched for (last time accessed 15 June 2020) in the Medline/PubMed, ScienceDirect/Scopus, and Web of Sciences databases using the following keywords and phrases: berry consumption and diabetes, berry polyphenolic compounds and diabetes, berry intake and glucose metabolism, berries and high-fat diets, berries and high-glycemic diets, metabolic fingerprinting of berries, lipid metabolism and berries, glycemic control, human clinical trials with berries, in vitro/in vivo studies using berries, and individual berry names. The search using these keywords and phrases resulted in more than 3000 articles in said scientific databases, as illustrated in detail in Figure 1. All articles not in line with the objectives of this review article were not considered. Additionally, the articles that were found more than once in these databases were counted once, and after removal of these duplicate and irrelevant records, nearly 2645 publications were thoroughly screened for inclusion eligibility. Finally, 336 publications were found relevant and fit to be reviewed. Only studies examining berries or berry product consumption relative to metabolic syndrome conditions or otherwise DM respective and berry fingerprinting were included. The schematic flow diagram for the selection of studies in this work is presented in Figure 1.

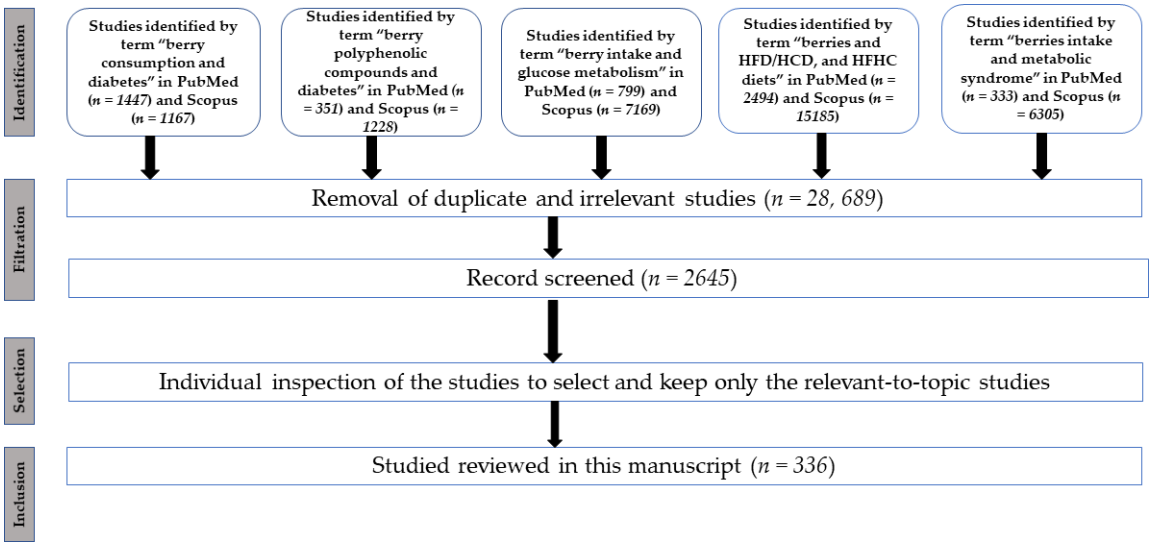

Figure 1. Schematic representation of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram collection and selection of studies included in this review. Adapted from Moher, Liberati [8].

3. Blueberries

Blueberries (BlBs) top the list of five fruits recommended by the Food and Agriculture Organization of the United Nations (FAO) against diabetes, cancer, liver disease, anemia, and cardiovascular disease (CVD). Initially, the in vitro antidiabetic activity of BlBs were reported by Barberis et al. [9] and Martineau et al. [10]. Barberis et al. described the reduced amount of glucose absorption in the Caco-2TC7 monolayer human intestinal cell line in the supplementation of phosphate-buffered-saline (PBS) containing BlB juice (BlBJ) prior to glucose stimulation. Martineau et al. [10] used insulin-dependent/independent 3T3-L1, C2C12, and TC-tet cell lines. The overnight incubation of these cells with BlB extracts (BlBEs) enhanced glucose uptake even in the absence of insulin compared to the vehicle-delivered control cell cultures [10]. The basal secretion of insulin from TC-tet cells increased 2.5 times to 7.5 times with increasing glucose amounts from 6 mM to 10 mM. A significant increase in glucose-stimulated insulin secretion (GSIS) was also seen after treating cells with BlBEs [10]. The BlBE adipogenic effects were also examined by assessing lipid formation and accumulation in pre-adipocytes, and BlB treatment was almost as effective as the positive control, rosiglitazone, for lipid accumulation. BlB consumption downregulated

the HFD-induced upregulation of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), leptin, and inflammatory genes (L-6, TNF-α, inducible nitric oxide synthase (iNOS)), monocyte chemo-attractant protein-1 (MCP-1) (an inflammatory cytokine), peroxisome proliferator-activated receptors γ (PPAR-γ), and fatty acid synthase (FAS) [11,12].

Hypoglycemic and hypolipidemic potential of BlBs or its polyphenol rich products has also been checked in many in vivo studies; Grace et al. [13] fed *streptozotocin* (STZ)-induced diabetic rats diets supplemented with phenolic- and anthocyanin-enriched BlBES. Anthocyanin-enriched diets increased hypoglycemic activity (51%) compared with phenolic-enriched diets (33%) and metformin-treated controls (32%), suggesting that anthocyanins modulated hyperglycemic and hyperlipidemic activities [13]. The supplementation of BlBE increased the beneficial glucose metabolism involved peroxisome proliferator response element (PPRE) (1.3–1.8%), glucose transporter 2 (GLUT-2) (1.5%), and PPAR-γ (1.4%) activities, and reduced the proinflammatory nuclear factor (NF)-κB activity [14]. Furthermore, an increase in the intercellular levels of the mRNA of glucose transporter (GLUT4), insulin receptor substrate-1/2 (IRS-1/IRS-2) (insulin response mediators regarding glucose metabolism), and AMP-activated protein kinase (AMPK) (a key regulator of mitochondrial biogenesis and cellular energy homeostasis) were observed in skeletal muscles, indicating increased glucose uptake [15,16]. BlB metabolites, especially anthocyanins, also promoted glucagon-like peptide-1 (GLP-1) expression and PPAR activity; GLP-1 increases glucose-dependent insulin secretion and pancreatic β-cell proliferation, whereas PPAR and nuclear fatty acid receptors improve IR [17]. Few studies have also shown improved insulin resistance (I) but with conflicting results in reduced BW gains [16,18,19]. However, in human clinical trials, improved insulin sensitivity without BW changes was observed [20]. Thus, insulin sensitivity may increase following BlB consumption, but BlBE may be less effective for modulating weight loss. Besides the BlBE, a few studies have also used the whole BlB fruit to determine its antidiabetic and anti-obesity potential in a group of people at high risk of T2DM (Table 1). BlB smoothie supplementation significantly reduced insulin resistance (IR) [21]. The ad libitum consumption of 100% pure BlBJ showed hypoglycemic activity, suppressing fatty acid synthase (FAS)- and β-oxidation-related gene expression in HFD-fed C57BL/6 mice (Table 1) [12]. Alcohol-free fermented juice, containing 30% BlBJ and 70% blackberry juice, reduced epididymal fat pad weights, percent fat mass, plasma triglyceride, and total cholesterol (TC) levels as well as mean adipocyte diameters and improved fasting blood glucose and GTT levels [22]. In another study, BlBJ consumption increased glucose uptake and inhibited adipogenesis by reducing adiponectin levels in KKKy mice [23]. In addition to BlBJ, BlB powder consumption in sugar-matched/sugar-non-matched smoothies extended the post-prandial glucose response and reduced peak postprandial glucose levels [24]. Diabetes and obesity are inter-linked via chronic inflammatory conditions, where macrophages infiltrate and accumulate in adipose tissue, triggering pro-inflammatory cytokine secretion [25]. BlB supplementation reduced these (pro)-inflammatory cytokine secretions (i.e., NF-κB, interleukin (IL)-10, tumor necrosis factor (TNF)-α, and IL-6 expression) in obese and diabetic mice [25]. BlBE consumption also showed excellent anti-inflammatory effects against soluble vascular cell adhesion molecule-1 (sVCAM-1) (inflammatory biomarker), MCP-1, C-reactive protein (CRP) (acute inflammatory protein), and vascular endothelium [26].

Oxidative stress increases reactive oxygen species (ROS), chemokines, nitric oxides (NOs), adhesion molecules, nuclear factor (IκBα) production, and glycation prior or after diabetes. Human aortic endothelial cells (HAECs) treated with purified BlB anthocyanins (hippuric acid, hydroxyhippuric acid, isovanillic acid-3-sulfate, benzoic acid-4-sulfate, and vanillic acid-4-sulfate) demonstrated reduced ROS, chemokine, NO, adhesion molecule, and IκBα production [27]. In a human clinical trial, post-exercise blueberry BlB consumption decreased manganese superoxide dismutase (Mn-SOD) levels [28]. Li et al. [29] reported anti-oxidative and anti-inflammatory cytokine marker suppression by 19 and 31%, respectively, in adipocytes and macrophages co-cultured with piceatannol, a BlB-derived bioactive compound. Piceatannol also ameliorated malfunctioning insulin-stimulated glucose uptake by upregulating Akt phosphorylation (crucial for IRS activation and hence increasing insulin sensitivity) and forkhead box O1 (FOXO1) (a transcription factor). Pterostilbene, a PPAR-α agonist found in BlB, promoted fatty acid catabolism by upregulating (up to 3%) of AMPK, carnitine palmitoyl transferase-1 (CPT-1) (an enzyme for long-chain fatty acid-LCFA oxidation), acyl-CoA oxidase (ACOX) (enzyme of β-oxidation system), and uncoupling protein-2 (UCP-2) (a protein involved in glucose disposal, insulin secretion, and cellular energy dissipation) expression. AMPK is associated with mitochondrial energy production, and AMPK activation regulates liver cell gluconeogenesis by suppressing glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK) (gene involved in glyceroneogenesis and gluconeogenesis), FOXO1, PPAR-γ coactivator 1α (PGC-1α), and glucose production. FOXO1 regulates PEPCK, PGC-1α, and G6Pase expression, thus affecting glucose release [30].

The integration of BlB polyphenols with a HFD also attenuated HFD rendered disorders and dysbiosis. The BlB powder supplementation improved the systematic inflammation and insulin sensitivity by modulating the gut microbial population in rat fed on a HFD [31]. In human, BlB intervention offered the prebiotic-effect by increasing the relative abundance of beneficial fermentative bacterium *Bifdobacterium* spp [32]. The BlB-derived anthocyanins also improved the IMBD restoration by decreasing the population of *E. coli* [33]. More recently, Rodríguez-Daza and Daoust [34] also witnessed that BlB-derived proanthocyanidins did not significantly improve the dysbiosis symbolic Firmicutes:Bacteroidetes ratio, but its supplementation did improve the population of genera (Akkermansia, Adlercreutzia, an unknown genus of order Clostridiales, Peptostreptococcaceae, and Ruminococcaceae) considered responsible for the maintenance and restoration of the colon mucosal barrier. The health promoting role of BlB and its byproducts can be explained further by a comprehensive metabolite profile for BlB/BlBE [10,35–39] and is shown in Table 2. The metabolic fingerprinting reveals BlB/BlBE as a rich source of antioxidative, antidiabetic, anti-inflammatory anthocyanins, proanthocyanidins, epicatechins, aglycons, glycosides, catechins, phenolic acids, chlorogenic acids, caffeic acid derivatives, and quercetin derivatives. Collectively, these studies demonstrated that BlB supplementation protected against HFD/HCD-induced IR hyperglycemia, pro-inflammatory responses, oxidative stress, adipocyte death, and improved insulin sensitivity, with mixed results for HFD-induced BW gain. The identified anthocyanins associated with these activities include glucosides, galactosides, and arabinosides of cyanidin, delphinidin, malvidin, peonidin, and petunidin.

Table 1. A comprehensive list of berry interventions and their consequent effects on various levels.

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
				(I) Blueberries (BlB) (Animal studies)			
1	RCT	C57bl/6J mice (*n* = NS)	12 wk	BlB anthocyanins 300–500 mg/kg.BW.day	LFD (20% kcal from lard fat) and HFD (70%kcal from lard fat)	Lower the blood glucose level and dyslipidemia markers	[13]
2	RCT	Male C57BL/6 mice (*n* = 24)	8 wk	4% (*w*/*w*) F/D whole BlB powder with HFD provided ad libitum	HFD (60% kcal from fat)	Offer protection against HFD-induced obesity, adipose tissue macrophages inflammatory gene expression, and oxidative stress	[11]
3	RCT	C57/Bl6 mice (*n* = 200)	12 wk	5% or 10% (*w*/*w*) of whole BlB with HFD provided ad libitum	HFD (45% kcal from fat)	Reduced HFD-induced cellular inflammatory cytokines, chemokines, interleukins, and proinflammatory interferon gamma -producing T-cells	[18]
4	RCT	Male Zucker Fatty and Zucker Lean rats (*n* = 48)	8 wk	4% (*w*/*w*) F/D whole BlB powder with HFD provided ad libitum	HFD (45% kcal from fat)	Hypolipidemic, Hyper-insulinemic, hypoglycemic and anti-inflammatory	[40]
5	RCT	C57BL/6 mice (*n* = 48)	12 wk	Ad libitum 100% BlBJ with HFD provided ad libitum	LFD (20% kcal from fat) and HFD (45%kcal from fat)	Reduced expressions of inflammatory and FA synthesis genes. Reduced IR and plasma dyslipidemia markers	[12]
6	RCT	C57BL/6 mice (*n* = 72)	8 wk	65.1 ± 1.6 mg cyanidin-3-*O*-glucoside/L (from 30% BlB + 70% blackberry juice available ad libitum)	HFD (60% kcal from fat)	Anti-obesity, hypoglycemic, antidiabetic	[22]
7	RCT	C57BL/6 and KKAy mice (*n* = 20)	4 wk	BlBJ (40–80 mL/kg per day in drinking water)	Normal chow diet	Improved glucose tolerance, reduced glycemic response suggesting increased insulin sensitivity	[23]
8	RCT	Obese Zucker rat (*n* = 20)	8 wk	8% wild BlB diet (WB) provided ad libitum	NA	Downregulated expression and plasma concentrations of NF-kB, TNFα, IL-6, CRP in liver and adipose tissues	[25]
				Human studies			
9	SB and RCT	Obese men and women (*n* = 66)	8 wk	50 g F/DBlB per day	NA	Reduction in plasma oxidized LDL and other plasma lipid oxidation products	[20]
10	DB, PC, RCT	overweight or obese individuals (*n* = 30)	4 wk	4 g of inulin/day from BlB (equivalent to two cups of whole BlB)	NA	Improvement in glycemic response, insulin sensitivity, satiety, serum lipid parameters, and fecal markers of gut microbiota	[41]
11	DB, PC, RCT	Diabetic patients (*n* = 58)	24 wk	160 mg of BlB anthocyanins twice daily	NA	Reduced serum concentration of LDL-C, TG, apolipoprotein, apolipoprotein C-III, lipid and protein oxidation markers with strengthening the inherent antioxidative system	[42]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
12	DB, PC, RCT	Healthy adults (n = 44)	6 wk	45 g/day F/D BlBpowder	12-oz yogurt and skim milk-based smoothie	Improvement in endothelial function in subjects with metabolic syndrome	[21]
13	DB, CO, RCT,	Healthy human beings (n = 17)	4 wk	310–724 mg/kg.BW.day BlBanthocyanin	Sugar-matched smoothie	Extend the postprandial glucose response beyond the period observed for a sugar-matched control	[24]
				(I) Bilberries (BB) (Animal studies)			
1	RCT	Male KK-Ay mice (n = 16)	5 wk	27 g of BB extract/kg diet daily	NA	Activation of AMP-activated protein kinase (AMPK) resulting in increased insulin sensitivity, upregulation of glucose transporter GLUT4, suppression of glucose production in liver	[15]
2	RCT	diabetic groups of Wistar rats (n = 32)	4 wk	BB extracts 2 g/day by gavage	Normal chow diet	Increased serum insulin, reduced TC, VLDL-C, LDL-C, and TG levels, and prevented HDL-C decline	[17,43]
3	RCT	Brown Norway (BN) rats (n = 96)	6 wk	BB extract 100 mg/kg.BW.day	Normal chow diet	Prevent diabetic retinopathy	[44]
4	RCT	Male KM mice (n = 60)	5 d	BB extract (containing 42.04% anthocyanins) 200 mg/kg BW·day)	Normal chow diet	Reduced the live damage and oxidative stress markers (ALT, MDA, NO) with improvements in enzymatic antioxidative system (GSH)	[45]
5	RCT	Male Wistar rats (n = 15)	12 wk	40 mg/kg.day BB extracts in 5 mL drinking water	Normal chow diet	Prevent capillary albumin filtration	[46]
6	RCT	Goto-Kakizaki (GK) rat (n = NS)	4 wk	BB decoction with rodent chow	powdered rodent chow	Improved mitochondrial respiratory and biogenesis parameters	[47]
				(Human studies)			
7	DB, CO, RCT	T2DM Male volunteer subjects (n = 8)	24 h	A single capsule of 0 × 47 g BB extract (36% *w*/*w*)	NA	Decrease in the incremental AUC for both glucose and insulin without alterations in GLP1, glucagon, amylin, and anti-inflammatory peptides	[48]
8	CO, DB, RCT design	Obese/Overweight/diabetic men and women (n = 16)	3 wk	3 × 0.47 g of Mirtoselect capsules per day, a standardized BB extract (36% *w*/*w*) anthocyanins)	NA	Reduced activity of digestion enzymes without alterations in anti-inflammatory markers, vascular health markers and reducing capacity	[49]
9	RCT	Healthy men and women (n = 9)	1 d	10% BB in fermented drink up to 300 g/day	White bread	Lower the insulin response than glycemic response	[50]
10	RCT	Healthy men and women (n = 62)	4 wk	BB juice 330 mL/day	NA	Anti-inflammatory	[51]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
11	Parallel RCT	Healthy men (n = 40)	8 wk	Fresh BB 100 g/day of BB	NA	Increased intestinal bioavailability of antioxidative and antidiabetic compounds	[52]
12	RCT	Healthy men and women (n = 27)	8 wk	Fresh BB 400 g/day	NA	Reduction in the low-grade inflammation with different cytoplasmic ribosomal proteins, Toll-like receptor, and B-cell receptor signaling pathways	[53]
				(I) Cranberries (CrB) (Animal studies)			
1	RCT	36 C57Bl/6J male mice	8 wk	CrB extracts 200 mg/kg BW on daily basis by gavage	HFD (65% lipids, 15% proteins and 20% carbohydrates)	Attenuated HFD-induced obesity, TC and TG accumulation, oxidative stress, with improvements in glycemic response, insulin sensitivity, HOMAIR, alleviate intestinal inflammation	[54]
2	RCT	Male Fischer rats (n = 24)	16 m	2% whole CrB powder standard NIH-31 standard rodent chow available *ad libitum*	NA	Increased β-cell glucose responsiveness; age related decline in in basal plasma insulin concentrations was delayed by cranberry	[55]
				Human studies			
3	CO, RCT	Obese participants (n = 25)	2–4 h	Cranberries (40 g)	HF breakfast (70 g fat, 974 kcal)	Improved postprandial glycemic control, reduction in lipid oxidation products and inflammatory cytokines	[56]
4	PC, DB, RCT	T2DM men and women (n = 30)	12 wk	3 capsules of CrB extract/day (1 capsule = 500 mg)	NA	Decrease in the TC:HDLC ratio	[57]
5	single CO RCT	Healthy men and women (n = 12)	OTCS	Dextrose sweetened normal calorie CrB juice (NCCBJ; 27% CBJ, *v/v*; 130 Cal/240 mL) and low-calorie CrB juice (LCCBJ; 27%, v/v CrBJ;19 Cal/240 mL)	5 g Vanilla Crisp Power Bar (contained 230 Cal, 2.5 g total fat, 3 g dietary fiber, 20 g sugars, 22 g other carbo-hydrates, and 9 g protein	Improved metabolic response towards glucose	[58]
6	RCT	Non-diabetic men and women (n = 187)	OTCS	low-calorie 27% CrBJ (19 Cal/240 mL); normal-calorie 27%CrBJ (140 Cal/240 mL) at weight-adjusted serving size (480 mL/70 kg)	NA	Improved glycemic response	[59]
7	RCT	T2DM men and women (n = 13)	OTCS	Raw CrB (55 g, 21 cal, 1 g fiber); Sweetened dried CrB (40 g, 138 cal, 2.1 g fiber); Sweetened dried CrB containing less sugar (SDC-LS, 40 g, 113 cal, 1.8 g fiber + 10 g polydextrose)	White bread (57 g, 160 cal, 1 g fiber)	favorable glycemic and insulinemic response	[60]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
8	CS Nutrition Examination Survey (n = 10 891)	Healthy men and women	2 days	Average 2-day CrBJ intake 158 to 404 mL	Routine diet	Lowered the weight-gain, TC, and proinflammatory serum CRP levels	[61]
9	DB, CO, RCT	Healthy men and women (n = 12)	OTCS	37·5 g of CrB in addition to 37.5 g × 3 of other berries (bilberries, strawberries, blueberries) + 35 g added sugar	NA	Hypoglycemic and hypo-insulinemic	[62]
10	Parallel RCT	Diabetic men and women (n = 48)	8 wk	200 × 2 mL RCCJ was enriched with omega-3 fatty acid (180 mg EPA + 120 mg DHA) on daily basis	usual diet and physical activity during the study	Anti-dyslipidemic and hypoglycemic	[63]
11	Parallel DB RCT	T2DM male patients (n = 58)	12 wk	1 cup (240 mL) CrB juice daily	NA	Antiglycation, antidiabetic, reducing CVD risk factors in T2DM male patients	[64]
12	Parallel DB, PC RCT	Healthy men and women (n = 56)	8 wk	480 mL (80 kcal) whole CrB juice daily	Complete diets in addition to Background diets consisted of typical American foods (HFD) and 3–5 servings of fruits or vegetables daily (328– 618 g/d depending on energy intake)	Anti-dyslipidemic, hypoglycemic, improved HOAM-IR	[65]
13	RCT	Patients with metabolic syndrome (n =55)	60 d	0·7 L/day (J (20 kcal) of reduced-energy CrB juice containing 66 mg proanthocyanidins; total phenolics of 104 and 0·12 mg folic acid	NA	An increase in adiponectin and folic acid and a decrease in homocysteine, decreased lipoperoxidation and protein oxidation levels	[66]
14	CO, DB	Obese yet healthy men (n = 35)	4 wk	500 mL CrB juice/day	NA	Improved augmentation in obese men	[67]
15	DB, CO	Obese men (n = 30)	12 wk	Increasing doses of low-calories CrBJ during three successive periods of 4 wk (wk 1–4: 125 mL/day, wk 5–8: 250 mL/day, and wk 9–12: 500 mL/day)	NA	Improved antioxidative defense system	[68]
16	DB, CO	Obese men (n = 30)	12 wk	Increasing doses of low-calories CrBJ during three successive periods of 4 wk (wk 1–4: 125 mL/day, wk 5–8: 250 mL/day, and wk 9–12: 500 mL/day)	NA	Decrease in plasma OxLDL, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin concentrations	[69]
17	CO, DB	Abdominally obese men (n = 30)	12 wk	Increasing doses of low-calories CrBJ during three successive periods of 4 wk (wk 1–4: 125 mL/day, wk 5–8: 250 mL/day, and wk 9–12: 500 mL/day)	NA	Increased plasma HDL-cholesterol concentrations	[70]
18	RCT	Healthy men (n = 21)	2 wk	CrBJ 7 mL/kg.BW.day	NA	Reduction in plasma OxLDL levels and Improved antioxidative defense system	[71]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
(I) Raspberries (RB) (Animal studies)							
1	RCT	Weanling male Syrian golden hamsters	12 wk	RBJ 275 mL/day (1 mL = 0.6 g of berries)	semi-purified hyperlipidic diet (0.5% cholesterol and 15% lard)	Hypo-cholestrolemic and antioxidative	[72]
2	RCT	Male Wistar rats (n = 30)	10 d	Dose of ellagitannins enriched RB extracts equivalent to daily consumption of 125 g of fresh fruit by a human healthy adult of 70 kg (i.e., 20 mg/kg BW.day orally)	NA	Protection from the ethanol induced oxidative stress and inflammatory biomarkers	[73]
3	RCT	male Lewis rats (n = 24)	30 d	RB extracts at 30–120 mg/kg.BW	NA	Inhibition of inflammation, pannus formation, cartilage damage, and bone resorption	[74]
4	RCT	CD1 male mice (n = 36)	4 wk	RB infusion by gavage (100 mg/kg BW.day)	NA	Improved antioxidative defense system	[75]
5	RCT	obese diabetic (db/db) mice (n = 30)	8 wk	5.3% RB supplementation along agar-based diet finally containing polyphenolics (963 mg extractable GAE/kg agar-based diet)	agar-based diet	Hyper-cholestrolemic and diabetes-induced oxidative stress	[76]
6	RCT	Male Zucker Fatty rats (n = NS)	12 wk	20 g of diet per day containing RB (2% red raspberry F/D powder)	NA	Upregulation of the expression of myocardial adiponectin receptor 1 and apolipoprotein E, improving the plasma cholesterol and triglyceride homeostasis	[77]
7	RCT	Male Wistar rats (n = 42)	5 d	313 g whole RB with/without *Lactobacillus plantarum* HEAL19 (HEAL19 at 10^9 cfu) per day with diet	Normal chow diet	Increased intestinal SCFA load and anti-inflammatory	[78]
8	RCT	Male F-344 rats (n = NS)	6 wk	AIN-76A diet containing either 5% whole BRB powder, 0.2% BRB anthocyanins, or 2.25% of the residue fraction provided ad libitum	NA	Anti-dysbiosis, anti-inflammatory, anti-obesity	[79]
9	RCT	Male db/db mice with C57BL/6J Background (n = 48)	8 wk	150 mg/kg.BW.day per mice RB derived pelargonidin-3-*O*-glucoside	NA	Hypoglycemic, anti-inflammatory, anti-obesity	[80]
10	RCT	Specific-pathogen free C57BL/6 mice (n = 20)	7 wk	AIN-76A diet with 10% black raspberry powder provided ad libitum	NA	Hypo-glycemic, anti-metabolic syndromic	[81]
11	RCT	Male db/db mice (n = 30)	8 wk	10% F/D RB in a isocaloric standard diet	Isocaloric standard diet	Hypo-cholestrolemic, antioxidative, improved insulin sensitivity	[82]
12	RCT	C57BL/6J mice (n = NS)	10 wk	Energy-containing RB foods (juice and puree concentrate and whole fruit powder) containing 10% raspberry and HFD supplemented with 0.2% (w/w) RB extract provided ad libitum	HFD (45% energy from fat) + high-carbohydrate food (35% energy from starch)	Anti-obesity and antidiabetic	[83]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
13	RCT	C57BL/6J, C57BL/Ks db/db, and db/+ male mice (*n* = NS)	8 wk	0.2% Cyanidin 3-glucoside in HFD	HFD (58% of calories from coconut hydrogenated fat)	Anti-obesity, anti-inflammatory, improvement in the insulin sensitivity	[84]
14	RCT	Male Sprague Dawley rats (*n* = 40)	8 wk	Application of RB derived EA (0.1–10 mg/mL) on ischemic stomach (1.5 mL/100 g.BW) in an in an ex vivo chamber	NA	Gastric protective action against gastric lesions induced by NH_4OH, due to anti-oxidative activity of EA	[85]
15	RCT	Male Wistar rats (*n* = 22)	4 wk	Oral administration of 10–20 mg/kg.BW of RB derived elagic acid	NA	Anti-inflammatory and anti-oxidative	[86]
16	RCT	Male Wistar rats AMPK α1−/− (*n* = 12)	10 wk	5% supplementation of RB extracts (contains polyphenols at ~11 g gallic acid equivalent (GAE)/kg of DW) along HFD	HFD (60% from fat)	reduced ectopic lipid storage, alleviated inflammation responses, improved whole-body insulin sensitivity, and promoted mitochondrial biogenesis	[87]
17	RCT	Male mice (C57BL/6) (*n* = 40)	12 wk	5% F/D RB powder in HFD provided ad libitum	HFD (60% energy from fat)	Anti-dyslipidemic, hypoglycemic	[88]
18	RCT	Male KK-Ay mice (*n* = NS)	5 weeks	Cyanidin 3-glucoside 2 g/kg.BW.day in the normal chow diet	NA	Anti-obesity, anti-inflammatory, improvement in the insulin sensitivity	[89]
19	RCT	Male mice (C57BL/6) (*n* = 40)	12 wk	3% RB seed floor (equivalent to 0.03% ellagic acid) in HFD and HFD + High-sucrose diet	HFD (41% energy from fat) HFD + High-sucrose diet (37% energy from sucrose)	Anti-dyslipidemic, hypoglycemic, attenuated hepatic ER and oxidative stresses, as well as adipocyte inflammation	[90]
				Human studies			
20	PC, CO, RCT	Healthy men and women (*n* = 20)	4 wk	High-carbohydrate bars (120–123 g) containing freeze-dried black RB (10% (LOW-Rasp) or 20% (HIGH-Rasp)), One bar each day after overnight fasting.	macronutrient-matched high-carbohydrate cereal bars (45% total sugars)		[91]
21	RCT	Healthy men and women (*n* = 12)	NS	100 g RB along the designated diet	High-carbohydrate food in the form of pancakes (50 g available carbohydrate from 333 kcal pancake)	Alter postprandial hyperglycemia to sustainable glycemic response	[92]
22	3 randomized, controlled, CO,	Healthy women (*n* = 13–20)	OTCS	150 g whole berries puree along each meal study 1: white bread + strawberries, bilberries, or lingonberries study 2: white bread + h raspberries, cloudberries, or chokeberries study 3: white bread or rye bread + mix berries consisting of equal amounts of strawberries, bilberries, cranberries, and blackcurrants	White bread or rye bread with 50 g available starch	Reduced the postprandial insulin response, improved the glycemic profile, improved postprandial glucose metabolism.	[93]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
23	CO, RCT	T2DM men and women (n = NS)	12 wk	250 g frozen red raspberries puree with each breakfast	NA	Anti-dyslipidemic, anti-inflammatory, anti-obesity	[94]
				(I) Mulberries (MBs) (Animal studies)			
1	Randomized block design	Male C57BL/6 mice (n = 60)	8 wk	MB anthocyanins at 200 mg/kg HFD provided ad libitum	HFD (45% kcal from fat)	Anti-dyslipidemia, anti-inflammatory, anti-obesity	[95]
2	RCT	Male db/db mice with C57BL6/J genetic background (n = 50)	8 wk	MB fruit extracts 50 and 125 mg/kg BW every day orally by gavage	NA	Antioxidative and hypoglycemic	[96]
3	RCT	male adult Wistar rats (n = 70)	6 wk	MB fruit wine 400 mL/70 kg of body weight daily	NA	Antioxidative and hypoglycemic	[97]
4	RCT	male Sprague–Dawley rats (n = 50)	8 wk	MB fruit derived cyanidin-3-*O*-β-D-glucopyranoside (10 mg/kg.BW. daily) orally by gavage	NA	Antidiabetic cystopathy	[98]
5	RCT	Adult diabetic male Wistar rats (n = 12)	6 wk	M ȝ polysaccharides (200 mg/kg.BW daily)	HFD	Improved oral glucose tolerance/insulin resistance, bioactivities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), were increased	[99]
6	RCT	Male Gold Syrian hamsters (n = NS)	12 wk	Water extracts of MB fruit at 1–2% (*w*/*w*) in HFD provided ad libitum	HFD (1% cholesterol and 10% corn oil)	Hypolipidemic	[100]
7	RCT	Male C57BL/6 mice (n = 48)	12 wk	Anthocyanin from MB of 40–200 mg/kg of HFD	HFD (45% kcal from fat)	Inhibit body weight gain, reduce the resistance to insulin, lower the size of adipocytes, attenuate lipid accumulation and decrease the leptin secretion.	[101]
8	RCT	Male Syrian golden hamsters (n = 32)	12 wk	Water extracts of MB fruit at 0.5–2% (w/w) in HFD provided ad libitum	HFD (10% corn oil + 0.1% cholesterol)	Anti-obese and hypolipidemic effects	[102]
9	RCT	Male Wistar rats (n = 32)	4 wk	5–10% (w/w) mulberry fruit polysaccharide fractions in HFD provided ad libitum	HFD (10% lard, 1% cholesterol, 0.5% sodium cholate, and 88.5% commercial diet)	Hypolipidemic and improved the enzymatic antioxidant system	[103]
10	RCT	Male C57BL/6 mice (n = 48)	6 wk	0.5–2% (w/w) water extracts of MB fruit in high-fat (35% kcal from fat) ethanol rich liquid diet (36%kcal from ethanol) provided ad libitum	high-fat (35% kcal from fat) ethanol rich liquid diet (36%kcal from ethanol)	Anti-obesity, hypoglycemic, antioxidative, anti-inflammatory	[104]
11	RCT	Male Sprague-Dawley rats (n = 40)	10 wk	MB fruit extracts 100 or 200 mg/kg.BW.day	HFD (1% cholesterol, 18% lipid (lard), 40% sucrose)	Anti-dyslipidemic, antioxidative, ameliorates nonalcoholic fatty liver disease (NAFLD)	[105]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
12	RCT	Female Wistar rats (n = 48)	20 wk	Microencapsulated 50 to 250 mg/kg.BW.day mulberry fruit extracts (microencapsulated) with HCHF	High-carbohydrate high-fat (HCHF) diet which contained total energy around 4.62 kcal/g (fat 31.54%, protein 20.25%, and carbohydrate 48.21%).	Anti-inflammatory, antioxidative, improved metabolic syndrome	[106]
13	RCT	Male, C57BL/6J mice (n = 12)	13 wk	20% MB powder in HFD provided ad libitum	HFD, 60% calories from fat	Anti-obesity, antidiabetic, increase of *Bacteroidetes*/*Firmicutes* ratio	[107]
14	RCT	db/m mice (n = 50)	NS	MB fruit polysaccharide fractions (200–800 mg/kg.BW)	NA	Improved antioxidant enzymatic defense system, antihyperglycemic and antihyperlipidemic effects	[108]
15	RCT	Male C57BL6/J genetic background (db/db) mice (n = 60)	8 wk	Mulberry fruit extract 25–250 mg/kg BW daily	NA	Upregulation of gluconeogenesis pathway	[109]
16	RCT	Adult diabetic male Wistar (n = 40)	7 wk	MB fruit polysaccharide fractions MFP50 and MFP90 (400 mg/kg.BW)	HFD	Antihyperglycemic and antihyperlipidemic effects	[110]
				(I) Lingonberries (LB) (Animal studies)			
1	RCT	Male C57BL/6 mice (n = NS)	8 wk	LB extracts (125, 250, and 500 mg/kg) in HFD provided ad libitum	HFD (35% fat, 20% protein, and 36.5% carbohydrate)	Attenuates hepatic steatosis hyperglycemia, hyperlipidemia. Improves insulin signaling	[111]
2	RCT	SHR rats (n = NS)	8 wk	Cold-compressed LB juice provided ad libitum	NA	Reduced hypertension and pro-inflammatory markers	[112]
3	RCT	Male C57BL/6JBomTac mice (n = 120)	13 wk	20% (*w*/*w*) F/D LB in HFD provided ad libitum	HFD (45 kcal% fat)	Significantly reduced body fat, lipid accumulation, and plasma levels of the inflammatory marker PAI-1, as well as mediated positive effects on glucose metabolism homeostasis.	[113]
4	RCT	Male C57BL/6JBomTac mice (n = NS)	11 wk	20% (*w*/*w*) F/D LB in HFD provided ad libitum	HFD (45 kcal% fat)	Reduced plasma levels of markers of endotoxemia and inflammation	[114]
5	RCT	Male Apoe-/- mice (n = 35)	8 wk	44% lingonberry + HFD	HFD (38 kcal% fat)	Decreased triglyceridemia and reduced atherosclerosis	[115]
6	RCT	Male C57BL/6JBomTac mice (n = NS)	11 wk	20% (*w*/*w*) F/D LB in HFD provided ad libitum	HFD (45 kcal% fat)	Improvement in glycaemia, reduction in inflammation and hepatic steatosis	[116]
7	RCT	C57BL/6JBomTac (n = NS)	13 wk	20% (*w*/*w*) freeze-dried LB + blackcurrants, bilberries or açai berry in HFD provided ad libitum	HFD (45 kcal% fat)	Downregulation of inflammatory pathways, NF-κB, STAT3 and mTOR as possible targets for antidiabetic therapy	[117]
8	RCT	Male ApoE−/− mice (n = 50)	8 wk	Two LB polysaccharide fractions 15–60 g/ kg BW with HFD daily	HFD (38 kcal% fat)	Hypoglycemic, hypolipidemic, altered caecal microbiota composition	[118]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
				Human studies			
9	RCT	Scandinavian type 2 diabetes patients (n = 30)	12 wk	Recommended daily intake of LB/berries/fruits	Okinawan-based Nordic diet of about 1,900 kcal/day	Improved metabolic and anthropometric parameters	[119]
10	CO, DB, RCT	Healthy normal-weight nonsmoking men (n = NS)	6 d	Glycemic diet + 40 g lingonberry powder Lipemic diet + 60 g lingonberry powder	Glycemic diet: 200 g yoghurt (lactose-free and fat-free non-flavored natural yoghurt + 50 g glucose) Lipemic diet: 200 g Yoghurt (lactose-free and fat-free non-flavored natural yoghurt + 35 canola oil)	Nullified the glycemic effect of the sugars present in the meals without affecting the postprandial lipemic response	[120]
11	CO, DB, RCT	13 Healthy, over-weight, non-smoking male and female volunteers	Single meal challenge	100 g lingonberry	Hyperlipidic and hypercaloric meals (38 kcal% fat)	Reduced glycemic response, rarified the increase of cholesterolemia	[121]
12	RCT	Normal, healthy subjects (n = 9)	12 wk	LB polysaccharides + fibers (2 g/Kg of oat bread)	Oat bread	In reduced glucose and C-peptide response	[122]
13	SB, CO, RCT	Healthy women volunteers (n = 20)	2-h meal tests	Diet 1: 150 g whole LB puree containing 35 g sucrose per day Diet 2: 300 mL LB nectar (equal to 150 g fresh berries) containing 35 g sucrose	NA	Optimized postprandial metabolic responses to sucrose with delayed digestion and absorption of sucrose/glucose	[123]
14	RCT	Healthy non-smoking males (n = 14)	2-h meal tests	60 g of LB juice press residue corresponding to 270 g of fresh LB with standard diet	Standard diet: white wheat bread, cucumber, water, and a banana	Gut microfloral metabolism of polyphenols resulting in increased levels of hippuric acid and 4-hydroxyhippuric acid	[124]
				(I) Blackberries (BBR) (Animal studies)			
1	RCT	Male Wistar rats (n = 32)	5 wk	Anthocyanin-enriched fraction (AF) and Ellagitannin-enriched fraction (EF) equivalent to (4 mg cyanidin eq/kg BW) and 2.68 mg EA eq/kg BW respectively	NA	Reinforce the antioxidative defense system and lipid oxidation markers	[125]
2	RCT	C57BL/6 mice (n = 60)	12 wk	BBR extracts at 200 mg/kg food BBR extracts: cyanidin-3-glucoside (51.24%), cyanidin-3-rutinoside (42.31%), and peonidin-3glucoside (6.91%)	HFD (45% kcal from fat)	Anti-inflammatory, anti-hypertensive, anti-hypercholesterolemia, antioxidative	[126]
3	RCT	Male DIO C57BL/6J mice (n = 40)	12 wk	6.3%, (w/w) BBR extracts in HFD provided ad libitum	HFD (45% kcal from fat)	Anti-obesity, Anti-inflammatory, anti-hypertensive,	[127]
4	RCT	Male Wistar rats (n = 24)	17 wk	25 mg/kg.BW BBR extracts in HFD provided ad libitum	HFD (45% kcal from fat)	Anti-obesity, anti-inflammatory, anti-dyslipidemic	[128]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
5	RCT	Male diabetic Sprague-Dawley rats (n = 40)	40 d	Microfiltrated 12.5–25% BBR juices		Reduced glycaemia (−10.4%), TG (−4.6%) and TC (21.0%), lipid peroxidation, attenuation of oxidative stress	[129]
6	RCT	Male Wistar strain rats (n = 40)	4 wk	Normal standard diet with 0.98% BBR polyphenols and 6% BBR fiber	Normal chow diet	Anti-inflammatory and anti-dyslipidemic	[130]
7	RCT	Female obese (BKS(D)-Leprdb/J72) and lean (C57BL/6J) mice (n = 24)	10 wk	Aged or fresh BBR supplemented at 10% (w/w) of diet provided ad libitum	Normal chow diet	Increased in total beneficial bacterial population	[131]
8	RCT	Male C57BL/6J mice (n = 72)	10 wk	Alcohol-free blueberry–blackberry fermented beverage (AFFB) a) AFFB [70% blackberry and 30% blueberry, 8.4 mg cyanidin-3-*O*-glucoside (C3G) eq./kg.BW)/day]; (b) dose 0.1 × ostamberlite extract (PAE), 1.1 mgC3G eq./kg BW/day; (c) dose 1 × PAE, 9.0 mg C3G eq./kg BW/day; (d) dose 2 × PAE, 18.9 mg C3G eq./kg BW/day	HFD (60.3% fat, 21.3% carbohydrate and 18.4% protein)	Reduced percent fat mass, mean adipocyte diameters, epididymal fat pad weights, and plasma TG and TC.	[22]
				Human studies			
9	RCT	Diabetic and obese men and women (n = 152)	1 wk	Consumption of daily recommended amount of low glycemic index fruit (0.7–1.4 servings/day)	NA	Anti-dyslipidemic	[132]
10	open, single-center RCT	Healthy human subjects (n = 6)	4 h	200 mL of BBR juice equivalent to 400 mg of cyanidin equivalent/50 kg of body weight	NA	Improved plasma and urine antioxidant system	[133]
11	RCT	Dyslipidemic patients (n =72)	8 wk	300 mL of BBR juice (equivalent to 316 mg/100 g polyphenols) of BBR with pulp every day	NA	Increased apo A-1 and HDL-C along reduction in apo B and hsCRP	[134]
				(I) Strawberries (SB) (Animal studies)			
1	RCT	Diabetic male albino Wistar rats (n = 36)	4 wk	Aqueous, alcoholic and hydro-alcoholic SB extract (2 g/kg b.w.day	NA	Reduced expression level of genes involving glucose, lipid metabolism with improvement in glucose metabolism and liver function	[135]
2	RCT	Male Wistar rats (n = 20)	12 wk	HFD supplemented with 0.2% irradiated/non-irradiated SB extracts	HFD (47.5% kcal from fat)	Reduction in the oxidative damage in brain and peripheral tissues	[136]
3	RCT	Male C57BL/6J mice (n = 36)	24 wk	HFD supplemented with 2.6% freeze-dried SB	HFD containing approximately 20% higher in energy density compared to the low-fat diets	Reduction in the HFD led increase of FBS, adhesion molecule-1, leptin, E-selectin, resistin, and plasminogen activator protein-1	[137]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
4	RCT	Male Wistar rats (n = 48)	8 wk	Supplementation of the diet with a 6% w/w (equivalent to a 5 g/kg 65 BW dose) of a F/D SB-BlB (5:1) powder (FDSB)	High-fat-sucrose diet (D12451, Research Diet)	Anti-obesogenic and anti-inflammatory effects	[138]
5	RCT	Male Wistar rats (n = 24)	16 wk	AIN93-modified diet with lyophilized SB extract at 10 g/kg of diet	AIN93-modified diet	Improvement of oxidative stress biomarkers, mitochondrial performance, antioxidant enzyme activities, reduction of DNA damage and ROS concentration	[139]
6	RCT	Male Wistar rats (n = 20)	12 wk	Supplementation of 0.2% SB	HFD (47.5% calories from fat)	Antioxidative, anti-stress	[140]
7	RCT	German Landrace pigs (n = 48)	4 wk	205–745 g of SB with normal feed per day	Lin: eed oil (15 g/day) enriched feed	Anti-stress and antioxidative	[22]
8	RCT	db/db mice homozygous for the diabetes spontaneous mutation (Leprdb) with C57BL/6J background (n = 24)	10 wk	2.35% F/D SB powder in the diet pellets (w/w) (equivalent to two human servings of SB i.e., ~160 g SB)	NA	Increased *Bacteriodetes* to *Firmicutes* ratio	[141]
9	RCT	Male CD-1 mice (n = 60)	8 wk	5% (w/w) of diet freeze-dried whole SB powder	AIN93G diet	Increased *Bacteriodetes* to *Firmicutes* ratio	[142]
				(Human studies)			
10	DB, RCT, parallel study	Insulin resistant and obese males and females (n = 41)	6 wk	Beverage containing 1·84 g of a mixture of dry SB and CrB providing 333 mg of polyphenols on daily basis (also equivalent to 112 g consumption of fresh berry fruit)	NA	Improved insulin sensitivity and release	[143]
11	CO, SB, PC, RCT	Hyperlipidemic men and women (n = 24)	12 wk	SB beverage containing 10 g/serving of freeze-dry SB powder providing 338 mg of polyphenols daily (also equivalent to 110 g consumption of fresh berry fruit)	HFD consisting of typical breakfast food items (i.e., bagel, cream cheese, whole milk, egg, margarine, cantaloupe)	Reduced postprandial lipemia and oxidative stress markers	[144]
12	CO, RCT	Healthy males and females (n = 30)	5 d	20 g of five types SB jams each with sugar of different glycemic index	60 g white bread slice	Non-significant reduction in the postprandial glucose level	[145]
13	CO DB RCT	Healthy males and females (n = 16)	3 wk	60 g of three types SB jams each with sugar of different glycemic index and polyphenolic contents		Strawberry jam with high sugar level produced less levels of FFA.	[146]
14	DB RCT	T2DM males and female subjects (n = 36)	6 wk	Two cups of F/D SB beverage containing 25 g × 2 = 50 g	NA	Reduction in LDL-C and LDL-C/TC and LDL-C/HDL-C ratio	[147]
15	SB, CO parallel, RCT	Obese and overweight men and women (n = 24)	6 wk	SB beverage containing 10 g/serving of freeze-dry SB powder providing 96 mg of polyphenols on daily (also equivalent to 100 g consumption of fresh berry fruit)	High-carbohydrate-fat diet	Attenuation of diet-induced inflammatory markers	[148]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
16	Single-center, CO, SB, PC,	Men and women (n = 26)	OTCS	SB Milk based beverage containing 10 g/305 mL of F/D SB powder	high-carbohydrate, moderate-fat meal (HCFM)	Reduced postprandial insulin and inflammatory response	[149]
17	Four-arm, SB, PC, CO, RCT	Males and females with insulin resistance (n = 23)	NS	SB milkshake containing 10–40 g freeze-dried SB powder where 10 g freeze dried powder = 110 g fresh strawberries	Standard western type meal	Reduced lipid oxidation and post-meal insulin demand	[150]
18	Observatory study	Healthy men and women (n = 247)	20 years	Dietary flavonoids intake (47–560 mg/day) from fruits and berries	-	Flavonoid Compounds in Driving Patterns of Microbial Community Assembly	[151]
19	RCT	Obese men and women (n = 66)	12 wk	SB beverage containing 25–50 g freeze-dry SB powder daily	HFD (50% calories from fat)	Increased the glutathione level, serum catalase activity, and plasma antioxidant capacity	[152]
20	DB RCT	T2DM patients (n = 40)	6 wk	50 g of freeze-dried SB powder (equivalent to 500 g fresh strawberries) each day	NA	Reduction in the markers of lipid peroxidation (MDA), inflammatory markers (CRP). Reducing trend in HbA1c.	[153]
				(I) Goji berries (GB) (Animal studies)			
1	RCT	Alloxan-induced hyperglycemic/hyperlipidemic adult rabbits (n = 35) and male mice (n = 24)	10 d	Water decoction (0.25 g/kg BW day), crude GB polysaccharides (10 mg/kg BW day), and purified GB polysaccharides (10 mg/kg BW day)	NA	Hypoglycemic and hypolipidemic effect with increased plasma antioxidant capacity	[154]
2	RCT	Male Wistar rats (n = 70)	8 wk	Ethanolic and aqueous GB extracts at 50 mg/kg b.w. or 100 mg/kg BW daily	HFD	Significantly reduced liver damage and oxidative changes	[155]
3	RCT	Diabetic male mice of original Kun-ming strain (n = NS)	4 wk	GB polysaccharides (20–40 mg/kg BW day) orally	NA	Hypoglycemic and hypolipidemic	[156]
4	randomized block design	Obese male Sprague-Dawley rats (n = 60)	8 wk	GB anthocyanins at 50–200 mg/kg BW.day	HFD	Reduced body-weight-gain with anti-inflammatory properties	[157]
5	RCT	STZ-diabetic Male Wistar rats (n = NS)	8 wk	GB polysaccharides (10 mg/kg, BW.day)	NA	Increased antioxidative scavenging and antioxidant enzymes. Increased activity of protein kinase C (PKC)	[158]
6	RCT	STZ-induced diabetic Sprague-Dawley male rats (n = 60)	8 wk	Water decoction of GB (5 g/kg.BW.day)	NA	Protective effects in diabetic retinopathy	[159]
8	RCT	Male Wistar rats (n = 16)	4 wk	GB polysaccharides 10 mg/kg BW.day dissolved in physiological saline	High-fat-sucrose diet	hypoglycemic and improving hyperinsulinemia	[160]
9	RCT	Diabetic male C57BL/6J mice (n = 48)	7 wk	GB polysaccharides 100–500 mg/kg BW.day by gastric perfusion	HFD	Hypoglycemic effects with increased insulin-sensitizing, glucose metabolism, insulin secretion, and promoting pancreatic cell proliferation.	[26]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
10	RCT	Swiss Albino rat (n = 30)	3 wk	Water-soluble polysaccharides (galactomannan) 250–500 mg/kg BW.day by oral gavage	NA	Hypolipidemic, reduced lipid oxidation, increased insulin-sensitizing and serum antioxidant level	[161]
11	RCT	Diabetic Wistar rats (n = 48)	8 wk	Water-soluble GB polysaccharides 250–500 mg/kg BW.day by oral gavage	HFD and HCD (12% protein, 5% fat, 67% carbohydrate, 5% cholesterol, and 5% other additives)	Reduced serum level of IL-2, IL-6, TNF-α, IFN-α, MCP-1, and ICAM-1 with increased activities of SOD and GSH-Px activities	[162]
12	RCT	Postnatal Royal College of Surgeons (RCS) rats (n = 60)	4 wk	Whole GB powder 1 mg/kg of per day	NA	Reduced Caspase-2 activity in experimental group at 25th post-neonatal day	[163]
13	RCT	Male IL-10-deficient mice (n = 14)	10 wk	Diet supplemented with 1% GB	Normal diet	Increased gut population of SCFA producing bacteria	[164]
				Human studies			
14	RCT	Kunming mice of clean grade (n = 14)	2 wk	GBPS at a dose of 0.1 mL/10 g body weigh daily via intragastric administration	Normal diet	Increased gut population of SCFA producing bacteria, *Firmicutes*, *Akkermansia, Lactobacillus*, and *Prevotellaceae*	[165]
15	DB, PC, RCT	Healthy males and females (n = 50)	30 d	Intake of 120 mL of GB juice (equivalent to 1632 mg/ daily serving (120 mL) of goji berry polyphenols	Traditional Chinese diet rich in carbohydrate	Increased serum levels of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) with reduced level of MDA	[166]
16	RCT	Metabolic syndrome patients (n = NS)	45 d	14 g of GB with meals	Normal diet	Reduction in transaminases, waist circumference with improvements in lipid profile, glutathione and catalase level.	[167]
17	RCT	Male and female C57BL/b6N mice (n = 56)	8 wk	GB polysaccharides (1–10 mg/kg BW day) orally	NA	Increased hepatic antioxidant enzymes, y inhibited cytochrome P450 2E1, nitric oxide metabolism and lipid peroxidation	[168]
18	DB, CO, RCT	healthy overweight men (n = NS)	Single meal challenge	meal containing 25 g of dried GB fruit	Ready-made meal with a fixed macronutrient composition (30–40% fat, 40–50% carbohydrates, and 13–16% proteins)	No-single-dose-effect on substrate oxidation and prospandial-energy-expenditure	[169]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
(I) Acai berries (AB) (Animal studies)							
1	RCT	Male mice of the C57BL/6 strain (n = NS)	12 wk	AB seed extracts 300 mg/kg.BW.day by intragastric gavage	HFD (60% calorie from fat)	Reduced expressions of lipogenic proteins (SREBP-1c, pACC, ACC, HMG-CoA reductase) with increased expression of pAMPK, pACC/ACC, and cholesterol transporters (ABCG5 and ABCG8)	[170]
2	RCT	Zebrafish (n = 70)	5 wk	HC diet supplemented with10% w/w of AB puree powder	high cholesterol (HC) diet (47.5% crude protein, 6.5% crude fat, 4% cholesterol, 2.0% crude fiber, 10.5% crude ash)	Reduced oxidative markers with lipid lowering effects	[171]
3	RCT	Oxidatively damaged sod1/sod1 mutant strains *Drosophila melanogaster* (n = 120)	5-6d	AB supplemented sugar-yeast (SY) medium to a final concentration of 0.25%, 0.5%, 1% or 2% (w/v) of the food	SY medium	Increased transcript level of gluconeogenesis gene phosphoenolpyruvate carboxykinase (Pepck) with reduction in oxidative stress	[172]
4	RCT	ApoE-deficient (ApoE 2/2) male mice (n = 23)	12 wk	AIN-93M diet formulated to contain 2% F/D açai' pulp + exercise in progressive treadmill for 30 min daily at a speed of 12 m/min, 0% incline	AIN-93M diet	Hepatic superoxide dismutase activity, mRNA expression of monocyte chemotactic protein-1, percentages of hepatic lipid droplets	[173]
5	RCT	STZ-induced diabetic Male Wistar rats (n = NS)	45 d	AB seed extracts 200 mg/kg.BW.day in drinking water	NA	Reduced oxidative damage by reducing the expression of caspase-3, IL-6, TNF-α and MCP-1	[174]
6	RCT	Female Fischer rats (n = 32)	6 wk	Hypercholesterolemic diet (25% soy oil and 1% cholesterol) supplemented with 2% AB (dry wt/wt)	Hypercholesterolemic diet (25% soy oil and 1% cholesterol)	Reduced expression of cholesterol biosynthesis genes HMG CoA-R, EBP-2, ApoB100, LDL-R, ABCG8, and CYP7A1	[175]
7	RCT	STZ-induced diabetic Male Wistar rats (n = 40)	9 wk	AB seed extracts 200 mg/kg.BW.day by intragastric gavage	HFD (55% calorie from fat)	Hypoglycemic and hypolipidemic with reduced expression of TNF-α and activating the insulin-signaling pathway in muscle and adipose tissue	[176]
8	RCT	Diabetic female Fisher rats (n = NS)	30 d	Standard AIN-93 diet supplemented with 2% (*w/w*) AB pulp	AIN-93	Modulate ROS production by neutrophils and improve the liver oxidant/antioxidant balance	[177]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
				(Human studies)			
9	CO, DB, RCT	Overweight healthy males (n = 23)	Single day meal challenge	Frozen AB pulp (150 g) was prepared in a smoothie with 50 g banana	50 g banana and matched for fat with 1.5 g hexadecanoic acid [palmitic acid (16:0)] and 8.5 g sunflower oil [30% (9Z)-Octadec-9-enoic acid (oleic acid [18:1]), 60% (9Z,12Z)-9,12-Octadecadienoic acid (linoleic acid [18:2]), and 10% palmitic acid (16:0)],	Lower incremental area under the curve (iAUC) for total peroxide oxidative status after açai and increased the iAUC for insulin	[178]
10	RCT	Male Swiss mice (n = 32)	12 wk	A single daily dose freeze-dried AB pulp (3 g/kg) via gavage	HFD (32% lard and 1% cholesterol)	Attenuated hepatic steatosis and reduced lipid accumulation	[179]
11	four-way CO	Healthy men and women (n = 11)	Single dose study	100% clarified AB juice/pulp 7 mL/kg BW of each study	NA	Increased plasma antioxidant capacity without affecting generation of reactive oxygen species, and uric acid concentrations in plasma	[180]
12	open label pilot study	Overweight adults (n = 10)	30 d	Intake of 100 g AB pulp twice daily	NA	Postprandial increase in the AUC of plasma glucose with reduced TC, LDL-C, and LDL-C/HDL-C	[181]
				(I) Chokeberries (CB) (Animal studies)			
1	RCT	C57BL/6JmsSlc and KK-Ay male mice (N = 10, EACH GROUP)	4 wk	CB provided ad libitum	Normal chow diet	Duction of glucose-dependent insulinotropicpolypeptide (GIP) level	[182]
2	RCT	STZ-induced-diabetic-male ICR mice (n = 32)	4 wk	CB extract (10–100 mg/kg.BW) daily administered orally	NA	Hypoglycemic, hypolipidemic, antioxidative	[183]
3	RCT	C57BL/6N mice (n = 20)	12 wk	CB powder dissolved in water (50 mg/kg daily)	HFD (60 kcal% Lard)	Reduced the body and liver weight, lipid accumulation, PPARγ2, FAS, hepatic TG and leptin. Serum transaminases, indicators for liver antioxidant capacity were significantly increased.	[184]
4	RCT	Male C57BL/6J (n = 60)	8 wk	C 3 extracts (100 mg/kg.BW) dissolved in 0.5% carboxymethyl cellulose	HFD (containing 60% kcal fat)	Attenuated weight-gain, increase in serum TG, TC, LDL-C and better glucose tolerance	[185]
5	RCT	Male Wistar rats (n = NS)	6 wk	Aronia melanocarpa fruit juice (AMFJ) at doses 10 and 20 mL/kg	NA	Hypoglycemic, hypolipidemic	[186]
6	RCT	Polish Merino lambs (n = 24)	12 wk	1 0-300 g of chokeberry pomace per each kg of the complete feed mixture	Complete feed mixture	Hypoglycemic, hypolipidemic	[187]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
7	RCT	Middle-aged non-medicated subjects with MS (n = 38) an healthy volunteers (n = 14)	8 wk	CB extracts 100 mg/kg.BW three times daily	NA	Beneficial changes in lipid profile, coagulation parameters, inhibition of platelet aggregation	[188]
8	RCT	Male Wistar rats (n = 24)	4 wk	Diet was supplemented by the extract from CB fruits (0.2% W/W) added at the expense of corn starch	Standard casein diet enriched with 0.5% of cholesterol. Exp group: the diets were modified by 8% of lard and 65% of fructose added at the expense of soybean oil and maize starch,	Maltase and sucrase, e improvement of antioxidant status, cholesterol-lowering,	[189]
9	RCT	Male Wistar rats (n = NS)	4 wk	CB juice 10 mL/kg.BW.day	NA	Hypoglycemic, hypolipidemic, antioxidative	[190]
10	RCT	Male Wistar rats (n = 72)	8 wk	CB juice 50 mL/kg.BW.day	High-carbohydrate, high-fat + purple maize flour (HPM)	Reduced Inflammatory cell infiltration, visceral adiposity index, total body fat mass, improved glucose tolerance	[191]
11	RCT	Male Wistar rats (n = 36)	6 wk	CBE at 100 or 200 mg/kg BW.day	Fructose rich diet containing (g/kg diet): casein, 207; DL-methionine, 3·0; fructose, 600; lard, 50; cellulose, 79·8;	Elevated plasma adiponectin levels and inhibited plasma TNF-α and IL6. Increased in the expression level of glucose and lipid metabolizing genes	[192]
12	RCT	Male Wistar albino rats (n = 60)	4 wk	Standardized Aronia extract (SAE) 0.45 mL/kg.BW day) for 4 weeks	HFD (25% fat, 15% protein, 51% starch, and 5% fiber)	Reduced serum level of TC, TG, LDL-C, with increased serum levels of SFA and PUFA.	[193]
				(Human studies)			
13	CO open-label trial	T2DM patients (n = 35)	12 wk	Oral CB juice supplementation (150 mL/day, three times a day for 50 mL)	NA	Significantly improved the renal /hematological and lipid parameters (TG, TC, LDL-C, LDL-C/HDL-C) in diabetic patients	[194]
14	RCT	Healthy female volunteers (n = 29)	12 wk	100 mL of polyphenol-rich organic CB juice per day	NA	Reduced TBARS, pro-oxidantantioxidant balance, increase in paroxonase-1 activity	[195]
15	RCT	Apparently healthy women (n = 25)	12 wk	Consume 100 mL of polyphenol-rich organic CB juice daily	NA	Increased SOD and GPX activities, C22:6n-3, PUFAs, total PUFAs and unsaturation index and decrease in n-6:n-3 ratio	[196]
16	RCT	Healthy volunteers and 25 patients with metabolic syndrome (n = 22)	8 wk	CB extract (3 × 100 mg/day)	NA	Improvement in serum lipids, and oxidative status (GSH-Px, SOD, TBARS)	[197]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
17	RCT	Healthy subjects (n = 33)	4 wk	Consume 200 mL of polyphenol-rich organic CB juice daily (containing 386 ± 9.7 mg of total phenolics expressed as gallic acid equivalents per 100 g)	NA	Positive effects on BP and lipid status in hypertensive subjects	[198]
18	RCT	Diabetic Wistar white male rats (n = 48)	16 wk	dose of polyphenols extracts 0.040 g/kg BW every 2 day	NA	Reduced TNF-α and IFN-γ levels	[199]
19	RCT	Healthy, non-smoking volunteers (n = 11)	3 wk	CrB juice between meals (250 mL per day) (560 mg GAE/100 mL)	NA	Increased serum antioxidant capacity with no significant change in the blood lipid profile	[200]
20	RCT	Men with the diagnosed mild hypercholesterolemia (n = 58)	6 wk	CB juice between meals (250 mL per day) (560 mg GAE/100 mL)	NA	Improved lipid profile with reduced lipid peroxides (LPO), C-reactive high sensitivity protein (hsCRP), homocysteine,	[201]
21	3-arm, DB, parallel RCT	Healthy male volunteers (n = 66)	12 wk	CB extract" capsules (containing 116 mg total (poly)phenols). CB whole fruit" capsules (containing the equivalent to 10 g of the whole CB fruit, and 12 mg of total (poly)phenols)	NA	Increased *Anaerostipes, Bifidobacterium*, *Faecalibacterium*, and *Clostridium* genera	[202]
				(I) Black Currants (BCT) (Animal studies)			
1	RCT	Old male Sprague-Dawley (SD) rats (n = NS)	Single meal challenge test	BCE 5 mg kg.BW (1 mg D3R kg.BW) / /	Normal diet with IP administration of glucose solution (2 g/kg)	Improved hyperglycemic and hypoinsulinemic condition	[203]
2	RCT	Male KK-Ay (n = 16)	7 wk	BC extracts (2 g/Kg.diet) (equivalent to delphinidine-3-glucoside (D3R) 2 g/Kg.diet)	NA	Improved glucose tolerance with increased GLP-1 concentration, and upregulation of AMPK-α and prohormone convertase 1/3(GLP-1 precursor)	[204]
3	RCT	Male C57BL/6J mice (n = 48)	8 wk	Diet supplemented with 1% BC powdered extract (32% anthocyanins)	HFD (60 kcal% fat diet)	Protective effect of BC anthocyanins against obesity and associated insulin resistance.	[205]
4	RCT	Male C57BL/6J mice (n = 24)	12 wk	HF/HC diet supplemented with 0.1% of BCE (containing 25% anthocyanins and 40% polyphenols) by weight	AIN-93M high fat/high cholesterol (HF/HC) diet (16% fat, 0.25% cholesterol by weight; 55.7%, 125.5% and 31.8% energy from carbohydrate, protein and fat, respectively; 4529 kcal/Kg	Reduced BW and adipocyte size of the epididymal fat, energy expenditure and mitochondrial biogenesis genes	[206]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
5	RCT	Male New Zealand white rabbits (n = 20)	4 wk	Diet supplemented with 1.5% BC polyphenolic extract	HFD (10% lard) was 17% from protein, 32% from fat and 51% from carbohydrates	Reduced concentration of putrefactive metabolites, β-glucuronidase activity, ameliorated hyperlipidemia, and antioxidative capacity	[207]
6	RCT	Sprague–Dawley male rats (n = 40)	4 wk	2 mL of BC extract (containing 30 mg BC /kg BW) or 2 mL of CAM30 extract (containing 13.4 mg CAM30/kg body weight), respectively, three times weekly by oral gavage	NA	Reduced β-glucuronidase activity and undesirable bacteria in the caeca. Increased lactobacilli and bifidobacterial gut species	[208]
7	RCT	Male Sprague-Dawley (SD) rats (n = 40)	8 wk	BC extract 100–300 mg/kg.BW.day administered orally	High-fructose (HF) diet (60% fructose diet)	Improvements in hypertension, dyslipidemia, insulin resistance, and obesity	[209]
8	RCT	Male Sprague-Dawley rats (n = 128)	6 wk	Diets with dietary fiber and BC extracts (Currantex 30) (containing total anthocyanin 32% (w/w))	NA	Increased intestinal population of SCFA and total beneficial bacterial population	[210]
				(Human studies)			
9	RCT	Healthy volunteers (n = 30)	2 wk	BC extracts (1500 mg/day; 375 mg × 4 capsules) BC powder CAM30 (672 mg/day; 168 mg × 4 capsules)	NA	Increased intestinal population of SCFA and total beneficial bacterial population	[211]
10	DB, CO, RCT	Healthy subjects (n = 26)	Single meal challenge test	Apple and BC polyphenol-rich drinks (1200 mg apple polyphenols (AE), [illegible] anthocyanins (AE+BE))	Standardized high-carbohydrate meal 100 g of white bread	Reduced Postprandial insulin, C-peptide and GIP, GLUT and SGLT1-mediated glucose transport	[212]
11	DB, CO, RCT	Healthy subjects (n = 22)	Single meal challenge test	Low-sugar-BC drink containing 300–600 mg anthocyanins	Standardized high-carbohydrate meal 100 g of white bread	Reduced postprandial insulinemia, glycemia, and incretin secretion	[213]
12	RCT	Healthy participants (n = 17)	6 d	BC powder 6 g/day with water	NA	Improved postprandial AUC of glucose and insulin	[214]
13	DB, CO, RCT	Endurance-trained females (n = 16)	7 d	BC extract 600 mg/day	NA	Increased fat oxidation	[215]
14	RCT	Healthy sedentary male and female participants (n = 40)	Single meal challenge test	BC juice 200 mL/participant	standardized meal bar to consume for breakfast at least 1 h prior to starting the trial.	Supported positive affective responses	[216]
15	parallel, four-arm, study design + DB, CO parallel trial	Healthy individuals (n = 24) (n = 32)	A single meal challenge study	Two opaque gelatin capsules containing BC anthocyanin (3.2 mg/kg total anthocyanins)	NA	Dose-dependent increase in plasma anthocyanins and recovery from exercise-induced oxidative stress	[217]

Table 1. *Cont.*

No.	Study Design	Study Subject	Duration	Berry Interventions	Intervention Diet	Significant Findings	Ref.
				(I) Maqui berries (MqB) (Animal studies)			
1	open exploratory study	Pre-diabetic volunteers (n = 43)	Single dose study	A single dose of Delphinol Capsules bearing either 60, 120, or 180 mg Delphinol on each day with one-week washout period	NA	Dose dependently lowered basal insulinemia and glycemia	[218]
2	RCT	Male balb/c mice (n = NS)	7 d	MqB extracts (25, 50 and 100 mg/kg.BW)	NA	Ameliorate the oxidative stress condition	[219]
3	RCT	Male C57BL ßJ mice	12 wk	MqB anthocyanins (ANC), Labrasol/water: 66/34 + ANC (LAB + ANC)	HFD (60% calories from fat)	Decreased glucose production, down-regulation of gluconeogenic enzyme	[220]
4	DB, CO, RCT	Fifty overweight volunteer smokers (n = 42)	4 wk	3 capsules of 150 mg standardized maqui berry extract containing 54 mg of anthocyanin daily (equivalent to 162 mg anthocyanins/day)	NA	Reduced levels of Ox-LDL in the anthocyanin group	[221]
5	RCT	Male C57BL/6Nhsd mice (n = 18)	4 wk	MqB derived Delphinidine (15 mg/kg.BW) daily	High-fat diet and high-carbohydrate drinking water (45% kcal from fat)	Reduced TG accumulation with no effect on metabolic alterations related glucose metabolism	[222]
6	Prospective observational study	Middle-aged participants (n = 21)	8 wk	Two tablets per day of an MCN (Eonlipid) (containing maqui, 300 mg in each tablet)	NA	Improvement of most atherogenesis and oxidative stress biomarkers	[223]
7	CO, RCT	Healthy male subjects (n = 11)	ONCS	Intake of 250 mL of the MqB drink containing an number of total polyphenols ~1000 µmol equivalents of gallic acid	Meals containing food-grade glucose and rice, containing 50 g of carbohydrates by each meal	Reduced glycemic indexed for high-carbohydrate diets.	[224]
8	RCT	C57BL/6J littermates' male mice (n = 23)	16 wk	HFD supplemented with 4–5 mg of MqB polyphenols/ 10–15 kcal per day	HFD (45% calories from fat)	Reduced body-weight-gain, improved glucose tolerance and insulin resistance. Differential expression of genes involved in fatty acid oxidation, de novo lipogenesis, thermogenesis, and multilocular lipid droplet formation	[225]

Note: Acai berry, AB; AB juice, ABJ; ATP-binding cassette sub-family G member 8, ABCG8; AMP-activated-proteins kinase-α, AMPK-α; AB extracts, ABE; ATP-binding cassette sub-family G member 5, ABCG5; vascular cell adhesion molecule-1 VCAM-1; Apolipoprotein B, ApoB; Area under curve, AUC; Bilberry, BB; BB juice, BBJ; BB extracts, BBE; Black currant, BCT; BCT juice, BCTJ, BCT extracts, BCTE; Blueberry, BlB; BlB juice BlBJ; BlB extracts, BlBE; body weight, BW; C-reactive high sensitivity protein hsCRP; catalase CAT; Cytochrome P450 Family 7 Subfamily A Member 1, CYP7A1; Chokeberry, CB; cranberry, CrB; Cross-over, CO; cross-sectional, CS; cyanidin-3-*O*-glucoside, C3G; day, d; Double-blind, DB; Freeze-dried, F/D; High-fat-diet, HFD; low-fat-diet, LFD; glucose transporter 1, GLT; glucagon-like-peptide 1, GLP1; Glutathione peroxidase GPx; glutathione reductase GSH-x; 3-hydroxy-3-methylglutaryl-CoA, HMG-CoA; interferon alpha *IFN*-α; Intercellular Adhesion Molecule 1, ICAM-1; interleukin, IL; Lingonberry, LB; Low-fat-diet, LFD; Low-Density Lipoprotein (LDL) Receptor (LDL-R); Monocyte Chemoattractant Protein 1 (MCP-1); Mulberry, MB; Maqui berry, MqB; Ox-LDL, nonalcoholic fatty liver disease (NAFLD); oxidized low-density-lipoproteins; oxLDL-C; polyunsaturated fatty acids, PUFA; thiobarbituric acid reactive substances (TBARS); total glyceraldehyde, TG; total cholesterol, TC; Tumor necrosis factor, TNF-α; single-blinded, SB; Superoxide dismutase, SOD; one-time-challenge-study, OTCS; placebo-controlled, PC; Peroxisome proliferator-activated receptor-α, PPARα; phosphoenolpyruvate carboxykinase (Pepck); Raspberry, RB; Randomized controlled trial, RCT; respiratory quotient (RQ), short-chain fatty acids, SCFA; sodium glucose transporter protein, SGLT; Sterol regulatory element-binding protein, SREBP-1c; weeks, wk.

Table 2. A comprehensive list of potential health promoting individual anthocyanins and phenolic compounds with their quantities found in berries or berry products.

Compounds	Bilberry (mg/100 g fw)	Blueberry (mg/100 g fw)	Cranberry (mg/100 g fw)	Raspberry (mg/100 g dw)	Mulberry (mg/100 g fw)	Lingonberry (mg/g DE)	Blackberry (mg/g DE)	Strawberry (mg/100 g fw)	Goji Berry (mg/100 g dw)	Acai Berry (mg/100 g dw)	Black Chokeberry (mg/100 g dw)	Black Currant (mg/100 g fw)	Maqui Berry (mg/100 g fw)
References	[38,51]	[10,35–39]	[226–229]	[230–232]	[233–235]	[236,237]	[131,238,239]	[240,241]	[62,123,242,243]	[244–246]	[247]	[248,249]	[219,250,251]
Cyanidin	18–290	-	-	-	-	-	-	27–175	27.5	-	-	-	22.8–26.0
Delphinidin	29–280	-	-	-	-	-	-	-	-	-	-	-	105.0–120.3
Quercetin	1.5–8	0.07 *	104	-	0.3–10.04	-	-	0.09–0.54	-	39.02	37-400	-	-
Myricetin	nd-3	-	69	-	-	-	-	0.05–0.77	-	-	-	-	-
p-Coumaric acid	1–9	-	0.25	67.03–2792.6	0.3–4.2	0.13	-	2.64	0.07–0.22	-	-	-	-
m-Coumaric acid	7–30	-	-	-	0.3–14.2	-	0.93	-	-	-	-	-	-
Sinapic acid	-	-	0.211	-	-	-	-	0.61	-	-	-	-	-
Gallic acid	-	-	-	3–72.2	3.8–8.6	-	-	26.5–47.54	-	701.6	-	-	75
Ascorbic acid	-	-	0.011	2.4–5.34	-	-	-	-	-	-	-	-	-
Ferulic acid	-	-	0.087	-	5.3–294	-	-	0.95	753.6	2.46	-	-	-
Chlorogenic acid	-	3.08 *	-	177.4	4.3–22.3	-	-	0.35–1.10	-	37.65	-	-	-
Protocatechuic acid	-	-	-	-	3	-	-	-	-	-	-	-	-
5-*O*-Caffeoylquinic Acid	-	-	-	-	283–1735	-	5.57–8.88	-	8.4–37.9	-	346–413	-	-
1,3-di-*O*-Caffeoylquinic Acid	-	-	-	-	0.2–0.3	-	0.15–0.22	-	0.6–4.27	-	13–508	-	-
Caffeic acid	-	-	0.15	2.41–5.31	1.3–9.2	0.26	-	0.52	0.76–1.52	8.12	-	-	-
Protocatechuic acid	4–8	-	-	-	-	-	-	-	-	-	-	-	-
Ellagic acid	-	-	120	1151.7	23.9	-	2.012	2.72	-	-	-	-	-
Benzoic acid	-	-	4.7	-	-	3.79	-	-	-	-	-	-	-
p−Hydroxyphenylacetic acid	-	-	0.007	-	4.3–12.9	-	-	-	-	-	-	-	-
2,3-Dihydroxybenzoic acid	-	-	0.003	-	12.9	-	-	-	-	28.18	-	-	-
2,4-Dihydroxy benzoic acid	-	-	0.04	-	-	-	-	-	0.13–0.51	3.37	-	-	-
Vanillic acid	-	-	0.05	3–4.41	-	-	-	2.91–3.1	4–6.37	57.7	-	-	-
Trans-cinnamic acid	-	-	0.02	-	-	-	-	-	-	-	-	-	-
O-Hydroxycinnamic acid	-	-	0.089	-	-	-	-	-	-	-	-	-	-
p-Hydroxybenzoic acid	-	-	0.021	-	-	-	-	-	-	172	-	-	-
Resveratrol	1–12	-	-	-	-	0.13	-	-	-	-	-	-	-
Epigallocatechin	-	-	1.5	-	25.6	-	-	-	-	-	-	-	-
(+/−)-Catechins	6–7	-	4.5	129.3	-	-	-	19.56–135.19	106.6	49.1	593	-	-
(+/−)Epicatechin	6–7	-	4.5	791.7	0.2–24	-	-	1.07	-	44.6	6767	-	-
Gallocatechin gallate	-	-	0.4	-	10.2–63.7	-	-	-	-	-	-	-	-
Epigallocatechin gallate	-	-	1.9	-	4.5–8.4	-	-	5.65	-	-	-	-	-
Delphinidin 3-galactoside	167.1	23.4	-	-	-		-	-	-	-	-	52 ***	-
Delphinidin 3-glucoside	169.1	15.4	-	-	-		26.8–29.40	-	-	-	-	839 ***	389.9

Table 2. *Cont.*

Compounds	Bilberry (mg/100 g fw)	Blueberry (mg/100 g fw)	Cranberry (mg/100 g fw)	Raspberry (mg/100 g dw)	Mulberry (mg/100 g fw)	Lingonberry (mg/g DE)	Blackberry (mg/g DE)	Strawberry (mg/100 g fw)	Goji Berry (mg/100 g dw)	Acai Berry (mg/100 g dw)	Black Chokeberry (mg/100 g dw)	Black Currant (mg/100 g fw)	Maqui Berry (mg/100 g fw)
Cyanidin 3-galactoside	122.6	4.2	2	-	-		-	-	-	-	105–2407	-	-
Delphinidin 3-arabinoside	152.3	-	-	-	-		-	-	-	-	-	-	-
Cyanidin 3-glucoside	130.4	2.6	0.1	15.02–53.94	4.72		-	-	-	998.74	5–113	327 **	679
Petunidin 3-galactoside	50	11.7	-	-	-		-	-	-	-	-	103 ***	-
Cyanidin 3-arabinoside	110.6	3.5	1.4	-	-		-	-	-	-	215–1148	-	-
Petunidin 3-glucoside	101.9	12.4	-	-	-		10.02–15.25	-	-	21	-	-	-
Peonidin 3-galactoside	13.3	1.8	2.8	-	-		-	-	-	-	-	-	-
Petunidin 3-arabinoside	23.9	9.3	-	-	-		-	-	-	-	-	-	-
Peonidin 3-glucoside	56.7	2.1	0.3	-	-		2.04–3.62	-	-	193	-	71 ***	-
Malvidin 3-galactoside	27.5	34.9	-	-	-		-	-	-	-	-	-	-
Peonidin 3-arabinoside	4.5	1	1.1	-	-		-	-	-	-	-	-	-
Malvidin 3-glucoside	67.7	31.2	-	-	-		9.49–10.57	-	-	-	-	-	-
Malvidin 3-arabinoside	12.8	34.7	-	-	-		-	-	-	-	-	-	-
Quercetin-3-galactoside	-	-	70.4	-	-		-	-	-	-	-	-	-
Quercetin-3-α-arabinopyranoside	-	-	34.4	-	-		-	-	-	-	-	-	-
Quercetin-3-rhamnoside	-	-	41.6	-	-		-	-	-	-	-	-	-
Kaempferol-3-glucoside	-	-	5.6	-	-		-	5.12–17.67	-	-	-	-	-
Myricetin 3-α-arabinofuranoside	-	-	37.5	-	-		-	-	-	-	-	-	-
Quercetin 3-*O*-glucuronide	-	-	-	717.57	-		-	9.4–39	-	-	-	-	-
Quercetin pentoside	-	-	-	252	-		-	-	-	-	-	-	-
Cyanidin-3-*O*-sophoroside	-	-	-	43.27–800.3	-		-	-	-	-	-	-	-
Cyanidin-3-*O*-rutinoside	-	-	-	5.49–104.58	2.73		-	-	-	433.98	-	1693 ***	-
Pelargonidin-3-glucoside	-	-	-	-	0.14		-	-	-	-	-	-	-
Quercetin 3-*O*-rutinoside	-	-	-	-	192–398		-	-	0.9–23.2	-	-	1.8–2.37	-
Quercetin 3-*O*-galactoside	-	-	-	-	0.2–345		-	-	-	-	-	-	-
Quercetin 3*O*-glucoside	-	-	-	-	72.4–345.7		0.23–0.88	9.8–25.1	16.9–90.9	44–3756	-	1.5–2.0	-
Kaempferol 3-*O*-glucoside	-	-	-	-	35.5–478		-	5.96–14.39	0.5–1.94	-	-	-	-
Pelargonidin 3-*O*-rutinoside	-	-	-	-	17.8–290		-	-	-	-	-	-	-

Table 2. *Cont.*

Compounds	Bilberry (mg/100 g fw)	Blueberry (mg/100 g fw)	Cranberry (mg/100 g fw)	Raspberry (mg/100 g dw)	Mulberry (mg/100 g fw)	Lingonberry (mg/g DE)	Blackberry (mg/g DE)	Strawberry (mg/100 g fw)	Goji Berry (mg/100 g dw)	Acai Berry (mg/100 g dw)	Black Chokeberry (mg/100 g dw)	Black Currant (mg/100 g fw)	Maqui Berry (mg/100 g fw)
Delphinidin-*O*-(pentosyl)hexoside	-	-	-	-	-	-	0.82–1.88	-	-	-	-	-	-
Delphinidin-*O*-rhamnoside	-	-	-	-	-	-	2.14	-	-	-	-	-	-
Malvidin-*O*-pentoside	-	-	-	-	-	-	1.08–2.13	-	-	-	-	-	-
Malvidin-*O*-rhamnoside	-	-	-	-	-	-	0.13–0.63	-	-	-	-	-	-
Caffeoylisocitrate	-	-	-	-	-	-	0.35	-	-	-	-	-	-
Caffeic acid-*O*-hexoside	-	-	-	-	-	-	0.4–0.56	-	-	-	-	-	-
Myricetin-*O*-hexoside	-	-	-	-	-	-	0.19–0.29	-	-	-	-	29	-
Pelargonidin-3-glucoside	-	-	-	-	-	-	-	17.82–20.85	-	-	-	-	-
Pelargonidin-3-malonylglucoside	-	-	-	-	-	-	-	5.51–8.16	-	-	-	-	-
Pelargonidin-3-glucoside	-	-	-	-	-	-	-	114–348	-	17.58	-	-	-
Pelargonidin-3-rutinoside	-	-	-	-	-	-	-	18–62	-	-	-	-	

Note: Atmospheric-pressure chemical ionization, APCI; Diode array detector, DAD; dry extracts, DE; dry weight, dw; Electron spray ionization, ESI; fresh weight, fw; Hexahydroxydiphenoyl, HHDP; High pressure liquid chromatography, HPLC; Liquid chromatography, LC; Lycium barbarum glycoprotein, LbGp; Lycium barbarum polysaccharides, LBP/LBPC/LBPA/LBPF; Mass spectrometry, MS; Nuclear magnetic resonance, NMR; reverse phase, RP; photodiode array detector, PDA; Quadrupole Time-of-Flight Mass Spectrometry, QTOF-MS; Ultra High pressure liquid chromatography, UPLC. * mg compound/mg extract. ** mg/100 g of sample dw. *** nmol/g.

4. Bilberries

Bilberries (BBs, *Vaccinium myrtillus*) are rich in quercetin, anthocyanins, tannins, catechins, vitamins, and pectins [252]. However, the most important classes of compounds considered responsible for the therapeutic role of BB/BB extracts (BBEE) are phenolic acids and anthocyanins. The majority of compounds belonging to these two classes are presented in Table 2. The phenolics of blueberries varied widely and comprised of 0.3% of fresh fruits, which usually ranged from 48 to 304 mg/100 g of fresh fruit. Among the phenolic acids, the most abundant phenolic acids were ascorbic acid, chlorogenic acids, and 3-caffeoylquinic acid followed by caffeic, ferulic, ellagic, and gallic acids. Among the free phenolic acids, chlorogenic acids and ascorbic acids are of prime importance with reference to their health promoting activities [39]. Additionally, at least 15 different BB anthocyanins have been identified including the antidiabetic anthocyanin aglycones, which constituted >70% of the total anthocyanin of BB (Table 2) [17,38,39]. BB anthocyanins showed excellent in vitro α-amylase and α-glucosidase inhibitory activities, reducing or preventing intestinal glucose absorption, and redirecting lipoprotein metabolism regulator enzymatic activities [43]. BB anthocyanins also inhibited advanced glycation end-product (AGE) formation, a severe diabetic complication. The main bioactive compounds considered responsible for inhibiting AGE activity were chlorogenic acid, quercetin-3-galactoside, quercetin-3-arabinoside, quercetin-3-glucoside, quercetin glycoside, quercetin-3-rhamnoside, myricetin glycoside 4, myricetin, and procyanidin b2 biomarkers [253]. BB polyphenols regulate hexose transport via GLUT2 and Na-glucose co-transporter 1 (SGLT-1), which assists glucose uptake. In other studies, GLUT2-mediated hexose transport was impeded by BB-derived flavones [48,254]. Cermak et al. [255] also reported that quercetin-3-*O*-glucoside and quercetin-4-*O*-glucoside decreased intestinal hexose absorption by inhibiting SGLUT1 in pig jejunum brush-border-membrane vesicles.

In one of the in vivo studies, supplementation with bilberry extract (BBE) reduced fasting blood sugars (FBS), total glyceraldehyde (TG), TC, and LDL-C levels. BB ingestion increased islet of Langerhans size and minimized retinopathy prognosis. BBE ingestion improved insulin sensitivity and hypoglycemia by upregulating AMPK, which upregulated GLUT4, PPAR-α, ACOX, and carnitine palmitoyltransferase-1 and ACPT-1A, which is synonymous to the suppression of glucose production and increased insulin sensitivity [15]. In another crossover study, the lyophilized BBE showed an 18% decrease in (incremental rise of) plasma glucose levels in overweight/obese diabetic humans, accompanied by decreased plasma insulin levels [48]. Recently, Alnajjar et al. [49] also reported that BBE anthocyanins reduced plasma glucose, oral glucose tolerance test (OGTT), TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, TG, and inflammatory adipokine [leptin, TNF-α, and high-sensitivity CRP (hs-CRP)] levels, without affecting the plasma Trolox equivalent antioxidant capacity (TEAC). The anti-inflammatory role of BB was also witnessed when BB juice (BBJ) consumption in healthy adults also reduced NF-κB-regulated inflammatory mediator expression (CRP, IL-6, IL-15, and monokine induced by gamma-interferon) and increased plasma levels of quercetin (by 32–51%) and *p*-coumaric acid [51]. Later on, Kolehmainen et al. [53] examined the anti-inflammatory mechanism associated with BB consumption and reported the regulation of cytoplasmic ribosomal protein expression and the toll-like receptor (TLR) signaling and β-cell receptor signaling pathways, with decreased proinflammatory macrophage and monocyte functional gene expression including C-C chemokine receptor 2 and monocyte-to-macrophage differentiation. Kim et al. [127] also reported that daily BBE consumption reduced vascular permeability by reducing vascular endothelial growth factor levels in diabetic rats, in addition to restoring tight junction protein expression including claudin-5, zonula occludens-1, and occludin [127].

An accumulated number of evidence has also suggested that BB(E) intake is also helpful in relieving the oxidative stress and oxidative stress-related complications in obese and (pre)-diabetic subjects (Table 1). BBE administration alleviated stress-induced liver damage by decreasing plasma alanine aminotransferase (ALT), malondialdehyde (MDA), and nitric oxide (NO) levels and increasing glutathione (GSH) and vitamin C levels [45]. Capillary albumin filtration (CAF) is an early diabetic complication, associated with neuropathy and hypertension. BB anthocyanins

prevented experimentally-induced-CAF, improving vision and retinopathy, and remarkable CAF reductions were observed among diabetic patients [46,48,256]. The suggested mechanism for inhibiting CAF involves BB anthocyanosides, which reduced aldose reductase activity and acted as strong antioxidants or pro-reductants, inhibiting AMP and guanosine monophosphate phosphodiesterase by scavenging superoxide anions [256]. Albumin retention (AR) was assessed by the isotopic CAF test in STZ-induced diabetic rats after anthocyanoside-rich BBE administration [46], and BBE treatment was found to reduce and maintain reduced AR (14% to 1.3%) and low-frequency/high-frequency (LF/HF) ratio values in diabetic rats, without toxic effects [47]. BB-derived phenols increased the population of beneficial SCOA-producing gut bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.) and reduced bacterial metabolic syndrome biomarker genera including Enterobacteria. The dysbiosis symbolic Firmicutes/Bacteroidetes ratio, IR, and obesity-led-dysbiosis also decreased following BB consumption [49]. BB added to a fermented oatmeal drink caused a high glucose response, with a significantly reduced insulin index (Table 1) [50].

5. Cranberries

Cranberries (CrBs, *Vaccinium macrocarpon*) have also been intensively investigated for their proclaimed favorable cardiometabolic and dysmetabolic syndrome effects, likely due to phytochemicals such as oligosaccharides, procyanidins, and anthocyanins. A comprehensive list of potential well-known antioxidative, antidiabetic, and anti-inflammatory compounds found in CrB (products) or CrB extracts (CrBE) used in clinical or non-clinical interventional studies are listed in Table 2 [226–228]. The purified fractions of procyanidins were more antidiabetic potent than the anthocyanin and oligosaccharide fractions [257]. With respect to individual compounds, quercetin-3-galactoside, 5-caffeoylquinic acid, and quercetin-3-rhamnoside were the major compounds comprising 75–77% of total flavonols of cranberry whilst 4-caffeoylquinic acid, 3-caffeoylquinic acid, quercetin-3-arabinopyranoside, myricetin3-galactoside, quercetin, quercetin-3-arabinofuranoside, and quercetin-3-benzoylgalactoside were found in the least amounts. Many authors have initially described the in vitro antidiabetic/antiglycation activities of cranberry extracts or its products [226,257]. Barrett et al. [227] isolated ellagitannins and proanthocyanidins and demonstrated their dose-dependent inhibition of α-amylase and glucoamylase activities. CrB powder from stress-adapted portions of cranberry juice (CrB-JSB) showed increased α-amylase and glucoamylase activities compared with CrB powder, and CrB-JSB (200 mg/mL) also showed anti-hypertensive properties by inhibiting the angiotensin I-converting enzyme (ACE-1) activity [228]. Podsedek et al. [258] found that CrB extracts inhibited pancreatic lipase activities more potently than other berries, but digestive enzyme inhibitory activities were less potent. Purified CrB proanthocyanidins and oligosaccharides also reduced the levels of HbAC1 levels from 7.05% to 5.75, 5.55, and 5.45% in the hemoglobin-glucose assay, whereas the recommended HbAC1 value should be below 7%, according to the American Diabetes Association. Reduced glucose-induced AGE formation during middle glycation stages was also observed during the human serum albumin (HSA)-methylglyoxal and HSA-glucose assays [257]. CrB-derived phenolic-rich extracts decreased fluorescent AGE generation by almost 60%, which was more effective than the other berry anti-AGE activities of raspberries, apples, grapes, and strawberries. The CrB anthocyanin and procyanidin fractions also decreased fluorescent AGE generation in an arginine-methylglyoxal model by 53.3 to 56.8% [226]. The CrB oligosaccharide-rich fraction showed concentration-dependent anti-glycation activity, which reduced AGE formations by 53.3 to 56.8%, respectively, almost as strongly as the reference compound [259].

The hypoglycemic, hypo-insulinemic, and hypolipidemic properties of CrB or its byproducts have also been reported in many clinical interventions (Table 1) [5,56–60,260]. Low-calorie dried cranberry (LCDC, 40 g) consumption after HFD reduced hyperglycemic and hyperlipidemic conditions, halted increases in IR/HOMA-IR and inflammatory biomarkers (TNF-α IL-6, IL-2, IL-10, IL-18, malondialdehyde-MDA) in adipose tissue, and lowered plasma lipid oxidation and oxidative stress biomarker levels in the treated group [56]. After testing LCDC, sweetened, dried CrBs (SWDC)

consumed by non-insulinemic diabetic patients also reduced plasma glucose levels when compared with white bread (WB) and unsweetened dried CrBs (USCB) [260]. The plasma insulin peak following SWDC consumption appeared earlier than the insulin peaks for WB or USCB consumption and was significantly lower than those for WB and USCB. Bread consumption induced higher insulin and postprandial glucose responses, which could be diminished by incorporating CrBs [58–60,260]. CrB extracts (CrBEs) also halted visceral adiposity and weight gain in HFD-fed C57BL/6J mice, and improved HFD-induced hypercholesterolemia, hypertriglyceridemia, antioxidant defense mechanisms, and hepatic oxidative stress and normalized the NF-κB/IκB ratio [54]. Long-term CrBE consumption effects were also investigated [55,63], and the addition of CrBE to normal chow delayed age-related basal plasma insulin concentration declines [63]. CrBE supplementation also improved glucose responsiveness and increased insulin concentrations (7.6%) in rats, without significant HOMA-IR changes. CrBEs also induced duodenal homeobox 1 and insulin expression within islets, which enhanced insulin release, suggesting insulinotropic effect of cranberry intervention [55]. CrBEs showed the anti-obesity effect by inducing the LDL receptor expression, resulting in increased hepatic cholesterol uptake and promoted cholesterol binding to bile acids, causing increased fecal cholesterol excretion [57]

CrBJ consumption was also examined in randomized clinical studies (Table 1). Healthy adults who consumed CrB juice (CrBJ) also showed reduced proinflammatory CRP levels [61]. Daily CrBJ supplementation for 60 days increased paraoxonase-1 (PON-1) and apolipoprotein (Apo)A-I expression (dysfunctioning of PON-1 and apoA-I results in glycation in T2DM patients) accompanied by decreased blood glucose and ApoB levels in T2DM patients. CrBJ inhibited GLUT-4-mediated gastric glucose uptake and aldose reductase, α-amylase, and α-glucosidase activities and protected LDL-C against oxidation [60,64,228]. Moreover, both routine-calorie CrBJ (RCCJ) and high-calorie CrBJ (HCCJ) are enriched in hexoses and sugars, which could limit their use by diabetic individuals. Therefore, low-calorie CrBJ (LCCJ) was examined in glycemic and insulinemic T2DM patients by Wilson et al. [58,59] and Novotny et al. [65]. LCCJ consumption did not affect LDL-C, HDL-C, or TC levels; however, ApoA-I, ApoA-II, ApoB, and TG levels were reduced in the treated group. Individuals with higher baseline TG or HOMA-IR values experienced more pronounced drops in TG and HOMA-IR than others [65]. Serum HbA1c levels were reduced by 11.4% and 6.02% following RCCJ and RCCJ enriched with omega-3 fatty acid consumption. Omega-3 fatty acid-enriched RCCJ also increased HDL-C levels by 21.1% compared with the baseline [63]. Additionally, folic acid consumption combined with LCCJ decreased plasma homocysteine levels and increased adiponectin and folic acid levels without any change in inflammatory biomarker levels (IL-6, IL-10, IL-18, and TNF-α) [66].

In summary, CrB consumption exerted antimetabolic syndromic effects by downregulating GLUT2 and GLUT4 expression and increasing hepatic cholesterol uptake. Diet-induced weight gain and low-grade inflammation were counteracted by the prevention of TG accumulation and strengthened antioxidative defense mechanisms. The other proposed possible mechanisms of action of CrB, or its products, consumption include reduction and inhibition of ACE-I activity and oxidative stress, accompanied by improvements in endothelium-dependent vasodilation. Furthermore, CrB-derived bioactive compounds including quercetin, inhibited microsomal TG transfer protein (MTP), preventing ApoB-containing lipoprotein assembly. Quercetin also lowered proinflammatory CRP expression in a transgenic mouse model and decreased cytokine-induced CRP expression in Hep3β cells and Chang liver cells [68,69,261], which was analogous to weight loss- and polyunsaturated fatty acid (PUFA)-rich Mediterranean diet-induced CRP suppression [65,261]. Additionally, CrB consumption has beneficial effects on the gut microbiome. HFD reduced Bacteroidetes and increased Firmicutes populations in C57Bl/6J mice, which was reversed by CrBEs intake. CrBEs also increased the Akkermansia gut population, which may prevent HFD-induced increases in circulating pro-inflammatory lipopolysaccharides (LPS) [54].

6. Raspberries

Raspberries (RBs), especially red RBs (*Rubus idaeus* L.), are rich in fiber and potent therapeutic phytochemicals that have rendered raspberries as a functional food for metabolic syndrome [199]. The phytochemicals of raspberries provide the healthy and protective affects to its consumers by influencing the cell signaling pathways that affect transporters, receptors, cellular events, and gene expression. These health promoting RB phytochemicals belong to ellagitannins and anthocyanins (Table 2) [262]. Among these two classes, RB anthocyanins are major contributors to health promoting bioactivities. The anthocyanins of RB are cyanidin-based, but with dissimilar glycosidic units. The pelargonidin-based anthocyanins are only found in RB and strawberries with a sophoroside unit attachment unique to raspberries. Ellagitannins are hydrolyzable tannins that represent another major RB phytochemical group, which are hexahydroxydiphenoyl esters with quinic acid or glucose cores. Glucose cores can attach to galloyl groups, and further arrangements within hexahydroxydiphenoyl molecules yield the ellagic acids. Numerous in vitro studies have described that RB extracts (RBE) reduced lipid oxidation, LDL-oxidation, ROS generation, and DNA damage, associated with upregulated CAT and SOD enzymatic antioxidant activities [73]. Hypoglycemic studies revealed that RBEs inhibited α-amylase, with mixed effects on α-glucosidase, and aglycones and anthocyanin promoted GSIS from pancreatic cells [263,264]

Fresh RB extracts (RBEs) and freeze-dried RB powder have also been employed for in vivo evaluation (Table 1), in which oxidative stress was found to be relieved as decreased protein and lipid oxidation and damage was seen [74,75]. RB freeze-dried powder fed to obese and diabetic mice reduced ROS levels in erythrocytes by 0.87% when compared to the controls, indicating the ROS-neutralizing role of RB powder bioactive constituents during homeostasis. The RB intervention reduced ROS levels by increasing the glutathione peroxidase (GPx)/SOD ratio (2%) and GPx activity (2.13%) when compared to the placebo controls. Upregulated GPx activity also inhibited lipid peroxidation and protected against diabetes by delaying perturbed metabolism development [76]. RB juice (RBJ) given to hypercholesterolemic golden Syrian hamsters reduced plasma LDL-C levels and increased hepatic GSHPx and SOD activities by 30% and 25%, respectively [72]. Polyphenol-rich black RBs have also been combined with HCD foods for sustainable postprandial glycemic control, reducing plasma free fatty acid (FFA) and oxidative stress marker levels. RBs, combined with HCD, blunted postprandial insulinemia and ex vivo LDL-oxidation during the postprandial state, hindering glucose uptake (Table 1) [91]. Purified hydrolyzable RB tannin supplementation in rat gastritis models also demonstrated increased endogenous antioxidant defense system components and decreased inflammatory biomarkers and conditions. RB ellagic acid suppressed the specific immunoglobulin antibody response in cytotoxic cells without affecting other immunoglobulin parameters. Reduced lipid peroxidation, neutrophil infiltration, and iNOS overexpression were observed in ex vivo gastritis and Crohn's disease models [85,86]. A recent study showed that RBE consumption mitigated carcinogenic acrylamide-induced liver toxicity in male Wistar rats. RB treatment increased plasma antioxidants enzyme levels and reduced acrylamide-induced hepatic ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma-glutamyltransferase (γ-GT) activities [265].

Limited human clinical trials have been performed with RBs, but the antidiabetic effects of RBEs and purified compounds have been examined in diabetic rat models (Table 1) [87,88]. Numerous anthocyanin and polyphenolic components have been hypothesized to affect starch digestion, altering postprandial glucose levels [263]. RB anthocyanin also enhanced insulin sensitivity, upregulated adiponectin expression, downregulated inflammatory cytokines, and altered AMPK phosphorylation, which is a T2DM therapeutic target [264]. A clinical trial examined RB intake with a HCD and reported no postprandial insulin and glucose response alterations [92,93]. In another study following HC-bar consumption, RB intake increased postprandial glucose levels, without changing peak glucose concentrations, and diminished postprandial insulinemia [91]. RB effects on IR and the underlying mechanisms in skeletal muscles were studied by Zhao et al. [87]. AMPK inactivation led to skin lipid

accumulation and insulin sensitivity loss. This study found that AMPK-α1 is important for AMPK activation, and dietary RB powder inclusion increased insulin sensitivity by upregulating cytochrome C protein in AMPK-$\alpha1^{+/+}$ rats [87]. The supplementation of 5% RB with HFD improved insulin sensitivity by increasing IRS-1 phosphorylation at Tyr 612 and increasing the p-Akt/Akt ratio. RB intake also attenuated nod-like receptor pyrin containing 3 (NLRP3) inflammasome activation, which is a major contributor to metabolic syndrome. NLPR3 activation, combined with caspase 1, forms caspase 1p20 and caspase 1p10. Caspase 1p20 activation releases IL-1β and IL-18. RB consumption downregulated NLPR3, caspase 1p20, IL-1β, and IL-18 expression in HFD-fed mice [88]. Recently, Zou et al. [266] also reported that 5% RB powder supplementation with HFD suppressed TNF-α, L-6, IL-1β, and NF-κB p65 expression and increased GLUT4 expression and IRS-1 and Akt phosphorylation. RB powder also increased mitochondrial biogenesis genes (PGC-1α and Nrf1) and mitochondrial abundance markers (cytochrome c, citrate synthase, and cytochrome c oxidase subunit IV) [266].

The health-promoting effect of raspberry supplementation on the glycerophospholipids metabolism is also evident (Table 1). The addition of 10% freeze-dried RB to an isocaloric diet increased plasma HDL-C (1.5%) and insulin sensitivity and decreased abdominal fat (38%), blood TG, cholesterol, ROS (19%), and LDL-C (0.3%). Similarly, RB-derived cyanidin-3-glucoside upregulated GLUT4 expression, without affecting insulin sensitizer adiponectin [89]. Ellagic acid, which is unique to RB, increased insulin secretion and decreased FBS, HbA1c, and glycated urinary albumin levels. RB inclusion in HFD/HCD diminished impaired insulin tolerance and inflammatory cytokines. RB seed flour, combined with a HCD, downregulated the lipogenic gene expression of lipoprotein lipase (LPL), stearoyl CoA desaturase-1 (SCD-1), and diacylglycerol acyltransferases 2 (DGAT2) and gluconeogenesis promoting genes including PEPCK, G6Pase, sterol regulatory element-binding protein 1c (SREBP-1c), and carbohydrate response element-binding protein (ChREBP) (Table 1) [90]. RB ketones also prevented HFD/HCD-induced BW gains, reduced visceral and adipose tissue, reduced hepatic TG contents, and increased norepinephrine-induced lipolysis in white adipocytes, suppressing lipid accumulation by enhancing lipolysis and fatty acid oxidation [267]. RB supplementation in diabetic patients substantially lowered postprandial glucose levels, without affecting plasma insulin levels after a fatty meal challenge [94]. RB consumption also reduced TG levels [71]. Conflicting results regarding RB interventions and effects on metabolic syndrome biomarkers have been reported. Noratto et al. [76] found an insignificant difference in the weight gain between diabetic mice fed with and without RBs. Similarly, Kirakosyan et al. [77] and Norrato et al. [76] reported no RB intervention effects on LDL-C, fasting blood insulin, IκBα, and PPAR-γ levels. Contrasting results may be due to higher baseline weights of the subjects. However, Kirakosyan et al. showed that RB intake reduced glucose metabolisms and insulin signaling mRNA levels including MAP2K1, glycogen synthase (GYS1), hexokinase, IκBβ, phosphatidylinositol-4,5-bisphosphate 3-kinase, mechanistic target of rapamycin (mTOR), Chuk (involved in innate immunity), C-X-C chemokine receptor type 4 (involved in inflammation), LPL, GYS1, MAP2K1(involved in apoptosis), nicotinamide phosphoribosyltransferase, ApoE, PPAR-γ, and PPAR-α (involved in glucose and lipid dynamics) (Table 1) [77].

RB intake also increased gut *Lactobacillus*, which is a healthy gut marker, and increased beneficial gut intestinal SCFAs, which are colonic epithelial cell substrates and improve gut health [78]. RB consumption increased SCFA-producing bacterial populations including *Bacteroides*, *Butyricimonas*, *Ruminococcus, Akkermansia, Clostridium butyricum, Mucispirillum, Oscillibacter, Ruminococcaceae,* and *Lachnospiraceae,* which improved metabolic syndromic conditions during metformin T2DM treatment [268]. Furthermore, RB consumption time- and dose-dependently increased the gut microbial population of *Anaerostipes*, *Ruminococcus*, *Akkermansia*, *Coprobacillus*, *Allobaculum*, *Anaerovorax*, *Dorea*, *Asaccharobacter*, *Anaerotruncus*, *Coprobacillus*, *Desulfovibrio*, *Victivallis*, and *Mucispirilum,* and decreased the microbial population of *Acetivibrio*, *Anaerotruncus*, *Bifidobacterium*, *Lactococcus*, *Prabacteroides*, *Streptococcus*, *Turicibacter*, and *Acetivibrio*. Increased beneficial microbial communities as above-mentioned can reduce inflammation, obesity, metabolic syndrome, and dysbiosis [79]. Su et al. [80] reported that RB-derived pelargonidin-3-*O*-glucoside increased the gut population of

Prevotella and improved the Bacteroidetes/Firmicutes ratio. Another more recent report concluded that there was a favorable higher population of *Akkermansia muciniphila* and Bacteroidetes/Firmicutes ratios in pathogenic free mice fed on black RB powder [81]. Conclusively, RB consumption showed antidiabetic effects, inhibiting glucosidase and amylase activities, strengthening the endogenous antioxidant defense system, reducing inflammatory biomarkers, activating AMPK, GLUT2/GLUT4, IRS-1 phosphorylation, downregulating lipogenesis and gluconeogenesis genes, and increasing epithelial mucus barrier protecting and SCOA-producing bacterial populations (Table 1) [83,84].

7. Mulberries

Mulberries (MBs, *Morus alba*/*Morus rubra*) are rich in cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin-3-glucoside, and other anthocyanins comprising 78% of the MB polyphenolic compounds (Table 2) [269]. These purified anthocyanins from MB showed excellent glucose-lowering properties in HepG2 cells, increasing PPAR-α and AMPK phosphorylation (activation) and the p-mTOR/mTOR ratio (synonymous with the activation of insulin receptors and insulin-like growth factor 1 receptors). During metabolic syndrome, IRS-1 inactivation increases the p-p38/p38 ratio (subfamily of MAPK, which requires inflammatory cytokines for activation) and reduces PGC-1α expression (a regulator of energy homeostasis and mitochondrial biogenesis), which were abolished or reversed with MB anthocyanins treatment [269]. In addition to anthocyanins, polyphenol-rich MB methanolic extracts also showed excellent α-glucosidase inhibitory activities due to quercetin 3-*O*-rutinoside, chlorogenic acid, and cyanidin 3-*O*-glucoside [235]. Cyanidin glycosides in MBs also reportedly possess potent anti- α-glucosidase activity, which inhibit the enzyme by affecting α-glucosidase α-helix contents via cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R) domain matching [270]. HepG2 cells treated with the five most abundant MB polyphenols including C3G, 1-deoxynojirimycin, resveratrol, C3R, and oxyresveratrol showed improved glucose consumption and postprandial glucose disposal through increased glucokinase activity [271]. Another study found that 1,5-dicaffeoylquinic and dihydroquercetin acid protected cells against glucotoxicity [29]. MB extracts (MBEs) upregulated PGC-1α (38%) and FOXO1 (40%) (regulator of PEPCK and G6Pase enzymes) and downregulated PEPCK (79%) and G6Pase (37%) expression in IR model cells. MBEs also upregulated AKT2 (crucial for IRS activation and hence increasing insulin sensitivity) and glycogen synthase kinase (GSK)3β levels, with significantly increased p-AKT/AKT ratios (hence reduced IR) and increased GSK3β phosphorylation and glycogen synthase 2 (GSY2) activation [272].

In in vivo studies, MB polyphenols and polysaccharides reduced ROS levels and enhanced reductant enzymatic activities including GPx, SOD, and CAT while reducing IL-8, TNF-α, COX-2, and IL-6 release in STZ-induced diabetic mice (Table 1) [273]. MB anthocyanins also attenuated HFD-induced decreased hepatic SOD and GPx activities [95]. Yan et al. [269] reported that MB anthocyanins alleviated hypoglycemia by inhibiting ROS generation, promoting AMPK phosphorylation, activating tuberous sclerosis 2, (reducing the mTOR and ACC signaling), reducing p38-MAPK and PGC-1α expression, and increasing mitochondria and matrix metalloprotease (MMP) abundance in diabetic mice (Table 1) [97]. MB wine consumption by diabetic mice also reversed glycemic status, with reduced oxidative stress markers, proteinuria, non-esterified fatty acid contents, and lipid peroxidation and improved antioxidant defense systems [97]. MB-derived and purified cyanidin-3-*O*-β-D-glucopyranoside intervention also circumvented diabetic cytopathy by reducing oxidative stress markers of DNA modification including 8-hydroxy-2-deoxyguanosine and increasing the axonal transport of nerve growth factor [98].

The oral MBE supplementation also improved insulin signaling by decreased GSK3β, and increased GSY2, AKT, increasing p-AKT/AKT ratios in skeletal, hepatic, and adipocytes tissues of diabetic mice [272]. Oral MB fruit intake in diabetic mice also improved insulin sensitivity by upregulating (up to 3%) the IRS-1, p-IRS01/IRS-1, p-AMPK/AMPK, CCAAT-enhancer-binding proteins (C/EBP), sterol regulatory element-binding protein 1 (SREBP-1c), and PGC-1α [269,274]. Ren et al. [99] further reported that MB consumption normalized glucose metabolism by

abolishing protein-tyrosine phosphatase 1B expression and activating the phosphoinositide-3-kinase (PI3K)/AKT pathway. MB anthocyanin-induced p38-AMPK-PGC-1α pathway upregulation increased thermogenesis gene activity. Anthocyanin components also downregulated lipogenesis genes including hydroxymethylglutaryl coenzyme A reductase (HMG-CA-R), SREBP-1c, and FAS [100] and activated scavenger receptor class B type 1 and ATP-binding cassette transporter (ABCA1), which transfer cholesterol.

MBEs combined with HFD demonstrated excellent anti-obesity and hypolipidemic properties. MBE supplementation reduced BW gains by 41.3% in HFD-fed diabetic male C57BL/6 mice. Serum TG, TC, HDL-C, and LDL-C levels in HFD + MBE-fed mice were lower than those in HFD-fed diabetic mice, but higher than the MBE-fed controls. Liver injury parameters (ALT and AST) were reduced in HFD + MBE-fed mice, with reduced adipose and hepatic liver lipid droplet sizes [101]. MB fruit consumption lowered TG, TC, LDL-C, and FFA levels in other studies (Table 1) [102,103]. MB-derived anthocyanin consumption decreased serum levels of inflammatory markers (IL-6, IL-1α, iNOS, TNF-α, IFN-γ, and NF-κB), thiobarbituric-acid-reactive substances (TBARS) (a lipid oxidation marker), hyperlipidemic markers (TC, glucose, TG, and leptin), insulin, and hepatic AST, ALP, and ALT levels, downregulated FAS, and increased heme oxygenase-1 (HO-1) (a cytoprotective enzyme) and antioxidant enzyme levels in HFD-fed male C57BL/6 mice (Table 1) [95,104]. Aqueous MBEs employed the hypolipidemic and hypoglycemic effects by activating the AMPK, increasing the p-AMPK/AMPK ratio (hence improving mitochondrial biogenesis), and downregulated FAS, acetyl coenzyme A carboxylase (ACC), glycerol-3-phosphate acyltransferase (GPAT), and SREBP-1 [104]. MBEs in HFD-fed male Sprague-Dawley rats prevented non-alcoholic fatty liver disease (NAFLD) by downregulating lipid/cholesterol homeostasis-related genes (FAS, ACC, GPAT, and SREBP-1) and suppressing the lipid oxidation biomarkers MDA and 4-hydroxynonenal [105,106]. Hu et al. [275] demonstrated that MBE increased nuclear factor erythroid-2-related factor 2 (Nrf2) phosphorylation and nuclear translocation, activating the Nrf2/antioxidant response element signaling pathway, which increased quinone oxidoreductase 1, HO-1, and NAD(P)H expression and promoted antioxidant enzymatic activities, thus protecting hepatocytes against palmitic acid-induced lipo-toxicity and oxidative stress.

Gut microbiota regulates dietary energy harvesting, glucose homeostasis, and lipid metabolism, especially in brown adipose tissues (BAdT). Mitochondria-rich BAdT activation can increase energy expenditure following MB-induced UCP1 upregulation and oxidative phosphorylation downregulation, releasing energy as heat. MB powder consumption reversed HFD-induced gut microbiome changes, increasing the Bacteroidetes/Firmicutes ratio and Bacteroidetes populations (*Porphyromonadaceae, Parabacteroide, S24-7, Prevotellaceae*, *Alloprevotella*, *Rikenellaceae*, *Alistipes*, *Rikenella*) and decreasing the *Proteobacteria* (*Alphaproteobacteria, Brevundimonas, Devosia, Rhodobacteraceae*, *Polymorphobacter*, *Deltaproteobacteria*, *Desulfovibrio*, *Arenimonas*), and Firmicutes (*Clostridia, Lachnospiraceae, Eubacterium, Coprococcus, Ruminococcaceae*, *Oscillibacter*, *Ruminiclostridium*) populations [107,108]. At the genus-level, MB fruit supplementation promoted SCOA/SCFA-producing and IMBD-restoration-supportive genera *Lactobacillus, Bacteroidales, Bacteroides, Allobaculum,* and *Akkermansia* growth, and suppressed *Corynebacterium, Staphylococcus, Aerococcus, Jeotgalicoccus, Facklamia,* and *Enterococcus* growth. *Allobaculum* and *Lactobacillus* protect against metabolic syndrome, and both genera increased in diabetic rats after MB intake [108]. Approximately 60 metabolites were identified in MB including flavonols, phenolic acids, flavonoids, lignans, and organic acids (Table 2) [234]. In short, MB fruit consumption upregulated/activated glucose-consumption-related pathways and insulin-sensitivity-related pathways (p-AKT/AKT ratio, glucokinase, PGC-1α, FOXO1, IRS-1, p-IRS-1/IRS-1, p-AMPK/AMPK, C/EBP, and Bacteroidetes/Firmicutes ratio) and downregulated lipogenesis-related pathways (FAS, ACC, GPAT, and SREBP-1) in skeletal, hepatic, and adipocyte tissues.

8. Lingonberries

Lingonberry (LB, *Vaccinium vitis-idaea*) alleviates metabolic syndrome including frequent urination and fatigue. In in vitro studies, LB extracts (LBEs) increased glucose uptake in C2C12 skeletal

muscle cells by modulating AMPK activity [276]. LB polysaccharides inhibited α-glucosidase activity (by 118–136%) more strongly than the referenced acarbose [277]. In in vitro digestibility assays, LB polyphenols (7% *w*/*v*) were added to white rice, which significantly reduced glucose release [278]. Ethanolic LBEs demonstrated antiglycation activity, with AGE inhibition majorly mediated by LB cyanidin-3-galactoside, quercetin-3-galactoside, and (+)-catechin [279]. In J774 macrophages, LBEs significantly inhibited LPS-modulated NO production, without substantial effects on COX-2 or iNOS expression. Proinflammatory cytokine (IL-6, IL-1β, and TNF-α) expression was reduced by TNF-α downregulation, IκB receptor degradation inhibition, and reduced extracellular signal-related kinase 1/2 phosphorylation [280]. However, in RAW 264.7 macrophages and activated 3T3-L1 adipocytes, LBEs mitigated oxidative stress by suppressing COX-2, iNOS, TNF-αα, IL-6, MCP-1, and IL-1β expression [281].

In in vivo studies, LB consumption also improved hyperinsulinemic, hyperglycemic, and dyslipidemic conditions (Table 1) [111]. LBE consumption reduced blood glucose levels (17–25%), obesity-induced hepatic steatosis (50–60%), and plasma TG, TC, and LDL-C levels (12–18%) associated with increased GLUT4 expression and AMPK and Akt phosphorylation, increasing glucose metabolism and hepatic fatty acid oxidation [111]. LB juice (LBJ) improved low-grade inflammation and endothelial function by increasing NO availability, which is necessary for the inhibition of adhesion molecules, MCP-1, ACE-1, COX-2, and other pro-inflammatory markers [112]. The LB-rich Okinawan-based Nordic diet improved anthropometric (BW, body mass index (BMI), and waist circumference) and metabolic (HOMA-IR, IR, FBS, TG, CRP, TC, and HDL-C) parameters [119]. Linderborg et al. [120] demonstrated that LB powder consumption compensated for additional glucose and lipid consumption. LBJ intake prevented HFD-induced BW gains in C57BL/6JBomTac mice. LB supplementation reduced FBS, fasting insulin, and HOMA-IR levels (Table 1) [113,114]. Hepatic lipid accumulation and liver function parameters (ALT, TG, and cholesterol) decreased after LB supplementation, more strongly than other berries [113,114]. In a recent hyperlipidic and hypercaloric meals challenge study, the LB supplementation halted increased cholesterolemia and decreased the glycemic response, CRP, and postprandial endotoxemia [121]. In an atherosclerosis ApoE$^{-/-}$ mouse model, whole LB consumption upregulated bile acid synthesis gene Cyp7a1, increased the cecal propionic-acid-producing bacteria proportions, and decreased triglyceridemia and atherosclerosis [115]. The insulinemic and glycemic response following oat bread consumption was also checked. The LB polysaccharide and fiber consumption, following bread consumption, reduced glucose and CRP responses [122]. Whole LB and LB nectar intake reduced postprandial glucose and insulin levels after 35 g sucrose intake, and insulin levels increased more rapidly following LB than after glucose intake. Postprandial glucose levels were also reduced following LBJ consumption. Insulin and FFA changes after LBJ consumption were similar to those observed after whole fruit consumption (Table 1) [113,114,123].

Urinary metabolomics revealed that a LBJ-containing diet increased 4-hydroxyhippuric acid and hippuric acid excretion, whereas 4-deoxythreonic acid, 3-hydroxybutanoic acid, dimethylamine, creatinine, and citric acid excretion reduced, likely due to high polyphenolic compound and benzoic acid contents in LBJ (Table 2) [124,282]. Plasma lipidomics data showed that LB consumption increased health-promoting lyso-phosphatidylethanolamines, (LPE) (16:0), lysophosphatidylcholine (LPC) (20:5), (16:1), and (22:5), and phosphatidylcholines (PC) (33:2), (32:2), (35:6), (34:4), (36:6), and (36:5), whereas obesity and diabetes symbolic sphingomyelins (SM) (34:1), (33:1), (40:3), and (38:2) were reduced. Quinate levels also increased, and plasma alanine and glucose levels decreased significantly [116]. LBE and powder supplementation of HFD downregulated the expression levels of macrophage marker endothelial growth factor-like module containing mucin-like, hormone receptor-like 1 (EMR1), and LPS-sensing TLR4 (member of the toll-like receptor family activation of which results in signaling the NF-κB pathway and inflammatory cytokine production) and upregulated tight junction-associated occluding (an integral membrane protein whose modulation is associated with cellular proliferation, differentiation, signal transduction, and migration) and proglucagon (a precursor of glucagon from α-pancreatic cells). The HFD-fed control microbiome showed the upregulation of the ATP-binding

cassette (ABC) transporter, cell motility, membrane transporter, bacterial chemotaxis, bacterial motility, the two-component system, flagellar assembly, transcription, and signal transduction genes, compared with the LB-treated group [283]. LB consumption enriched genes associated with lipid metabolism, nutrient transport, energy, nucleotides, and amino acids (Table 1) [113,114,117]. At the phyla level, LB supplementation affected the diversity and population of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia*. The relative abundance of *Bacteroidetes* increased, and the relative abundance of *Firmicutes* decreased significantly, reducing the obesity and diabetes symbolic *Firmicutes*/*Bacteroidetes* ratio [113,114,117]. At the genus level, HFD increased *Firmicutes* genera populations including *Lachnospiraceae, Oscillospira,* and *Ruminococcus*. The abundance of *Bacteroidetes* increased following LB supplementation, due to unknown members of the S24-7 family. LB supplementation increased *Parabacteriodes*, *Odoribacter*, and *Akkermansia* populations. The principal component analysis confirmed LB extract-induced gut microbial profile variations. HFD increased the population density of the genera *Oscillospira* and *Ruminococcus* and the *Lachnospiraceae* family, microbes associated with diabetes pathogenesis progression [284], which was prevented by LB fruit/powder/extract consumption [285]. *Akkermansia* population increases were associated with the abundance of *Akkermansia muciniphila* species, which are known beneficial gut microbacteria that counteract HFD-induced adipose tissue inflammation, endotoxemia, BW gain, and IR in C57BL/6 mice [286]. Liquid chromatography (LC)-tandem mass spectrometry (MS/MS)-based LB fingerprinting identified several bioactive compounds responsible for antioxidative, antidiabetic, and anti-inflammatory properties. These bioactive compounds primarily belong to anthocyanidins, flavonols, glycosides, catechins, and different conjugates of ferulic and caffeoyl acid (Table 2). Depending on aglycon weight, cyaniding-containing compounds were the major bioactive compounds followed by proanthocyanidins, which represent phenolic compounds in LB [236,237].

9. Blackberries

Blackberries (*Rubus grandifolius* L and *Rubus fruticosus* L.) are consumed fresh or as juices, jams, and liquors. Blackberries are enriched in health-promoting compounds (Table 2) belonging to flavanals, flavanones, flavonols (kaempferol and quercetin glycosides), anthocyanins, hydroxycinnamic acids, and caffeic acid conjugates. The high-performance liquid chromatography (HPLC)-electrospray ionization (ESI)-mass spectrometry (MS)-based *Rubus grandifolius* L. metabolic profiling revealed 50 phytochemicals including anthocyanins, hydroxycinnamic acids, flavonols, flavanones, and ellagitannins (Table 2) [131,238,239]. These blackberry-derived compounds offered an antidiabetic and anti-obesity role by inhibiting digestive enzymes (α- and β-glucosidase, aldose reductase, lipase, and α-amylase) and exhibiting anti-glycation abilities. The blackberry α-glucosidase and α-amylase inhibitory activity was superior to the reference compounds, Acarbose and 1-Deoxynojirimycin (1-DNJ) [287]. Anthocyanins are considered to be the primary mediator of blackberry extract anti-digestive activities, and glycosides are the primary inhibitors of α-glucosidase activity. The interaction between glycosides and enzymes is considered to be competitive, suggesting that glycosides bind to enzymatic active sites [288]. Cytidine glycosides from leaf and fruit *R. grandifolius* extracts reduced aldose reductase activity, which is responsible for AGE accumulation in diabetic patients via dicarbonyl activity [289]. The recorded anti-glycation activity of BB fruit extracts was IC_{50} = 1.87 mg/mL, and ellagitannins and flavonols were the most prominent anti-glycation agents [131,238,239]. HepG2 cells incubated with gut microbial-fermented blackberry metabolites (GMBB) and gastrointestinal-digested BB slurry (GIDBB) showed improved glucose uptake. Increased HepG2 uptake also increased glycogen synthesis. GIDBB and GMBB also maintained the desired cellular redox status by neutralizing ROS and restoring the mitochondrial membrane potential. GIDBB and GMBB supplementation restored glutathione levels, strengthening the oxidative defense system [290].

In in vivo studies, blackberry-derived purified anthocyanin-enriched and ellagitannin-enriched fractions decreased lipid peroxidation markers (TBARS and MDA) and increased hepatic and

brain antioxidant enzyme activities (CAT, GSH, SOD, and GPx) [125]. Similarly, blackberry extract consumption attenuated the HFD-induced effects in an obesity-prone mouse model and prevented the increase in metabolic and lipidemic parameters, while reinforcing endogenous and exogenous antioxidant enzyme systems (Table 1) [126]. LPL activity, plasma glucose, insulin, and acyl-carnitines were also upregulated after blackberry consumption. Antioxidative enzyme system reinforcement correlated with the anti-inflammatory and anti-dyslipidemia potential of blackberry extracts [127]. The glycemic and lipidemic-controlling mechanisms of blackberry extracts were mediated through the downregulation of lipogenesis factors (FAS, SCD-1, microsomal triglyceride transfer protein, diglycerides acyltransferase, and adipose triglyceride lipase), energy coupling/uncoupling proteins (UCP-1, UCP-2, and UCP-3), pro-inflammatory cytokines (PPAR-α, Nrf2, IL-6, and TNF-α), and fatty acid β-oxidation genes (CPT-1a and ACOX-1) (Table 1) [127], which were maintained by long-term and chronic blackberry extract consumption. Additionally, the increases in total monounsaturated fatty acid contents of adipocytes, plasma brain-derived neurotrophic factor levels, and pro-inflammatory leptin levels in HFD-fed controls were counteracted by blackberry extract consumption [128]. Human clinical trials were also run, in which healthy human subjects were given BB fruits in addition to HFD, resulting in reduced fat accumulation and increased fat oxidation. Blackberry consumption lowered postprandial glucose and lipid levels by activating AMPK and BAdTs. [291]. Pulpy blackberry juice consumption by dyslipidemic patients decreased ApoB and hs-CRP, increased ApoA-1 and HDL-C, and left other lipid parameters unaffected [134]. In healthy subjects, blackberry juice increased exogenous and endogenous antioxidant enzymes. Cyanidin, ascorbate, total ellagic acid, urate, and R-tocopherol contributed to increased plasma and urine antioxidant capacities [125,133]. Daily blackberry consumption reduced dyslipidemia and insulinemic parameters in diabetic and obese adults [132]. Blackberry polyphenolic compounds inhibit digestive enzyme activities, physically interacting with hexose absorption transporters and modulating transporter expression at the genomic level [292]. Blackberry compounds may also modulate peripheral glucose use, damaged pancreatic cell regeneration, and enhance blood glucose withdrawal by increasing insulin sensitivity (Table 1) [109,129].

Blackberry juice was also examined in STZ-induced-diabetic male Sprague-Dawley and hamster rats (Table 1) [129]. Blackberry juice significantly reduced food and water intake, reducing the BWs of both control and diabetic rats [129]. Blackberry nectar supplementation of a cholesterolemic diet reduced hyperlipidemic parameters and hepatic lipid peroxidation [181]. Blackberry juice consumption effectively reduced triacylglycerols (−43.5%), glucose (−48.6%), and cholesterol (−28.6%) levels without side effects. Blackberry juice consumption limited lipid peroxidation in the plasma (−7.5%) and kidneys (−19.5%). Similarly, alcohol-free fermented blackberry juice (AFBBJ) was used to supplement HFD in obese C57BL/6J mice [270], which significantly reduced fat-mass gain and FBS and decreased plasma TG, TC, LDL-C, and HOMA-IR levels, while increasing β-cell function (HOMA-β) [22]. Liver function tests revealed no change in ALT, but AST increased in AFBBJ-treated mice. Genomic sequencing approaches revealed pancreatic gene upregulation, responsible for amino acid and glucose metabolism and insulin secretion regulation [22].

The intestinal bioavailability of blackberry polyphenols and resulting impact on gut microflora have also been recently investigated. The low-absorption and cecal accumulation of BB polyphenols were the main reasons for positive health effects. The cecal microbial fermentation of blackberry polyphenols generates antidiabetic and antioxidative blackberry metabolites including C3G, 2,4,6-trihydroxybenzoic acid, coumarin, and caffeic acid. The increased cecal glycoside concentration and secondary metabolites improved glucose consumption (Table 1) [290]. The increased cecal SCFA concentration suggested an increase in SCFA-producing bacteria; however, the relative abundance of different bacterial groups was not reported [130]. Blackberry treatment altered the gut microfloral composition by increasing cecal Bacteriodetes over Firmicutes. *Lactobacillus johnsonii* was abundant in both blackberry-treated and control groups, whereas *Lachnospiraceae* dominated the blackberry group, promoting glycoside metabolism. However, *Clostridiales*, *Enterococcus faecalis*, and *Bifidobacterium pseudolongum* were more dominant in the control groups [131].

10. Strawberries

Strawberry (*Fragaria* × *ananassa*) consumption has been associated with decreased risk and occurrence of metabolic syndrome, cancer, diabetes, chronic inflammation, and hypertension. The credit of these health-promoting activities goes to its rich phytochemical contents (Table 2). Many studies analytically analyzed the crude and fractionated phytochemical contents of strawberry and found strawberry rich in antioxidative, anti-obesity, antiglycation, anti-inflammatory, and antidiabetic compounds from flavanols, flavonols, anthocyanins, hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, ellagic acid and ellagic acid glycosides, and ellagitannins (Table 2). The most surplus glucose-lowering acid moieties were malonic and *p*-coumaric acid and the most identified flavonols of strawberry were derivatives of kaempferol and quercetin glycosides. The red-coloration-granting and anti-oxidative anthocyanins of strawberries were mostly the derivatives of pelargonidin and cyanidin [240]. The hydrolysis of ellagitannins gave rise to the most important antidiabetic phytochemical called ellagic acid, which comprised more than 50% of the total polyphemolic components of strawberry. The level of ellagic acid is about 3–10 times higher in the strawberry than other berries, fruits, and nuts. It is one of the constituents due to which strawberry can regarded as a functional food [293]. In in vitro studies, strawberry ethanolic extracts inhibited pancreatic lipase activity more strongly than reference orlistat. Aqueous and ethanolic strawberry extracts inhibited adipocyte cell division and inhibited inflammatory mediator (β-hexosaminidase and histamine) release by 61.8 to 80% [294]. Strawberry polyphenolic compounds interact with glucose transporters such as SGLTI and GLUT2 and attenuate glucose uptake due to polyphenol compound competition for transporter active sites [295]. HPLC-diode array detector (DAD)-MS analysis and statistical correlations showed the contribution of pelargonidin-3-*O*-glucoside to glucose uptake inhibition. Strawberry extracts effectively inhibited uptake and transport of glucose up to 5% in HepG2 cultures [295]. Da Silva Pinto et al. [296] showed that the strawberry extract α-glucosidase inhibitory activity was superior to the α-amylase inhibitory activity. Strawberry-derived ellagitannin consumption (>50 mg/mL) sufficiently inhibited ACE activity [296]. Methanolic strawberry extracts activated p-AMPK/AMPK expression in HepG2 cells, resulting in fatty acid and cholesterol regulatory gene inactivation and phosphorylation including HMG-CoA-R and ACC. Activated p-AMPK/AMPK expression increased LDL receptor expression including PGC-1α and sirtuin 1 (a NAD^+-dependent deacetylase that inhibit hepatic lipogenesis, stimulating FA β-oxidation, and maintaining cholesterol and bile acid levels) in HepG2 cells [297].

Numerous in vivo studies have also cited the health promoting activities of strawberry or its byproducts in animal models and human clinical trials. The intake of aqueous, alcoholic, and hydro-alcoholic strawberry extracts improved the serum glucose level, liver function (decreased serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase), lipid profile (decreased LDL-C, LDL-C/HDL-C, and LDL-C/TC ratio), and lipid oxidation markers (decreased MDA and CAT) [136,137]. Genes associated with glucose, cholesterol, and lipid metabolism [FAS, ACC, CPT-1A, malonyl-CoA, acyltransferase, ACC-α (ACACA), and acyl-CoA synthetase long-chain family member 1] were also downregulated by strawberry treatment [135]. Paquette et al. [143] used the hyperinsulinemic-euglycemic clamp methodology to examine improved insulin sensitivity and secretion after strawberry extract consumption, but did not detect improvements in fasting insulin and glucose concentrations. In animal studies, HFD supplementation with strawberry prevented weight gain without influencing food and water intake. Strawberry beverage consumption protected against postprandial lipemia by reducing TG (14%), TC (5%), and LDL-C levels (5%) in hyperlipidemic patients following HFD [144]. Sugar-rich strawberry jam consumption also attenuated glycemic index and postprandial glucose level increases in diabetic human subjects [145,298]. Strawberry jam consumption showed favorable lipid and sugar metabolism results, even compared with low-sugar strawberry jam [146]. Strawberry consumption with HCD also controlled postprandial glucose levels, affected glucose and insulin responses, and GLP-1 expression. Regular strawberry beverage and juice consumption decreased blood pressure, TC, and the TC/HDL-C ratio in

diabetic patients. T2DM and CVD risk factors were also ameliorated (Table 1) [66,114,123,147]. Strawberry extracts reduced IL-6 and plasminogen activator inhibitor 1 (PAI-1) (a risk factor for atherosclerosis) levels in obese individuals after HFD/HCD, without influencing TNF-α, CRP, platelet aggregation, or fasting insulin and glucose levels [148]. In another similar study, the postprandial insulin level and inflammatory response (hs-CRP and IL-6) were reduced with increased plasma pelargonidin sulfate and pelargonidin-3-*O*-glucosidein levels after strawberry powder consumption with high-carbohydrate, moderate-fat meals [149]. In another recent study, strawberry-blueberry powder, consumed with a HFD/HCD, reduced BW gains (12.7%), visceral fat mass (18%), retroperitoneal and subcutaneous white adipose tissues (up to 10.45–16.5%), postprandial insulin and glucose levels, IR, and inflammatory markers (MCP-1, TNF-α, IL6, CRP, and PPAR-α), in male Wistar rats and C57BBL/6J mice (Table 1) [19,138]. Strawberry-blueberry powder exerted anti-adipogenic effects by regulating lipid metabolizing genes including PPAR-α and C/EBPα. Inflammatory and lipogenesis-related gene expression were reduced including TNF-α, IL6, and C/EBPα, adipogenesis-driver transcription factors (PPAR-γ), adiponectin, adipocyte fatty acid-binding protein, SREBF1, leptin, SCD-1, and FAS [138]. In another dose-response checking study, the intake of strawberry against the Western-type-meal reduced the oxidized low-density lipoproteins and post-meal insulin demand in insulin resistant patients [150].

Oxidative stress is a leading cause of metabolic syndrome and diabetes. Strawberry powder supplementation in an isoenergetic diet containing the oxidative-inducing antibiotic drug doxorubicin reversed doxorubicin-induced decreases in the antioxidants retinol and α-tocopherol and upregulated liver antioxidant enzymes including GPx, CAT, GSH, SOD, and GST (Table 1). Plasma hepatic stress biomarker levels including protein carbonyls and hydroperoxide were reduced by strawberry intake [139,152]. Strawberry-based foods containing carbohydrate, fat, and lipids increased total antioxidant levels (1.26 to 1.45 mmol/l) of the subjects while decreasing HbA1C (from 7.00 to 6.72%) levels. The plasma hs-CRP and MDA levels also decreased from 3.36 to 2.76 nmol/mL and 3.36 to 2.76 nmol/mL, respectively [153]. Strawberry powder intake prevented HFD- and stress-induced decreases in γ-aminobutyric acid levels and reduced oxidative stress and lipid oxidation markers, in male Wistar rats [140]. Fresh strawberry consumption reduced linseed oil-induced DNA damage and plasma oxidative marker levels and increased the plasma antioxidant status of pigs [299].

Strawberry intake effects on gut microbial ecology in diabetic subjects increased phylogenetic species richness (α-diversity) and global microbial composition (β-diversity) variations at the genus and operational taxonomic unit levels. *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* were significantly altered after the strawberry intervention. Strawberry intake significantly increased the abundance of beneficial *Bacteroides* and *Actinobacteria* and decreased *Akkermansia*, *Verrucomicrobia*, *Dehalobacterium,* and *Dorea* (Firmicutes). At the genus level, the abundance of SCOA-producing *Lactobacillus* and "prebiotic-effect-giving" *Bifidobacterium* increased, whereas *Dehalobacterium*, *Dorea*, SMB53, and *Turicibacter* remained unaltered [141]. Additionally, a specific relationship between ingested flavonoids and microbial community patterns was identified [151]. Dietary flavanol and flavanone intake were positively associated with *Eggerthela lenta*. Flavonols and flavanol monomer intake was positively associated with *Adlercreutzia equolifaciens* (involved in phytochemical degradation) and inversely associated with *Flavonifractor plauti* (Gram-negative poorly understood) populations [151]. Whole strawberry powder intake increased the α-diversity of colonic inflammatory CD-1 mice, increasing *Bifidobacterium* and *Lactobacillus* and reducing pro-inflammatory *Akkermansia*, *Dorea*, and *Bilophila* [142]. The polyphenolic compounds that affected gut microbiota compositions in strawberry fruit extracts were flavanols, flavonols anthocyanins, hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, ellagic acid, ellagic acid glycosides, and ellagitannins (Table 2) [240,241].

11. Goji Berries

Goji berry (GB, *Lycium Barbarum)* is a functional food and alternative therapeutic tool for T2DM treatment [155]. The major GB therapeutic phytochemicals include polysaccharides (5–8%), carotenoids (0.03–0.5%), and phenolic compounds (traces). The compounds belonging to these classes have been listed in Table 2 [123,242]. The GB is considered the best source of dipalmitin zeaxanthin carotenoids. These carotenoids showed effective protection against diabetic-induced-retinopathy [300]. The in vitro hypoglycemic tests showed the inhibitory capability of GB carotenoids was 9.6 to 82.6% and 5.7 to 15.3% for α-glucosidase and α-amylase enzymes, respectively [242]. In GB polyphenolic compounds, phenolic acids (24.7%) and flavonoids (75.3%) are major phytochemical classes. The major therapeutic flavonoids in GB are squercetin-3-*O*-rutinoside (from 7.1 to 232.7 mg/kg) and quercetin-3-*O*-hexoside (from 169.1 to 1107.7 mg/kg) whereas phenolic compounds include caffeoylquinic acid (0.34 μg/g), caffeic acid (3.73 μg/g), *p*-coumaric acid (6.06 μg/g), chlorogenic acid (12.4 μg/g), kaempferol-3-*O*-rutinoside (11.3 μg/g), quercetin-diglucoside (66.0 μg/g), and rutin (42.0 μg/g) [242]. As GB polysaccharides (GBPS) are major contributors of health-endowing activities and have been vastly investigated, this section will primarily focus on GBPS. GBPS are considered to be therapeutic in alternative medicine with immunomodulation, antioxidant, neuroprotection, anti-tumor, antidiabetic, radioprotection, anti-osteoporosis, hepatoprotection, and anti-fatigue activities. The GBPS biological activities depend on their molecular weight, chemical structure, and chain conformation [154,301]. The GBPS are among a few plant-based bioactive compounds that have shown simultaneous hypoglycemic and hypolipidemic properties. Due to hypoglycemic and anti-hyperlipidemic properties, GBPS may be a potent T2DM inhibitor, delaying disease prognosis, even after disease development. Antidiabetic assays showed impressive lipid and glucose reducing effects [155,302]. Acidic GBPS treatment in rat insulinoma cells decreased oxidative stress biomarkers and increased antioxidant enzyme systems. GBPS treatment of IR alloxan-treated-HepG2 cells protected against oxidative stress and improved cell survival and proliferation [302]. Similarly, the GBPS was further checked for possibly hampering glucose uptake in the gut and intestine. The GBPS intensively reduced glucose absorption in a dose-dependent manner by competing for intestinal absorption [303]. Rat insulinoma cells incubated with GBPS rescued damaged pancreatic cells, improved the survival rate, and encouraged insulin secretion. The IR cell model was supplemented with purified GBPS, which upregulated glucose consumption. GBPS was easily translocated and transported across the Caco-2 intestinal cell membrane through the SGLT-1 transporter, producing a hypoglycemic effect. Therefore, GBPS is a plant-based bioactive compound that shows simultaneous hypoglycemic and hypolipidemic properties [303]. Purified GBPS fractions showed dose-dependent hypoglycemic activities, resulting in increased glucose uptake [156,303]. Besides GBPS, GB carotenoids have also shown antidiabetic and α-glucosidase and α-amylase enzyme inhibitory activities [242].

The hypolipidemic effects of GB intake have been studied by in vivo approaches (Table 1), but human clinical trials for GB have been limited, with most studies performed using small sample sizes in China. GB consumption effectively reduced serum lipid peroxide species in diabetic patients. Reductions in waist circumference, TG, transaminase, and TC levels were reported in metabolic syndrome patients following routine GB intake. Lipid profile improvements were accompanied by increased GSH and CAT enzymatic activities [167]. GB anthocyanins reduced BW gain (17.4 to 38.7%) by increasing fecal fatty acid contents and downregulating IL-6, TNF-α, IFN-γ, NF-κB, and iNOS gene expression [157]. GBPS decoction treatment of alloxan-induced, diabetic, obese rabbits effectively reduced blood glucose levels. GBPS substantially decreased serum TG (−4.27%), TC (−3.5%), LDL-C levels, and increased HDL-C serum levels (0.78) [154]. The hypoglycemic and hypolipidemic effects of GBPS were later confirmed by the works of Zhao et al. [158]. Supplementation of HFD with GBPS decreased HOMA-IR, fasting and postprandial insulin and glucose levels, serum TG, TC, and LDL-C levels, and weight gain [158].

The oxidative stress relieving effect of GBPS was also checked (Table 1). The effect of GBPS treatment on the kidneys of STZ-induced diabetic rats increased kidney antioxidant enzymes including

CAT, SOD, GBPx, GST, and GSH [170]. The supplementation of GB in the form of GB milkshakes increased plasma zeaxanthin and antioxidant levels by 57 and 26%, respectively. GB juice (GBJ) also increased GSH peroxidase (GSH-Px) and SOD by 9.87% and 8.7%, respectively and decreased MDA levels by 5.95% [166]. GBPS intake also protected against glaucoma, which was confirmed in retinal ganglion cells, and disrupted intraocular pressure [159]. GBPS administration to C57BL/6 mice reversed oxidative stress, dyslipidemia, and diabetic changes. GBPS administration downregulated nitrotyrosine and MDA expression and increased antioxidant enzymes such as CAT, GPx, and Cu/Zn SOD. GBPS intake also diminished pro-inflammatory biomarkers including TNF-α, IL-1β, iNOS, and COX-2. Following pro-inflammatory marker reduction, liver injury biomarkers, called chemokines, were also reduced. The liver regeneration process was also observed following GB intake, enhancing liver regeneration biomarkers [168,304].

With respect to hypoglycemic effect specifically (Table 1), Zhao et al. [160] confirmed the antidiabetic characteristics of GBPS, which increased GLUT-4 expression in the skeletal muscle plasma membrane. Purified GBPS in pancreatic cells increased glucose uptake and metabolism, insulin secretion, and proliferation. The enhanced glucose metabolism mechanism was associated with increased hepatic hexokinase and pyruvate kinase expression/activity (Table 1) [26,161]. GBPS may block the ATP-sensitive K^+ channel, activate glycogen synthetase and insulin-like growth factor, enhance peripheral glucose utilization, or inhibit glucagon releasing factors in pancreatic α-cells [197]. In a recent single meal challenge study, increased glucose and lipid consumption were observed in GB-treated patients, associated with increased respiratory quotients, oxygen usage, and carbon dioxide release. However, no single-dose effects on substrate oxidation and postprandial-energy-expenditure were reported [169]. Du et al. [162] compared GBPS with metformin and reported similar normalization effects on blood glucose and insulin levels. This study also reported reduced IL-2, IL-6, TNF-α, intercellular adhesion molecule-1 (ICAM-1), MCP-1, and blood urea/nitrogen levels, inhibited albuminuria, and reversed histopathological alterations. GBPS treatment in HFD/HCD-fed rats also demonstrated hypoglycemic and hypolipidemic effects [115]. Ni et al. [163] examined the potential neuroprotective effects of aqueous GB extracts. Retinal apoptosis causes photoreceptor degradation and diabetic retinopathy (DN), and GB carotenoid supplementation in rats hampered caspase-2-induced apoptosis, protecting photoreceptors [163]. Prolonged or chronic hyperglycemia downregulates luteolin and zeaxanthin-metabolizing gene expression, causing retinopathy. GB carotenoids protected against diabetes-induced retinopathy. GB supplementation upregulated carotenoid metabolism genes and retina biogenesis in STZ-induced diabetic rats [300]. GB also contains taurine, a non-essential amino acid, and GB-derived taurine enhanced PPAR-γ activity and elevated cAMP levels, hampering the prognosis of DN with reversal of epithelial barrier impairments [300].

GBPS, polyphenol, and carotenoid effects on the gut microbiome were also studied (Table 1). Fermentation and simulated digestion experiments revealed that GBPS was digested and degraded only in the distal gut, releasing monosaccharides and promoting beneficial SCOA-producing bacterial growth. Monosaccharides with side chains are more susceptible to degradation than monosaccharides with linked backbones. GBPS greatly increased SCFA-producing gut microbiota and increased Bacteroidetes (including *Prevotella* and *Bacteroides*) and Actinobacteria (containing *Collinsella* and *Bifidobacterium*) populations, whereas *Megamonas* and *Megasphaera* (Firmicutes) populations were decreased. Furthermore, SCOA/SCFA-producing, prebiotic-effect-giving, proteolytic microflora such as *Bacteroides*, *Phascolarctobacterium*, *Bifidobacterium*, *Prevotella*, *Clostridium XlVb*, *Oscillibacter Collinsella*, and *Lactococcus* were prominent following GBPS treatment [305]. In another study, dietary GB supplementation also increased health-promoting secondary metabolite and SCOA-producing *Actinobacteria*, *Lachnospiraceae*, *Clostridium* XIVb, *Sporobacter*, *Pseudoflavonifractor*, *Butyriccicoccus*, *Anaerotruncus, Anaerosporobacter,* and *Ruminococcaceae* populations without affecting *Akkemansia*, *Mucispirillum*, *Bacteriodes*, and *Desulfovirio*. Butyryl-Coenzyme A CoA transferase is an important butyrate gene, and GBPS supplementation increased its expression in butyrate-producing bacteria such as the *Clostridium cluster* XIVa group including *Lachnospiraceae*, *Faecailbacterium prausnitzii,* and

Ruminococcaceae [164]. The GBPS prebiotic effects increased the populations of *Firmicutes*, *Akkermansia*, *Proteobacteria*, *Lactobacillus*, and *Prevotellaceae* [165].

12. Acai Berries

Acai berry (AB, *Euterpe oleracea*) is native to South America and has high phytochemical contents. The dominant antidiabetic phenolic acid constituents in AB include ferulic acid, anthocyanin-3-glycosides, *p*-hydroxybenzoic acid, epicatechin, protocatechuic acid, gallic acid, ellagic acid, catechin, *p*-coumaric acid, vanillic acid, and gallotannins (Table 2) [246]. Anthocyanin and flavonoids are prominent therapeutic polyphenols including C3G and C3R [244,245]. AB juice (ABJ) is richer in polyphenols and flavonoids than other berry juices, resulting in increased antioxidant capacities [200]. In in vitro studies, the isotonic ABJ pancreatic lipase inhibitory activity was significantly positively correlated with anthocyanin contents. Isotonic ABJ also reduced adipogenesis and lipid accumulation in 3T3-L1 adipocytes and inhibited α-glucosidase activity [306]. Isotonic ABJ also inhibited Cu-mediated LDL oxidation and oxidized or acetylated LDL uptake. AB puree also showed antiglycation activities at a concentration 0.1 mg/mL, which was 89% stronger than the control [171]. Polyphenols in ABJ affect adipogenesis, preventing obesity, weight gain, inflammation, and diabetes [307].

In in vivo studies, AB fruit proved to be a very useful therapeutic agent for circumventing oxidative stress, and controlling dyslipidemic and metabolic syndrome conditions (Table 1). The supplementation of AB fruit effectively prevented protein oxidation as increased protein sulfhydryl groups were observed, with decreased protein oxidation biomarker carbonyl proteins. A single AB pulp dose enhanced plasma antioxidant capacity 7-fold 3 h after its consumption. Plasma anthocyanins reached maximum levels 2.2 h after AB pulp consumption [179,180]. In another in vivo study, AB pulp supplementation in oxidatively damaged mutant *Drosophila melanogaster*, in combination with HFD, reversed HFD-induced oxidative stress damage and prolonged the lifespan expectancy by 22% [172,308]. AB supplementation with exercise improved hepatic oxidation status by reducing inflammatory MCP-1 expression, SOD activity, redox-sensitive signaling pathway activation, ROS generation, and ROS stress [173]. To elucidate the antidiabetic and antioxidative molecular mechanism of AB, AB-mediated transcript-level changes were examined in 12 genes associated with JNK, nutrient sensing, and insulin-like signaling pathways [309]. PEPCK genes, involved in glyceroneogenesis and gluconeogenesis, were reduced in the AB pulp group. Cholesterolemic diet consumption decreased lethal/essential or life gene (lefl2) expression, which was reversed by AB fruit consumption. Two JNK targets, metallothionein A, and glutathione S transferase D1, which have antioxidant activities, were upregulated after AB consumption without affecting the remaining JNK downstream target genes (Ferritin 1 heavy chain homolog, Ice, Heat shock protein 68, and Puckered). Moreover, AB ingestion promoted longevity by intensifying stress response pathway activity and suppressing PEPCK genes [172,309]. Treatment with AB seed extracts also reduced blood pressure, the hypertension biomarker renin, and DN biomarker levels (creatinine, urea, creatin, and albumin). Diabetes onset leads to oxidative stress and hypertension, decreasing the number of glomeruli per area per kidney, a major DN marker. AB seed extracts reduced kidney volume expansion and prevented a decrease in the number of glomeruli per area per kidney [174]. AB seed extracts substantially reduced renal injury (resulting in reduced urea and creatine excretion), hampering renal fibrosis progression. The diabetes-induced glomerular filtration barrier injury markers, podocin and nephrin, decreased in diabetic male Wistar rats, whereas AB seed extract treatment restored these levels. AB seed extract treatment also reduced renal proinflammatory cytokines and oxidative stress biomarkers, reinforcing the anti-oxidative defense system [174]. The effects of exercise and AB seed-rendered extract consumption in STZ and HFD-induced diabetic rats reduced HbA1C, glycemia, serum insulin, HOMA-IR, serum TG, TC, LDL-C, and HDL-C levels [176]. Insulin signaling components (insulin receptors, pAKT, and AKT) in skeletal muscles were upregulated following AB seed extract consumption and exercise [176]. Reduced adiponectin levels are observed in T2DM, associated with deregulated sugar and lipid

metabolism, and AB seed extracts reversed this effect. AB seeds induced increased GLUT-4 expression and glucose uptake due to AMPK activation [176] and increased GLP-1 and incretin levels with reduced leptin and inflammatory cytokine expression, which were not observed in HFD-fed rats treated with exercise alone. Increased GLP-1 and incretin expression promotes insulin secretion, suppressing gastric emptying, and glucagon synthase [176,310]. The same research group then used the AB seed extracts to check the anti-obesity features in the C57BL/6 mice strain fed on HFD. HFD supplemented with the AB seed extract prevented weight gain in mice [311]. Adiponectin levels, which are responsible for lipid metabolism, decreased in HFD-fed mice and were restored by AB seed extract supplementation. AB seed extracts increased glucose and lipid metabolizing protein expression including pAMPK/AMPK, pACC/ACC, HMG-CoA, and various transporters including ATP-binding cassette sub-family G member 5-ABCG5 and ATP-binding cassette sub-family G member 8-ABCG8, while reducing SREBP-1c expression. Similarly, protein and lipid oxidation products including carbonyl proteins and MDA were reduced by strengthening the anti-oxidative enzyme system [311].

Regarding glucose-lowering effect, recently, the human AB fruit consumption with normal meals decreased FBS and mean plasma insulin levels after one month. Plasma TG, TC, and LDL-C levels, and the LDL-C/HDL-C ratio also decreased, with increased plasma HDL-C levels [181]. The AB consumption with HFD enhanced fecal cholesterol contents, with no influence on low-grade-inflammation biomarkers [113]. Freeze-dried AB fruit pulp reversed the HFD-induced alterations in PEPCK expression [312]. Aqueous ethanolic AB extracts restored mitochondrial complex I function by modulating NADH:ubiquinone oxidoreductase core unit 7 and 8 expression. NLRP3 (a component of inflammasome) and caspase 1/caspase 3/caspase 8 (Interleukin-1 converting enzyme family, which initiates inflammatory response) were downregulated in oxidative-agent-treated macrophages [313]. AB supplementation also interfered with hepatic cholesterolemic metabolism. AB attenuated the high-cholesterol diet effects by reducing weight gain, TC and LDL-C levels, and key regulatory gene expression associated with the cholesterol biosynthesis pathway including HMG CoA-R, EBP-2, ApoB100, LDL-R, ABCG8, and CYP7A1 [175]. Intensive feeding with freeze-dried AB pulp attenuated HFD-induced hepatic steatosis by improving IR, adiponectin expression, adiponectin receptor 2, SREBP-1c, PPAR-α, and its target gene, CPT. Fat accumulating gene expression including UCP-2 and fatty acid translocase were reduced by AB treatment [179]. Both lipid accumulation and oxidation were reduced in zebrafish fed with a high-cholesterol diet, and reduced serum TC, LDL-C, and MDA levels were observed in AB-treated zebrafish [171]. Aside from lipid oxidation inhibition, the AB intake also prevented amino acid oxidation after HCD, reducing protein carbonyls and sulfhydryl groups, which are important protein damage biomarkers. Reduced arylesterase and PON activities and reduced hepatic ALT, AST, and ALP levels demonstrated improved hepatic operation [175]. AB powder also improved anti-inflammatory mechanisms after HFD by improving glucose intolerance and reducing IL-6 and TNF-α concentrations in epididymal adipose tissue [312].

A comprehensive study examining AB intake on the gut microflora is currently lacking. Simulated digestion studies examining AB polyphenols inhibited the growth of symbiotic and saccharolytic *Bacteroides*, *Prevotella*, and *Clostridium histolyticum*. AB polyphenols showed favorable effects on the intestinal SCFA bacteria population including LAB [178]. Guergoletto et al. [177] noted increased intestinal populations of obesity-protecting bacteria (i.e., *Bifidobacterium* spp., *Eubacterium rectale–Clostridium coccoides* group, *Bacteroides spp—Prevotella group,* and *FOS-Raftilose* P95). However, AB polyphenols showed no considerable effects on *Enterococcus spp* and *C. histolyticum* [177].

Conclusively, AB exerted antidiabetic, anti-obesity, antioxidative, and anti-inflammatory actions by reducing the expression of PPAR-γ and its modulators (C/EBP-ß, C/EBP-δ, and other C/EBP family members, Kruppel-like factor, and SREBP1C) Moreover, decreased expression level of transcriptomic factors such as C/EBPß (−0.41%), C/EBPα (−0.66%), Kruppel like factor (−0.83%), and SREBP1C (−0.24%) were also seen [125,133]. AB also reduced the expression levels of lipogenic genes FAS (−0.5%), aP2 (−0.7%), LPL (−0.7%), and FATP1 (−0.55%). Low-grade-inflammation biomarkers including leptin and total PAI decreased with increasing anti-inflammatory and anti-adipogenic

adiponectin levels [170,172,309,314]. The expression levels of the pro-inflammatory factors NF-κB, TNF-α, MCP-1 (−0.81%), IL-6 (−0.48%), IL-8 (−0.05%), IL-1ββ (−0.03%), and INF-β(−0.49%) were also reduced. TNF-α activates NF-κB and interleukins (IL-2 and IL-6), which was prevented by ABJ polyphenols [170,313,314].

13. Chokeberries

Chokeberries (black chokeberry (BCB), *Aronia melanocarpa*, red chokeberry (RCB), *Aronia arbutifolia*) can be consumed as whole fruit, jam, wine, juice, syrup, tea, soft spreads, chili starters, salsa, beer, extracts, gummies, ice cream, and tinctures. CB consumption was used to treat colds in America and to treat hyperglycemia, metabolic syndrome, and hypertension in Europe and Russia. In in vitro bioassays, CB extract (CBE) showed significant α-glucosidase inhibitory activity compared with the referenced antidiabetic drug acarbose. Purified anthocyanins (cyanidin 3-galactoside, cyanidin 3-arabinoside, cyanidin 3-glucoside, and cyanidin 3-xyloside) were the strongest antidiabetic compounds compared with isolated dimeric and trimeric procyanidins. BCB juice (BCBJ) also inhibited α-glucosidase, dipeptidyl peptidase (DPP) IV, and ACE activities by 75, 35, and 95% in a dose-dependent manner, respectively [182]. BCB fermentation and digestion increase polyphenol bioaccessibility. Fermented and digested *Aronia* kefir showed stronger α-glucosidase (IC_{50} = 152.53 ± 15.24 mg kefir/mL) and pancreatic α-amylase inhibitory (IC_{50} = 146.52 ± 5.37 mg kefir/mL) activities than non-fermented *Aronia* (IC_{50} = 365.16 ± 370 48.84 mg and 196.21 ± 5.50 mg, respectively) [315]. BCBJ relieved oxidative stress in βTC3 cells by restoring the anti-oxidative enzyme pool and insulin secretion, as comprehensively explained in Figure 2 [316]. The oxidative-stress-induced reduction in insulin secretion was restored by the BCB extract (BCBE) treatment under basal glucose conditions [316]. BCBE treatment of pancreatic cells nullified cytokine (IL-1β and IFN-γ)-induced effects and decreased oxidative stress production [183]. BCBE pretreatment (0.001, 0.01, 0.1, or 1 mg/mL) of diabetic hepatic cells line RINm5F) reduced cytokine-induced-oxidative stress from 19.3–0.39 μM to 14.9–0.35 μM [183]. Similarly, BCBE pretreatment of HAECs nullified the TNF-α-induced ICAM-1 and VCAM-1 expression by 35 and 45%, respectively, in a dose-dependent manner. BCBEs also prevented NF-κB p65 phosphorylation, which activates the pro-inflammatory transcription factor NF-κB [317,318].

Addressing the anti-inflammatory potential of CB, in in vivo clinical studies, Kardum et al. [195,196] administered CBJ to patients with pharmacologically incurable grade I hypertension and high blood pressure, resulting in decreased systolic/diastolic blood pressure, with a stronger effect associated with long-term consumption. CBEs also reduced systolic/diastolic blood pressure [197], particularly in congenital heart disease patients [198]. Following hypertension, inflammation is another diabetes complication and numerous studies have cited the anti-inflammatory potential of BCB or its juice consumption. Increased PPAR-γ2 expression was attenuated by BCBEs, reducing downstream lipid metabolizing PPAR-γ2 target expression such as PGE receptor and LPL, decreasing intracellular lipid droplet accumulation [184]. Regular BCBJ consumption improved chronic inflammatory conditions, lowering IFN-γ and TNF-α levels [195,196,198]. The immunomodulatory effects of BCB intake have also been discussed in the literature in STZ-induced male Wistar rats. DM causes immune imbalances because damaged pancreatic cells trigger macrophage and T lymphocyte infiltration, which lesion β-cells. BCB consumption by STZ-induced male Wistar rats reduced fibrinogen, TNF-α, and IFN-γ levels, which returned to their normal values 72 h post-administration of BCB [199].

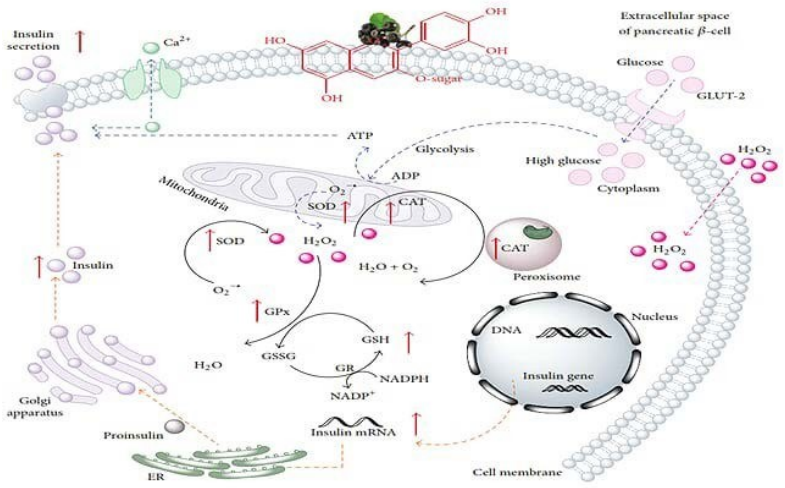

Figure 2. Schematic presentation of chokeberry anthocyanin-induced insulin secretion and antioxidant enzyme pathways in pancreatic β-cells under high-glucose-induced stress conditions. Glucose is transported across the cell membrane via glucose transporter (i.e., GLUT-2), followed by glycolysis and pyruvate production. Afterward, pyruvate is used for the generation of ATP in mitochondria. Here, in connection with the electron transport chain, radicals, like superoxide anion ($O_2^{\bullet-}$), are also produced and simultaneously neutralized by the enzymatic antioxidant SOD. SOD converts the $O_2^{\bullet-}$ into harmless O_2 and another radical H_2O_2. In addition to H_2O_2 diffusion through the cell membrane, H_2O_2 is also scavenged by CAT and GPx resulting in water and oxygen production. Chokeberry-derived anthocyanins strengthen this inherent enzymatic antioxidant system (i.e., SOD, CAT, and GPx), which can more actively neutralize the radicals generated during glucose metabolism. H_2O_2-stimulated reduction of GSH is also ameliorated by chokeberry anthocyanins. Chokeberry anthocyanins also replenish the pool of insulin by increasing the insulin gene expression. Proinsulin, a precursor of insulin, folded in the endoplasmic reticulum, is transported to the Golgi apparatus. Chokeberry anthocyanins can also influence the opening of the voltage-gated Ca^{2+} channels, leading to an increased fusion of insulin granules with the cell membrane (Source: Rugina et al. [316]).

Regarding hypoglycemic response, BCBJ consumption also modulated circulating lipid levels including TG, TC, and LDL-C in mild hypertensive patients (Table 1) [185,198]. BCBJ consumption also reduced serum TG, TC, and LDL-C levels in hypercholesterolemic healthy subjects [128]. Long-term BCB consumption was recommended for desirable hypoglycemic and hypolipidemic effects [128,185,198]. Valcheva-Kuzmanova et al. [186] demonstrated up to 39% reduced postprandial serum TG levels in STZ-induced diabetic rats after BCBJ consumption and reported encouraging results for both diabetic and healthy rats. However, Lipińska and Józ´wik [187] showed pronounced hypolipidemic effects only in diabetic Polish Merino lambs including significantly decreased serum LDL-C and increased HDL-C levels, without significant effects on serum TC levels. In addition to preventing increased plasma glucose, homocysteine, and fibrinogen levels, reduced serum lipid levels (TG, TC, and LDL-C) were observed in STZ-induced diabetic rats [201]. Hepatic steatosis and NAFLD were prevented by BCB treatment in HFD-fed diabetic C57BL/6N mice. Daily BCBE administration prevented increased body, liver, and epididymis weights [188]. Several possible mechanisms have been proposed in the literature referring to the lipid-lowering property of BCB consumption. The BCB hypoglycemic effect may be associated with increased cynidine-induced lipid metabolism, reduced catechin-induced cholesterol absorption, and the flavonoid-influenced downregulation of cholesterol synthesis enzymes including HMG-CA-R, cholesterol acyltransferase, and acyl-CoA [185,188].

The anti-oxidative, anti-obesity, and anti-diabetic potential of BCB was checked in the various diabetic model mice (Table 1), where BCB increased serum insulin secretion with reduced pro-inflammatory cytokine expression (MAPKs, NF-κB, COX-2, and iNOS) in a dose-dependent manner [183]. Jurgoński et al. [189] fed BCBE to high-fructose-diet-fed STZ-induced diabetic rats and

showed increased maltase and sucrase activity, and decreased lactase production in the small intestinal mucosal membrane. Daily BCBJ consumption lowered postprandial glucose levels after OGTT, regardless of gender, and reduced ACE, α-glucosidase, and DPP IV activities in a dose-dependent manner [182]. Valcheva-Kuzmanova et al. [186] showed lower postprandial glucose levels (up to 44%) in STZ-induced diabetic rats after BCB consumption, and Lipińska and Jóźwik [187] demonstrated a pronounced FBS decrease in BCB-treated Polish merino lambs. Postprandial OGTT results for BCB-treated mice decreased, with improved intraperitoneal ITT results [185]. Similarly, consumption by STZ-induced diabetic mice reduced serum TBARS levels and mitigated lipid peroxidation (by 29–50%) and kidney hypertrophy [190]. Following CCl_4 administration, the decreased concentration of CAT, GPx, and GR were increased by 117%, 56% and 44%, respectively, after the intake of BCBJ. Protein carbonyls, protein oxidation biomarkers, decreased by 22% after BCBJ consumption in male Wistar rats [190]. BCBJ consumption by the KK-Ay and C57BL/6JmsSlc mice reduced BW, white adipose tissue weight, α-glucosidase and DPP IV activity, and blood TG levels. Mesenteric, epididymal, subcutaneous, and retroperitoneal white adipose tissue weights were reduced by 26%, 27%, 48%, and 38% compared with those in control animals [39]. Bhaswant et al. [191] administered BCBJ to male Wister HFD- and HCD-fed rats and observed reduced BW gain and feed conversion efficiency. Total body fat mass, BMI, abdominal fat (epididymal, omental fat pads, and retroperitoneal), and visceral adiposity index reductions were more pronounced in Wistar rats fed with BCBJ than in those fed with biofunctional purple maize flour. BCBJ consumption also reduced liver injury biomarkers (ALP, AST, and ALT), although these levels remained within the normal range [191]. In another study, male Wistar rats were fed high-fructose diets containing BCBE, resulting in increased plasma HDL-C and adiponectin levels [192]. IRS-1/2 and PI3K regulatory subunit protein expression increased by 2.3-, 1.8-, and 1.5-times, respectively, along with inhibiting the phosphatase and tensin homolog (Pten) (−0.61%) expression. The expression level of glucose uptake, transportation (GLUT1 and GLUT4) and gluconeogenesis (GYS) was uplifted by 1.5 times compared to high-fructose fed control rats. BCB consumption inhibited lipogenesis and lipid accumulation by reducing fatty acid-binding protein, FAS, and LPL (lipogenesis protein) by 0.6–0.7%. Improved glucose and lipid metabolism and increased glucose and lipid regulatory metabolizing protein expression (adiponectin and PPAR-γ) were also observed [192]. Cynidine 3, 5-diglucoide was identified as a DPP IV inhibitor. DPP IV cleaves incertins including GLP-I and glucose-dependent-insulinotropic polypeptide at their N-terminal regions, resulting in decreased insulin secretion [182,186]. Cyanidin glycosides including 3-galactoside, 3-glucoside, cyanidin 3-*O*-β-glucoside3-arabinoside, and 3-xyloside enhance glucose uptake and GLUT4 translocation. Diabetes-associated hyperlipidemic complications were improved by regulating the FOXO1-mediated adipose TG lipase transcription [185].

BCB contains high levels of anthocyanins (1958.18 mg/100 g FW), proanthocyanidins (522–1002 mg/100 g FW), and hydroxycinnamic acids (187.9 mg/100 g FW) including chlorogenic acid and neochlorogenic acid [212,247]. Cynidine-3-*O*-glucoside, cynidine-3-*O*-galactoside, cynidine-3-*O*-xyloside, and cynidine-3-*O*-arabinoside are the primary antidiabetic and anti-oxidative anthocyanin compounds in BCBJ (Table 2). No studies have examined the CB consumption effects on gut microflora in diabetic/obese individuals, although CB consumption has been examined in healthy individuals [202]. Chronic BC capsule treatment influenced the intestinal diversity of health promoting and SCOA-producing *Anaerostipes*, *Bifidobacterium*, *Faecalibacterium*, and *Clostridium* genera. CBE capsules increased the relative abundance of *Anaerostipes*, whereas whole CB capsules increased *Bacteroides* and *Clostridium* XiV populations. Correlation analysis between gut microbial genera and plasma polyphenolic contents revealed that *Prevotella*, *Dialister*, *Desulfovibrio,* and *Bifidobacteria* were responsible for the increased levels of nine, eight, seven, and six health promoting plasma CB metabolites, respectively, including derivatives of benzoic acid, hippuric acid, phenylacetic acid, cinnamic acid, caffeic acid, flavonols, (iso)ferulic acid, benzaldehydes, and pyrogallol [202].

14. Black Currants

Black currant (BCT, *Ribes nigrum* L.) is cultivated primarily in Europe, New Zealand, and Australia. BCT is a rich source of anthocyanins that represent 95% of polyphenolic compounds, with the remaining 5% including other minor polyphenol classes. Delphinidin-3-rutinoside (D3R) is the major BCT antidiabetic anthocyanin compound that improves glucose tolerance. In BCT nectar, cynidine and delphinidin rutinosides are the dominating anthocyanins, followed by glucoside compounds [93,319]. A full list of other therapeutic BCT compounds are presented in Table 2. GLP-1 and AMPK are the primary BCT polyphenolic compound targets. BCT extract (BCTE) consumption increased GLP-1 secretion. GLP-1, an incretin, promotes pancreatic β-cell division and glucose-dependent insulin release [212,213,289]. BCTEs contain approximately 70% anthocyanins (especially rutinosides and glucosides of delphinidin and cyanidin) and are considered to be effective α-glucosidase inhibitors [289]. Apple and BCT juice (BCTJ) treatment in human Caco-2 cells reduced sodium-independent and total glucose uptake by 46 and 51%, respectively. In oocytes, apple and BCTJ-derived phloretin and phlorizin effectively reduced glucose uptake by 58 and 85%, respectively [213]. The BCT polysaccharide BCP-I also showed remarkable antiglycation activities due to its inhibitory effects on Amadori products [320]. BCT powder incorporation into high-glycemic-indexed food decreased glucose release and increased antioxidant capacities [321].

In addition to in vitro studies, glucose and lipid lowering effect of BC extracts or its screened anthocyanins have also been investigated enormously in various in vivo studies (Table 1). The intake of major BCT anthocyanin consumption, in combination with intraperitoneal glucose administration, prevented increased serum glucose concentrations with the simultaneous increase in serum insulin levels [203]. Improved hyperglycemia and hypoinsulinemia are caused by the GLP-activation-induced increase in insulin secretion. BCT powder, administered for six days before OGTT, improved postprandial plasma insulin and glucose levels in healthy human subjects [214]. BCTE consumed with a normal diet by KK-Ay mice induced hypoglycemia and modulated basal GLP-1 concentrations without affecting plasma insulin levels, food intake, or BW [204]. Proglucagon cleaving agent proprotein convertase subtilisin/Kexin type 1, which processes proglucagon into GLP-1, increased. BCTE treatments also increased AMPK phosphorylation in skeletal muscles, upregulating insulin-independent glucose uptake pathways by increasing downstream target expression including GLUT-4 and the translocating plasma membrane [204]. Previously, Esposito et al. [205] also conducted an anti-diabetic study using 1% BCT powder, which decreased rat BWs, irrespective of dietary fat contents. Microbiological fecal analyses showed increased fecal anthocyanin contents, especially in lean animals. These results suggested that gut microflora more actively transform polyphenolic metabolites in lean animals rather than in obese animals. BCT supplementation reversed the postprandial glucose levels associated with HFD; however, the postprandial glucose level continued to rise due to gut microbiota disruption. Similarly, BCT improved HFD-induced insulin, but the gut microflora disruption increased IR. These results signified the importance of gut microflora during the BCT polyphenol metabolization and biotransformation [205]. The supplementation of 0.1% BCTE in HFD reduced retroperitoneal and epididymal adipose fat. BCTE hypolipidemic characteristics were verified by upregulated lipogenic/lipid metabolizing genes in adipocytes including UCP-2, UCP-3, mitochondrial transcription factor A (TFAM), PPAR-α, SREBP-1c, FAS, and SCD-1, and fatty acid oxidation genes including CPT-1α and 1β [206]. Repressed inflammatory marker expression in macrophages has also been reported. Reduced IKKε (an enzyme complex that is involved in propagating the cellular response to inflammation) and TANK-binding kinase 1 (a member of IKK subfamily, which activates in response to lipopolysaccharides) expression was observed in the BCT-treated group, compared with upregulation in the HFD group [206,212]. BCTJ/nectar waste extract (pomace) was much richer in anthocyanins than in BCT pulp. Phytochemically, BCT pomace extracts are rich in D3G, D3R, cyanidin-3-rutinoside, glycosides, and flavonol aglycones. HFD supplemented with BCT pomace extracts did not affect food intake or BW. Fat in the diet increases small intestinal digesta viscosity, whereas BCT pomace polyphenolic extracts made this digesta more acidic [207]. The polyphenolic-rich

BCTE also reduced cecal tissue mass and increased ammonia contents. HFD reduced bacterial glycolytic enzyme activities such as α- and β-galactosidases and α- and β-glucosidases, which were restored by BCT pomace extract. BCT supplementation reduced β-glucuronidase activity, which is associated with reduced pressure on the intestinal detoxification mechanism [208]. BCT supplementation reduced the cecal putrefactive SFCA concentration, regardless of diet [207,208]. BCTE consumption increased mean fat oxidation during prolonged cycling exercise by endurance-trained females with reduced mean carbohydrate oxidation [215]. However, the opposite outcome was observed when BCTJ was consumed before exercise, without significant effects on blood lactate, glucose, and MDA levels [216].

In addition to HFD, the high-fructose-diet or HCD were also involved in the hyperglycemic, hyperlipidemic, and metabolic syndrome conditions. BCTE administration with high-fructose-diet prevented increases in liver weight, BW, and epididymal fat pad weight. OGTT results improved, with decreased p-AMPK and IRS-1 levels in the BCTE-treated group. BCTE supplementation also decreased high-fructose-diet-induced hyperglycemic marker expression and reduced atherosclerosis risk by diminishing ICAM-1, VCAM-1, E-selectin, endothelin, and eNOS expression levels in aortic tissues [209]. Consumption of an anthocyanin-rich sugar-free BCT drink with a normal-carbohydrate diet delayed the glycemic and insulinemic response with reduced incretin and GLP-1 expression [212,213]. The consumption of BB, BCT, CrB, and strawberries restricted post-meal blood insulin and glucose fluctuations induced by HFD/HCD. LB combined with BCT (whole or nectar) ameliorated postprandial insulinemic and glycemic control and response [62,123,267]. The irreversible hydrolysis of sucrose into fructose and glucose under high temperature and low pH conditions produces invertase sugars. BCT nectar, sweetened with invertase sugars, reduced postprandial blood glucose levels and the maximal blood glucose level by 33 and 87%, respectively. The nectar x time interaction also revealed lower insulin secretion at 15 and 30 min of post-nectar-consumption and expulsion of insulin from the baseline was cut by 13% compared to the reference [62,123,267].

Regarding oxidative stress and diabetes-related complications, ample amounts of evidence have suggested that anthocyanins from BC exert anti-hypertensive, anti-inflammatory, anti-fibrotic, and anti-hepatic steatosis effects by limiting lipogenesis and gluconeogenesis (Table 2) [217]. BCT-derived purified extracts administered to hepatic steatosis model C57BL/6J mice did not prevent BW loss, but serum ALT and AST levels increased. BCT anthocyanin supplementation decreased hepatic TG and TC accumulation [304]. Histological analysis showed that microvascular steatosis, inflammatory cell infiltration, and hepatocyte ballooning were reduced by (up to 50%) BCT anthocyanins. Hepatic stellate cells produce collagen during fibrogenesis. Reduced α-smooth muscle actin and upregulated carbamoyl phosphate synthase 1 suggest hepatic stellate cell inhibition, inhibiting fibrosis and non-alcoholic hepatic steatosis. BCTE treatment increased mitochondrial biogenesis and decreased the AMPK/pAMPK ratio and pivotal mitochondrial biogenesis regulators including PGC-1α and β, Nrf-1 and -2, and TFAM. Mitochondrial fatty acid β-oxidation occurs due to mitochondrial oxidative phosphorylation, which was reversed through effects on PPAR-α, CPT-1, and medium-chain acyl CoA dehydrogenase expression [62,93,123,217,304,319].

15. Maqui Berries

Maqui berries (MB) (*Aristotelia chilensis)* have recently gained attention due to their high content of polyphenolic compounds. The stated phytochemical composition of MB was 138 ± 0.4 mg/100 g fresh weight with 35% relative abundance of delphinidin [218]. Di Lorenzo et al. [219] analyzed the MqB composition (Table 2). MqB is rich in anthocyanins including 84% diglycosylated and 16% monoglycosylated anthocyanins [251,322,323]. The in vitro sugar hydrolyzing enzymes inhibitory activities of MB extracts were reported by Rubiliar and his colleagues. Rubiliar et al. [324] reported α-amylase and α-glucosidase inhibitory activities, resulting in decreased postprandial glucose levels and improved glucose tolerance [324]. Crude and purified MqB extracts (MqBEs) reduced MDA production and minimized oxidative damage [250]. An isotonic soft drink containing lyophilized MqB, acai, and blackthorn berry powders [152] demonstrated pancreatic lipase and α-glucosidase inhibitory activities,

which were superior to the control, acai-, and blackthorn-based beverages. Likewise, the in vitro anti-diabetic assay showed the inhibition of α-glucosidase activity by 90% compared to the lemon juice control (80%), whilst the recorded inhibitory α-glucosidase activity of tested commercial isotonic drinks was around 50% [306]. The MqBE anti-diabetic and anti-lipidemic potentials were further examined in RAW264.7 mouse monocytes and 3T3-L1 mouse pre-adipocytes [220,325]. MBEs reduced adipocyte formation by promoting MMP-2 and MMP-9 (endopeptidases). GST treatment decreased GSH, SOD, and CAT expression, which was reversed by MqBE treatment in macrophages. LPS treatment increased IL-6, MCP-1, TNF-α, and galectin-3 with decreased adiponectin expression, which was countered and reversed by MqBEs in macrophages [220]. Furthermore, a dose of 100 and 180 μM MqB delphinidin inhibited sodium palmitate-induced-TG-accumulation by 50 and 59%, respectively, in Hep2G cells [222].

With respect to in vivo antidiabetic and anti-obesity potential of MqB (Table 1), Rojo et al. [220] fed C57BL/BJ mice anthocyanin-rich MqBEs, which significantly decreased plasma glucose levels following glucose ingestion. Anthocyanin-rich MqBEs also reduced G6Pase and increased insulin sensitivity. Glucose uptake was upregulated in L6 skeletal muscle cells, without toxic effects [220]. Delphinidin 3-sambubioside-5-glucoside, a signature MqB biomarker, showed an equivalent capacity to metformin for normalizing blood glucose levels [326]. Lipid accumulation was inhibited by 4–11% by MqBE treatment in 3T3-L1 mice; however, lipogenesis was inhibited by 6–38% during adipocyte differentiation. The lipogenesis inhibitor protein, preadipocyte factor 1, was upregulated in MqB-treated 3T3-L1 mice. MqB supplementation also exerted an anti-inflammatory response by reducing ROS expression by 9.8 to 61.8%. The expression of COX-2 and production of PGE2 was also evaluated in the RAW 264.7 macrophages to understand the anti-inflammatory mechanism of MqB. MqB inhibited PGE2 expression and reduced COX-2 expression (by 16.2–62%), inhibiting LPS-induced iNOS/NO production and COX-2/PGE2 pathway activation in macrophages [218,326]. MqB delphinidin anthocyanins inhibited glucose uptake and transport from the rat duodenum by inhibiting SGLT-1. The inclusion of MqB-derived 35% anthocyanins and 25% delphinidin glycosides in a rice-chicken diet effectively reduced postprandial glucose levels. Purified delphinidin anthocyanin supplementation with a normal diet reduced fasting glucose and insulin levels [218,326]. MqB anthocyanins, in capsular form (3 × 150 mg per day), decreased oxidized LDL-C and 8-iso-prostaglandin F2α, a urinary excretion oxidative stress marker [221,326]. Furthermore, MqB-derived-delphinidin treatment effectively increased AMPK phosphorylation. Gene expression analysis showed that sodium palmitate exposure upregulated lipid accumulating genes such as SREBF1, CPT1-A, patatin-like phospholipase domain containing 2, and FASN, which were reduced by delphinidin treatment. Delphinidin supplementation limited weight gain in HFD-fed C57BL/6Nhsd mice, but not increased liver weight. Glucose homeostasis variations induced by HFD/HCD were also minimized by delphinidin treatment [222]. Hidalgo et al. [327] showed that delphinidin supplementation in rat jejunum tissues/cells reduced the short circuit current generated by glucose addition to an Ussing chamber. Delphinidin halted 3-*O*-methyl-glucose incorporation in the mouse intestine, with effects similar to the inhibition of electrogenic glucose transportation by SGLT-1 [328]. In response to delphinidin treatment and FFA1 activation, the Gαq/11 subunit was coupled with inositol trisphosphate, propionyl l-carnitine, and diacylglycerol upregulation, which modulates intracellular Ca^{2+} from the endoplasmic reticulum. In previous studies, delphinidin treatment also caused intracellular Ca^{2+} release and prevented 3-*O*-methyl-glucose uptake by FFA1 activation. Therefore, delphinidin may represent a new ligand class that can reduce intestinal glucose uptake through FFA1 activation and increased cAMP expression [327].

MqB juice (MqBJ) consumption limited oxidation in human subjects (Table 2). The copper-triggered LDL-C oxidation time lag increased with MqBJ consumption because anthocyanins chelate copper. LDL-C oxidation time is proportional to the MqBJ anti-oxidative capacity. H_2O_2 treatment-induced increased oxidative stress was reduced by MqBJ treatment in human umbilical vein endothelial cells [329]. A pilot study showed that the daily MqBE consumption with folic acid and berberine

effectively reduced TC, LDL-C, oxidized cholesterol glycemia, free radical levels, and increased serum antioxidant capacity. Furthermore, the insulinemia, microalbuminuria, HDL, CRP, and TG values increased. MqB treatment counteracted hyperlipidemia, hyperglycemia, and ROS production in metabolic syndrome patients. An MqB polyphenol-based-nutraceutical reversed low-grade-inflammation, oxidative stress, and atherosclerogenesis in pre-diabetic patients [223]. MqBE and purified anthocyanin consumption showed positive outcomes for post-stroke stress and depression in diabetic mice. MqBEs and anthocyanins can mitigate anhedonia in humans. Anhedonic mice consumed less sucrose with increased water intake, which was mitigated by MqBE or purified anthocyanins in a dose-dependent-manner. Stroke and stress biomarkers such as TBARS, SOD, CAT, and GSH levels decreased following MqBE/anthocyanin treatment in stroke model mice [219].

16. Conclusions

This review aimed to collect and discuss scientific evidence regarding the positive role of berry consumption on the prevention of diabetes and its complications. Available human, animal, and in vitro studies were collected and comprehensively presented. This review demonstrated that berry product consumption represents a reliable and effective method for preventing and managing metabolic hyperglycemic and hyperlipidemic conditions. Variations in postprandial glucose and insulin levels could be reversed and normalized in diabetic subjects following post-meal berry consumption as supplements for HFD/HCD. Berry anthocyanins promoted glucose uptake and metabolism by activating pAMPK/AMPK, GLUT-4, and SGLUT-1, and inhibited weight gain and pro-inflammatory responses, downregulating lipogenesis genes (adipogenic transcription factors and PPAR-γ2) and pro-inflammatory cytokine production. Berry consumption also showed glucose-lowering and insulin sensitivity improvements, which are closely associated with hypoinsulinemia, insulin signaling activation (in adipose and skeletal muscles), the adiponectin-AMPK pathway, and GLP-1 upregulation. Regarding the relation of gut microbial ecosystem and DM, berry intake not only counteracted the deleterious HFD/HCD effects, but also favored the population of health promoting fermentative, SCOA/SCFA-producing, obesity-preventing, glycolytic, proteolytic, and secondary metabolites metabolizing microflora. The primary potential health-promoting classes of bioactive compounds found in berries include glycosides, glucosides, catechins, epicatechins, proanthocyanidins, cynidines, delphinidins, quercetin, myricetin, malvidins, petunidin, flavanols, flavonols, caffeic acids, chlorogenic acids, phenolic acids, ferulic acids, *p*-coumaric acids, vanillic acids, ellagic acids, hydroxycinnamic acid derivatives, and polysaccharides. Based on the reviewed papers, to obtain these health-endowing effects, the daily recommended dose of whole berry varies from 200 to 400 g of berry intake for a 70 kg BW middle aged person.

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Abbreviations

1-Deoxynojirimycin (1-DNJ); Acai berry (AB); acetyl coenzyme A carboxylase (ACC); acyl-CoA oxidase (ACOX); adhesion molecules nuclear factor (IκBα); advanced glycation end-product (AGE); albumin retention (AR); alcohol-free fermented blackberry juice (AFBBJ); alkaline phosphatase (ALP); aminotransferase (ALT); AMP-activated protein kinase (AMPK); angiotensin I-converting enzyme (ACE-1); apolipoprotein A (Apo)A-I;

aspartate aminotransferase (AST); ATP-binding cassette (ABC); ATP-binding cassette transporter (ABCA1) Bilberry juice (BBJ); BB/BB extracts, (BBEE); Bilberries, (BBs); Black currant (BCT); BlB extracts (BlBEs); BlB juice (BlBJ); Blueberries (BlBs); body weights (BW); brown adipose tissues (BAdT); capillary albumin filtration (CAF); carbohydrate response element-binding protein (ChREBP); cardiovascular disease (CVD); carnitine palmitoyl transferase-1 (CPT-1); Cranberries (CrBs); cranberries juice (CrB-JSB); CrB extracts (CrBE); CrB extracts (CrBEs); CrB juice (CrBJ); C-reactive protein (CRP); Diabetes mellitus (DM); diabetic retinopathy (DN); diacylglycerol acyltransferases 2 (DGAT2); fasting blood sugars (FBS); fatty acid synthase (FAS); Food and Agriculture Organization of the United Nations (FAO); forkhead box O1 (FOXO1); free fatty acid (FFA); gamma-glutamyltransferase (γ-GT); gastrointestinal-digested BB slurry (GIDBB); GB polysaccharides (GBPS); glucagon-like peptide-1 (GLP-1); glucose tolerance test (GTT); glucose transporter (GLUT4); glucose transporter 2 (GLUT-2); glucose-6-phosphatase, (G6Pase); glucose-stimulated insulin secretion (GSIS); glutathione (GSH); glycerol-3-phosphate acyltransferase (GPAT); glycogen synthase (GYS1); glycogen synthase 2 (GSY2); Goji berry (GB); high-carbohydrate diets, (HCD); high-fat diets, (HFD); high-sensitivity CRP (hs-CRP); Human aortic endothelial cells, (HAECs); human serum albumin (HSA); inducible nitric oxide synthase (iNOS); insulin receptor substrate-1/2 (IRS-1/IRS-2); insulin resistance, (IR); intercellular adhesion molecule-1 (ICAM-1); intestinal mucosal barrier dysfunction, (IMBD); lactate dehydrogenase (LDH); Lingonberry (LB); lipopolysaccharides (LPS); Low-calorie dried cranberry, (LCDC); low-density lipoprotein cholesterol (LDL-C); lysophosphatidylcholine (LPC); lyso-phosphatidylethanolamines, (LPE); malondialdehyde (MDA); manganese superoxide dismutase, (Mn-SOD); Maqui berries (MB); microbial-fermented blackberry metabolites (GMBB); microsomal TG transfer protein (MTP); mitochondrial transcription factor A (TFAM); monocyte chemo-attractant protein-1 (MCP-1); Mulberries (MBs); Na-glucose co-transporter 1 (SGLT-1); nitric oxide (NO); nitric oxides (NOs); nod-like receptor pyrin containing 3 (NLRP3); non-alcoholic fatty liver disease (NAFLD); paraoxonase-1 (PON-1); peroxisome proliferator response element (PPRE); peroxisome proliferator-activated receptors γ (PPAR-γ); phosphatidylcholines (PC); polyunsaturated fatty acid (PUFA); PPAR-γ coactivator 1α (PGC-1α); proinflammatory nuclear factor (NF)-κB; Raspberries (RBs); RB extracts (RBE); reactive oxygen species (ROS); short-chain fatty acids, (SCFA); short-chain organic acids, (SCOA); soluble vascular cell adhesion molecule-1 (sVCAM-1); sphingomyelins (SM); sterol regulatory element-binding protein 1c (SREBP-1c); streptozotocin (STZ); Toll-like receptors, (TLR); total cholesterol (TC); total glyceraldehyde (TG); Trolox equivalent antioxidant capacity (TEAC); Type 1 diabetes mellitus, (T1DM); type 2 diabetes mellitus, (T2DM); unsweetened dried CrBs (USCB); white bread (WB); World Health Organization, (WHO).

References

1. World Health Organization (WHO). *Organisation for Economic Co-Operation and Development (OECD)*; World Health Organization: Geneva, Switzerland, 2016.
2. Nathan, D.M.; Buse, J.B.; Davidson, M.B.; Heine, R.J.; Holman, R.R.; Sherwin, R.; Zinman, B. Management of Hyperglycemia in Type 2 Diabetes: A consensus algorithm for the initiation and adjustment of therapy: A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* **2006**, *29*, 1963–1972. [CrossRef]
3. Enwere, O.; Salako, B.; Falade, C. Prescription and cost consideration at a diabetic clinic in Ibadan, Nigeria: A report. *Ann. Ib. Postgrad. Med.* **2010**, *4*. [CrossRef]
4. Evans, D.B.; Etienne, C. Health systems financing and the path to universal coverage. *Bull. World Health Organ.* **2010**, *88*, 402. [CrossRef] [PubMed]
5. Wang, D.D.; Hu, F.B. Precision nutrition for prevention and management of type 2 diabetes. *Lancet Diabetes Endocrinol.* **2018**, *6*, 416–426. [CrossRef]
6. Ley, S.H.; Hamdy, O.; Mohan, V.; Hu, F.B. Prevention and management of type 2 diabetes: Dietary components and nutritional strategies. *Lancet* **2014**, *383*, 1999–2007. [CrossRef]
7. Mursu, J.; Virtanen, J.; Tuomainen, T.P.; Nurmi, T.; Voutilainen, S. Intake of fruit, berries, and vegetables and risk of type 2 diabetes in Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Am. J. Clin. Nutr.* **2013**, *99*, 328–333. [CrossRef]
8. Moher, D. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS ONE Med.* **2009**, *6*, e1000097.
9. Barberis, A.; Garbetta, A.; Cardinali, A.; Bazzu, G.; D'Antuono, I.; Rocchitta, G.; Fadda, A.; Linsalata, V.; D'Hallewin, G.; Serra, P.A.; et al. Real-time monitoring of glucose and phenols intestinal absorption through an integrated Caco-2TC7cells/biosensors telemetric device: Hypoglycemic effect of fruit phytochemicals. *Biosens. Bioelectron.* **2017**, *88*, 159–166. [CrossRef]
10. Martineau, L.C.; Couture, A.; Spoor, D.; Benhaddou-Andaloussi, A.; Harris, C.; Meddah, B.; LeDuc, C.; Burt, A.; Vuong, T.; Le, P.M.; et al. Anti-diabetic properties of the Canadian lowbush blueberry Vaccinium angustifolium Ait. *Phytomedicine* **2006**, *13*, 612–623. [CrossRef]

11. DeFuria, J.; Bennett, G.; Strissel, K.J.; Perfield, J.W.; Milbury, P.E.; Greenberg, A.S.; Obin, M.S. Dietary blueberry attenuates whole-body insulin resistance in high fat-fed mice by reducing adipocyte death and its inflammatory sequelae. *J. Nutr.* **2009**, *139*, 1510–1516. [CrossRef]
12. Wu, T.; Tang, Q.; Gao, Z.; Yu, Z.; Song, H.; Zheng, X.; Chen, W. Blueberry and mulberry juice prevent obesity development in C57BL/6 Mice. *PLoS ONE* **2013**, *8*, e77585. [CrossRef] [PubMed]
13. Grace, M.H.; Ribnicky, D.M.; Kuhn, P.; Poulev, A.; Logendra, S.; Yousef, G.G.; Raskin, I.; Lila, M.A. Hypoglycemic activity of a novel anthocyanin-rich formulation from lowbush blueberry, Vaccinium angustifolium Aiton. *Phytomedicine* **2009**, *16*, 406–415. [CrossRef]
14. Huang, W.; Yao, L.; He, X.; Wang, L.; Li, M.; Yang, Y.; Wan, C. Hypoglycemic activity and constituents analysis of blueberry (Vaccinium corymbosum) fruit extracts. *Diabetes Metab. Syndr. Obes. Targets Ther.* **2018**, *11*, 357–366. [CrossRef] [PubMed]
15. Takikawa, M.; Inoue, S.; Horio, F.; Tsuda, T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via Activation of AMP-activated protein kinase in diabetic mice. *J. Nutr.* **2010**, *140*, 527–533. [CrossRef] [PubMed]
16. Nair, A.R.; Elks, C.M.; Vila, J.; Del Piero, F.; Paulsen, D.B.; Francis, J. A Blueberry-enriched diet improves renal function and reduces oxidative stress in metabolic syndrome animals: Potential mechanism of TLR4-MAPK signaling pathway. *PLoS ONE* **2014**, *9*, e111976. [CrossRef] [PubMed]
17. Nakajima, J.I.; Tanaka, I.; Seo, S.; Yamazaki, M.; Saito, K. LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. *J. Biomed. Biotechnol.* **2004**, *2004*, 241–247. [CrossRef]
18. Mykkänen, O.T.; Huotari, A.; Herzig, K.-H.; Dunlop, T.W.; Mykkänen, H.; Kirjavainen, P.V. Wild blueberries (Vaccinium myrtillus) alleviate inflammation and hypertension associated with developing obesity in mice fed with a high-fat Diet. *PLoS ONE* **2014**, *9*, e114790. [CrossRef]
19. Prior, R.L.; Wu, X.; Gu, L.; Hager, T.J.; Hager, A.; Howard, L.R. Whole berries versus berry anthocyanins: Interactions with dietary fat levels in the C57BL/6J mouse model of obesity. *J. Agric. Food Chem.* **2008**, *56*, 647–653. [CrossRef]
20. Basu, A.; Du, M.; Leyva, M.J.; Sanchez, K.; Betts, N.M.; Wu, M.; Aston, C.E.; Lyons, T.J. Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *J. Nutr.* **2010**, *140*, 1582–1587. [CrossRef]
21. Stull, A.J.; Cash, K.C.; Champagne, C.M.; Gupta, A.K.; Boston, R.C.; Beyl, R.A.; Johnson, W.D.; Cefalu, W.T. Blueberries improve endothelial function, but not blood pressure, in adults with metabolic syndrome: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients* **2015**, *7*, 4107–4123. [CrossRef]
22. Johnson, M.H.; Wallig, M.; Luna-Vital, D.A.; De Mejia, E.G. Alcohol-free fermented blueberry–blackberry beverage phenolic extract attenuates diet-induced obesity and blood glucose in C57BL/6J mice. *J. Nutr. Biochem.* **2016**, *31*, 45–59. [CrossRef] [PubMed]
23. Vuong, T.; Benhaddou-Andaloussi, A.; Brault, A.; Harbilas, D.; Martineau, L.C.; Vallerand, D.; Ramassamy, C.; Matar, C.; Haddad, P.S. Antiobesity and antidiabetic effects of biotransformed blueberry juice in KKAy mice. *Int. J. Obes.* **2009**, *33*, 1166–1173. [CrossRef] [PubMed]
24. Bell, L.; Lamport, D.J.; Butler, L.T.; Williams, C. A study of glycaemic effects following acute anthocyanin-rich blueberry supplementation in healthy young adults. *Food Funct.* **2017**, *8*, 3104–3110. [CrossRef]
25. Vendrame, S.; Daugherty, A.; Kristo, A.S.; Riso, P.; Klimis-Zacas, D. Wild blueberry (*Vaccinium angustifolium)* consumption improves inflammatory status in the obese Zucker rat model of the metabolic syndrome. *J. Nutr. Biochem.* **2013**, *24*, 1508–1512. [CrossRef] [PubMed]
26. Zhu, J.; Liu, W.; Yu, J.; Zou, S.; Wang, J.; Yao, W.; Gao, X. Characterization and hypoglycemic effect of a polysaccharide extracted from the fruit of Lycium barbarum L. *Carbohydr. Polym.* **2013**, *98*, 8–16. [CrossRef]
27. Bharat, D.; Cavalcanti, R.R.M.; Petersen, C.; Begaye, N.; Cutler, B.R.; Costa, M.M.A.; Ramos, R.K.L.G.; Ferreira, M.R.; Li, Y.; Bharath, L.P.; et al. Blueberry metabolites attenuate lipotoxicity-induced endothelial dysfunction. *Mol. Nutr. Food Res.* **2017**, *62*, 1700601. [CrossRef]
28. Bloedon, T.; Vendrame, S.; Bolton, J.; Lehnhard, R.; Riso, P.; Klimis-Zacas, D. The effect of wild blueberry (Vaccinium angustifolium) consumption on oxidative stress, inflammation, and DNA damage associated with exercise. *Comp. Exerc. Physiol.* **2015**, *11*, 173–181. [CrossRef]
29. Li, F.; Zhang, B.; Chen, G.; Fu, X. The novel contributors of anti-diabetic potential in mulberry polyphenols revealed by UHPLC-HR-ESI-TOF-MS/MS. *Food Res. Int.* **2017**, *100*, 873–884. [CrossRef]

30. Ren, G.; Rimando, A.M.; Mathews, S.T. AMPK activation by pterostilbene contributes to suppression of hepatic gluconeogenic gene expression and glucose production in H4IIE cells. *Biochem. Biophys. Res. Commun.* **2018**, *498*, 640–645. [CrossRef]
31. Lee, S.I.; Keirsey, K.; Kirkland, R.I.; Grunewald, Z.; Fischer, J.G.; De La Serre, C.B. Blueberry supplementation influences the gut microbiota, inflammation, and insulin resistance in high-fat-diet–fed rats. *J. Nutr.* **2018**, *148*, 209–219. [CrossRef]
32. Vendrame, S.; Guglielmetti, S.; Riso, P.; Arioli, S.; Klimis-Zacas, D.; Porrini, M. Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. *J. Agric. Food Chem.* **2011**, *59*, 12815–12820. [CrossRef] [PubMed]
33. Polewski, M.A.; Esquivel-Alvarado, D.; Wedde, N.S.; Kruger, C.G.; Reed, J.D. Isolation and characterization of blueberry polyphenolic components and their effects on gut barrier dysfunction. *J. Agric. Food Chem.* **2019**, *68*, 2940–2947. [CrossRef] [PubMed]
34. Rodríguez-Daza, M.-C.; Daoust, L.; Boutkrabt, L.; Pilon, G.; Varin, T.; Dudonné, S.; Levy, É.; Marette, A.; Roy, D.; Desjardins, Y. Wild blueberry proanthocyanidins shape distinct gut microbiota profile and influence glucose homeostasis and intestinal phenotypes in high-fat high-sucrose fed mice. *Sci. Rep.* **2020**, *10*, 1–16. [CrossRef] [PubMed]
35. Yang, H.; Pang, W.; Lu, H.; Cheng, D.; Yan, X.; Cheng, Y.; Jiang, Y. Comparison of metabolic profiling of cyanidin-3-o-galactoside and extracts from blueberry in aged mice. *J. Agric. Food Chem.* **2011**, *59*, 2069–2076. [CrossRef]
36. Harris, C.; Burt, A.J.; Saleem, A.; Le, P.M.; Martineau, L.C.; Haddad, P.S.; Bennett, S.A.L.; Arnason, J.T. A single HPLC-PAD-APCI/MS method for the quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. *Phytochem. Anal.* **2007**, *18*, 161–169. [CrossRef]
37. Lätti, A.K.; Kainulainen, P.S.; Hayirlioglu-Ayaz, S.; Ayaz, F.A.; Riihinen, K.R. Characterization of anthocyanins in caucasian blueberries (*Vaccinium arctostaphylos* L.) native to Turkey. *J. Agric. Food Chem.* **2009**, *57*, 5244–5249. [CrossRef]
38. Može, S.; Polak, T.; Gašperlin, L.; Koron, D.; Vanzo, A.; Ulrih, N.P.; Abram, V. Phenolics in slovenian bilberries (Vaccinium myrtillus L.) and blueberries (*Vaccinium corymbosum* L.). *J. Agric. Food Chem.* **2011**, *59*, 6998–7004. [CrossRef]
39. Shi, M.; Loftus, H.; McAinch, A.J.; Su, X.Q. Blueberry as a source of bioactive compounds for the treatment of obesity, type 2 diabetes and chronic inflammation. *J. Funct. Foods* **2017**, *30*, 16–29. [CrossRef]
40. Seymour, E.M.; Tanone, I.I.; Urcuyo-Llanes, D.E.; Lewis, S.K.; Kirakosyan, A.; Kondoleon, M.G.; Kaufman, P.B.; Bolling, S.F. Blueberry intake alters skeletal muscle and adipose tissue peroxisome proliferator-activated receptor activity and reduces insulin resistance in obese rats. *J. Med. Food* **2011**, *14*, 1511–1518. [CrossRef]
41. Rebello, C.J.; Burton, J.; Heiman, M.; Greenway, F.L. Gastrointestinal microbiome modulator improves glucose tolerance in overweight and obese subjects: A randomized controlled pilot trial. *J. Diabetes Complicat.* **2015**, *29*, 1272–1276. [CrossRef]
42. Li, D.; Zhang, Y.; Liu, Y.; Sun, R.; Xia, M. Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. *J. Nutr.* **2015**, *145*, 742–748. [CrossRef] [PubMed]
43. Asgary, S.; Rafieian-Kopaei, M.; Sahebkar, A.; Shamsi, F.; Goli-Malekabadi, N. Anti-hyperglycemic and anti-hyperlipidemic effects of Vaccinium myrtillusfruit in experimentally induced diabetes (antidiabetic effect of *Vaccinium myrtillusfruit*). *J. Sci. Food Agric.* **2015**, *96*, 764–768. [CrossRef] [PubMed]
44. Kim, J.; Kim, C.-S.; Lee, Y.M.; Sohn, E.; Jo, K.; Kim, J.S. Vaccinium myrtillus extract prevents or delays the onset of diabetes—Induced blood–retinal barrier breakdown. *Int. J. Food Sci. Nutr.* **2015**, *66*, 236–242. [CrossRef] [PubMed]
45. Bao, L.; Yao, X.S.; Yau, C.C.; Tsi, D.; Chia, C.-S.; Nagai, H.; Kurihara, H. Protective effects of bilberry (*Vaccinium myrtillus* L.) extract on restraint stress-induced liver damage in mice. *J. Agric. Food Chem.* **2008**, *56*, 7803–7807. [CrossRef]
46. Cohen-Boulakia, F.; Valensi, P.E.; Boulahdour, H.; Lestrade, R.; Dufour-Lamartinie, J.-F.; Hort-Legrand, C.; Behar, A. In vivo sequential study of skeletal muscle capillary permeability in diabetic rats: Effect of anthocyanosides. *Metabolism* **2000**, *49*, 880–885. [CrossRef]

47. Ferreira, F.M.; Peixoto, F.; Nunes, E.; Sena, C.; Seiça, R.; Santos, M.S. MitoTeas: Vaccinium myrtillus and Geranium robertianum decoctions improve diabetic Goto–Kakizaki rats hepatic mitochondrial oxidative phosphorylation. *Biochim. Biophys. Acta* **2010**, *1797*, 79–80. [CrossRef]
48. Hoggard, N.; Cruickshank, M.; Moar, K.M.; Bestwick, C.; Holst, J.J.; Russell, W.R.; Horgan, G. A single supplement of a standardised bilberry (*Vaccinium myrtillus* L.) extract (36 % wet weight anthocyanins) modifies glycaemic response in individuals with type 2 diabetes controlled by diet and lifestyle. *J. Nutr. Sci.* **2013**, *2*, e22. [CrossRef]
49. Alnajjar, M.; Barik, S.K.; Bestwick, C.; Campbell, F.; Cruickshank, M.; Farquharson, F.; Holtrop, G.; Horgan, G.; Louis, P.; Moar, K.-M.; et al. Anthocyanin-enriched bilberry extract attenuates glycaemic response in overweight volunteers without changes in insulin. *J. Funct. Foods* **2020**, *64*, 103597. [CrossRef]
50. Granfeldt, Y.; Björck, I. A bilberry drink with fermented oatmeal decreases postprandial insulin demand in young healthy adults. *Nutr. J.* **2011**, *10*, 57. [CrossRef]
51. Karlsen, A.; Paur, I.; Bøhn, S.K.; Sakhi, A.K.; Borge, G.I.; Serafini, M.; Erlund, I.; Laake, P.; Tonstad, S.; Blomhoff, R. Bilberry juice modulates plasma concentration of NF-κB related inflammatory markers in subjects at increased risk of CVD. *Eur. J. Nutr.* **2010**, *49*, 345–355. [CrossRef]
52. Erlund, I.; Marniemi, J.; Hakala, P.; Alfthan, G.; Meririnne, E.; Aro, A. Consumption of black currants, lingonberries and bilberries increases serum quercetin concentrations. *Eur. J. Clin. Nutr.* **2003**, *57*, 37–42. [CrossRef] [PubMed]
53. Kolehmainen, M.; Mykkänen, O.; Kirjavainen, P.V.; Leppänen, T.; Moilanen, E.; Adriaens, M.E.; Laaksonen, D.E.; Hallikainen, M.; Puupponen-Pimia, R.; Pulkkinen, L.; et al. Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. *Mol. Nutr. Food Res.* **2012**, *56*, 1501–1510. [CrossRef] [PubMed]
54. Anhê, F.F.; Roy, D.; Pilon, G.; Dudonné, S.; Matamoros, S.; Varin, T.V.; Garofalo, C.; Moine, Q.; Desjardins, Y.; Levy, E.; et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increasedAkkermansiaspp. population in the gut microbiota of mice. *Gut* **2014**, *64*, 872–883. [CrossRef] [PubMed]
55. Zhu, M.; Hu, J.; Perez, E.; Phillips, D.; Kim, W.; Ghaedian, R.; Napora, J.K.; Zou, S. Effects of long-term cranberry supplementation on endocrine pancreas in aging Rats. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2011**, *66*, 1139–1151. [CrossRef]
56. Schell, J.; Betts, N.M.; Foster, M.; Scofield, R.H.; Basu, A. Cranberries improve postprandial glucose excursions in type 2 diabetes. *Food Funct.* **2017**, *8*, 3083–3090. [CrossRef]
57. Lee, I.T.; Chan, Y.C.; Lin, C.W.; Lee, W.J.; Sheu, W.H.-H. Effect of cranberry extracts on lipid profiles in subjects with Type 2 diabetes. *Diabet. Med.* **2008**, *25*, 1473–1477. [CrossRef]
58. Wilson, T.; Meyers, S.; Singh, A.; Limburg, P.; Vorsa, N. Favorable glycemic response of Type 2 diabetics to low-calorie cranberry juice. *J. Food Sci.* **2008**, *73*, H241–H245. [CrossRef]
59. Wilson, T.; Singh, A.P.; Vorsa, N.; Goettl, C.D.; Kittleson, K.M.; Roe, C.M.; Kastello, G.M.; Ragsdale, F.R. Human glycemic response and phenolic content of unsweetened cranberry juice. *J. Med. Food* **2008**, *11*, 46–54. [CrossRef]
60. Wilson, T.; Luebke, J.L.; Morcomb, E.F.; Carrell, E.J.; Leveranz, M.C.; Kobs, L.; Schmidt, T.P.; Limburg, P.J.; Vorsa, N.; Singh, A.P. Glycemic responses to sweetened dried and raw cranberries in humans with Type 2 diabetes. *J. Food Sci.* **2010**, *75*, H218–H223. [CrossRef]
61. Duffey, K.J.; Sutherland, L.A. Adult consumers of cranberry juice cocktail have lower C-reactive protein levels compared with nonconsumers. *Nutr. Res.* **2015**, *35*, 118–126. [CrossRef]
62. Törrönen, R.; Sarkkinen, E.; Niskanen, T.; Tapola, N.; Kilpi, K.; Niskanen, L. Postprandial glucose, insulin and glucagon-like peptide 1 responses to sucrose ingested with berries in healthy subjects. *Br. J. Nutr.* **2011**, *107*, 1445–1451. [CrossRef] [PubMed]
63. Javid, A.Z.; Maghsoumi-Norouzabad, L.; Ashrafzadeh, E.; Yousefimanesh, H.A.; Zakerkish, M.; Angali, K.A.; Ravanbakhsh, M.; Babaei, H. Impact of cranberry juice enriched with Omega-3 Fatty acids adjunct with nonsurgical periodontal treatment on metabolic control and periodontal status in Type 2 patients with diabetes with periodontal Disease. *J. Am. Coll. Nutr.* **2017**, *37*, 71–79. [CrossRef] [PubMed]
64. Shidfar, F.; Heydari, I.; Hajimiresmaiel, S.J.; Hosseini, S.; Shidfar, S.; Amiri, F. The effects of cranberry juice on serum glucose, apoB, apoA-I, Lp (a), and Paraoxonase-1 activity in type 2 diabetic male patients. *J. Res. Med. Sci.* **2012**, *17*, 355–360. [PubMed]

65. Novotny, A.; Baer, J.; Khoo, D.J.; Gebauer, C.; Charron, S.K. Cranberry juice consumption lowers markers of cardiometabolic risk, including blood pressure and circulating c-reactive protein, triglyceride, and glucose concentrations in adults. *J. Nutr.* **2015**, *145*, 1185–1193. [CrossRef] [PubMed]
66. Simão, T.N.C.; Lozovoy, M.A.B.; Simão, A.N.C.; Oliveira, S.R.; Venturini, D.; Morimoto, H.K.; Miglioranza, L.H.S.; Dichi, I. Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome. *Br. J. Nutr.* **2013**, *110*, 1885–1894. [CrossRef]
67. Ruel, G.; Lapointe, A.; Pomerleau, S.; Couture, P.; Lemieux, S.; Lamarche, B.; Couillard, C. Evidence that cranberry juice may improve augmentation index in overweight men. *Nutr. Res.* **2013**, *33*, 41–49. [CrossRef]
68. Ruel, G.; Pomerleau, S.; Couture, P.; Lemieux, S.; Lamarche, B.; Couillard, C. Plasma matrix metalloproteinase (MMP)-9 levels are reduced following low-calorie cranberry juice supplementation in men. *J. Am. Coll. Nutr.* **2009**, *28*, 694–701. [CrossRef]
69. Ruel, G.; Pomerleau, S.; Couture, P.; Lemieux, S.; Lamarche, B.; Couillard, C. Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. *Br. J. Nutr.* **2008**, *99*, 352–359. [CrossRef]
70. Ruel, G.; Pomerleau, S.; Couture, P.; Lemieux, S.; Lamarche, B.; Couillard, C. Favourable impact of low-calorie cranberry juice consumption on plasma HDL-cholesterol concentrations in men. *Br. J. Nutr.* **2006**, *96*, 357–364. [CrossRef]
71. Ruel, G.; Pomerleau, S.; Couture, P.; Lamarche, B.; Couillard, C. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. *Metabolism* **2005**, *54*, 856–861. [CrossRef]
72. Suh, J.H.; Romain, C.; González-Barrio, R.; Cristol, J.P.; Teissedre, P.L.; Crozier, A.; Rouanet, J.M. Raspberry juice consumption, oxidative stress and reduction of atherosclerosis risk factors in hypercholesterolemic golden Syrian hamsters. *Food Funct.* **2011**, *2*, 400–405. [CrossRef] [PubMed]
73. SanGiovanni, E.; Vrhovšek, U.; Rossoni, G.; Colombo, E.; Brunelli, C.; Brembati, L.; Trivulzio, S.; Gasperotti, M.; Mattivi, F.; Bosisio, E.; et al. Ellagitannins from rubus berries for the control of gastric inflammation: In vitro and in vivo studies. *PLoS ONE* **2013**, *8*, e71762. [CrossRef]
74. Jean-Gilles, D.; Li, L.; Ma, H.; Yuan, T.; Chichester, I.C.O.; Seeram, N.P. Anti-inflammatory effects of polyphenolic-enriched red raspberry extract in an antigen-induced arthritis rat model. *J. Agric. Food Chem.* **2011**, *60*, 5755–5762. [CrossRef] [PubMed]
75. Gião, M.; Pestana, D.; Faria, A.; Guimarães, J.T.; Pintado, M.; Calhau, C.; Azevedo, I.; Malcata, F.X. Effects of extracts of selected medicinal plants upon hepatic oxidative stress. *J. Med. Food* **2010**, *13*, 131–136. [CrossRef] [PubMed]
76. Noratto, G.; Chew, B.P.; Atienza, L.M. Red raspberry (*Rubus idaeus* L.) intake decreases oxidative stress in obese diabetic (db/db) mice. *Food Chem.* **2017**, *227*, 305–314. [CrossRef] [PubMed]
77. Kirakosyan, A.; Seymour, E.M.; Kondoleon, N.; Gutiérrez, E.; Wolforth, J.; Bolling, S. The intake of red raspberry fruit is inversely related to cardiac risk factors associated with metabolic syndrome. *J. Funct. Foods* **2018**, *41*, 83–89. [CrossRef]
78. Jakobsdottir, G.; Blanco, N.; Xu, J.; Ahrné, S.; Molin, G.; Sterner, O.; Nyman, M. Formation of short-chain fatty acids, excretion of anthocyanins, and microbial diversity in rats fed blackcurrants, blackberries, and raspberries. *J. Nutr. Metab.* **2013**, *2013*, 1–12. [CrossRef] [PubMed]
79. Pan, P.; Lam, V.; Salzman, N.H.; Huang, Y.W.; Yu, J.; Zhang, J.; Wang, L. Black raspberries and their anthocyanin and fiber fractions alter the composition and diversity of gut microbiota in F-344 rats. *Nutr. Cancer* **2017**, *69*, 943–951. [CrossRef] [PubMed]
80. Su, H.; Xie, L.; Xu, Y.; Ke, H.; Bao, T.; Li, Y.; Chen, W. Pelargonidin-3-O-glucoside derived from wild raspberry exerts antihyperglycemic effect by inducing autophagy and modulating gut microbiota. *J. Agric. Food Chem.* **2019**. [CrossRef]
81. Tu, P.; Bian, X.; Chi, L.; Gao, B.; Ru, H.; Knobloch, T.J.; Weghorst, C.M.; Lu, K. Characterization of the functional changes in mouse gut microbiome associated with increased akkermansia muciniphila population modulated by dietary black raspberries. *ACS Omega* **2018**, *3*, 10927–10937. [CrossRef]
82. Atienza, L.E.; Garcia, P.; Noratto, G. Effects of raspberry on biomarkers of diabetes, cardiovascular disease (cvd) and oxidative stress in obese diabetic (db/db) mice. *FASEB J.* **2015**, *29*, LB283.

83. Luo, T.; Miranda-Garcia, O.; Adamson, A.; Sasaki, G.; Shay, N. Development of obesity is reduced in high-fat fed mice fed whole raspberries, raspberry juice concentrate, and a combination of the raspberry phytochemicals ellagic acid and raspberry ketone. *J. Berry Res.* **2016**, *6*, 213–223. [CrossRef]
84. Guo, H.; Xia, M.; Zou, T.; Ling, W.; Zhong, R.; Zhang, W. Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. *J. Nutr. Biochem.* **2012**, *23*, 349–360. [CrossRef]
85. Iino, T.; Tashima, K.; Umeda, M.; Ogawa, Y.; Takeeda, M.; Takata, K.; Takeuchi, K. Effect of ellagic acid on gastric damage induced in ischemic rat stomachs following ammonia or reperfusion. *Life Sci.* **2002**, *70*, 1139–1150. [CrossRef]
86. Rosillo, M.A. Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. *Biochem. Pharmacol.* **2011**, *82*, 737–745. [CrossRef] [PubMed]
87. Zhao, L.; Zou, T.; Gomez, N.A.; Wang, B.; Zhu, M.-J.; Du, M. Raspberry alleviates obesity-induced inflammation and insulin resistance in skeletal muscle through activation of AMP-activated protein kinase (AMPK) α1. *Nutr. Diabetes* **2018**, *8*, 39. [CrossRef] [PubMed]
88. Zhu, M.; Kang, Y.; Xue, Y.; Liang, X.; García, M.P.G.; Rodgers, D.; Kagel, D.R.; Du, M. Red raspberries suppress NLRP3 inflammasome and attenuate metabolic abnormalities in diet-induced obese mice. *J. Nutr. Biochem.* **2017**, *53*, 96–103. [CrossRef]
89. Sasaki, R.; Nishimura, N.; Hoshino, H.; Isa, Y.; Kadowaki, M.; Ichi, T.; Tanaka, A.; Nishiumi, S.; Fukuda, I.; Ashida, H.; et al. Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice. *Biochem. Pharmacol.* **2007**, *74*, 1619–1627. [CrossRef]
90. Kang, I.; Espín, J.C.; Carr, T.P.; Tomás-Barberán, F.A.; Chung, S. Raspberry seed flour attenuates high-sucrose diet-mediated hepatic stress and adipose tissue inflammation. *J. Nutr. Biochem.* **2016**, *32*, 64–72. [CrossRef]
91. Smith, T.J.; Karl, J.P.; Wilson, M.A.; Whitney, C.C.; Barrett, A.; Farhadi, N.F.; Chen, C.Y.O.; Montain, S.J. Glycaemic regulation, appetite and ex vivo oxidative stress in young adults following consumption of high-carbohydrate cereal bars fortified with polyphenol-rich berries. *Br. J. Nutr.* **2019**, *121*, 1026–1038. [CrossRef]
92. Clegg, M.E.; Pratt, M.; Meade, C.M.; Henry, C.J. The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response. *Br. J. Nutr.* **2011**, *106*, 335–338. [CrossRef] [PubMed]
93. Törrönen, R.; Kolehmainen, M.; Sarkkinen, E.; Poutanen, K.; Mykkänen, H.; Niskanen, L. Berries reduce postprandial insulin responses to wheat and rye breads in healthy women. *J. Nutr.* **2013**, *143*, 430–436. [CrossRef] [PubMed]
94. Schell, J.; Betts, N.M.; Lyons, T.J.; Basu, A. Raspberries improve postprandial glucose and acute and chronic inflammation in adults with Type 2 Diabetes. *Ann. Nutr. Metab.* **2019**, *74*, 165–174. [CrossRef] [PubMed]
95. Wu, T.; Yin, J.; Zhang, G.; Long, H.; Zheng, X. Mulberry and cherry anthocyanin consumption prevents oxidative stress and inflammation in diet-induced obese mice. *Mol. Nutr. Food Res.* **2016**, *60*, 687–694. [CrossRef] [PubMed]
96. Yan, F.; Zheng, X. Anthocyanin-rich mulberry fruit improves insulin resistance and protects hepatocytes against oxidative stress during hyperglycemia by regulating AMPK/ACC/mTOR pathway. *J. Funct. Foods* **2017**, *30*, 270–281. [CrossRef]
97. Srikanta, A.H.; Kumar, A.; Sukhdeo, S.V.; Peddha, M.S.; Govindaswamy, V. The antioxidant effect of mulberry and jamun fruit wines by ameliorating oxidative stress in streptozotocin-induced diabetic Wistar rats. *Food Funct.* **2016**, *7*, 4422–4431. [CrossRef]
98. Ha, U.S.; Bae, W.J.; Kim, S.J.; Yoon, B.I.; Jang, H.; Hong, S.H.; Lee, J.Y.; Hwang, S.Y.; Kim, S.W. Protective effect of cyanidin-3-O-β-D-glucopyranoside fraction from mulberry fruit pigment against oxidative damage in streptozotocin-induced diabetic rat bladder. *Neurourol. Urodyn.* **2012**, *32*, 493–499. [CrossRef]
99. Ren, C.; Zhang, Y.; Cui, W.; Lu, G.; Wang, Y.; Gao, H.; Huang, L.; Mu, Z. A polysaccharide extract of mulberry leaf ameliorates hepatic glucose metabolism and insulin signaling in rats with type 2 diabetes induced by high fat-diet and streptozotocin. *Int. J. Biol. Macromol.* **2015**, *72*, 951–959. [CrossRef]
100. Liu, L.K.; Chou, F.-P.; Chen, Y.C.; Chyau, C.C.; Ho, H.H.; Wang, C.J. Effects of mulberry (*Morus albal.*) extracts on lipid homeostasis in vitro and in vivo. *J. Agric. Food Chem.* **2009**, *57*, 7605–7611. [CrossRef]

101. Wu, T.; Qi, X.; Liu, Y.; Guo, J.; Zhu, R.; Chen, W.; Zheng, X.; Yu, T. Dietary supplementation with purified mulberry (Morus australis Poir) anthocyanins suppresses body weight gain in high-fat diet fed C57BL/6 mice. *Food Chem.* **2013**, *141*, 482–487. [CrossRef]
102. Du, Q.; Zheng, J.; Xu, Y. Composition of anthocyanins in mulberry and their antioxidant activity. *J. Food Compos. Anal.* **2008**, *21*, 390–395. [CrossRef]
103. Peng, C.H.; Liu, L.K.; Chyau, C.C.; Wang, C.J.; Chuang, C.M.; Huang, C.N. Mulberry water extracts possess an anti-obesity effect and ability to inhibit hepatic lipogenesis and promote lipolysis. *J. Agric. Food Chem.* **2011**, *59*, 2663–2671. [CrossRef] [PubMed]
104. Tang, C.C.; Huang, H.P.; Lee, Y.J.; Tang, Y.H.; Wang, C.J. Hepatoprotective effect of mulberry water extracts on ethanol-induced liver injury via anti-inflammation and inhibition of lipogenesis in C57BL/6J mice. *Food Chem. Toxicol.* **2013**, *62*, 786–796. [CrossRef] [PubMed]
105. Yang, D.K.; Joo-Yong, L. Mulberry fruit extract ameliorates nonalcoholic fatty liver disease (NAFLD) through inhibition of mitochondrial oxidative stress in rats. *Evid. Based Complement. Altern. Med.* **2018**, *2018*, 8165716. [CrossRef] [PubMed]
106. Wattanathorn, J.; Kawvised, S.; Thukham-Mee, W. Encapsulated Mulberry Fruit Extract Alleviates Changes in an Animal Model of Menopause with Metabolic Syndrome. *Oxidative Med. Cell. Longev.* **2019**, *2019*, 5360560. [CrossRef]
107. Sheng, Y.; Liu, J.; Zheng, S.; Liang, F.; Luo, Y.; Huang, K.; Xu, W.; He, X. Mulberry leaves ameliorate obesity through enhancing brown adipose tissue activity and modulating gut microbiota. *Food Funct.* **2019**, *10*, 4771–4781. [CrossRef] [PubMed]
108. Chen, C.; You, L.; Huang, Q.; Fu, X.; Zhang, B.; Liu, R.-H.; Li, C. Modulation of gut microbiota by mulberry fruit polysaccharide treatment of obese diabetic db/db mice. *Food Funct.* **2018**, *9*, 3732–3742. [CrossRef]
109. Pierre, W. Hypoglycemic and hypolipidemic effects of Bersama engleriana leaves in nicotinamide/streptozotocin-induced type 2 diabetic rats. *BMC Complement. Altern. Med.* **2012**, *12*, 264. [CrossRef]
110. Jiao, Y.; Wang, X.; Jiang, X.; Kong, F.; Wang, S.; Yan, C. Antidiabetic effects of Morus alba fruit polysaccharides on high-fat diet- and streptozotocin-induced type 2 diabetes in rats. *J. Ethnopharmacol.* **2017**, *199*, 119–127. [CrossRef]
111. Eid, H.M.; Ouchfoun, M.; Brault, A.; Vallerand, D.; Musallam, L.; Arnason, J.T.; Haddad, P.S. Lingonberry (*Vaccinium vitis-idaea* L.) exhibits antidiabetic activities in a mouse model of diet-induced obesity. *Evid. Based Complement. Altern. Med.* **2014**, *2014*, 1–10. [CrossRef]
112. Kivimäki, A.S.; Ehlers, P.I.; Siltari, A.; Turpeinen, A.M.; Vapaatalo, H.; Korpela, R. Lingonberry, cranberry and blackcurrant juices affect mRNA expressions of inflammatory and atherothrombotic markers of SHR in a long-term treatment. *J. Funct. Foods* **2012**, *4*, 496–503. [CrossRef]
113. Heyman-Lindén, L.; Axling, U.; Blanco, N.; Sterner, O.; Holm, C.; Berger, K. Evaluation of beneficial metabolic effects of berries in high-fat fed C57BL/6J Mice. *J. Nutr. Metab.* **2014**, *2014*, 1–12. [CrossRef] [PubMed]
114. Heyman-Lindén, L.; Kotowska, D.; Sand, E.; Bjursell, M.; Plaza, M.; Turner, C.; Holm, C.; Fåk, F.; Berger, K. Lingonberries alter the gut microbiota and prevent low-grade inflammation in high-fat diet fed mice. *Food Nutr. Res.* **2016**, *60*, 29993. [CrossRef] [PubMed]
115. Matziouridou, C.; Marungruang, N.; Nguyen, T.D.; Nyman, M.; Fak, F. Lingonberries reduce atherosclerosis inApoe-/-mice in association with altered gut microbiota composition and improved lipid profile. *Mol. Nutr. Food Res.* **2016**, *60*, 1150–1160. [CrossRef]
116. Al-Hamimi, S.; Heyman-Lindén, L.; Plaza, M.; Turner, C.; Berger, K.; Spégel, P. Alterations in the plasma metabolite profile associated with improved hepatic function and glycemia in mice fed lingonberry supplemented high-fat diets. *Mol. Nutr. Food Res.* **2016**, *61*, 1600442. [CrossRef]
117. Heyman-Lindén, L.; Seki, Y.; Storm, P.; Jones, H.A.; Charron, M.J.; Berger, K.; Holm, C. Berry intake changes hepatic gene expression and DNA methylation patterns associated with high-fat diet. *J. Nutr. Biochem.* **2016**, *27*, 79–95. [CrossRef]
118. Marungruang, N.; Kovalenko, T.; Osadchenko, I.; Voss, U.; Huang, F.; Burleigh, S.; Ushakova, A.A.; Skibo, G.; Nyman, M.; Prykhod'Ko, O.; et al. Lingonberries and their two separated fractions differently alter the gut microbiota, improve metabolic functions, reduce gut inflammatory properties, and improve brain function in ApoE−/− mice fed high-fat diet. *Nutr. Neurosci.* **2018**, *23*, 1–13. [CrossRef]

119. Huang, F.; Nilholm, C.; Roth, B.; Linninge, C.; Hoglund, P.; Nyman, M.; Ohlsson, B. Anthropometric and metabolic improvements in human type 2 diabetes after introduction of an Okinawan-based Nordic diet are not associated with changes in microbial diversity or SCFA concentrations. *Int. J. Food Sci. Nutr.* **2017**, *69*, 729–740. [CrossRef]
120. Linderborg, K.M.; Järvinen, R.; Lehtonen, H.M.; Viitanen, M.; Kallio, H. The fiber and/or polyphenols present in lingonberries null the glycemic effect of the sugars present in the berries when consumed together with added glucose in healthy human volunteers. *Nutr. Res.* **2012**, *32*, 471–478. [CrossRef]
121. Furlan, C.; Valle, S.C.; Maróstica, M.R.; Östman, E.; Björck, I.; Tovar, J. Effect of bilberries, lingonberries and cinnamon on cardiometabolic risk-associated markers following a hypercaloric-hyperlipidic breakfast. *J. Funct. Foods* **2019**, *60*, 103443. [CrossRef]
122. Rokka, S.; Ketoja, E.; Järvenpää, E.; Tahvonen, R. The glycaemic and C-peptide responses of foods rich in dietary fibre from oat, buckwheat and lingonberry. *Int. J. Food Sci. Nutr.* **2013**, *64*, 528–534. [CrossRef] [PubMed]
123. Törrönen, R.; Kolehmainen, M.; Sarkkinen, E.; Mykkänen, H.; Niskanen, L. Postprandial glucose, insulin, and free fatty acid responses to sucrose consumed with blackcurrants and lingonberries in healthy women. *Am. J. Clin. Nutr.* **2012**, *96*, 527–533. [CrossRef] [PubMed]
124. Lehtonen, H.-M.; Lindstedt, A.; Järvinen, R.; Sinkkonen, J.; Graça, G.; Viitanen, M.; Kallio, H.; Gil, A.M. 1H NMR-based metabolic fingerprinting of urine metabolites after consumption of lingonberries (*Vaccinium vitis-idaea)* with a high-fat meal. *Food Chem.* **2013**, *138*, 982–990. [CrossRef] [PubMed]
125. Hassimotto, N.M.A.; Lajolo, F.M. Antioxidant status in rats after long-term intake of anthocyanins and ellagitannins from blackberries. *J. Sci. Food Agric.* **2010**, *91*, 523–531. [CrossRef]
126. Wu, T.; Gao, Y.; Guo, X.; Zhang, M.; Gong, L. Blackberry and blueberry anthocyanin supplementation counteract high-fat-diet-induced obesity by alleviating oxidative stress and inflammation and accelerating energy expenditure. *Oxidative Med. Cell. Longev.* **2018**, *2018*, 1–9. [CrossRef]
127. Kim, B.; Gil Lee, S.; Park, Y.-K.; Ku, C.S.; Pham, T.X.; Wegner, C.J.; Yang, Y.; Koo, S.I.; Chun, O.K.; Lee, J.Y. Blueberry, blackberry, and blackcurrant differentially affect plasma lipids and pro-inflammatory markers in diet-induced obesity mice. *Nutr. Res. Pract.* **2016**, *10*, 494–500. [CrossRef]
128. Meireles, M.; Rodríguez-Alcalá, L.M.; Marques, C.; Norberto, S.; Freitas, J.; Fernandes, I.; Mateus, N.; Gomes, A.; Faria, A.; Calhau, C. Effect of chronic consumption of blackberry extract on high-fat induced obesity in rats and its correlation with metabolic and brain outcomes. *Food Funct.* **2016**, *7*, 127–139. [CrossRef]
129. Azofeifa, G.; Quesada, S.; Navarro, L.; Hidalgo, O.; Portet, K.; Pérez, A.M.; Vaillant, F.; Poucheret, P.; Michel, A. Hypoglycaemic, hypolipidaemic and antioxidant effects of blackberry beverage consumption in streptozotocin-induced diabetic rats. *J. Funct. Foods* **2016**, *26*, 330–337. [CrossRef]
130. Kosmala, M.; Jurgoński, A.; Juskiewicz, J.; Karlińska, E.; Macierzynski, J.; Rój, E.; Zduńczyk, Z. Chemical composition of blackberry press cake, polyphenolic extract, and defatted seeds, and their effects on cecal fermentation, bacterial metabolites, and blood lipid profile in rats. *J. Agric. Food Chem.* **2017**, *65*, 5470–5479. [CrossRef]
131. Salyer, J.; Park, S.H.; Ricke, S.C.; Lee, S.O. Analysis of microbial populations and metabolism of anthocyanins by mice gut microflora fed with blackberry powder. *J. Nutr. Food Sci.* **2013**, *3*. [CrossRef]
132. Jenkins, D.J.A.; Srichaikul, K.; Kendall, C.W.C.; Sievenpiper, J.L.; Abdulnour, S.; Mirrahimi, A.; Meneses, C.; Nishi, S.; He, X.; Lee, S.; et al. The relation of low glycaemic index fruit consumption to glycaemic control and risk factors for coronary heart disease in type 2 diabetes. *Diabetologia* **2010**, *54*, 271–279. [CrossRef]
133. Hassimotto, N.M.A.; Pinto, M.D.S.; Lajolo, F.M. Antioxidant status in humans after consumption of blackberry (Rubus fruticosusL.) juices with and without defatted milk. *J. Agric. Food Chem.* **2008**, *56*, 11727–11733. [CrossRef] [PubMed]
134. Vafa, M.; Aghababaee, S.K.; Shidfar, F.; Tahavorgar, A.; Gohari, M.; Katebi, D.; Mohammadi, V. Effects of blackberry (*Morus nigra* L.) consumption on serum concentration of lipoproteins, apo A-I, apo B, and high-sensitivity-C-reactive protein and blood pressure in dyslipidemic patients. *J. Res. Med. Sci.* **2015**, *20*, 684–691. [CrossRef] [PubMed]
135. Mandave, P.C.; Khadke, S.; Karandikar, M.; Pandit, V.; Ranjekar, P.; Kuvalekar, A.; Mantri, N. Antidiabetic, lipid normalizing, and nephroprotective actions of the strawberry: A potent supplementary fruit. *Int. J. Mol. Sci.* **2017**, *18*, 124. [CrossRef] [PubMed]

136. Oviedo-Solís, C.I.; Sandoval-Salazar, C.; Lozoya, E.; Maldonado-Aguilera, G.A.; Aguilar-Zavala, H.; Beltrán-Campos, V.; Pérez-Vázquez, V.; Ramírezemiliano, J. Ultraviolet light-C increases antioxidant capacity of the strawberry (Fragaria x ananassa) in vitro and in high-fat diet-induced obese rats. *Food Sci. Nutr.* **2017**, *5*, 1004–1014. [CrossRef]
137. Parelman, M.A.; Storms, D.H.; Kirschke, C.P.; Huang, L.; Zunino, S.J. Dietary strawberry powder reduces blood glucose concentrations in obese and lean C57BL/6 mice, and selectively lowers plasma C-reactive protein in lean mice. *Br. J. Nutr.* **2012**, *108*, 1789–1799. [CrossRef]
138. Aranaz, P.; Romo-Hualde, A.; Zabala, M.; Navarro-Herrera, D.; De Galarreta, M.R.; Gil, A.G.; Martínez, J.A.; Aranaz, P.; Gonzalez, C.J. Freeze-dried strawberry and blueberry attenuates diet-induced obesity and insulin resistance in rats by inhibiting adipogenesis and lipogenesis. *Food Funct.* **2017**, *8*, 3999–4013. [CrossRef]
139. Diamanti, J.; Mezzetti, B.; Giampieri, F.; Alvarez-Suarez, J.M.; Quiles, J.L.; Gonzalez-Alonso, A.; Ramirez-Tortosa, M.D.C.; Granados-Principal, S.; Gonzáles-Paramás, A.M.; Santos-Buelga, C.; et al. Doxorubicin-induced oxidative stress in rats is efficiently counteracted by dietary anthocyanin differently enriched strawberry (Fragaria×ananassaDuch.). *J. Agric. Food Chem.* **2014**, *62*, 3935–3943. [CrossRef]
140. Sandoval-Salazar, C.; Oviedo-Solís, C.I.; Lozoya, E.; Aguilar-Zavala, H.; Solís-Ortiz, S.; Pérez-Vázquez, V.; Balcón-Pacheco, C.D.; Ramírezemiliano, J. Strawberry intake ameliorates oxidative stress and decreases GABA levels induced by high-fat diet in frontal cortex of rats. *Antioxidants* **2019**, *8*, 70. [CrossRef]
141. Petersen, C.; Wankhade, U.D.; Bharat, D.; Wong, K.; Mueller, J.E.; Chintapalli, S.V.; Piccolo, B.D.; Jalili, T.; Jia, Z.; Symons, J.D.; et al. Dietary supplementation with strawberry induces marked changes in the composition and functional potential of the gut microbiome in diabetic mice. *J. Nutr. Biochem.* **2019**, *66*, 63–69. [CrossRef]
142. Han, Y.; Song, M.; Gu, M.; Ren, D.; Zhu, X.; Cao, X.; Li, F.; Wang, W.; Cai, X.; Yuan, B.; et al. Dietary intake of whole strawberry inhibited colonic inflammation in dextran-sulfate-sodium-treated mice via restoring immune homeostasis and alleviating gut microbiota dysbiosis. *J. Agric. Food Chem.* **2019**, *67*, 9168–9177. [CrossRef]
143. Paquette, M.; Larqué, A.S.M.; Weisnagel, S.J.; Desjardins, Y.; Marois, J.; Pilon, G.; Dudonné, S.; Marette, A.; Jacques, H. Strawberry and cranberry polyphenols improve insulin sensitivity in insulin-resistant, non-diabetic adults: A parallel, double-blind, controlled and randomised clinical trial. *Br. J. Nutr.* **2017**, *117*, 519–531. [CrossRef] [PubMed]
144. Burton-Freeman, B.; Linares, A.; Hyson, D.; Kappagoda, T. Strawberry modulates LDL oxidation and postprandial lipemia in response to high-fat meal in overweight hyperlipidemic men and women. *J. Am. Coll. Nutr.* **2010**, *29*, 46–54. [CrossRef] [PubMed]
145. Kurotobi, T.; Fukuhara, K.; Inage, H.; Kimura, S. Glycemic index and postprandial blood glucose response to Japanese strawberry jam in normal adults. *J. Nutr. Sci. Vitaminol.* **2010**, *56*, 198–202. [CrossRef] [PubMed]
146. Ibero-Baraibar, I.; Cuervo, M.; Navas-Carretero, S.; Abete, I.; Zulet, M.A.; Martínez, J.A. Different postprandial acute response in healthy subjects to three strawberry jams varying in carbohydrate and antioxidant content: A randomized, crossover trial. *Eur. J. Nutr.* **2013**, *53*, 201–210. [CrossRef] [PubMed]
147. Amani, R.; Moazen, S.; Shahbazian, H.; Ahmadi, K.; Jalali, M.T. Flavonoid-rich beverage effects on lipid profile and blood pressure in diabetic patients. *World J. Diabetes* **2014**, *5*, 962–968. [CrossRef]
148. Ellis, C.L.; Edirisinghe, I.; Kappagoda, T.; Burton-Freeman, B. Attenuation of meal-induced inflammatory and thrombotic responses in overweight men and women after 6-week daily strawberry (Fragaria) intake. *J. Atheroscler. Thromb.* **2011**, *18*, 318–327. [CrossRef]
149. Edirisinghe, I.; Banaszewski, K.; Cappozzo, J.; Sandhya, K.; Ellis, C.L.; Tadapaneni, R.K.; Kappagoda, C.T.; Burton-Freeman, B.M. Strawberry anthocyanin and its association with postprandial inflammation and insulin. *Br. J. Nutr.* **2011**, *106*, 913–922. [CrossRef]
150. Park, E.; Edirisinghe, I.; Wei, H.; Vijayakumar, L.P.; Banaszewski, K.; Cappozzo, J.C.; Burton-Freeman, B. A dose-response evaluation of freeze-dried strawberries independent of fiber content on metabolic indices in abdominally obese individuals with insulin resistance in a randomized, single-blinded, diet-controlled crossover trial. *Mol. Nutr. Food Res.* **2016**, *60*, 1099–1109. [CrossRef]
151. Ivey, K.L.; Chan, A.T.; Izard, J.; Cassidy, A.; Rogers, G.B.; Rimm, E.B. Role of dietary flavonoid compounds in driving patterns of microbial community assembly. *mBio* **2019**, *10*, e01205-19. [CrossRef]

152. Basu, A.; Morris, S.; Nguyen, A.; Betts, N.M.; Fu, D.; Lyons, T.J. Effects of dietary strawberry supplementation on antioxidant biomarkers in obese adults with above optimal serum lipids. *J. Nutr. Metab.* **2016**, *2016*, 1–9. [CrossRef] [PubMed]
153. Moazen, S.; Amani, R.; Rad, A.H.; Ahmadi, K.; Jalali, M.-T.; Shahbazian, H. Effects of freeze-dried strawberry supplementation on metabolic biomarkers of atherosclerosis in subjects with Type 2 Diabetes: A randomized double-blind controlled trial. *Ann. Nutr. Metab.* **2013**, *63*, 256–264. [CrossRef] [PubMed]
154. Luo, Q.; Cai, Y.; Yan, J.; Sun, M.; Corke, H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. *Life Sci.* **2004**, *76*, 137–149. [CrossRef]
155. Cui, B.K.; Liu, S.; Lin, X.; Wang, J.; Li, S.H.; Wang, Q.-B.; Li, S. Effects of Lycium barbarum aqueous and ethanol extracts on high-fat-diet induced oxidative stress in rat liver tissue. *Molecules* **2011**, *16*, 9116–9128. [CrossRef] [PubMed]
156. Jing, L.; Cui, G.; Feng, Q.; Xiao, Y. Evaluation of hypoglycemic activity of the polysaccharides extracted from Lycium Barbarum. *Afr. J. Tradit. Complement. Altern. Med.* **2009**, *6*, 579–584. [CrossRef] [PubMed]
157. Yin, J.; Wu, T. Anthocyanins from black wolfberry (*Lycium ruthenicum Murr*.) prevent inflammation and increase fecal fatty acid in diet-induced obese rats. *RSC Adv.* **2017**, *7*, 47848–47853. [CrossRef]
158. Zhao, R.; Li, Q.W.; Li, J.; Zhang, T. Protective effect of Lycium barbarum polysaccharide 4 on kidneys in streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol.* **2009**, *87*, 711–719. [CrossRef]
159. Hu, C.; Lee, Y.J.; Colitz, C.M.; Chang, C.J.; Lin, C.T. The protective effects of *Lycium barbarum* and *Chrysanthemum morifolum* on diabetic retinopathies in rats. *Veter. Ophthalmol.* **2012**, *15*, 65–71. [CrossRef]
160. Zhao, R.; Gao, X.; Zhang, T.; Li, X. Effects of Lycium barbarum. polysaccharide on type 2 diabetes mellitus rats by regulating biological rhythms. *Iran. J. Basic Med. Sci* **2016**, *19*, 1024–1030.
161. Al-Fartosy, A.J.M. Protective effect of galactomannan extracted from Iraqi *Lycium Barbarum* L. fruits against alloxan-induced diabetes in rats. *Am. J. Biochem. Biotechnol.* **2015**, *11*, 73–83. [CrossRef]
162. Du, M.; Hu, X.; Kou, L.; Zhang, B.; Zhang, C. Lycium barbarum Polysaccharide mediated the antidiabetic and antinephritic effects in diet-streptozotocin-induced diabetic sprague dawley rats via regulation of NF-κB. *BioMed Res. Int.* **2016**, *2016*, 1–9. [CrossRef]
163. Ni, T.; Wei, G.; Yin, X.; Liu, X.; Liu, D. Original article Neuroprotective effect of Lycium barbarum on retina of Royal College of Surgeons (RCS) rats: A preliminary study. *Folia Neuropathol.* **2013**, *2*, 158–163. [CrossRef] [PubMed]
164. Kang, Y.; Yang, G.; Zhang, S.; Ross, C.F.; Zhu, M. Goji berry modulates gut microbiota and alleviates colitis in IL-10-deficient mice. *Mol. Nutr. Food Res.* **2018**, *62*, 1800535. [CrossRef] [PubMed]
165. Zhu, W.; Zhou, S.; Liu, J.; McLean, R.J.; Chu, W. Prebiotic, immuno-stimulating and gut microbiota-modulating effects of Lycium barbarum polysaccharide. *Biomed. Pharmacother.* **2020**, *121*, 109591. [CrossRef]
166. Amagase, H.; Sun, B.; Borek, C. Lycium barbarum (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. *Nutr. Res.* **2009**, *29*, 19–25. [CrossRef]
167. Zanchet, M.Z.D.S.; Nardi, G.M.; Bratti, L.D.O.S.; Filippin, F.B.; Locatelli, C. Lycium Barbarum reduces abdominal fat and improves lipid profile and antioxidant status in patients with metabolic syndrome. *Oxidative Med. Cell. Longev.* **2017**, *2017*, 1–12. [CrossRef]
168. Xiao, J.; Liong, E.C.; Ching, Y.P.; Chang, R.C.C.; So, K.F.; Fung, M.L.; Tipoe, G.L. Lycium barbarum polysaccharides protect mice liver from carbon tetrachloride-induced oxidative stress and necroinflammation. *J. Ethnopharmacol.* **2012**, *139*, 462–470. [CrossRef]
169. Driessche, J.J.V.D.; Plat, J.; Plasqui, G.; Mensink, R.P. A single dose of goji berries does not affect postprandial energy expenditure and substrate oxidation in healthy, overweight men. *J. Nutr. Metab.* **2019**, *2019*, 1–6. [CrossRef]
170. De Oliveira, P.R.B.; Da Costa, C.A.; De Bem, G.F.; Cordeiro, V.S.C.; Santos, I.B.; De Carvalho, L.C.R.M.; Conceição, E.P.; Lisboa, P.C.; Ognibene, D.T.; Sousa, P.J.C.; et al. Euterpe oleracea mart.-derived polyphenols protect mice from diet-induced obesity and fatty liver by regulating hepatic lipogenesis and cholesterol Excretion. *PLoS ONE* **2015**, *10*, e0143721. [CrossRef]
171. Kim, J.Y.; Hong, J.-H.; Jung, H.K.; Jeong, Y.S.; Cho, K.H. Grape skin and loquat leaf extracts and acai puree have potent anti-atherosclerotic and anti-diabetic activity in vitro and in vivo in hypercholesterolemic zebrafish. *Int. J. Mol. Med.* **2012**, *30*, 606–614. [CrossRef]

172. Sun, X.; Seeberger, J.; Alberico, T.; Wang, C.; Wheeler, C.T.; Schauss, A.G.; Zou, S. Açai palm fruit (*Euterpe oleracea Mart*.) pulp improves survival of flies on a high fat diet. *Exp. Gerontol.* **2010**, *45*, 243–251. [CrossRef] [PubMed]
173. De Castro, C.A.; Natali, A.J.; Cardoso, L.M.; Ferreira-Machado, A.B.; Novello, A.A.; Da Silva, K.A.; Tafuri, N.F.; Da Matta, S.L.P.; Pedrosa, M.L.; Peluzio, M.D.C.G. Aerobic exercise and not a diet supplemented with jussara açaí (*Euterpe edulis Martius*) alters hepatic oxidative and inflammatory biomarkers in ApoE-deficient mice. *Br. J. Nutr.* **2014**, *112*, 285–294. [CrossRef]
174. Cordeiro, V.D.S.C.; De Bem, G.F.; Da Costa, C.A.; Santos, I.B.; De Carvalho, L.C.R.M.; Ognibene, D.T.; Da Rocha, A.P.M.; De Carvalho, J.J.; De Moura, R.S.; Resende, A.C. Euterpe oleracea Mart. seed extract protects against renal injury in diabetic and spontaneously hypertensive rats: Role of inflammation and oxidative stress. *Eur. J. Nutr.* **2017**, *57*, 817–832. [CrossRef]
175. De Souza, M.O.; e Silva, L.S.; Magalhães, C.L.D.B.; De Figueiredo, B.B.; Costa, D.C.; Silva, M.E.; Pedrosa, M.L. The hypocholesterolemic activity of açaí (*Euterpe oleracea Mart*.) is mediated by the enhanced expression of the ATP-binding cassette, subfamily G transporters 5 and 8 and low-density lipoprotein receptor genes in the rat. *Nutr. Res.* **2012**, *32*, 976–984. [CrossRef] [PubMed]
176. De Bem, G.F.; Costa, C.A.; Santos, I.B.; Cordeiro, V.D.S.C.; De Carvalho, L.C.R.M.; De Souza, M.A.V.; Soares, R.D.A.; Sousa, P.J.D.C.; Ognibene, D.T.; Resende, A.C.; et al. Antidiabetic effect of Euterpe oleracea Mart. (açaí) extract and exercise training on high-fat diet and streptozotocin-induced diabetic rats: A positive interaction. *PLoS ONE* **2018**, *13*, e0199207. [CrossRef] [PubMed]
177. Guergoletto, K.B.; Costabile, A.; Flores, G.; Garcia, S.; Gibson, G.R. In vitro fermentation of juçara pulp (*Euterpe edulis*) by human colonic microbiota. *Food Chem.* **2016**, *196*, 251–258. [CrossRef] [PubMed]
178. AlQurashi, R.M.; Alarifi, S.N.; Walton, G.E.; Costabile, A.; Rowland, I.R.; Commane, D.M. In vitro approaches to assess the effects of açai (*Euterpe oleracea*) digestion on polyphenol availability and the subsequent impact on the faecal microbiota. *Food Chem.* **2017**, *234*, 190–198. [CrossRef]
179. Guerra, J.F.D.C.; Maciel, P.S.; De Abreu, I.C.M.E.; Pereira, R.R.; Silva, M.; Cardoso, L.D.M.; Pinheiro-Sant'Ana, H.M.; Lima, W.G.; Silva, M.E.; Pedrosa, M.L. Dietary açai attenuates hepatic steatosis via adiponectin-mediated effects on lipid metabolism in high-fat diet mice. *J. Funct. Foods* **2015**, *14*, 192–202. [CrossRef]
180. Mertens-Talcott, S.U.; Rios, J.; Jilma-Stohlawetz, P.; Pacheco-Palencia, L.A.; Meibohm, B.; Talcott, S.T.; Derendorf, H. Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich açai juice and Pulp (*Euterpe oleracea Mart*.) in human healthy volunteers. *J. Agric. Food Chem.* **2008**, *56*, 7796–7802. [CrossRef]
181. Udani, J.; Singh, B.B.; Singh, V.J.; Barrett, M.L. Effects of Açai (Euterpe oleracea Mart.) berry preparation on metabolic parameters in a healthy overweight population: A pilot study. *Nutr. J.* **2011**, *10*, 45. [CrossRef]
182. Yamane, T.; Kozuka, M.; Konda, D.; Nakano, Y.; Nakagaki, T.; Ohkubo, I.; Ariga, H. Improvement of blood glucose levels and obesity in mice given aronia juice by inhibition of dipeptidyl peptidase IV and α-glucosidase. *J. Nutr. Biochem.* **2016**, *31*, 106–112. [CrossRef] [PubMed]
183. Jeon, Y.D.; Kang, S.H.; Moon, K.H.; Lee, J.H.; Kim, D.G.; Kim, W.; Kim, J.S.; Ahn, B.Y.; Jin, J.S. the effect of aronia berry on Type 1 Diabetes in vivo and in vitro. *J. Med. Food* **2018**, *21*, 244–253. [CrossRef]
184. Park, C.H.; Kim, J.H.; Lee, E.B.; Hur, W.; Kwon, O.-J.; Park, H.-J.; Yoon, S.K. Aronia melanocarpa extract ameliorates hepatic lipid metabolism through PPARγ2 downregulation. *PLoS ONE* **2017**, *12*, e0169685. [CrossRef] [PubMed]
185. Kim, S.; Baek, S.-K.; Bin Song, K. Physical and antioxidant properties of alginate films prepared from Sargassum fulvellum with black chokeberry extract. *Food Packag. Shelf Life* **2018**, *18*, 157–163. [CrossRef]
186. Valcheva-Kuzmanova, S.; Kuzmanov, K.; Tancheva, S.; Belcheva, A. Hypoglycemic effects of Aronia melanocarpa fruit juice in streptozotocin-induced diabetic rats. *Methods Find. Exp. Clin. Pharmacol.* **2007**, *29*, 101–105. [CrossRef]
187. Lipinska, P.; Atanasov, A.G.; Palka, M.; Józ´wik, A. Chokeberry pomace as a determinant of antioxidant parameters assayed in blood and liver tissue of polish merino and Wrzosówka lambs. *Molecules* **2017**, *22*, 1461. [CrossRef]
188. Sikora, J.; Broncel, M.; Markowicz, M.; Chałubiński, M.; Wojdan, K.; Mikiciuk-Olasik, E. Short-term supplementation with Aronia melanocarpa extract improves platelet aggregation, clotting, and fibrinolysis in patients with metabolic syndrome. *Eur. J. Nutr.* **2011**, *51*, 549–556. [CrossRef]

189. Jurgoński, A.; Juskiewicz, J.; Zduńczyk, Z. Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. *Plant. Foods Hum. Nutr.* **2008**, *63*, 176–182. [CrossRef]
190. Kujawska, M.; Ignatowicz, E.; Ewertowska, M.; Oszmiański, J.; Jodynis-Liebert, J. Protective effect of chokeberry on chemical-induced oxidative stress in rat. *Hum. Exp. Toxicol.* **2010**, *30*, 199–208. [CrossRef]
191. Bhaswant, M.; Shafie, S.R.; Mathai, M.L.; Mouatt, P.; Brown, L. Anthocyanins in chokeberry and purple maize attenuate diet-induced metabolic syndrome in rats. *Nutrients* **2017**, *41*, 24–31. [CrossRef]
192. Qin, B.; Anderson, R.A. An extract of chokeberry attenuates weight gain and modulates insulin, adipogenic and inflammatory signalling pathways in epididymal adipose tissue of rats fed a fructose-rich diet. *Br. J. Nutr.* **2011**, *108*, 581–587. [CrossRef] [PubMed]
193. Jakovljevic, V.; Milic, P.; Bradic, J.; Jeremic, J.; Zivkovic, V.; Srejovic, I.M.; Turnic, T.R.N.; Milosavljevic, I.; Jeremic, N.; Bolevich, S.; et al. Standardized aronia melanocarpa extract as novel supplement against metabolic syndrome: A rat model. *Int. J. Mol. Sci.* **2018**, *20*, 6. [CrossRef] [PubMed]
194. Milutinovic´, M.; Brankovic´, S.; C´ ujic´, N.; Šavikin, K.; Kostic´, M.; Kitic´, N.; Miladinovic´, B.; Kitic´, D. Antispasmodic effects of black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) extracts and juice and their potential use in gastrointestinal disorders. *J. Berry Res.* **2020**, *10*, 175–192. [CrossRef]
195. Kardum, N.; Konic´-Ristic´, A.; Šavikin, K.; Spasic, S.; Stefanovic, A.; Ivanisevic, J.; Miljkovic, M. Effects of polyphenol-rich chokeberry juice on antioxidant/pro-oxidant status in healthy subjects. *J. Med. Food* **2014**, *17*, 869–874. [CrossRef] [PubMed]
196. Kardum, N.; Takic´, M.; Šavikin, K.; Zec, M.; Zdunic´, G.; Spasic, S.; Konic´-Ristic´, A. Effects of polyphenol-rich chokeberry juice on cellular antioxidant enzymes and membrane lipid status in healthy women. *J. Funct. Foods* **2014**, *9*, 89–97. [CrossRef]
197. Broncel, M.; Kozirog, M.; Duchnowicz, P.; Koter-Michalak, M.; Sikora, J.; Chojnowska-Jezierska, J. Aronia melanocarpa extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. *Med. Sci. Monit.* **2010**, *16*.
198. Kardum, N.; Milovanovic´, B.; Šavikin, K.; Zdunic´, G.; Mutavdžin, S.; Gligorijevic´, T.; Spasic, S. Beneficial effects of polyphenol-rich chokeberry juice consumption on blood pressure level and lipid status in hypertensive subjects. *J. Med. Food* **2015**, *18*, 1231–1238. [CrossRef]
199. Badescu, M.; Badulescu, O.; Badescu, L.; Ciocoiu, M. Effects ofSambucus nigraandAronia melanocarpaextracts on immune system disorders within diabetes mellitus. *Pharm. Biol.* **2014**, *53*, 533–539. [CrossRef]
200. Nowak, D.; Gośliński, M.; Przygoński, K.; Wojtowicz, E. The antioxidant properties of exotic fruit juices from acai, maqui berry and noni berries. *Eur. Food Res. Technol.* **2018**, *244*, 1897–1905. [CrossRef]
201. Skoczynska, A.; Pore¸ba, R.; Steinmentz-Beck, A.; Martynowicz, H.; Affelska-Jercha, A.; Turczyn, B.; Wojakowska, A.; Jedrychowska, I. The dependence between urinary mercury concentration and carotid arterial intima-media thickness in workers occupationally exposed to mercury vapour. *Int. J. Occup. Med. Environ. Health* **2009**, *22*. [CrossRef]
202. Istas, G.; Wood, E.; Le Sayec, M.; Rawlings, C.; Yoon, J.; Dandavate, V.; Cera, E.D.; Rampelli, S.; Costabile, A.; Fromentin, E.; et al. Effects of aronia berry (poly)phenols on vascular function and gut microbiota: A double-blind randomized controlled trial in adult men. *Am. J. Clin. Nutr.* **2019**, *110*, 316–329. [CrossRef]
203. Tani, T.; Nishikawa, S.; Kato, M.; Tsuda, T. Delphinidin 3-rutinoside-rich blackcurrant extract ameliorates glucose tolerance by increasing the release of glucagon-like peptide-1 secretion. *Food Sci. Nutr.* **2017**, *5*, 929–933. [CrossRef] [PubMed]
204. Iizuka, Y.; Ozeki, A.; Tani, T.; Tsuda, T. Blackcurrant extract ameliorates hyperglycemia in Type 2 Diabetic mice in association with increased basal secretion of glucagon-like peptide-1 and activation of AMP-activated protein kinase. *J. Nutr. Sci. Vitaminol.* **2018**, *64*, 258–264. [CrossRef] [PubMed]
205. Esposito, D.; Damsud, T.; Wilson, M.; Grace, M.H.; Strauch, R.; Li, X.; Lila, M.A.; Komarnytsky, S. Black currant anthocyanins attenuate weight gain and improve glucose metabolism in diet-induced obese mice with intact, but not disrupted, gut microbiome. *J. Agric. Food Chem.* **2015**, *63*, 6172–6180. [CrossRef]
206. Benn, T.; Kim, B.; Park, Y.K.; Wegner, C.J.; Harness, E.; Nam, T.G.; Kim, D.O.; Lee, J.S.; Lee, J.Y. Polyphenol-rich blackcurrant extract prevents inflammation in diet-induced obese mice. *J. Nutr. Biochem.* **2014**, *25*, 1019–1025. [CrossRef] [PubMed]

207. Jurgoński, A.; Juskiewicz, J.; Zduńczyk, Z.; Matusevicius, P.; Kołodziejczyk, K. Polyphenol-rich extract from blackcurrant pomace attenuates the intestinal tract and serum lipid changes induced by a high-fat diet in rabbits. *Eur. J. Nutr.* **2014**, *53*, 1603–1613. [CrossRef]
208. Molan, A.; Liu, Z.; Kruger, M. The ability of blackcurrant extracts to positively modulate key markers of gastrointestinal function in rats. *World, J. Microbiol. Biotechnol.* **2010**, *26*, 1735–1743. [CrossRef]
209. Park, J.H.; Kho, M.C.; Kim, H.Y.; Ahn, Y.M.; Lee, Y.J.; Kang, D.G.; Lee, H.S. Blackcurrant suppresses metabolic syndrome induced by high-fructose diet in rats. *Evid. Based Complement. Altern. Med.* **2015**, *2015*, 1–11. [CrossRef]
210. Paturi, G.; Butts, C.A.; Monro, J.A.; Hedderley, D.I. Effects of blackcurrant and dietary fibers on large intestinal health biomarkers in rats. *Plant. Foods Hum. Nutr.* **2018**, *73*, 54–60. [CrossRef]
211. Molan, A.; Liu, Z.; Plimmer, G. Evaluation of the effect of blackcurrant products on gut microbiota and on markers of risk for colon cancer in humans. *Phytother. Res.* **2013**, *28*, 416–422. [CrossRef]
212. Castro-Acosta, M.L.; Stone, S.G.; Mok, J.E.; Mhajan, R.K.; Fu, C.I.; Lenihan-Geels, G.N.; Corpe, C.P.; Hall, W. Apple and blackcurrant polyphenol-rich drinks decrease postprandial glucose, insulin and incretin response to a high-carbohydrate meal in healthy men and women. *J. Nutr. Biochem.* **2017**, *49*, 53–62. [CrossRef] [PubMed]
213. Castro-Acosta, M.L.; Smith, L.; Miller, R.J.; McCarthy, D.I.; Farrimond, J.A.; Hall, W. Drinks containing anthocyanin-rich blackcurrant extract decrease postprandial blood glucose, insulin and incretin concentrations. *J. Nutr. Biochem.* **2016**, *38*, 154–161. [CrossRef] [PubMed]
214. Willems, M.; Parktin, N.; Widjaja, W.; Ajjimaporn, A. Effect of New Zealand blackcurrant extract on physiological responses at rest and during brisk walking in southeast asian men: A randomized, double-blind, placebo-controlled, crossover study. *Nutrients* **2018**, *10*, 1732. [CrossRef] [PubMed]
215. Strauss, J.A.; Willems, M.E.; Shepherd, S.O. New Zealand blackcurrant extract enhances fat oxidation during prolonged cycling in endurance-trained females. *Eur. J. Appl. Physiol.* **2018**, *118*, 1265–1272. [CrossRef] [PubMed]
216. Lomiwes, D.; Ha, B.; Ngametua, N.; Burr, N.; Cooney, J.M.; Trower, T.; Sawyer, G.M.; Hedderley, D.I.; Hurst, R.D.; Hurst, S.M. Timed consumption of a New Zealand blackcurrant juice support positive affective responses during a self-motivated moderate walking exercise in healthy sedentary adults. *J. Int. Soc. Sports Nutr.* **2019**, *16*, 1–14. [CrossRef] [PubMed]
217. Hurst, R.D.; Lyall, K.A.; Roberts, J.M.; Perthaner, A.; Wells, R.W.; Cooney, J.M.; Jensen, D.J.; Burr, N.; Hurst, S.M. Consumption of an anthocyanin-rich extract made from new zealand blackcurrants prior to exercise may assist recovery from oxidative stress and maintains circulating neutrophil function: A pilot study. *Front. Nutr.* **2019**, *6*, 73. [CrossRef]
218. Alvarado, J.L.; Leschot, A.; Olivera-Nappa, Á.; Salgado, A.-M.; Rioseco, H.; Lyon, C.; Vigil, P. Delphinidin-rich maqui berry extract (Delphinol®) lowers fasting and postprandial glycemia and insulinemia in prediabetic individuals during oral glucose tolerance tests. *BioMed Res. Int.* **2016**, *2016*, 9070537. [CrossRef] [PubMed]
219. Di Lorenzo, A.; Sobolev, A.P.; Nabavi, S.; Sureda, A.; Moghaddam, A.; Khanjani, S.; Di Giovanni, C.; Xiao, J.; Shirooie, S.; Sokeng, A.J.T.; et al. Corrigendum to Antidepressive effects of a chemically characterized maqui berry extract (*Aristotelia chilensis* (molina) stuntz) in a mouse model of Post-stroke depression. *Food Chem. Toxicol.* **2019**, *131*, 110641. [CrossRef]
220. Rojo, L.E.; Ribnicky, D.; Logendra, S.; Poulev, A.; Rojas-Silva, P.; Kuhn, P.; Dorn, R.; Grace, M.H.; Lila, M.A.; Raskin, I. In vitro and in vivo anti-diabetic effects of anthocyanins from maqui berry (*Aristotelia chilensis*). *Food Chem.* **2011**, *131*, 387–396. [CrossRef]
221. Davinelli, S.; Bertoglio, J.C.; Zarrelli, A.; Pina, R.; Scapagnini, G. A Randomized clinical trial evaluating the efficacy of an anthocyanin–maqui berry extract (Delphinol®) on oxidative stress biomarkers. *J. Am. Coll. Nutr.* **2015**, *34*, 28–33. [CrossRef]
222. Parra-Vargas, M.; Sandoval-Rodríguez, A.S.; Rodriguez-Echevarria, R.; Dominguez-Rosales, J.A.; Santos-Garcia, A.; Armendariz-Borunda, J. Delphinidin ameliorates hepatic triglyceride accumulation in human hepg2 cells, but not in diet-induced obese mice. *Nutrients* **2018**, *10*, 1060. [CrossRef] [PubMed]
223. Corsi, R.; Mosti, G.; Cavezzi, A.; Urso, S.U.; Dimitrova, G.; Fioroni, E.; Colucci, R.; Quinzi, V. A polyphenol-based multicomponent nutraceutical in dysmetabolism and oxidative stress: Results from a pilot study. *J. Diet. Suppl.* **2017**, *15*, 34–41. [CrossRef] [PubMed]

224. Ávila, F.; Jiménez-Aspee, F.; Cruz, N.; Gómez, C.; González, M.A.; Ravello, N. Additive effect of maqui (Aristotelia chilensis) and lemon (Citrus x limon) juice in the postprandial glycemic responses after the intake of high glycemic index meals in healthy men. *NFS J.* **2019**, *17*, 8–16. [CrossRef]
225. Sandoval, V.; Femenias, A.; Martínez-Garza, Ú.; Sanz-Lamora, H.; Castagnini, J.M.; Quifer-Rada, P.; Lamuela-Raventós, R.-M.; Marrero, P.F.; Haro, D.; Relat, J. Lyophilized maqui (*Aristotelia chilensis*) berry induces browning in the subcutaneous white adipose tissue and ameliorates the insulin resistance in high fat diet-induced obese mice. *Antioxidants* **2019**, *8*, 360. [CrossRef]
226. Wang, W.; Yagiz, Y.; Buran, T.J.; Nunes, C.D.N.; Gu, L. Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Res. Int.* **2011**, *44*, 2666–2673. [CrossRef]
227. Barrett, A.; Ndou, T.; Hughey, C.A.; Straut, C.; Howell, A.; Dai, Z.; Kaletunc, G. Inhibition of α-amylase and glucoamylase by tannins extracted from cocoa, pomegranates, cranberries, and grapes. *J. Agric. Food Chem.* **2013**, *61*, 1477–1486. [CrossRef]
228. Pinto, M.D.S.; Ghaedian, R.; Shinde, R.; Shetty, K. Potential of cranberry powder for management of hyperglycemia using in vitro models. *J. Med. Food* **2010**, *13*, 1036–1044. [CrossRef]
229. Pappas, E.; Schaich, K.M. Phytochemicals of cranberries and cranberry products: Characterization, potential health effects, and processing stability. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 741–781. [CrossRef]
230. Gulcin, I.; Topal, F.; Çakmakçı, R.; Bílsel, M.; Goren, A.C.; Erdogan, U.; Cakmakci, R. Pomological features, nutritional quality, polyphenol content analysis, and antioxidant properties of domesticated and 3 wild ecotype forms of raspberries (*Rubus idaeus* L.). *J. Food Sci.* **2011**, *76*, C585–C593. [CrossRef]
231. Sparzak, B. HPLC analysis of polyphenols in the fruits of *Rubus idaeus* L. (*Rosaceae*). *Nat. Prod. Res.* **2010**, *24*, 1811–1822. [CrossRef]
232. Krauze-Baranowska, M.; Głód, D.; Kula, M.; Majdan, M.; Hałasa, R.; Matkowski, A.; Kozłowska, W.; Kawiak, A. chemical composition and biological activity of Rubus idaeus shoots—A traditional herbal remedy of Eastern Europe. *BMC Complement. Altern. Med.* **2014**, *14*, 480. [CrossRef] [PubMed]
233. Mena, P.; Tassotti, M.; Martínez, J.J.; Hernández, F.; Del Rio, D.; Sánchez-Salcedo, E.M. Phytochemical evaluation of eight white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry clones grown in Spain based on UHPLC-ESI-MSn metabolomic profiles. *Food Res. Int.* **2016**, *89*, 1116–1122. [CrossRef]
234. Natic´, M.; Dabic´, D.C˘.; Papetti, A.; Akšic´, M.M.F.; Ognjanov, V.; Ljubojevic´, M.; Tešic´, Ž.L. Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruits grown in Vojvodina, North Serbia. *Food Chem.* **2015**, *171*, 128–136. [CrossRef]
235. Jin, Q.; Yang, J.; Ma, L.; Cai, J.; Li, J. Comparison of polyphenol profile and inhibitory activities against oxidation and α-glucosidase in mulberry (*Genus Morus*) cultivars from China. *J. Food Sci.* **2015**, *80*, C2440–C2451. [CrossRef]
236. Bujor, O.-C.; Giniès, C.; Popa, V.I.; Dufour, C. Phenolic compounds and antioxidant activity of lingonberry (*Vaccinium vitis-idaea* L.) leaf, stem and fruit at different harvest periods. *Food Chem.* **2018**, *252*, 356–365. [CrossRef]
237. Ieri, F.; Martini, S.; Innocenti, M.; Mulinacci, N. Phenolic distribution in liquid preparations of Vaccinium myrtillus L. and Vaccinium vitis idaea L. *Phytochem. Anal.* **2013**, *24*, 467–475. [CrossRef]
238. Spínola, V.; Pinto, J.; Castilho, P. Hypoglycemic, anti-glycation and antioxidant in vitro properties of two Vaccinium species from Macaronesia: A relation to their phenolic composition. *J. Funct. Foods* **2018**, *40*, 595–605. [CrossRef]
239. Spínola, V.; Pinto, J.; Llorent-Martínez, E.J.; Tomás, H.; Castilho, P. Evaluation of *Rubus grandifolius* L. (wild blackberries) activities targeting management of type-2 diabetes and obesity using in vitro models. *Food Chem. Toxicol.* **2019**, *123*, 443–452. [CrossRef]
240. Aaby, K.; Ekeberg, D.; Skrede, G. Characterization of phenolic compounds in strawberry (Fragaria × ananassa) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant Capacity. *J. Agric. Food Chem.* **2007**, *55*, 4395–4406. [CrossRef]
241. Šaponjac, V.T.; Gironés-Vilaplana, A.; Djilas, S.; Mena, P.; C´etkovic´, G.; Moreno, D.A.; C˘anadanovic´-Brunet, J.; Vulic´, J.; Stajc˘ic´, S.; Vinc˘ic´, M. Chemical composition and potential bioactivity of strawberry pomace. *RSC Adv.* **2015**, *5*, 5397–5405. [CrossRef]
242. Wojdyło, A.; Nowicka, P.; Ba¸belewski, P. Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. *J. Funct. Foods* **2018**, *48*, 632–642. [CrossRef]

243. Montesano, D.; Rocchetti, G.; Cossignani, L.; Lucini, L.; Simonetti, M.S.; Blasia, F. Italian Lycium barbarum L. berry: Chemical characterization and nutraceutical value. *Nat. Prod. Commun.* **2018**, *13*. [CrossRef]
244. Schauss, A.G.; Wu, X.; Prior, R.L.; Ou, B.; Huang, D.-J.; Owens, J.; Agarwal, A.; Jensen, G.S.; Hart, A.N.; Shanbrom, E. Antioxidant capacity and other bioactivities of the freeze-dried amazonian palm berry, euterpe oleraceaemart. (Acai). *J. Agric. Food Chem.* **2006**, *54*, 8604–8610. [CrossRef] [PubMed]
245. Schauss, A.G.; Wu, X.; Prior, R.L.; Ou, B.; Patel, D.; Huang, D.-J.; Kababick, J.P. Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, euterpe oleraceaemart. (Acai). *J. Agric. Food Chem.* **2006**, *54*, 8598–8603. [CrossRef] [PubMed]
246. Pacheco-Palencia, L.A. Absorption and biological activity of phytochemical-rich extracts from açai (*Euterpe oleracea Mart*.) pulp and oil in vitro. *J. Agric. Food Chem.* **2008**, *56*, 3593–3600. [CrossRef]
247. Sosnowska, D. Inhibitory effect of black chokeberry fruit polyphenols on pancreatic lipase—Searching for most active inhibitors. *J. Funct. Foods* **2018**, *49*, 196–204. [CrossRef]
248. Anttonen, M.J.; Karjalainen, R.O. High-performance liquid chromatography analysis of black currant (*Ribes nigrum* L.) fruit phenolics grown either conventionally or organically. *J. Agric. Food Chem.* **2006**, *54*, 7530–7538. [CrossRef]
249. Borges, G.; Degeneve, A.; Mullen, W.; Crozier, A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *J. Agric. Food Chem.* **2010**, *58*, 3901–3909. [CrossRef]
250. Cespedes, C.L.; Pavon, N.; Dominguez, M.; Alarcon, J.; Balbontin, C.; Kubo, I.; El-Hafidi, M.; Avila, J.G.; Dominguez-Lopez, M. The chilean superfruit black-berry *Aristotelia chilensis* (*Elaeocarpaceae*), Maqui as mediator in inflammation-associated disorders. *Food Chem. Toxicol.* **2017**, *108*, 438–450. [CrossRef]
251. Escribano-Bailón, M.T.; Alcalde-Eon, C.; Muñoz, O.; Rivas-Gonzalo, J.C.; Santos-Buelga, C. Anthocyanins in berries of Maqui [*Aristotelia chilensis* (Mol.) Stuntz]. *Phytochem. Anal.* **2005**, *17*, 8–14. [CrossRef]
252. Häkkinen, S.H.; Kärenlampi, S.O.; Mykkänen, H.M.; Törrönen, A.R. Influence of domestic processing and storage on flavonol contents in berries. *J. Agric. Food Chem.* **2000**, *48*, 2960–2965. [CrossRef] [PubMed]
253. Ferrier, J.; Djeffal, S.; Morgan, H.P.; Kloet, S.P.V.; Redžic´, S.; Cuerrier, A.; Balick, M.J.; Arnason, J.T. Antiglycation activity of *Vaccinium* spp. (*Ericaceae*) from the Sam Vander Kloet collection for the treatment of type II diabetes1This article is part of a Special Issue entitled 'A tribute to Sam Vander Kloet FLS: Pure and applied research from blueberries to heathland ecology'. *Botany* **2012**, *90*, 401–406. [CrossRef]
254. Buchert, J.; Koponen, J.M.; Suutarinen, M.; Mustranta, A.; Lille, M.E.; Törrönen, R.; Poutanen, K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. *J. Sci. Food Agric.* **2005**, *85*, 2548–2556. [CrossRef]
255. Cermak, R.; Landgraf, S.; Wolffram, S. Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. *Br. J. Nutr.* **2004**, *91*, 849–855. [CrossRef]
256. Morazzoni, P.; Livio, S.; Scilingo, A.; Malandrino, S. Vaccinium myrtillus anthocyanosides pharmacokinetics in rats. *Arzneimittelforschung* **1991**, *41*, 128–131.
257. Liu, H.; Liu, H.; Wang, W.; Khoo, C.; Taylor, J.; Gu, L. Cranberry phytochemicals inhibit glycation of human hemoglobin and serum albumin by scavenging reactive carbonyls. *Food Funct.* **2011**, *2*, 475. [CrossRef] [PubMed]
258. Podse¸dek, A.; Majewska, I.; Redzynia, M.; Sosnowska, D.; Koziolkiewicz, M. In vitro inhibitory effect on digestive enzymes and antioxidant potential of commonly consumed fruits. *J. Agric. Food Chem.* **2014**, *62*, 4610–4617. [CrossRef] [PubMed]
259. Sun, J.; Liu, W.; Ma, H.; Marais, J.P.J.; Khoo, C.; Dain, J.A.; Rowley, D.C.; Seeram, N.P. Effect of cranberry (*Vaccinium macrocarpon*) oligosaccharides on the formation of advanced glycation end-products. *J. Berry Res.* **2016**, *6*, 149–158. [CrossRef] [PubMed]
260. Wilson, T.; Bauer, B.A. Advising consumers about dietary supplements: Lessons from cranberry products. *J. Diet. Suppl.* **2009**, *6*, 377–384. [CrossRef] [PubMed]
261. Kaur, G.; Rao, L.V.M.; Agrawal, A.; Pendurthi, U.R. Effect of wine phenolics on cytokine-induced C-reactive protein expression. *J. Thromb. Haemost.* **2007**, *5*, 1309–1317. [CrossRef]
262. Marathe, P.H.; Gao, H.X.; Close, K.L. American diabetes association standards of medical care in diabetes 2017. *J. Diabetes* **2017**, *9*, 320–324. [CrossRef] [PubMed]
263. Grussu, D.; Stewart, D.; McDougall, G.J. Berry polyphenols inhibit α-amylasein Vitro: Identifying active components in rowanberry and raspberry. *J. Agric. Food Chem.* **2011**, *59*, 2324–2331. [CrossRef] [PubMed]

264. Tsuda, T.; Ueno, Y.; Yoshikawa, T.; Kojo, H.; Osawa, T. Microarray profiling of gene expression in human adipocytes in response to anthocyanins. *Biochem. Pharmacol.* **2006**, *71*, 1184–1197. [CrossRef] [PubMed]
265. Al-Salmi, F.A. Ameliorative and synergistic effect of red raspberry and lycopene against hepatotoxicity induced by acrylamide in male mice. *Int. J. Pharmacol.* **2019**, *15*, 166–176. [CrossRef]
266. Zou, T.; Kang, Y.; Wang, B.; De Avila, J.M.; You, J.; Zhu, M.-J.; Du, M. Raspberry supplementation reduces lipid accumulation and improves insulin sensitivity in skeletal muscle of mice fed a high-fat diet. *J. Funct. Foods* **2019**, *63*, 103572. [CrossRef]
267. Park, K.S. Raspberry ketone, a naturally occurring phenolic compound, inhibits adipogenic and lipogenic gene expression in 3T3-L1 adipocytes. *Pharm. Biol.* **2014**, *53*, 870–875. [CrossRef]
268. Lee, H.; Kim, J.; An, J.; Lee, S.; Kong, H.; Song, Y.; Choi, H.R.; Lee, S.J.; Chae, K.S.; Kwon, J.W.; et al. Amelioration of hyperglycemia by Rubus occidentalis (*black raspberry*) and increase in short-chain fatty acids producing bacteria. *J. Funct. Foods* **2019**, *54*, 433–439. [CrossRef]
269. Yan, F.; Chen, Y.; Azat, R.; Zheng, X. Mulberry anthocyanin extract ameliorates oxidative damage in HepG2 cells and prolongs the lifespan of caenorhabditis elegans through MAPK and Nrf2 pathways. *Oxidative Med. Cell. Longev.* **2017**, *2017*, 1–12. [CrossRef]
270. He, X.; Fang, J.; Ruan, Y.; Wang, X.; Sun, Y.; Wu, N.; Zhao, Z.; Chang, Y.; Ning, N.; Guo, H.; et al. Structures, bioactivities and future prospective of polysaccharides from Morus alba (*white mulberry*): A review. *Food Chem.* **2018**, *245*, 899–910. [CrossRef]
271. He, H.; Yu, W.G.; Yang, J.P.; Ge, S.; Lu, Y.H. Multiple Comparisons of glucokinase activation mechanisms of five mulberry bioactive ingredients in hepatocyte. *J. Agric. Food Chem.* **2015**, *64*, 2475–2484. [CrossRef]
272. Yan, F.; Dai, G.; Zheng, X. Mulberry anthocyanin extract ameliorates insulin resistance by regulating PI3K/AKT pathway in HepG2 cells and db/db mice. *J. Nutr. Biochem.* **2016**, *36*, 68–80. [CrossRef] [PubMed]
273. Wang, Y.; Xiang, L.; Wang, C.; Tang, C.; He, X. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. *PLoS ONE* **2013**, *8*, e71144. [CrossRef] [PubMed]
274. Zhang, H.; Ma, Z.F.; Luo, X.; Li, X. Effects of mulberry fruit (*Morus alba* L.) Consumption on health outcomes: A mini-review. *Antioxidants* **2018**, *7*, 69. [CrossRef] [PubMed]
275. Hu, D.; Bao, T.; Lu, Y.; Su, H.; Ke, H.; Chen, W. Polysaccharide from Mulberry Fruit (*Morus alba* L.) Protects against palmitic-acid-induced hepatocyte lipotoxicity by activating the Nrf2/ARE signaling pathway. *J. Agric. Food Chem.* **2019**. [CrossRef]
276. Harbilas, D.; Martineau, L.C.; Harris, C.S.; Adeyiwola-Spoor, D.C.; Saleem, A.; Lambert, J.; Caves, D.; Johns, T.; Prentki, M.; Cuerrier, A.; et al. Evaluation of the antidiabetic potential of selected medicinal plant extracts from the Canadian boreal forest used to treat symptoms of diabetes: Part II. *Can. J. Physiol. Pharmacol.* **2009**, *87*, 479–492. [CrossRef]
277. Ross, K.A.; Godfrey, D.; Fukumoto, L. The chemical composition, antioxidant activity and -glucosidase inhibitory activity of water-extractable polysaccharide conjugates from northern Manitoba lingonberry. *Cogent Food Agric.* **2015**, *1*. [CrossRef]
278. Quek, R.; Henry, C.J. Influence of polyphenols from lingonberry, cranberry, and red grape onin vitrodigestibility of rice. *Int. J. Food Sci. Nutr.* **2015**, *66*, 378–382. [CrossRef]
279. Beaulieu, L.-P.; Harris, C.S.; Saleem, A.; Cuerrier, A.; Haddad, P.S.; Martineau, L.C.; Bennett, S.A.; Arnason, J.T. Inhibitory effect of the cree traditional medicine wiishichimanaanh (*Vaccinium vitis-idaea*) on advanced glycation endproduct formation: Identification of active principles. *Phytotherapy Res.* **2009**, *24*, 741–747. [CrossRef]
280. Kylli, P.; Nohynek, L.; Puupponen-Pimia, R.; Westerlund-Wikström, B.; Leppanen, T.; Welling, J.; Moilanen, E.; Heinonen, M. Lingonberry (*Vaccinium vitis-idaea*) and European cranberry (*Vaccinium microcarpon*) proanthocyanidins: Isolation, identification, and bioactivities. *J. Agric. Food Chem.* **2011**, *59*, 3373–3384. [CrossRef]
281. Kowalska, K.; Olejnik, A.; Zielińska-Wasielica, J.; Olkowicz, M. Inhibitory effects of lingonberry (*Vaccinium vitis-idaea* L.) fruit extract on obesity-induced inflammation in 3T3-L1 adipocytes and RAW 264.7 macrophages. *J. Funct. Foods* **2019**, *54*, 371–380. [CrossRef]
282. Riihinen, K.; Kamal-Eldin, A.; Mattila, P.; González-Paramás, A.M.; Törrönen, A.R. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J. Agric. Food Chem.* **2004**, *52*, 4477–4486. [CrossRef] [PubMed]

283. Hildebrandt, M.A.; Hoffmann, C.; Sherrill–Mix, S.A.; Keilbaugh, S.A.; Hamady, M.; Chen, Y.; Knight, R.; Ahima, R.S.; Bushman, F.D.; Wu, G.D.; et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* **2009**, *137*, 1716–1724. [CrossRef] [PubMed]
284. Krych, L.; Nielsen, D.; Hansen, A.; Hansen, C. Gut microbial markers are associated with diabetes onset, regulatory imbalance, and IFN-γ level in NOD Mice. *Gut Microbes* **2015**, *6*, 101–109. [CrossRef] [PubMed]
285. Kameyama, K.; Itoh, K. Intestinal Colonization by a Lachnospiraceae Bacterium Contributes to the Development of Diabetes in Obese Mice. *Microbes Environ.* **2014**, *29*, 427–430. [CrossRef] [PubMed]
286. Everard, A.; Belzer, C.; Geurts, L.; Ouwerkerk, J.P.; Druart, C.; Bindels, L.B.; Guiot, Y.; Derrien, M.; Muccioli, G.G.; Delzenne, N.M.; et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 9066–9071. [CrossRef] [PubMed]
287. Sarkar, D.; Orwat, J.; Hurburt, T.; Woods, F.; Pitts, J.A.; Shetty, K. Evaluation of phenolic bioactive-linked functionality of blackberry cultivars targeting dietary management of early stages type-2 diabetes using in vitro models. *Sci. Hortic.* **2016**, *212*, 193–202. [CrossRef]
288. Sancho, R.A.S.; Pastore, G.M. Evaluation of the effects of anthocyanins in type 2 diabetes. *Food Res. Int.* **2012**, *46*, 378–386. [CrossRef]
289. Boath, A.S.; Grussu, D.; Stewart, D.; McDougall, G.J. Berry polyphenols inhibit digestive enzymes: A source of potential health benefits? *Food Dig.* **2012**, *3*, 1–7. [CrossRef]
290. Gowd, V.; Bao, T.; Chen, W. Antioxidant potential and phenolic profile of blackberry anthocyanin extract followed by human gut microbiota fermentation. *Food Res. Int.* **2018**, *120*, 523–533. [CrossRef]
291. Solverson, P.; Rumpler, W.V.; Leger, J.L.; Redan, B.W.; Ferruzzi, M.; Baer, D.J.; Castonguay, T.W.A.; Novotny, J. Blackberry feeding increases fat oxidation and improves insulin sensitivity in overweight and obese males. *Nutrients* **2018**, *10*, 1048. [CrossRef]
292. Williamson, G. Possible effects of dietary polyphenols on sugar absorption and digestion. *Mol. Nutr. Food Res.* **2012**, *57*, 48–57. [CrossRef] [PubMed]
293. Giampieri, F.; Forbes-Hernández, T.Y.; Gasparrini, M.; Alvarez-Suarez, J.M.; Afrin, S.; Bompadre, S.; Quiles, J.L.; Mezzetti, B.; Battino, M. Strawberry as a health promoter: An evidence based review. *Food Funct.* **2015**, *6*, 1386–1398. [CrossRef] [PubMed]
294. Zhu, Q. In vitro bioactivities and phytochemical profile of various parts of the strawberry (Fragaria × ananassa var. Amaou). *J. Funct. Foods* **2015**, *13*, 38–49. [CrossRef]
295. Manzano, S.; Williamson, G. Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Mol. Nutr. Food Res.* **2010**, *54*, 1773–1780. [CrossRef]
296. da Silva Pinto, M. Evaluation of antiproliferative, anti-Type 2 Diabetes, and antihypertension potentials of ellagitannins from strawberries (Fragaria × ananassa Duch.) using in vitro models. *J. Med. Food* **2010**, *13*, 1027–1035. [CrossRef]
297. Forbes-Hernández, T.Y.; Giampieri, F.; Gasparrini, M.; Afrin, S.; Mazzoni, L.; Cordero, M.D.; Mezzetti, B.; Quiles, J.L.; Battino, M. Lipid accumulation in HepG2 cells is attenuated by strawberry extract through AMPK activation. *Nutrients* **2017**, *9*, 621. [CrossRef]
298. Basu, A.; Fu, D.X.; Wilkinson, M.; Simmons, B.; Wu, M.; Betts, N.M.; Du, M.; Lyons, T.J. Strawberries decrease atherosclerotic markers in subjects with metabolic syndrome. *Nutr. Res.* **2010**, *30*, 462–469. [CrossRef]
299. Pajk, T.; Rezar, V.; Levart, A.; Salobir, J. Efficiency of apples, strawberries, and tomatoes for reduction of oxidative stress in pigs as a model for humans. *Nutrients* **2006**, *22*, 376–384. [CrossRef]
300. Kulczyński, B.; Gramza-Michałowska, A. Goji berry (*Lycium barbarum*): Composition and health effects—A review. *Pol. J. Food Nutr. Sci.* **2016**, *66*, 67–75. [CrossRef]
301. Jin, M.; Huang, Q.; Zhao, K.; Shang, P. Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. *Int. J. Biol. Macromol.* **2013**, *54*, 16–23. [CrossRef]
302. Zou, P.; Yang, X.; Huang, W.W.; Zhao, H.; Wang, J.; Xu, R.B.; Hu, X.L.; Shen, S.Y.; Qin, D. Characterization and bioactivity of polysaccharides obtained from pine cones of pinus koraiensis by graded ethanol precipitation. *Molecules* **2013**, *18*, 9933–9948. [CrossRef] [PubMed]
303. Tang, H.L.; Chen, C.; Wang, S.K.; Sun, G.J. Biochemical analysis and hypoglycemic activity of a polysaccharide isolated from the fruit of *Lycium barbarum* L. *Int. J. Biol. Macromol.* **2015**, *77*, 235–242. [CrossRef] [PubMed]
304. Tang, X.; Shen, T.; Jiang, X.; Xia, M.; Sun, X.; Guo, H.; Ling, W. Purified anthocyanins from bilberry and black currant attenuate hepatic mitochondrial dysfunction and steatohepatitis in mice with methionine and choline deficiency. *J. Agric. Food Chem.* **2015**, *63*, 552–561. [CrossRef] [PubMed]

305. Ding, Y.; Yan, Y.; Peng, Y.; Chen, D.; Mi, J.; Lu, L.; Luo, Q.; Li, X.; Zeng, X.; Cao, Y. In vitro digestion under simulated saliva, gastric and small intestinal conditions and fermentation by human gut microbiota of polysaccharides from the fruits of *Lycium barbarum*. *Int. J. Biol. Macromol.* **2019**, *125*, 751–760. [CrossRef]
306. Girones-Vilaplana, A.; Villaño, D.; Moreno, D.A.; García-Viguera, C. New isotonic drinks with antioxidant and biological capacities from berries (maqui, açaí and blackthorn) and lemon juice. *Int. J. Food Sci. Nutr.* **2013**, *64*, 897–906. [CrossRef]
307. Cheguru, P.; Chapalamadugu, K.C.; Doumit, M.E.; Murdoch, G.K.; Hill, R.A. Adipocyte differentiation-specific gene transcriptional response to C18 unsaturated fatty acids plus insulin. *Pflugers Arch.* **2012**, *463*, 429–447. [CrossRef]
308. Costa, A.G.V.; Garcia-Diaz, D.F.; Jimenez, P.; Silva, P.I. Bioactive compounds and health benefits of exotic tropical red–black berries. *J. Funct. Foods* **2013**, *5*, 539–549. [CrossRef]
309. Karpac, J.; Jasper, H. Insulin and JNK: Optimizing metabolic homeostasis and lifespan. *Trends Endocrinol. Metab.* **2009**, *20*, 100–106. [CrossRef]
310. Smith, A.C.; Mullen, K.L.; Junkin, K.A.; Nickerson, J.; Chabowski, A.; Bonen, A.; Dyck, D.J. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet-induced hyperglycemia. *Am. J. Physiol. Metab.* **2007**, *293*, E172–E181. [CrossRef]
311. Umpierrez, G.E.; Bailey, T.S.; Carcia, D.; Shaefer, C.; Shubrook, J.H.; Skolnik, N. Improving postprandial hyperglycemia in patients with type 2 diabetes already on basal insulin therapy: Review of current strategies. *J. Diabetes* **2017**, *10*, 94–111. [CrossRef]
312. Oyama, L.M.; Silva, F.; Carnier, J.; De Miranda, D.A.; Santamarina, A.B.; Ribeiro, E.B.; Nascimento, C.M.O.D.; De Rosso, V.V. Juçara pulp supplementation improves glucose tolerance in mice. *Diabetol. Metab. Syndr.* **2016**, *8*, 8. [CrossRef] [PubMed]
313. Machado, A.K.; Cadoná, F.C.; Assmann, C.E.; Andreazza, A.C.; Duarte, M.M.M.F.; Branco, C.D.S.; Zhou, X.; De Souza, D.V.; Ribeiro, E.E.; Da Cruz, I.B.M. Açaí (*Euterpe oleracea Mart.*) has anti-inflammatory potential through NLRP3-inflammasome modulation. *J. Funct. Foods* **2019**, *56*, 364–371. [CrossRef]
314. Martino, H.S.D.; Dias, M.M.D.S.; Noratto, G.; Talcott, S.; Mertens-Talcott, S.U. Anti-lipidaemic and anti-inflammatory effect of açai (*Euterpe oleracea Martius*) polyphenols on 3T3-L1 adipocytes. *J. Funct. Foods* **2016**, *23*, 432–443. [CrossRef]
315. Du, X.; Myracle, A.D. Fermentation alters the bioaccessible phenolic compounds and increases the alpha-glucosidase inhibitory effects of aronia juice in a dairy matrix followingin vitrodigestion. *Food Funct.* **2018**, *9*, 2998–3007. [CrossRef]
316. Rugina, D.; Diaconeasa, Z.M.; Isvoranu, C.; Bunea, A.; Socaciu, C.; Pintea, A. Chokeberry Anthocyanin Extract as Pancreaticβ-Cell Protectors in Two Models of Induced Oxidative Stress. *Oxidative Med. Cell. Longev.* **2015**, *2015*, 1–10. [CrossRef]
317. Iwashima, T.; Kudome, Y.; Kishimoto, Y.; Saita, E.; Tanaka, M.; Taguchi, C.; Hirakawa, S.; Mitani, N.; Kondo, K.; Iida, K. Aronia berry extract inhibits TNF-α-induced vascular endothelial inflammation through the regulation of STAT3. *Food Nutr. Res.* **2019**, *63*, 3361. [CrossRef]
318. Zapolska-Downar, D.; Bryk, D.; Małecki, M.; Hajdukiewicz, K.; Sitkiewicz, D. Aronia melanocarpa fruit extract exhibits anti-inflammatory activity in human aortic endothelial cells. *Eur. J. Nutr.* **2011**, *51*, 563–572. [CrossRef]
319. Törrönen, R.; Sarkkinen, E.; Tapola, N.; Hautaniemi, E.; Kilpi, K.; Niskanen, L. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. *Br. J. Nutr.* **2009**, *103*, 1094–1097. [CrossRef]
320. Xu, Y.; Niu, X.; Liu, N.; Gao, Y.; Wang, L.; Xu, G.; Li, X.; Yang, Y. Characterization, antioxidant and hypoglycemic activities of degraded polysaccharides from blackcurrant (*Ribes nigrum* L.) fruits. *Food Chem.* **2017**, *243*, 26–35. [CrossRef]
321. Hossain, A.K.M.M.; Mason, S.L.; Guo, X.-B.; Brennan, C.R. The combined effect of blackcurrant powder and wholemeal flours to improve health promoting properties of cookies. *Plant. Foods Hum. Nutr.* **2017**, *72*, 280–287. [CrossRef]
322. Cespedes, C.L.; Valdez-Morales, M.; Avila, J.G.; El-Hafidi, M.; Alarcon, J.; Paredes-López, O. Phytochemical profile and the antioxidant activity of Chilean wild black-berry fruits, Aristotelia chilensis (Mol) Stuntz (*Elaeocarpaceae*). *Food Chem.* **2010**, *119*, 886–895. [CrossRef]

323. Brauch, J.E.; Reuter, L.; Conrad, J.; Vogel, H.; Schweiggert, R.; Carle, R. Characterization of anthocyanins in novel Chilean maqui berry clones by HPLC–DAD–ESI/MSn and NMR-spectroscopy. *J. Food Compos. Anal.* **2017**, *58*, 16–22. [CrossRef]
324. Rubilar, M.; Jara, C.; Poo, Y.; Acevedo, F.; Gutiérrez, C.; Sineiro, J.; Shene, C. Extracts of Maqui (*Aristotelia chilensis*) and Murta (*Ugni molinae Turcz.*): Sources of antioxidant compounds and α-glucosidase/α-amylase inhibitors. *J. Agric. Food Chem.* **2011**, *59*, 1630–1637. [CrossRef] [PubMed]
325. Reyes-Farias, M.; Vasquez, K.; Fuentes, F.; Ovalle-Marin, A.; Parra-Ruiz, C.; Zamora, O.; Pino, M.; Quitral, V.; Jimenez, P.; Garcia, L.; et al. Extracts of Chilean native fruits inhibit oxidative stress, inflammation and insulin-resistance linked to the pathogenic interaction between adipocytes and macrophages. *J. Funct. Foods* **2016**, *27*, 69–83. [CrossRef]
326. Schreckinger, M.E.; Wang, J.; Yousef, G.; Lila, M.A.; De Mejia, E.G. Antioxidant capacity and in vitro inhibition of adipogenesis and inflammation by phenolic extracts of vaccinium floribundum and Aristotelia chilensis. *J. Agric. Food Chem.* **2010**, *58*, 8966–8976. [CrossRef]
327. Hidalgo, J. Delphinidin reduces glucose uptake in mice jejunal tissue and human intestinal cells lines through FFA1/GPR40. *Int. J. Mol. Sci.* **2017**, *18*, 750. [CrossRef]
328. Christensen, M.; Calanna, S.; Sparre-Ulrich, A.H.; Kristensen, P.L.; Rosenkilde, M.M.; Faber, J.; Purrello, F.; Van Hall, G.; Holst, J.J.; Vilsbøll, T.; et al. Glucose-dependent insulinotropic polypeptide augments glucagon responses to hypoglycemia in Type 1 Diabetes. *Diabetes* **2014**, *64*, 72–78. [CrossRef]
329. Miranda-Rottmann, S.; Aspillaga, A.A.; Pérez, D.D.; Vásquez, L.; Martinez, A.L.F.; Leighton, F. Juice and Phenolic Fractions of the BerryAristotelia chilensisInhibit LDL Oxidationin vitroand protect human endothelial cells against oxidative stress. *J. Agric. Food Chem.* **2002**, *50*, 7542–7547. [CrossRef]

3) **Hameed, Ahsan,** Edyta Adamska-Patruno, Joanna Godzien, Przemyslaw Czajkowski, Urszula Miksza, Karolina Pietrowska, Joanna Fiedorczuk, Monika Moroz, Witold Bauer, Julia Sieminska, Maria Górska, Adam Jacek Krętowski, and Michal Ciborowski. 2022. "The Beneficial Effect of Cinnamon and Red Capsicum Intake on Postprandial Changes in Plasma Metabolites Evoked by a High-Carbohydrate Meal in Men with Overweight/Obesity" *Nutrients* 14, no. 20: 4305. https://doi.org/10.3390/nu14204305

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Article

The Beneficial Effect of Cinnamon and Red Capsicum Intake on Postprandial Changes in Plasma Metabolites Evoked by a High-Carbohydrate Meal in Men with Overweight/Obesity

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Abstract: The relationship of high-carbohydrate (HC) meal intake to metabolic syndrome is still not fully explained. Metabolomics has the potential to indicate metabolic pathways altered by HC meals, which may improve our knowledge regarding the mechanisms by which HC meals may contribute to metabolic syndrome development. The fasting and postprandial metabolic response to HC or normo-carbohydrate (NC) meals with/without cinnamon + capsicum intake was evaluated using untargeted metabolomics and compared between normal-weight (NW) and overweight/obese (OW/OB) healthy men. Healthy male participants (age-matched) were divided into two groups (12 subjects per group). One was composed of men with normal weight (NW) and the other of men with overweight/obesity (OW/OB). On separate visits (with 2–3 week intervals), the participants received standardized HC or NC meals (89% or 45% carbohydrates, respectively). Fasting (0 min) and postprandial (30, 60, 120, 180 min) blood were collected for untargeted plasma metabolomics. Based on each metabolic feature's intensity change in time, the area under the curve (AUC) was calculated. Obtained AUCs were analyzed using multivariate statistics. Several metabolic pathways were found dysregulated after an HC meal in people from the OW/OB group but not the NW group. The consumption of HC meals by people with overweight/obesity led to a substantial increase in AUC, mainly for metabolites belonging to phospholipids and fatty acid amides. The opposite was observed for selected sphingolipids. The intake of cinnamon and capsicum normalized the concentration of selected altered metabolites induced by the intake of HC meals. A HC meal may induce an unfavourable postprandial metabolic response in individuals with overweight/obesity, and such persons should avoid HC meals.

Keywords: high-carbohydrate meal; cinnamon; capsicum; plasma metabolomics; overweight; obesity

1. Introduction

The world's obesity statistical data factsheet clearly shows that the global popula-tion with obesity has increased three-fold since 1975. There are 1.9 billion adults with overweight, and among them, 650 million with obesity [1]. Obesity is a multifactorial epidemic; however, the perceived energy imbalance between calories consumed and calo-ries expended is considered a major reason for weight gain. The presence of obesity is

a serious risk factor for type 2 diabetes mellitus (T2DM) development. Both conditions heavily impact the quality of human life [2]. Dietary intervention is considered the frontline approach for circumventing weight gain.

Since the beginning of the last decade, the dietary recommendations of macronutrient intake have been changing enormously for patients with overweight/obesity and/or T2DM. Initially, a low-calorie diet (LCD) comprising mainly fats with a limited carbohydrate restriction was considered ideal. This changed later to promotion of a high-carbohydrate diet (HCD) and low fat consumption following the recognition of the role of diabetes in developing cardiovascular disease [3]. Reducing plasma cholesterol level with a negligible increase in the fasting plasma glucose (FPG) was the rationale for advocating HCD with low-fat content [4]. However, the following of such diets failed to limit the incidences of metabolic syndrome, i.e., obesity, insulin resistance, and T2DM, which ultimately compelled the reconsideration of LCDs. Therefore, a high-fat diet (HFD) with low carbohydrate content is again gaining ground for the general reduction of/control over blood glucose level and body weight [3]. Numerous studies have been reported in the literature unveiling the effect of consuming HCD/LCD for humans [5,6]. All these intervention studies report comparable results of reduction in mean body weight, glycated haemoglobin (HbA1c), low-density lipoprotein cholesterol (LDL), insulin, high-density lipoprotein cholesterol (HDL), and fasting plasma glucose after HCD or LCD intake [5,6].

Excepting the different composition of macronutrients in food, there is an increased interest in the use of plant-based foods and dietary components for both healthy and vulner-able population groups [7]. Among them, cinnamon and capsicum are two of the most-used traditional plant-based spices that not only improve the physical cues of food but also grant favourable healthy effects to their consumers. Cinnamon has been extensively studied in the literature due to its therapeutic applications. Accumulated numbers of studies have demonstrated that cinnamon intake improves glycemic control, hyperlipidemia and insulin resistance by altering the expression of key regulatory genes in glucose-lipid metabolism pathways and reducing the production of proinflammatory prostaglandins, interleukins, and nitric oxides [8]. Similarly, capsicum, or chili pepper, is consumed routinely as a hot spice and mainly composed of capsaicin, capsiate, and other analogous bioactive agents such as capsinoids, dihydrocapsiate, and nordihydrocapsiate. With some exceptions, these compounds possess anti-obesity/anti-diabetic properties and could be new target com-pounds for the therapy and prevention of those diseases [9]. A recent metanalysis reported that capsicum intake significantly promoted satiety, energy expenditure, negative-energy balance, and fat oxidation, and improved glucose metabolism by increasing insulin sensi-tivity and adiponectin levels in prediabetic, diabetic, and gestational diabetic patients [10]. Moreover, capsicum or its phytocompounds attenuated the weight-gain and increase in LDL-cholesterol following the intake of high-fat/high-carbohydrate diets (HFD/HCD) and improved the plasma markers of glucose/lipid metabolism, obesity related systematic inflammation, and gut epithelial barrier function [11].

Evaluation of postprandial changes in plasma metabolites can show which metabolic pathways are modulated in the response to provided food. Such a short-term change can be useful to understand the long-term consequences of a diet rich in particular nutrients. Therefore, in the present study we used untargeted metabolomics to evaluate the short-term changes in plasma metabolites evoked by a single meal. In the first part of this study, we evaluated the short-term metabolic response of people with normal weight (NW) or with overweight/obesity (OW/OB) to high-carbohydrate (HC) and normo-carbohydrate (NC) meals. We observed postprandial differences in metabolite levels between subjects with OW/OB and NW after HC but not NC meals. Therefore, to evaluate the potential benefits of cinnamon/capsicum, in the second part of the study individuals with OW/OB received a HC meal with a cinnamon and capsicum capsule or a placebo capsule.

2. Materials and Methods

2.1. Ethics

This trial was registered at www.clinicaltrials.gov (accessed on 21 February 2021) as NCT03792685. The study protocols were approved by the local Ethics Committee (Medical University of Bialystok, Bialystok, Poland, R-I-002/35/2009 and R-I-002/314/2018), and before any study procedures, all of the participants signed informed consent. The study procedures were conducted following the ethical standards of human experimentation and the Declaration of Helsinki.

2.2. Participants and Study Design

The volunteers for these studies were of Polish-Caucasian origin. Only males were enrolled on the meal-challenge tests because of the possible sexual dimorphism of inves-tigated factors [12]. None of the participants suffered from T2DM or prediabetes. No other disorders or any treatments that might affect the results were reported. Subjects who followed any special diet or dietary pattern (vegetarian, high-fat, etc.) were excluded from the study. Participants were instructed to maintain their regular lifestyle throughout the study and to avoid alcohol, coffee, and excessive physical activity for at least one day before each test. During the meal test, men stayed in bed, in a quiet room with thermoneutral conditions (22–25 C).

Participants of the first study (n = 24), depending on BMI criteria, were classified as the OW/OB group (BMI > 25, n = 12) or the NW group (BMI < 25, n = 12). They took part in two meal-challenge test visits in a crossover design with 2–3 week intervals. Some of the participants refused to take part in both meal-challenge tests; therefore, all participants underwent a meal-challenge test with a HC meal and 18 with a NC meal. After fasting blood collection, subjects received a standardized HC meal (300 mL, Nutridrink Juice Style, Fat Free, Nutricia, Poland), providing 450 kcal (89% of energy from carbohydrate, 11% from protein, and 0% from fat), or NC meal (360 mL, Cubitan, Nutricia, Poland), providing 450 kcal (45% of energy from carbohydrate, 30% from protein, and 25% from fat).

In the second study, 20 individuals with OW/OB were enrolled into a double-blind, placebo-controlled trial consisting of two visits during which they received a HC meal with the capsule containing 2 g of cinnamon (Cinnamomum verum, Dary Natury Pvt., Ltd., Koryciny, Poland) and 200 mg of capsicum (Capsicum annum, Organic Cayenne Pepper ground, Lebensbaum, Diepholz, Germany) or with the placebo capsule (composed of maltodextrin only). Between the visits there was a wash-over period of around 1–3 weeks. The meal was composed of wheat roll (100 g), fruit jam (50 g), and juice (200 mL).

Graphical presentation of the study design is presented in Figure 1. The clinical and anthropometric characteristics of participants are shown in Table 1.

2.3. Study Procedures

At the screening visit, the demographic data and anthropometric measurements, body weight, body composition analysis, and blood collections for biochemical analyses were performed as described previously [13]. The meal-challenge test visits were conducted as described previously [14]. The metabolomic analyses were performed on plasma samples from the blood collected at fasting and 30, 60, 120, and 180 min after a meal.

2.4. Metabolomis Analysis

The metabolomic analyses were performed as described previously [15]. Briefly, metabolic fingerprinting was performed on an HPLC system (1290 Infinity, Agilent Tech-nologies, Santa Clara, CA, USA) coupled to an iFunnel Q-TOF (6550, Agilent Technologies, Santa Clara, CA, USA) mass spectrometer. Plasma samples were prepared and analyzed following previously described protocols [16]. The details of metabolomic data treatment as well as other calculations, statistical analyses, metabolite identification and pathway analysis are presented in the supplementary information file.

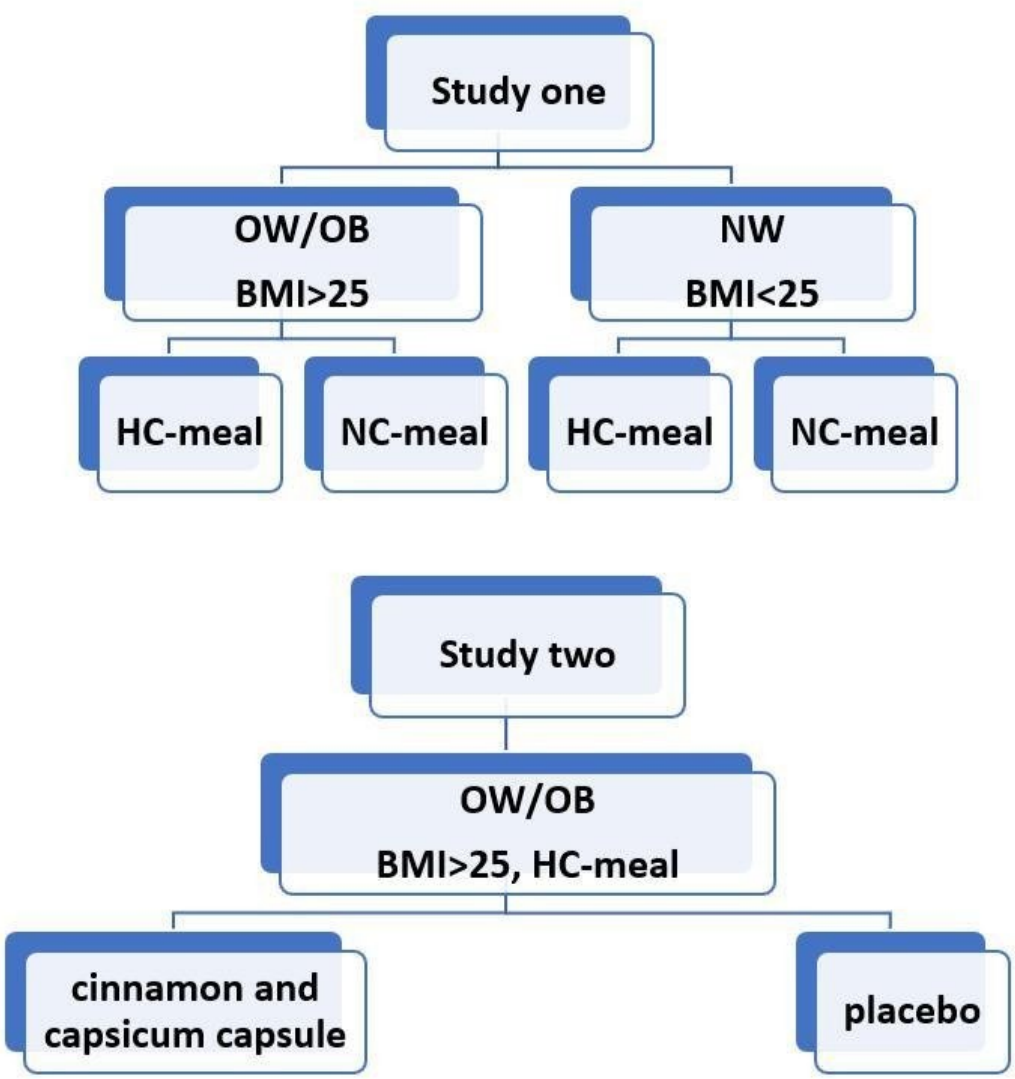

Figure 1. A graphical presentation of the study design.OW/OB: overweight/obese, BMI: body mass index, NW: normal weight, HC: high-carbohydrate, NC: normo-carbohydrate.

Table 1. Baseline characteristics of studied individuals.

Anthropometric Parameters	HC/NC Meal Study			Cinnamon/Capsicum Study	*p*-Value * for Comparison with OW/OB Group
	OW/OB	NW	*p*-Value *		
Age [years]	37.8 ± 6.3	35.3 ± 8.6	0.4	46 ± 8.4	0.02
BMI [kg/m^2]	30.8 ± 5.4	23.8 ± 1.6	0.0009	32.0 ± 4.3	0.2
Body Fat contents [%]	28.0 ± 6.3	17.0 ± 5.3	0.0001	31.4 ± 4.1	0.2
Fat free mass [%]	69.8 ± 12.2	66.3 ± 6.6	0.4	67.9 ± 7.5	0.6
WHR	0.998 ± 0.06	0.91 ± 0.06	0.003	1.03 ± 0.05	0.1
Fasting glucose concentration [mg/dL]	87.9 ± 5.8	84.2 ± 8.1	0.2	101.05 ± 8.98	0.0002
Fasting Insulin concentration [IU/mL]	12.7 ± 9.3	6.5 ± 1.7	0.06	13.8 ± 4.5	0.2
HOMA–IR	2.8 ± 2.1	1.3 ± 0.3	0.05	3.46 ± 1.2	0.05
HOMA–β	186.3 ± 121.1	157.5 ± 150.4	0.6	136.32 ± 56.3	0.4
HbA1c	5.3 ± 0.3	5.2 ± 0.3	0.3	5.4 ± 0.3	0.9

* For quantitative variables with normal distribution, the parametric *t*-test was used; for the other variables, the non-parametric Mann–Whitney test was applied. The data are represented as the mean ± STD, and *p*-values < 0.05 were considered significant. HC: high-carbohydrate, NC: normo-carbohydrate, OW/OB: individuals with overweight/obesity, NW: individuals with normal weight BMI: body mass index, WHR: waist-hip ratio, HOMA–IR: Homeostatic Model Assessment of Insulin Resistance, HOMA–β: Homeostatic Model Assessment of β–cell function, HbA1c: glycated haemoglobin.

3. Results

3.1. Baseline Characteristics of Subjects

Table 1 shows the baseline characteristics of the studied groups. The OW/OB group showed a greater mean BMI, body fat content, fat-free mass, and waist-hip ratio (WHR) than the NW group. The fasting glucose level was also higher for the OW/OB group. Moreover, the fasting insulin concentration and HOMA-IR values were almost double for the OW/OB group than the NW group. Participants with overweight/obesity taking part in the cinnamon/capsicum study were significantly older, had higher fasting plasma glucose and were more insulin resistant than those taking part in the NC/HC meal- challenge study. These results showed that people with overweight/obesity can be insulin resistant and are more prone to T2DM development.

3.2. Metabolomic Analyses

PLS-DA models were obtained to classify the patients. As can be seen (Figure 2), an evident separation of OW/OB and NW groups was observed in the fasting state. Data from both ion modes (Figure 3, panels A—negative and B—positive) indicate a clear separation of OW/OB and NW groups in postprandial changes in metabolite level evoked by a HC meal. However, in the case of the NC meal we were able to obtain an adequate quality PLS-DA model only for the data from the positive ion mode (Figure 3, panel C). Moreover, considering the parameters of the models, better group separation was achieved based on the HC meal (R2 = 0.998, Q2 = 0.837 for negative- and R2 = 0.994, Q2 = 0.706 for positive-ion mode) than for the NC meal data (R2 = 0.995, Q2 = 0.484 for positive-ion mode). Additionally, PLS-DA models were built to evaluate postprandial differences in metabolic profiles of people with OW/OB after intake of a HC meal accompanied with a cinnamon and capsicum or placebo capsule. As can be seen (Figure 4), based on the AUCs calculated for postprandial changes in the level of plasma metabolites, a clear discrimination between the placebo and cinnamon/capsicum intake is observed for data from negative (panel A) and positive (panel B) ion mode. Obtained PLS-DA models were used to select metabolites contributing the most to observed separation. Identification of significant metabolites was confirmed by MS/MS fragmentation (Table S1 available in the supplementary information file). Table 2 shows metabolites discriminating OW/OB and NW groups in the fasting state. Table 3 shows metabolites significantly discriminating studied groups after a HC or NC meal, and additionally the influence of cinnamon/capsicum on postprandial changes of these metabolites after a HC meal is also shown in this table. As can be seen, differences between the OW/OB and NW groups are mainly observed after a HC meal and some of these changes can be partially lifted by the cinnamon and capsicum intake. Table 4 shows metabolites significant in placebo vs. cinnamon/capsicum comparison. Table S2 (available in the supplementary information file) shows metabolites discriminating the OW/OB group from the NW group independently of the meal type.

Table 2. Significant serum metabolites discriminating individuals with overweight/obesity from lean individuals at baseline.

Metabolites	Monoisotopic Neutral Mass [Da]	RT [min]	OW/OB vs. NW		
			Change * [%]	VIP	Absolute p(corr)
Bilirubin	584.2621	8.0	−30	1.82	0.52
	584.2634	8.0	−34	2.12	0.41
Leucine (S)	131.0947	0.3	33	1.45	0.65
Valine	117.0788	0.2	−28	1.88	0.51
Piperidine	85.0892	0.3	41	1.80	0.73
Linoleamide (S)	279.2558	5.5	66	1.65	0.53
Dodecanamide (S)	199.1938	5.3	32	1.51	0.57
Palmitoleamide (S)	253.2406	6.3	79	2.17	0.68

Table 2. *Cont.*

Metabolites	Monoisotopic Neutral Mass [Da]	RT [min]	OW/OB vs. NW		
			Change * [%]	VIP	Absolute *p*(corr)
HETE	320.2348	5.8	43	1.78	0.49
LPC 18:1	507.3684	6.0	21	1.29	0.43
LPA 16:0	410.2430	5.7	20	1.39	0.51
LPI 16:0	572.2964	5.8	51	1.48	0.53
LPI 18:0	600.3278	6.8	31	1.15	0.43
LPI 18:1	598.3119	6.1	37	1.82	0.61
PC 36:5	779.5468	9.4	−45	1.98	0.64
PC 38:6	805.5624	9.8	−26	1.60	0.47
PC O–36:5 or P–36:4	765.5682	10.4	−30	1.83	0.52
PC 38:5	807.5777	10.1	−44	2.67	0.67

* Positive/negative value of percent of change means higher/lower intensity of metabolite in people with overweight/obesity in comparison to lean individuals. The *p*(corr) and VIP values were calculated based on respective PLS-DA models. Variables with VIP > 1.0 and absolute *p*(corr) > 0.4 were considered significant. RT: retention time, VIP: variable importance into the projection, *p*(corr): predictive loading value, S: the identity of these metabolites was confirmed by analysis of the standard, HETE: hydroxyeicosatetraenoic acid, LPC: lysophosphatidylcholine, LPA: lysophosphatidic acid, LPI: lysophosphatidylinositol, PC: phosphatidylcholine.

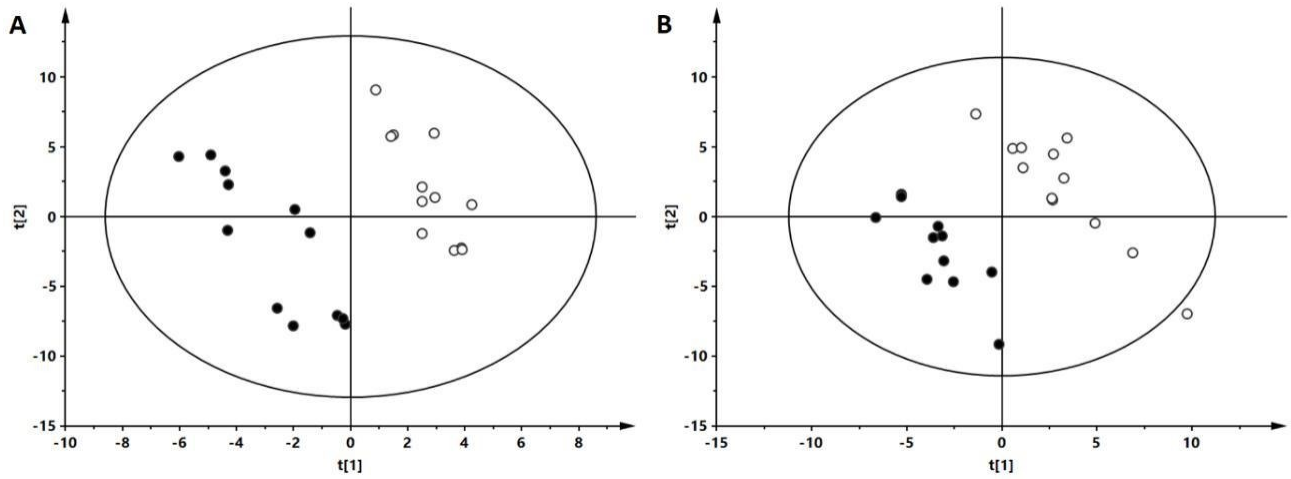

Figure 2. PLS-DA models based on plasma metabolite levels obtained before meal intake. Each panel shows results obtained for different data sets: panel A: ESI(-), R2 = 0.935, Q2 = 0.507; panel B: ESI(+), R2 = 0.987, Q2 = 0.647. R2: explained variance, Q2: predictive capability of the model. The OW/OB group is represented by solid circles, and the NW group by open.

3.3. Pathway Analysis

The results of pathway analysis performed for metabolites where AUCs were found significantly different between OW/OB and NW participants after a HC meal are presented in Figure 4 (panel A) and in Table S3 (panel A). Seven matching pathways were identified, among which four were statistically significant (arachidonic acid metabolism, glycerophos-pholipid metabolism, as well as linoleic and alpha-linoleic acid metabolism). In the cin-namon/capsicum study, five matching pathways were identified (Figure 5 and Table S3, panels B), all statistically significant. Interestingly, four of them were found significantly altered after HC meal intake by people from the OW/OB group. This shows that cinna-mon/capsicum taken by people with OW/OB regulates the metabolic pathways altered by a HC meal. An additional metabolic pathway altered by cinnamon/capsicum was the sphingolipid metabolism.

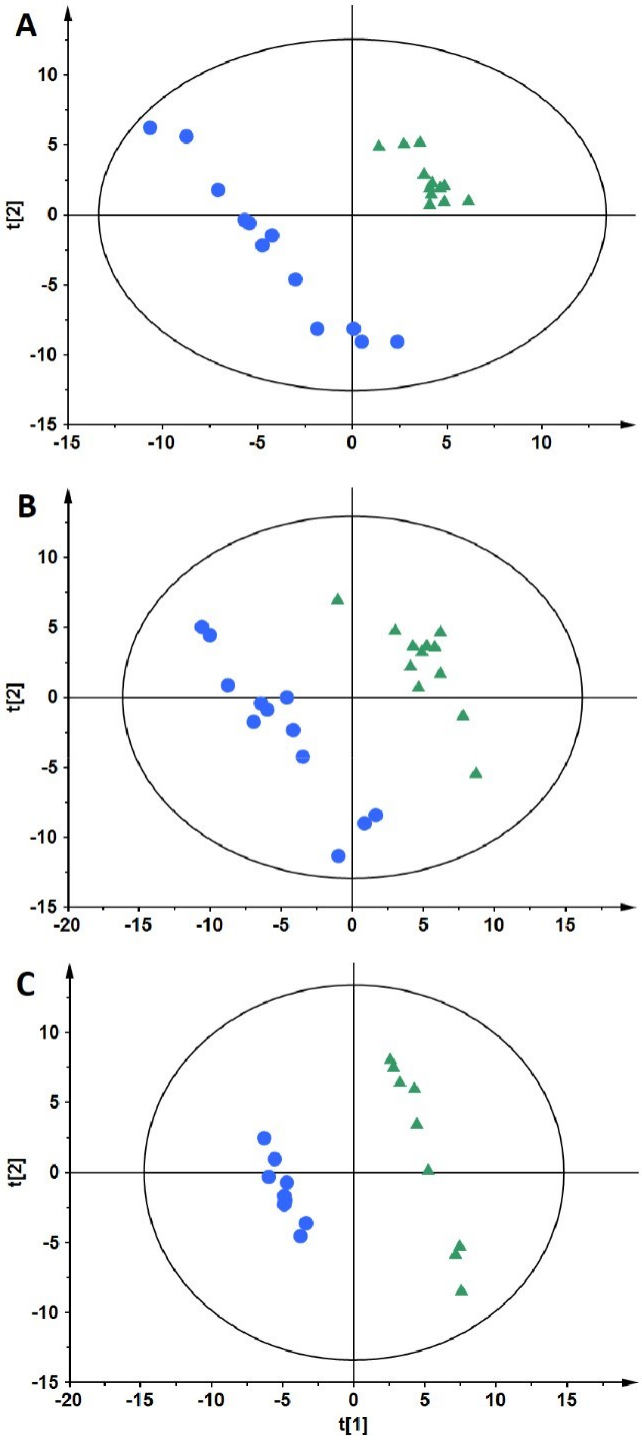

Figure 3. PLS-DA models based on postprandial AUCs for plasma metabolites obtained after HC meal intake (A,B) and NC meal intake (C). The OW/OB group is represented by blue circles and the NW group by green triangles. Each panel shows results obtained for different data sets: panel A: ESI(–), panels B and C: ESI(+).

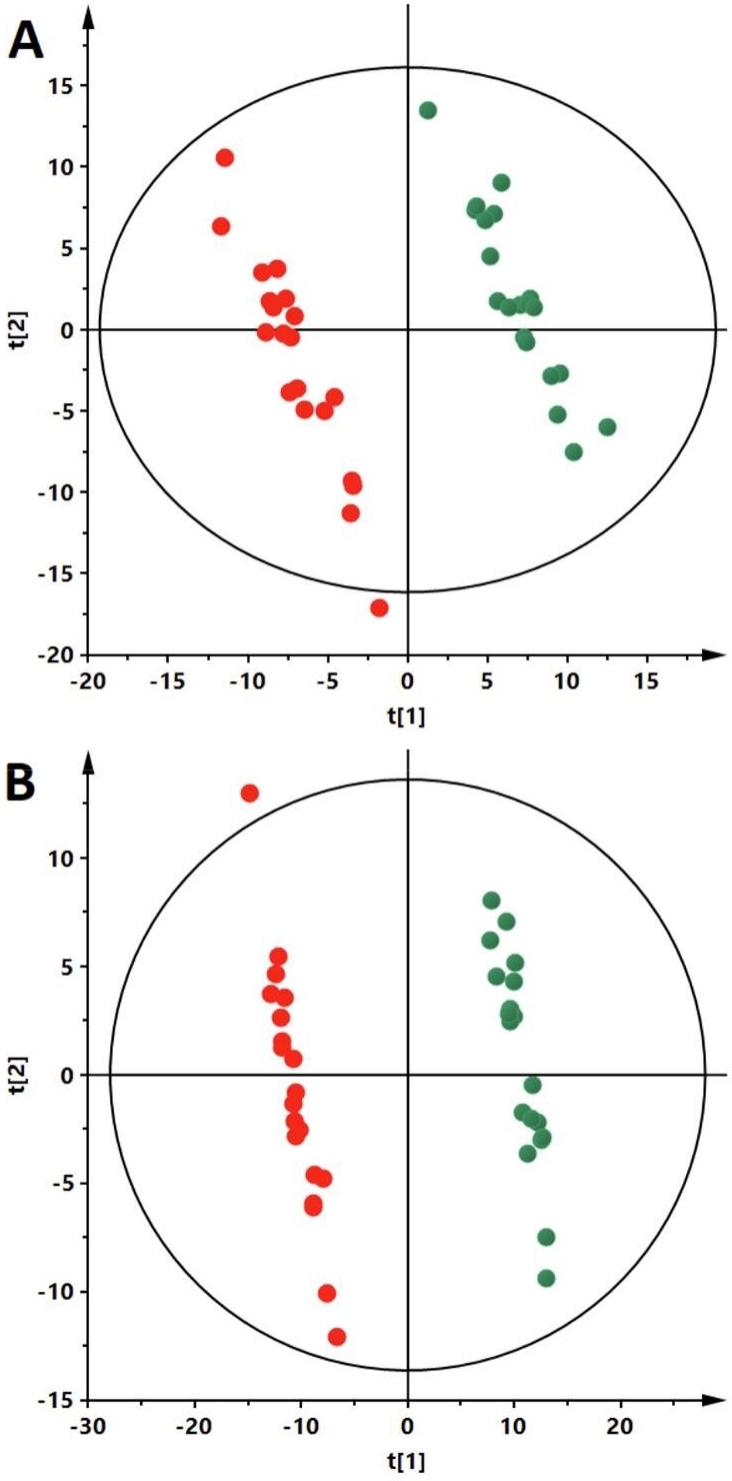

Figure 4. PLS-DA models based on postprandial AUCs for plasma metabolites obtained after HC meal taken with cinnamon/capsicum or placebo capsule. The data obtained from the samples collected during cinnamon/capsicum intervention are represented by red circles while during placebo intervention by green circles. Each panel shows results obtained for different data sets: panel A: ESI(-), R2 = 0.988, Q2 = 0.936; panel B: ESI(+), R2 = 0.997, Q2 = 0.973. R2: explained variance, Q2: predictive capability of the model.

Table 3. A list of meal–dependent metabolites that changed postprandially, discriminating people with overweight/obesity from lean individuals.

Metabolite	Monoisotopic Mass [Da]	RT [min]	NC Meal OW/OB vs. NW			HC Meal OW/OB vs. NW			Direction of AUC Change after Cinnamon/Capsicum Intake
			Change [%]	VIP	Absolute p(corr)	Change [%]	VIP	Absolute p(corr)	
Androsterone sulfate (S)	370.1814	3.9	8	NA	NA	114	**1.49**	**0.51**	↓
Indoxyl sulfate	213.0097	0.7	25	NA	NA	155	**2.71**	**0.67**	↓
Lactic acid	90.0319	0.3	10	NA	NA	73	**2.05**	**0.56**	Not changing
Uric acid	168.0282	0.2	16	NA	NA	145	**1.55**	**0.63**	↓
Hydroxy stearic acid	300.2659	7.4	17	NA	NA	54	**1.74**	**0.51**	↑
Hexanoylcarnitine	259.1779	2.2	16	0.42	0.12	84	**1.88**	**0.42**	ND
HETE	320.2348	5.8	11	NA	NA	129	**1.93**	**0.64**	ND
Sphinganine C17:0	287.282	4.2	11	0.54	0.10	49	**1.89**	**0.50**	↑
Sphinganine C16:0	273.2662	4.1	8	0.15	0.07	60	**1.79**	**0.56**	↑
Sphingosine C16:0	271.2509	4.5	12	0.88	0.27	40	**1.51**	**0.53**	Not changing
Sphingosine C18:3	295.2506	5.7	550	**2.63**	**0.55**	57	0.42	0.27	↓
Lauroyldiethanolamide	287.2456	5	4	0.26	0.08	71	**2.87**	**0.78**	↑
Linoleamide	279.2558	5.5	682	**3.75**	**0.77**	38	0.74	0.40	Not changing
Palmitoyl N-Isopropylamide	297.3025	7.8	69	**1.75**	**0.50**	36	0.67	0.39	↑
LPC 14:0	467.3007	5.1	2	0.22	0.14	185	**2.22**	**0.65**	↓
LPC O-15:0	467.3369	5.9	19	NA	NA	125	**1.02**	**0.65**	↑
LPC 16:0	495.3317	5.6	23	0.22	0.14	161	**1.79**	**0.56**	↓
LPC 17:0 sn-1	509.348	6.3	15	NA	NA	91	**1.96**	**0.51**	Not changing
LPC 17:0 sn-2	509.3481	6.2	14	NA	NA	94	**2.08**	**0.54**	Not changing
LPC 19:0	551.3587	6.0	22	NA	NA	92	**1.64**	**0.69**	↓
LPC 20:1	549.3789	6.3	36	0.28	0.02	274	**2.17**	**0.55**	↓
LPE 16:0	453.2856	5.6	23	NA	NA	255	**1.91**	**0.65**	Not changing
LPE O-16:0	439.3049	5.8	2	NA	NA	211	**1.96**	**0.66**	↑
LPE P-16:0 or LPE O-16:1	437.2904	5.8	30	NA	NA	195	**1.91**	**0.70**	↓
LPE P-19:1	477.3213	5.7	23	0.18	0.02	133	**1.67**	**0.67**	↓
LPE P-18:0 or LPE O-18:1	465.3216	5.9	25	NA	NA	113	**1.17**	**0.63**	↑
LPE P-20:0 or LPE O-20:1	493.3553	7.0	10	NA	NA	64	**1.39**	**0.53**	↓
LPE 20:3	503.3008	5.7	15	0.45	0.12	71	**2.49**	**0.53**	↑
LPA 22:4	486.2715	6.3	30	1.19	0.29	101	**1.53**	**0.52**	Not changing

Table 3. Cont.

Metabolite	Monoisotopic Mass [Da]	RT [min]	NC Meal OW/OB vs. NW			HC Meal OW/OB vs. NW			Direction of AUC Change after Cinnamon/Capsicum Intake
			Change [%]	VIP	Absolute p(corr)	Change [%]	VIP	Absolute p(corr)	
LPI 16:0	572.2965	5.8	41	NA	NA	224	**1.40**	**0.68**	ND
LPI 18:0	600.3276	6.5	22	NA	NA	98	**1.59**	**0.58**	ND
LPI 18:1	598.3119	6.1	26	NA	NA	76	**1.44**	**0.67**	↑
LPI 20:4	620.2964	5.6	22	NA	NA	41	**1.50**	**0.42**	↓
PC 32:1	731.547	10.1	73	1.52	0.34	116	**1.48**	**0.49**	↓
PC 38:5	807.5777	10.1	8	0.54	0.23	78	**1.06**	**0.49**	↓
SM 32:1	674.5368	8.3	19	0.97	0.26	48	**1.09**	**0.46**	↓

Positive/negative value of percent of change means higher/lower AUC of postprandial change of metabolite level in people with overweight/obesity in comparison to lean individuals. The p(corr) and VIP values were calculated based on respective PLS-DA models. Variables with VIP > 1.0 and absolute p(corr) > 0.4 were considered significant and are bolded. NA: values not available as it was not possible to build PLS-DA model based on this data set. ND: not enough quality data for this metabolite were recorded in the cinnamon/capsicum study. Not changing means that a difference between the AUC for placebo and cinnamon/capsicum was below 5%. HC: high-carbohydrate, NC: normo-carbohydrate, OW/OB: individuals with overweight/obesity, NW: individuals with normal weight, AUC: area under the curve, RT: retention time, p(corr): predictive loading value, VIP: variable importance into the projection, HETE: hydroxyeicosatetraenoic acid, LPA: lysophosphatidic acid, LPC: lysophosphatidylcholine, LPE: lysophosphoethanolamine, LPI: lysophosphatidylinositol, PC: phosphatidylcholine, SM: sphingomyelin.

Table 4. A list of metabolites significantly changing in the cinnamon/capsicum intervention study.

Metabolite	Monoisotopic Mass [Da]	RT [min]	Change [%]	VIP	Absolute *p*(corr)
Sphingosine-1-phosphate	379.2489	5.0	170	1.26	0.49
Sphinganine C17:0	287.282	4.2	1,758,409	2.11	0.88
Arachidonic Acid methyl ester	318.2559	8.0	688,141	1.32	0.58
Docosenamide	337.3343	7.4	159	2.43	0.99
LPC 14:0	**467.3007**	**5.1**	**−27**	**1.13**	**0.48**
LPC 16:0	**495.3328**	**5.5**	**−100**	**1.52**	**0.64**
LPC 18:2	519.3327	5.4	−8	1.79	0.79
LPC 20:1	**549.3789**	**6.3**	**−34**	**1.50**	**0.67**
LPE P-16:0	**437.2904**	**5.8**	**−17**	**1.22**	**0.54**
LPE P-19:1	**477.3213**	**5.7**	**−29**	**2.01**	**0.87**
LPE 20:3	**503.3013**	**5.6**	**1,134,744**	**1.36**	**0.56**
LPE 20:4	501.2858	5.3	−52	1.35	0.38
PC 32:4	757.5624	9.8	−59	1.05	0.44
PC36:2	807.5756	10.9	−99	1.38	0.54
PC 38:4	831.5747	10.2	−99	1.32	0.53
PC 40:6	855.5756	10.2	−90	1.62	0.66
PC 40:7	899.5623	10.5	2884	1.20	0.46
PC 16:0/20:4	963.5415	9.6	−88	1.50	0.42
PC O-36:2 or PC P-36:1	771.6079	8.5	17,503	2.12	0.86
PC O-40:5 or PC P-40:5	841.5964	10.2	−100	1.72	0.69
SM d34:2	846.4822	8.2	−27	2.43	0.86
SM d32:1	820.4666	8.0	−30	2.35	0.78

Positive/negative value of percent of change means higher/lower AUC of postprandial change of metabolite level after cinnamon/capsicum capsule intake in comparison to placebo capsule intake. Metabolites significant after HC meal in OW/OB vs. NW comparisons are bolded. The *p*(corr) and VIP values were calculated based on the PLS-DA models. Variables with VIP > 1.0 and absolute *p*(corr) > 0.4 were considered significant. RT: retention time, *p*(corr): predictive loading value, VIP: variable importance into the projection, LPC: lysophosphatidylcholine, LPE: lysophosphoethanolamine, PC: phosphatidylcholine, PE: phosphoethanolamine, SM: sphingomyelin. * Measured monoisotopic neutral mass (list of ions is provided in the supplementary material).

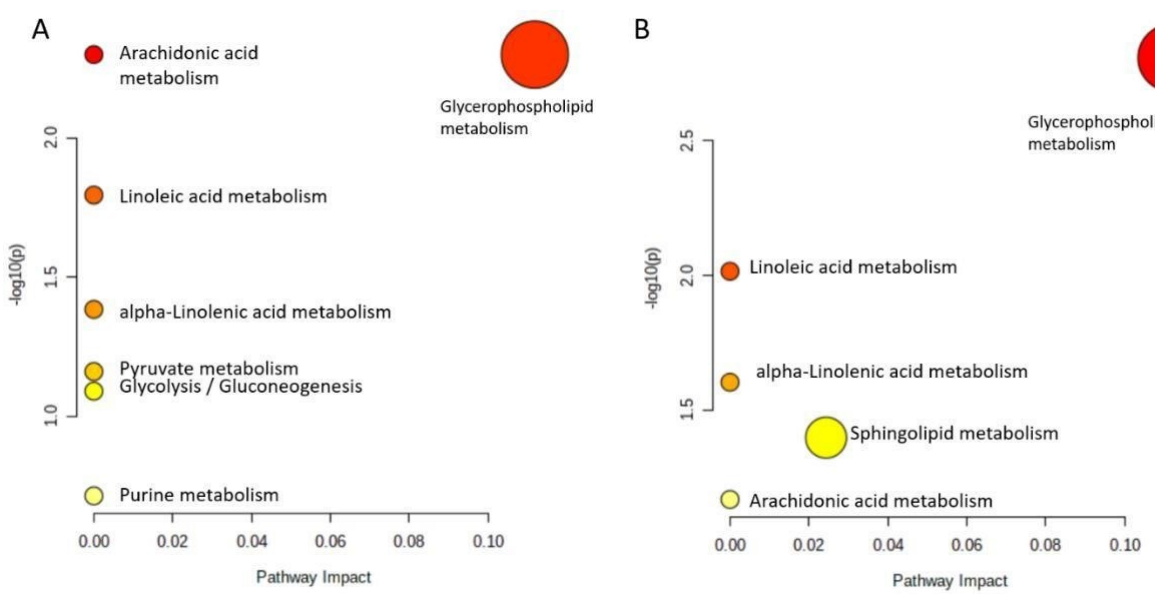

Figure 5. The metabolic pathways significantly matched with the discriminatory metabolites: (A) for meal–dependent metabolites that changed postprandially, discriminating people with overweight/obesity from lean individuals after a HC meal; (B) for metabolites significantly changing in the cinnamon/capsicum intervention study. The pathway impact value (x-axis) was obtained from pathway topological analysis, while the log(10) of the p-value (y-axis) was obtained from pathway enrichment analysis. The most significant pathways are characterized by a high -log10(p) value and the pathway impact value.

4. Discussion

Comprehensive untargeted metabolomic analysis of fasting and postprandial plasma samples was carried out to investigate and compare the effects of HC/NC meal intake on the plasma metabolome of people with overweight/obesity (OW/OB group) and people with normal weight (NW group). Metabolites discriminating individuals from OW/OB and NW groups in a fasting state (Table 2) clearly depicted the inherently different metabolic profiles of the two populations with different BMIs. Discriminating metabolites mainly belong to lipids from the following classes: phosphatidylcholine (PC), lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), lysophosphatidic acid (LPA), fatty acid amides (FAA), as well as hydroxyeicosatetraenoic acid. Additionally, changes in BCAA, bilirubin, and piperidine were noted. Metabolic profile discriminating at baseline people with overweight/obesity from people with normal weight mainly indicates dysregulated cellular lipid and amino acid metabolism. It is known that people with obesity have two-fold higher lipolytic fluxes. The increased lipolysis and lipolytic fluxes are mainly driven by increased activation/phosphorylation of hormone-sensitive lipase (HSL) and decreased protein levels of the adipose triglyceride lipase (ATGL) inhibitor G0S2. The increased expression levels of HSL and ATGL cause the enormous lipolysis per kilogram of body weight in people with overweight/obesity resulting in the alteration of the fasting metabolic profile [17]. In this study, several PCs were found decreased in individuals from the OW/OB group in comparison to those from the NW group. Similar findings were also reported by Bagheri et al. [18], observed reduced levels of long-chain PCs in the fasting plasma of individuals with overweight/obesity. The increased plasma level of LPC 18:1 in subjects from the OW/OB group is also in agreement with the work of Boulet et al. [19], observed a positive association between various LPC and different anthropometric variables, i.e., BMI, body-fat mass, as well as subcutaneous and visceral adipose tissue areas. Three LPIs (16:0, 18:0, and 18:1) were also found increased in participants from the OW/OB group in comparison to subjects from the NW group. LPI plays a role as an endogenous ligand of the cannabinoid receptor GPR55 and is involved in many physiological actions of adipose tissue biology. An increased plasma concentration of LPI is probably due to increased phosphatidylinositol hydrolysis via the actions of the calcium-dependent phospholipase A2 and calcium-independent phospholipase A1. The correlation of LPI and GPR55 showed that increased concentration of LPI augmented the GPR55 level in people with obesity, which is in turn in a positive relationship with weight, BMI, and percent of fat mass [20]. Other lipid entities where plasma level at baseline was found increased in participants with overweight/obesity compared to those from the NW group include FAA (i.e., dodecanamide, linoleamide, and palmitoleamide). Together with endocannabinoids and their metabolic enzymes, FAA constitute the endocannabinoid system. Many studies have signalled the significance of the correct functionality of this system to maintain and recover key physiological functions, including energy homeostasis [21].

Regarding amino acids, the fasting plasma level of two BCAA (leucine and valine) were also found to discriminate OW/OB and NW groups. The results obtained for leucine are in line with the previous studies reporting an increased concentration of BCAA in subjects with obesity in comparison to patients with normal weight [22]. However, a decreased valine level was observed, which can be explained by differences in ethnicity, sex, gene expression, and dietary patterns that influence the BCAA level [23]. The impaired catabolism of BCAA prompts obesity through reduced expression of branched-chain keto acid dehydrogenase and branched-chain aminotransferase. In short, this disrupted fasting plasma metabolic profile of individuals with overweight/obesity can be an early predictor of insulin resistance, impaired glucose tolerance, prediabetes, and even T2DM [24].

While comparing the metabolic response of both groups to specific meal types, the subjects from the OW/OB group showed substantially perturbed postprandial metabolic response mainly after HC meal consumption (Table 3). Most of these metabolites were phos-pholipids, sphingolipids, and FAA. Among others, the AUC of several phospholipids (LPEs, LPCs, and LPIs), indoxylsulfuric acid, lactic acid, or uric acid was significantly increased in individuals with OW/OB in comparison to lean subjects after the consumption of the HC, but not the NC meal.

For some metabolites (sphingosine 18:3, lauroyldiethanolamide, and palmitoyl N- isopropylamide), the AUC was found significantly increased after the NC, but not the HC meal, while for others (Table S2) significant differences in AUC between the studied groups were observed after both meals. For most metabolites, an increased postprandial AUC in the OW/OB group in comparison to the NW group was noted.

Many authors have reported similar results while investigating the effect of HC diet/meal intake on the metabolome of vulnerable populations compared to healthy ones. Gonzalez-Granda et al. [25] studied the effect of consuming high-fructose meals in obese-to-lean subjects and cited the marked increase in phospholipid (PCs: 30:0, 32:1, 34:1, 34:3, 36:3, 38:3, 40:4, 40:5) and diacyl-LPC (14:1, 16:1) levels in obese individuals after the intake of high-fructose diets. Most of the meal-type discriminatory metabolites (Table 2) were lipid entities, and postprandial AUC for these entities accelerated in OW/OB subjects in response to the HC meal. These results suggest that a population with higher BMI is more susceptible to dysregulation of lipid metabolism due to HC meal intake than lean and/or NW individuals. These findings are consistent with the results of another published study illustrating the lipidome of people with obesity and prediabetes, which reported perturbations in the levels of several PL, LPC, LPE, LPI, SM, and ceramides in people with obesity [26]. In another study, a diabetes-predicting model was published, indicating three phospholipids, i.e., LPC 18:2, PC 32:1, and PC 34:2, as early predictors of diabetes in the susceptible population [27]. These studies confirmed the positive association of HC meals and plasma/serum levels of phospholipids, fatty liver index, and weight gain. However, this relationship can be circumvented by adding healthy FA into a HC meal. The long-term consumption of hypocaloric diets with healthy fats, i.e., omega-3 olive oil, significantly improved the anthropometric measurements and fatty liver index, and decreased the PL in the obese subjects with metabolic features [28].

Tulipani et al. [29] reported an inverse relationship of LPCs 17:0, 18:1, and 18:2 with the BMI, body weight, hip circumference, and waist. This contradiction in findings might have arisen since OW/OB individuals were found largely normoglycemic in this study. Additionally, carbohydrate type, glycemic index, and quantity of intake were also consid-ered important for determining the overall blood glucose, lipid metabolism, and metabolic response [30].

HC meal consumption significantly exacerbated the postprandial AUC of hydroxy stearic acid and hydroxyeicosatetraenoic acid (HETE) in people from the OW/OB group in comparison to those from the NW group. The lipoxygenase and cyclooxygenase metabolize arachidonic acid to produce HETEs, leukotrienes, and prostaglandins [31]. HETE with its various isoforms (12(S)-HETE, 12(R)-HETE, and 12(S)-HpETE) have been found to acti-vate the inflammatory markers (tumour necrosis factor alpha-TNF or platelet-activating factor) and reduce the secretion of insulin by orchestrating the local immune response and apoptosis in insulin-producing pancreatic -cells [32]. A higher postprandial AUC of hydroxy stearic acid was also noticed in the OW/OB group. It is important to mention that low levels of hydroxy stearic acid have been found to employ cytostatic effects on highly proliferating cells, whereas its higher concentrations act as a strong inducer of apop-totic cell death by interfering with cell cycle kinetics via interacting with cdc2 kinase [33]. Sphingolipids (with the exception for sphingosine C18:3) are the group of metabolites for which postprandial AUCs were found decreased in OW/OB subjects in comparison to NW subjects in response to a HC meal. Information about postprandial changes in sphin-golipids is scarce. The level of sphingolipids was found to increase after a HFD in a study by Fujisawa, Takami [34]. Contrarily, sphingosine 18:3 was increased in the OW/OB group following a HC meal. Sphingosine is the key precursor of de novo biosynthesis pathways of ceramides. Increased plasma concentrations of sphingosine and ceramides were noted in obese and insulin-resistant Zucker rats, [35] and diabetic patients, [36]. Another metabolite for which postprandial change was meal-dependent is androsterone sulphate. The AUC for this metabolite was found to be significantly higher after the HC meal in OW/OB group participants. The relationship between steroid hormones and obesity and T2DM has been discussed in the literature [37]. A higher serum level of dehydroepiandrosterone, a precursor of androsterone, was found independently associated with a decreased risk of T2DM development in healthy men and postmenopausal women [38]. The postprandial AUC of indoxyl sulfate, a protein- bound uremic toxin known to induce oxidative stress and pro-inflammatory effects [39], was found to increase in individuals from the OW/OB group in comparison to those from the NW group after a HC meal. Indoxyl sulfate is also involved in forming advanced glycation end products, which in turn promote the pathogenesis of metabolic syndrome, cardiovascular diseases and chronic kidney disease [39]. These results confirmed that a HC meal can make people with OW/OB more vulnerable to metabolic syndrome, cardiovascular diseases, and chronic kidney disease. Moreover, we also noted higher postprandial AUC for lactic acid in response to HC meal intake in individuals from the OW/OB group, consistent with the fact that lactic acidosis is a common phenomenon in hyperglycemic human subjects [40].

As can be seen (Table 3), the different response of people with OW/OB in comparison to NW participants was mostly observed after the HC meal and discriminating metabolites were mostly lipids. It has been shown that ingested capsaicinoids can prevent low-fat, high-carbohydrate diet-induced obesity in rats [41]. Moreover, it has been shown that cinnamon extract regulates plasma levels of adipose-derived factors and expression of multiple genes related to carbohydrate metabolism and lipogenesis in fructose-fed rats [42]. The beneficial effects of both capsicum annum and cinnamon supplementation on the components of metabolic syndrome [10] and management of diabetes [43], respectively, have been re-viewed recently. As the literature data have indicated the different anti-diabetic/obesity mechanisms of cinnamon and capsicum action [10,43], we decided to combine them into one capsule and test their ability to diminish changes in plasma metabolome evoked by a HC meal in people with OW/OB. As can be seen in Figure 3, AUCs for postprandial changes in metabolite levels were different when the cinnamon/capsicum capsule was taken with a HC meal in comparison to placebo capsule intake. Metabolites significantly affected by cinnamon/capsicum intake are presented in Table 4. Additionally, in the last column of Table 3, we show how cinnamon/capsicum intake affected metabolites signif-icantly in the meal-type study. As can be seen, for the most significant metabolites after the HC meal, the opposite direction of change was observed in the cinnamon/capsicum intervention, which indicates that these spices may diminish the effect evoked by a HC meal. Although, considering exactly the same metabolites, only six that were significant after HC meal intake were observed to be significant after cinnamon/capsicum interven-tion (bolded in Table 4); performed pathway analyses (Figure 5 and Table S3) indicated that the metabolic pathways most affected by the HC meal were also affected by cinna-mon/capsicum intake with the HC meal. Although participants of both clinical studies were males with overweight or obesity, those from the cinnamon/capsicum study were older and more insulin resistant. It may explain why the lists of significant metabolites do not overlap more. However, high similarities of affected pathways, together with the opposite direction of change in metabolites affected by a HC meal in comparison to a HC meal taken with cinnamon/capsicum, indicate that these spices may regulate metabolic pathways perturbed by such a meal in individuals with OW/OB. Last but not least, these findings confirm the nutritional-therapeutic role of both cinnamon and capsicum, especially in vulnerable groups, which can be applied via including them in regular food recipes regardless of food cultures, gastronomy, and culinary types.

5. Conclusions

This study illustrated a comparison of changes in the postprandial metabolic response to HC meals in men with overweight/obesity. Several metabolites and metabolic path-ways were dysregulated after a HC meal in people from the OW/OB group, but not in those from the normal weight group. After the consumption of a HC meal by people with overweight/obesity, a substantial increase in AUC was mainly noted for different classes of lipids. To evaluate the possibility of diminishing an unfavourable postprandial metabolic response in individuals with overweight/obesity to a HC meal, another human intervention was performed. Ingestion of cinnamon/capsicum was shown to diminish metabolic changes evoked by a HC meal in men with OW/OB. The results were obtained from relatively small study groups composed of male subjects; therefore, a future work aiming to validate altered metabolites in a larger group of female and male participants or to evaluate the changes of regulatory genes and key enzymes involved in these pathways, should be performed. In addition to human studies, both in vitro and animal experiments could be performed to validate obtained results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14204305/s1, Table S1: A detailed fragmentation data of identified metabolites.; Table S2: Significant metabolites which postprandial change discriminate people with overweight/obesity and lean individuals independently of the meal type.; Table S3:A list of pathways affected by the discriminatory metabolites: (A) for meal–dependent metabolites changed postprandially discriminating people with overweight/obesity from lean individuals; (B) for metabolites significantly changing in the cinnamon/capsicum intervention study.

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Conflicts of Interest: The authors declared no conflict of interest.

References

1. WHO. WHO Mortality Database [Onlzine Database]; World Health Organization: Geneva, Switzerland, 2019.
2. Hameed, A.; Galli, M.; Adamska-Patruno, E.; Kr˛etowski, A.; Ciborowski, M. Select Polyphenol-Rich Berry Consumption to Defer or Deter Diabetes and Diabetes-Related Complications. Nutrients **2020**, 12, 2538. [CrossRef] [PubMed]
3. Jung, C.-H.; Choi, K.M. Impact of High-Carbohydrate Diet on Metabolic Parameters in Patients with Type 2 Diabetes. Nutrients **2017**, 9, 322. [CrossRef] [PubMed]
4. American Diabetes Association Nutrition Recommendations and Interventions for Diabetes: A position statement of the American Diabetes Association. Diabetes Care **2007**, 31, S61–S78. [CrossRef]
5. Guldbrand, H.; Dizdar, B.; Bunjaku, B.; Lindström, T.; Bachrach-Lindström, M.; Fredrikson, M.; Ostgren, C.J.; Nystrom, F.H. In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. Diabetologia **2012**, 55, 2118–2127. [CrossRef] [PubMed]
6. Hsu, W.C.; Lau, K.H.K.; Matsumoto, M.; Moghazy, D.; Keenan, H.; King, G.L. Improvement of insulin sensitivity by isoenergy high carbohydrate traditional Asian diet: A randomized controlled pilot feasibility study. PLoS ONE **2014**, 9, e106851. [CrossRef] [PubMed]
7. Palmnäs, M.; Brunius, C.; Shi, L.; Rostgaard-Hansen, A.; Torres, N.E.; González-Domínguez, R.; Zamora-Ros, R.; Ye, Y.L.; Halkjær, J.; Tjønneland, A.; et al. Perspective: Metabotyping—A Potential Personalized Nutrition Strategy for Precision Prevention of Cardiometabolic Disease. Adv. Nutr. **2019**, 11, 524–532. [CrossRef] [PubMed]
8. Ota, A.; Ulrih, N.P. An Overview of Herbal Products and Secondary Metabolites Used for Management of Type Two Diabetes. Front. Pharm. **2017**, 8, 436. [CrossRef] [PubMed]
9. Yuan, L.-J.; Qin, Y.; Wang, L.; Zeng, Y.; Chang, H.; Wang, J.; Wang, B.; Wan, J.; Chen, S.-H.; Zhang, Q.-Y.; et al. Capsaicin-containing chili improved postprandial hyperglycemia, hyperinsulinemia and fasting lipid disorders in women with gestational diabetes mellitus and lowered the incidence of large-for-gestational-age newborns. Clin. Nutr. **2016**, 35, 388–393. [CrossRef]
10. Jang, H.-H.; Lee, J.; Lee, S.-H.; Lee, Y.-M. Effects of Capsicum annuum supplementation on the components of metabolic syndrome: A systematic review and meta-analysis. Sci. Rep. **2020**, 10, 1–11. [CrossRef] [PubMed]
11. Hernández-Pérez, T.; Gómez-García, M.d.R.; Valverde, M.E.; Paredes-López, O. Capsicum annuum (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. Compr. Rev. Food Sci. Food Saf. **2020**, 19, 2972–2993. [CrossRef]

12. Lu, J.; Varghese, R.T.; Zhou, L.; Vella, A.; Jensen, M.D. Glucose tolerance and free fatty acid metabolism in adults with variations in TCF7L2 rs7903146. Metab. Clin. Exp. **2017**, 68, 55–63. [CrossRef] [PubMed]
13. Adamska, E.; Waszczeniuk, M.; Go´scik, J.; Golonko, A.; Wilk, J.; Pliszka, J.; Maliszewska, K.; Lipinska,´ D.; Milewski, R.; Wasilewska, A.; et al. The usefulness of glycated hemoglobin A1c (HbA1c) for identifying dysglycemic states in individuals without previously diagnosed diabetes. Adv. Med. Sci. **2012**, 57, 296–301. [CrossRef] [PubMed]
14. Adamska-Patruno, E.; Godzien, J.; Ciborowski, M.; Samczuk, P.; Bauer, W.; Siewko, K.; Gorska, M.; Barbas, C.; Kretowski, A. The Type 2 Diabetes Susceptibility PROX1 Gene Variants Are Associated with Postprandial Plasma Metabolites Profile in Non-Diabetic Men. Nutrients **2019**, 11, 882. [CrossRef] [PubMed]
15. Adamska-Patruno, E.; Samczuk, P.; Ciborowski, M.; Godzien, J.; Pietrowska, K.; Bauer, W.; Gorska, M.; Barbas, C.; Kretowski, A. Metabolomics Reveal Altered Postprandial Lipid Metabolism After a High-Carbohydrate Meal in Men at High Genetic Risk of Diabetes. J. Nutr. **2019**, 149, 915–922. [CrossRef]
16. Samczuk, P.; Luba, M.; Godzien, J.; Mastrangelo, A.; Hady, H.R.; Dadan, J.; Barbas, C.; Gorska, M.; Kretowski, A.; Ciborowski, M. –Gear mechanism‖ of bariatric interventions revealed by untargeted metabolomics. J. Pharm. Biomed. Anal. **2018**, 151, 219–226. [CrossRef]
17. Bak, A.M.; Møller, A.B.; Vendelbo, M.H.; Nielsen, T.S.; Viggers, R.; Rungby, J.; Pedersen, S.B.; Jørgensen, J.O.L.; Jessen, N.; Møller, N. Differential regulation of lipid and protein metabolism in obese vs. lean subjects before and after a 72-h fast. Am. J. Physiol. Metab. **2016**, 311, E224–E235. [CrossRef]
18. Bagheri, M.; Djazayery, A.; Farzadfar, F.; Qi, L.; Yekaninejad, M.S.; Aslibekyan, S.; Chamari, M.; Hassani, H.; Koletzko, B.; Uhl, O. Plasma metabolomic profiling of amino acids and polar lipids in Iranian obese adults. Lipids Health Dis. **2019**, 18, 1–9. [CrossRef]
19. Boulet, M.M.; Chevrier, G.; Grenier-Larouche, T.; Pelletier, M.; Nadeau, M.; Scarpa, J.; Prehn, C.; Marette, A.; Adamski, J.; Tchernof, A. Alterations of plasma metabolite profiles related to adipose tissue distribution and cardiometabolic risk. Am. J. Physiol. Metab. **2015**, 309, E736–E746. [CrossRef]
20. del Bas, J.M.; Caimari, A.; Rodriguez-Naranjo, M.I.; Childs, C.E.; Paras Chavez, C.; West, A.L.; Miles, E.A.; Arola, L.; Calder, P.C. Impairment of lysophospholipid metabolism in obesity: Altered plasma profile and desensitization to the modulatory properties of n–3 polyunsaturated fatty acids in a randomized controlled trial. Am. J. Clin. Nutr. **2016**, 104, 266–279. [CrossRef]
21. Iannotti, F.A.; Piscitelli, F.; Martella, A.; Mazzarella, E.; Allarà, M.; Palmieri, V.; Parrella, C.; Capasso, R.; Di Marzo, V. Analysis of the –endocannabinoidome‖ in peripheral tissues of obese Zucker rats. Prostaglandins Leukot. Essent. Fat. Acids **2013**, 89, 127–135. [CrossRef]
22. Libert, D.M.; Nowacki, A.S.; Natowicz, M.R. Metabolomic analysis of obesity, metabolic syndrome and type 2 diabetes: Amino acid and acylcarnitine levels change along a spectrum of metabolic wellness. Peer J. **2018**, 6, e5410. [CrossRef] [PubMed]
23. Zhao, X.; Han, Q.; Liu, Y.; Sun, C.; Gang, X.; Wang, G. The Relationship between Branched-Chain Amino Acid Related Metabolomic Signature and Insulin Resistance: A Systematic Review. J. Diabetes Res. **2016**, 2016, 1–12. [CrossRef]
24. Hameed, A.; Mojsak, P.; Buczynska, A.; Suleria, H.A.; Kretowski, A.; Ciborowski, M. Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM. J. Clin. Med. **2020**, 9, 2257. [CrossRef] [PubMed]
25. Gonzalez-Granda, A.; Damms-Machado, A.; Basrai, M.; Bischoff, S.C. Changes in Plasma Acylcarnitine and Lysophosphatidyl-choline Levels Following a High-Fructose Diet: A Targeted Metabolomics Study in Healthy Women. Nutrients **2018**, 10, 1254. [CrossRef]
26. Rhee, E.P.; Cheng, S.; Larson, M.G.; Walford, G.A.; Lewis, G.D.; McCabe, E.; Yang, E.; Farrell, L.; Fox, C.S.; O'Donnell, C.J.; et al. Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans. J. Clin. Investig. **2011**, 121, 1402–1411. [CrossRef]
27. Suvitaival, T.; Bondia-Pons, I.; Yetukuri, L.; Pöhö, P.; Nolan, J.J.; Hyötyläinen, T.; Kuusisto, J.; Oreši˘c, M. Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men. Metabolism **2018**, 78, 1–12. [CrossRef] [PubMed]
28. Cantero, I.; Abete, I.; del Bas, J.M.; Caimari, A.; Arola, L.; Zulet, M.A.; Martinez, J.A. Changes in lysophospholipids and liver status after weight loss: The RESMENA study. Nutr. Metab. **2018**, 15, 1–11. [CrossRef]
29. Tulipani, S.; Palau-Rodriguez, M.; Miñarro Alonso, A.; Cardona, F.; Marco-Ramell, A.; Zonja, B.; Lopez de Alda, M.; Muñoz-Garach, A.; Sanchez-Pla, A.; Tinahones, F.J.; et al. Biomarkers of Morbid Obesity and Prediabetes by Metabolomic Profiling of Human Discordant Phenotypes. Clin. Chim. Acta **2016**, 463, 53–61. [CrossRef]
30. Riccardi, G.; Rivellese, A.A.; Giacco, R. Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. Am. J. Clin. Nutr. **2008**, 87, 269S–274S. [CrossRef]
31. Persaud, S.J.; Muller, D.; Belin, V.D.; Kitsou-Mylona, I.; Asare-Anane, H.; Papadimitriou, A.; Burns, C.J.; Huang, G.C.; Amiel, S.A.; Jones, P.M. The Role of Arachidonic Acid and Its Metabolites in Insulin Secretion From Human Islets of Langerhans. Diabetes **2006**, 56, 197–203. [CrossRef]
32. Ma, K.; Nunemaker, C.S.; Wu, R.; Chakrabarti, S.K.; Taylor-Fishwick, D.A.; Nadler, J.L. 12-Lipoxygenase Products Reduce Insulin Secretion and {beta}-Cell Viability in Human Islets. J. Clin. Endocrinol. Metab. **2010**, 95, 887–893. [CrossRef]
33. Rodríguez, J.P.; Guijas, C.; Astudillo, A.M.; Rubio, J.M.; Balboa, M.A.; Balsinde, J. Sequestration of 9-Hydroxystearic Acid in FAHFA (Fatty Acid Esters of Hydroxy Fatty Acids) as a Protective Mechanism for Colon Carcinoma Cells to Avoid Apoptotic Cell Death. Cancers **2019**, 11, 524. [CrossRef] [PubMed]
34. Fujisawa, K.; Takami, T.; Fukui, Y.; Nagatomo, T.; Saeki, I.; Matsumoto, T.; Hidaka, I.; Yamamoto, N.; Okamoto, T.; Furutani-Seiki, M.; et al. Assessment of high-fat-diet-induced fatty liver in medaka. Biol. Open **2018**, 7, bio031534. [CrossRef]
35. Górska, M.; Dobrzyn´ A Fau-Zendzian-Piotrowska, M.; Zendzian-Piotrowska, M. Fau-Górski, J.; Górski, J. Effect of streptozotocin-diabetes on the functioning of the sphingomyelin-signalling pathway in skeletal muscles of the rat. Horm. Metab. Res. **2004**, 36, 14–21. [PubMed]

36. Górska, M.; Dobrzyn,´ A. Fau-Baranowski, M.; Baranowski, M. Concentrations of sphingosine and sphinganine in plasma of patients with type 2 diabetes. Med. Sci. Monit. **2005**, 11, 38.
37. Gambineri, A.; Pelusi, C. Sex hormones, obesity and type 2 diabetes: Is there a link? Endocr. Connect. **2019**, 8, R1–R9. [CrossRef]
38. Brahimaj, A.; Muka, T.; Kavousi, M.; Laven, J.S.E.; Dehghan, A.; Franco, O.H. Serum dehydroepiandrosterone levels are associated with lower risk of type 2 diabetes: The Rotterdam Study. Diabetologia **2017**, 60, 98–106. [CrossRef]
39. Lin, C.-J.; Lin, J.; Pan, C.-F.; Chuang, C.-K.; Liu, H.-L.; Sun, F.-J.; Wang, T.-J.; Chen, H.-H.; Wu, C.-J. Indoxyl Sulfate, Not P-Cresyl Sulfate, is Associated with Advanced Glycation end Products in Patients on Long-Term Hemodialysis. Kidney Blood Press. Res. **2015**, 40, 121–129. [CrossRef]
40. English, P.; Williams, G. Hyperglycaemic crises and lactic acidosis in diabetes mellitus. Postgrad. Med. J. **2004**, 80, 253–261. [CrossRef]
41. Sahin, K.; Orhan, C.; Tuzcu, M.; Sahin, N.; Ozdemir, O.; Juturu, V. Ingested capsaicinoids can prevent low-fat-high-carbohydrate diet and high-fat diet-induced obesity by regulating the NADPH oxidase and Nrf2 pathways. J. Inflamm. Res. **2017**, 10, 161–168. [CrossRef]
42. Qin, B.; Polansky Mm Fau-Anderson, R.A.; Anderson, R.A. Cinnamon extract regulates plasma levels of adipose-derived factors and expression of multiple genes related to carbohydrate metabolism and lipogenesis in adipose tissue of fructose-fed rats. Horm. Metab. Res. **2010**, 42, 187–193. [CrossRef] [PubMed]
43. Shang, C.; Lin, H.; Fang, X.; Wang, Y.; Jiang, Z.; Qu, Y.; Xiang, M.; Shen, Z.; Xin, L.; Lu, Y.; et al. Beneficial effects of cinnamon and its extracts in the management of cardiovascular diseases and diabetes. Food Funct. **2021**, 12, 12194–12220. [CrossRef] [PubMed]

9. Summary

Summary in English

Type 2 diabetes mellitus (T2DM) is a non-communicable and long-lasting hyperglycemic condition that has turned into a global epidemic. It is mostly managed by glycemic control via pharmacotherapeutic and evidence-based alternative approaches which come with a price and are causatives of financial strains on global healthcare systems. Therefore, precision nutrition-based approaches have recently been proposed as alternatives to prevent and/or treat T2DM and its complications. Precision nutrition is a way of personalization of dietary and nutritional recommendations. Although it is at the infancy stage, numerous dietary recommendations have already been suggested. Considering macronutrients consumption, several human studies have been reported unveiling comparable results of reduction in mean body weight, glycated haemoglobin, low-and high- density lipoprotein cholesterol, insulin, and fasting plasma glucose after high-carbohydrate (HC) and low-carbohydrate (LC) diet. Except macronutrients, inclusion of specific food additives/spices in a diet may exert a positive effect in the prevention of weight gain and T2DM development. An accumulated number of studies have demonstrated that cinnamon and capsicum have prebiotic, hypoglycemic, hypolipidemic, antioxidative, and anti-inflammatory effects and improve glycemic control, hyperlipidemia, and insulin resistance. However, the effects of different meal types and/or cinnamon and capsicum intake on plasma metabolome are unknown, which is also a prerequisite to unveil the mechanism behind weight management. Therefore, primarily, placebo-controlled randomized clinical trial was conducted to check the effect of HC and LC meals on plasma metabolome of lean and overweight/obese (OW/OB) participants. Secondly, another clinical trial was conducted in which the effects of cinnamon/capsicum intake on plasma metabolome affected by a HC meal consumption by OW/OB individuals was evaluated. The first study was performed on 24 male subjects divided into two subgroups (12 subjects per group): normal weight (35±9 years old) or OW/OB (38±6 years old). The participants with chronic diseases, a history of smoking and taking any medications were not allowed. The volunteers participated in two meal-challenge tests visits in a cross-over design with 2–3 weeks intervals. On the visit-day from each participant fasting blood sample was collected and after that subjects received a standardized HC or NC meal (both from Nutricia, Poland). In the second study a total of 20 OW/OB male subjects (BMI>25.0 kg/m^2, 46±8 years old) took part in the HC-meal challenge study with cinnamon and capsicum. The exclusion criteria, the protocols applied and meal-challenge test conditions were as in the first study. The participants received a meal and one of two investigated products (cinnamon+capsicum, or placebo, in a randomized order). An HC meal was composed of wheat roll (100g), fruit jam (50g) and juice (200ml). In both studies, additionally to the fasting blood sample, blood was collected 30, 60, 120, and 180 minutes after a meal. Collected blood was used to prepare plasma samples. Obtained plasma was fingerprinted using LC-QTOF-MS. Based on each metabolic feature‘s intensity change in time, the area under the curve (AUC) was calculated. Obtained AUCs were analyzed using multivariate statistics. The identity of significant metabolites was confirmed by analysis of metabolite's standards (if available) or based on the MS/MS spectra match. Several metabolic pathways were found dysregulated after an HC meal in people from the OW/OB group but not the NW group. The consumption of HC meals by people with overweight/obesity led to a substantial increase in AUC, mainly for metabolites belonging to phospholipids and fatty acid amides. The opposite was observed for selected sphingolipids. The intake of cinnamon and capsicum normalized the concentration of selected altered metabolites induced by the intake of HC meals. A HC meal may induce an unfavourable postprandial metabolic response in individuals with overweight/obesity, and such persons should avoid HC meals.

Summary in Polish (Streszczenie)

Cukrzyca typu 2 (T2DM) to niezakaźna choroba przewlekła charakteryzująca się hiperglikemią. Ze względu na jej dużą częstość występowania mówi się teraz o globalnej epidemii cukrzycy. Leczenie pacjentów z T2DM opiera się na farmakoterapii, co jest obciążeniem budżetu pacjentów i/lub systemu opieki zdrowotnej. Dlatego obecnie coraz szerzej bada się możliwość opracowania spersonalizowanych zaleceń dietetycznych jako alternatywę w zapobieganiu i/lub leczeniu T2DM i jej powikłań. Chociaż opracowanie strategii terapeutycznych na bazie spersonalizowanej dietoterapii wymaga jeszcze wiele pracy, to dostępny jest już szereg badań na ludziach w których oceniano konsekwencje spożycia poszczególnych makroskładników. Ich wyniki są podobne, zaobserwowano zmniejszenie średniej masy ciała, hemoglobiny glikowanej, cholesterolu, insuliny oraz stężenia glukozy na czczo zarówno u osób na diecie wysokowęglowodanowej (WW) jak i niskowęglowodanowej (NW). Oprócz makroskładników odżywczych włączenie do diety określonych dodatków do żywności/przypraw może mieć pozytywny wpływ na zapobieganie przyrostowi masy ciała i rozwojowi T2DM. Co raz więcej badań wskazuje, że cynamon i ostra papryka wykazują działanie prebiotyczne, hipoglikemiczne, hipolipidemiczne, przeciwutleniające i przeciwzapalne oraz poprawiają kontrolę glikemii, profil lipidowy i wrażliwość na insulinę. Jednak wpływ różnych rodzajów posiłków i/lub spożycia cynamonu i papryki na metabolom osocza jest nieznany, a badania tego typu mogą przyczynić się do poznania mechanizmów biochemicznych regulujących masę ciała i rozwój cukrzycy. W związku z powyższym przeprowadziliśmy dwa badania randomizowane kontrolowane placebo. W pierwszym zbadaliśmy wpływ posiłków WW i NW na metabolom osocza osób szczupłych i z nadwagą/otyłością (OW/OB). W drugim zaś wpływ spożycia cynamonu i papryki na metabolom osocza osób z OW/OB, które spożyły posiłek WW. Pierwsze badanie przeprowadzono na 24 mężczyznach, których podzielono na dwie podgrupy po 12 osób: i) prawidłowa masa ciała (35±9 lat), ii) OW/OB (38±6 lat). Do kryteriów wykluczających należały: występowanie choroby przewlekłej, palenie papierosów i przyjmowanie jakichkolwiek leków. Ochotnicy uczestniczyli w dwóch wizytach w odstępach 2–3 tygodni. W dniu wizyty od każdego uczestnika pobierano próbkę krwi na czczo. W pierwszym badaniu po pobraniu krwi uczestnik otrzymywał wystandaryzowany posiłek HC lub NC (Nutricia, Polska). W drugim badaniu wzięło udział łącznie 20 mężczyzn z OW/OB (BMI>25,0 kg/m2, 46±8 lat), którzy otrzymali posiłek WW poprzedzony przyjęciem kapsułki zawierającej cynamon i ostrą paprykę lub placebo. Kryteria wykluczenia, procedury i warunki przeprowadzenia testu z posiłkiem były takie same jak w pierwszym badaniu. W badaniu drugim posiłek WW składał się z bułki pszennej (100 g), dżemu owocowego (50 g) i soku (200 ml). W obu badaniach oprócz próbki krwi na czczo pobierano krew 30, 60, 120 i 180 minut po spożyciu posiłku. Pobraną krew wykorzystano do przygotowania próbek osocza. Otrzymane osocze poddano niecelowanej analizie metabolomicznej przy użyciu LC-QTOF-MS. Na podstawie zmiany intensywności każdej cechy metabolicznej w czasie obliczono pole pod krzywą (AUC). Uzyskane AUC analizowano przy użyciu statystyk wielowymiarowych. Identyfikacja istotnych statystycznie metabolitów została potwierdzona analizą standardów (jeśli były dostępne) lub na podstawie dopasowania widm MS/MS znajdujących się w ineternetowych bazach danych. Zaobserwowano zaburzenia kilku szlaków metabolicznych po spożyciu posiłku WW u osób z grupy OW/OB, ale nie u osób szczupłych. Spożywanie posiłków WW przez osoby z nadwagą/otyłością prowadziło do znacznego wzrostu AUC głównie takich metabolitów jak fosfolipidy i amidy kwasów tłuszczowych. W przypadku wybranych sfingolipidów zaobserwowano efekt odwrotny. Przyjęcie cynamonu i ostrej papryki przed spożyciem posiłku WW normalizowało poziom metabolitów, które ulegały zmianom wskutek spożycia posiłku WW. Spożywanie posiłków WW może wywołać niekorzystną poposiłkową odpowiedź metaboliczną u osób z nadwagą/otyłością i osoby takie powinny unikać tego rodzaju posiłków.

10. References

1. (WHO), W.H.O., *1.	WHO Mortality Database [online database]*. Geneva: World Health Organization, 2019.
2. Hameed, A., et al., *Select Polyphenol-Rich Berry Consumption to Defer or Deter Diabetes and Diabetes-Related Complications. LID - E2538 [pii] LID - 10.3390/nu12092538 [doi]*. (2072-6643 (Electronic)).
3. Jung, C.-H. and K.M. Choi, *Impact of High-Carbohydrate Diet on Metabolic Parameters in Patients with Type 2 Diabetes.* Nutrients, 2017. **9**(4): p. 322.
4. *Nutrition Recommendations and Interventions for Diabetes: A position statement of the American Diabetes Association.* Diabetes Care, 2007. **31**(Supplement 1): p. S61-S78.
5. Guldbrand, H., et al., *In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss.* Diabetologia, 2012. **55**(8): p. 2118-2127.
6. Hsu, W.C., et al., *Improvement of insulin sensitivity by isoenergy high carbohydrate traditional Asian diet: a randomized controlled pilot feasibility study.* PloS one, 2014. **9**(9): p. e106851-e106851.
7. Palmnäs, M., et al., *Perspective: Metabotyping—A Potential Personalized Nutrition Strategy for Precision Prevention of Cardiometabolic Disease.* Advances in Nutrition, 2019. **11**(3): p. 524-532.
8. Ao, Z., et al. *Spicy Food and Chili Peppers and Multiple Health Outcomes: Umbrella Review.* Molecular Nutrition & Food Research, 2022. 2200167.
9. Nuffer, W., et al. *Sweetly Improving Sugars? Reviewing Cinnamon's Effects on Blood Glucose.* Journal of Medicinal Food, 2022. Oct 7, doi: 10.1089/jmf.2022.0073.
10. Jang, H.-H., et al., *Effects of Capsicum annuum supplementation on the components of metabolic syndrome: a systematic review and meta-analysis.* Scientific reports, 2020. **10**(1): p. 20912-20912.
11. Ota, A. and N.P. Ulrih, *An Overview of Herbal Products and Secondary Metabolites Used for Management of Type Two Diabetes.* Frontiers in Pharmacology, 2017. **8**(436).
12. Yuan, L.-J., et al., *Capsaicin-containing chili improved postprandial hyperglycemia, hyperinsulinemia, and fasting lipid disorders in women with gestational diabetes mellitus and lowered the incidence of large-for-gestational-age newborns.* Clinical Nutrition, 2016. **35**(2): p. 388-393.
13. Hernández-Pérez, T., et al., *Capsicum annuum (hot pepper): An ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review.* Comprehensive Reviews in Food Science and Food Safety, 2020. **19**(6): p. 2972-2993.
14. Tebani, A. and Bekri S., *Paving the Way to Precision Nutrition Through Metabolomics*. Frontiers in Nutrition, 2019. **6** doi: 10.3389/fnut.2019.00041.
15. Adamska-Patruno, E., et al., *The Type 2 Diabetes Susceptibility PROX1 Gene Variants Are Associated with Postprandial Plasma Metabolites Profile in Non-Diabetic Men.* Nutrients, 2019. **11**(4): p. 882.
16. Adamska-Patruno, E., et al. *Metabolomics Reveal Altered Postprandial Lipid Metabolism After a High-Carbohydrate Meal in Men at High Genetic Risk of Diabetes*. Journal of Nutrition, 2019. **149**(6): p. 915-922.
17. Hameed, A., et al., *Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM.* Journal of Clinical Medicine, 2020. **9**(7).
18. Lu, J., et al., *Glucose tolerance and free fatty acid metabolism in adults with variations in TCF7L2 rs7903146.* Metabolism: clinical and experimental, 2017. **68**: p. 55-63.
19. Adamska, E., et al., *The usefulness of glycated hemoglobin A1c (HbA1c) for identifying dysglycemic states in individuals without previously diagnosed diabetes.* Advances in Medical Sciences, 2012. **57**(2): p. 296-301.
20. Samczuk, P., et al., *―Gear mechanism‖ of bariatric interventions revealed by untargeted metabolomics.* Journal of Pharmaceutical and Biomedical Analysis, 2018. **151**: p. 219-226.

21. Godzien, J., et al., *Rapid and Reliable Identification of Phospholipids for Untargeted Metabolomics with LC–ESI–QTOF–MS/MS.* Journal of Proteome Research, 2015. **14**(8): p. 3204-3216.
22. Wishart, D.S., et al., *HMDB 4.0: the human metabolome database for 2018.* (1362-4962 (Electronic)).
23. Bak, A.M., et al., *Differential regulation of lipid and protein metabolism in obese vs. lean subjects before and after a 72-h fast.* American Journal of Physiology-Endocrinology and Metabolism, 2016. **311**(1): p. E224-E235.
24. Bagheri, M., et al., *Plasma metabolomic profiling of amino acids and polar lipids in Iranian obese adults.* Lipids in health and disease, 2019. **18**(1): p. 94-94.
25. Boulet, M.M., et al., *Alterations of plasma metabolite profiles related to adipose tissue distribution and cardiometabolic risk.* (1522-1555 (Electronic)).
26. Iannotti, F.A., et al., *Analysis of the ―endocannabinoidome‖ in peripheral tissues of obese Zucker rats.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 2013. **89**(2-3): p. 127-135.
27. Gonzalez-Granda, A., et al., *Changes in Plasma Acylcarnitine and Lysophosphatidylcholine Levels Following a High-Fructose Diet: A Targeted Metabolomics Study in Healthy Women.* Nutrients, 2018. **10**(9): p. 1254.
28. Rhee, E.P., et al., *Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans.* The Journal of clinical investigation, 2011. **121**(4): p. 1402-1411.
29. Suvitaival, T., et al., *Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men.* Metabolism, 2018. **78**: p. 1-12.
30. Tulipani, S., et al., *Biomarkers of Morbid Obesity and Prediabetes by Metabolomic Profiling of Human Discordant Phenotypes.* Clinica Chimica Acta, 2016. **463**: p. 53-61.
31. Riccardi, G., A.A. Rivellese, and R. Giacco, *Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes.* The American Journal of Clinical Nutrition, 2008. **87**(1): p. 269S-274S.
32. Ma, K., et al., *12-Lipoxygenase Products Reduce Insulin Secretion and {beta}-Cell Viability in Human Islets.* The Journal of clinical endocrinology and metabolism, 2010. **95**(2): p. 887-893.
33. Górska, M., et al., *Effect of streptozotocin-diabetes on the functioning of the sphingomyelin-signalling pathway in skeletal muscles of the rat.* (0018-5043 (Print)).
34. Górska, M., M. Dobrzyń A Fau - Baranowski, and M. Baranowski, *Concentrations of sphingosine and sphinganine in plasma of patients with type 2 diabetes.* (1234-1010 (Print)).
35. Gambineri, A. and C. Pelusi, *Sex hormones, obesity and type 2 diabetes: is there a link?* Endocrine connections, 2019. **8**(1): p. R1-R9.
36. Brahimaj, A., et al., *Serum dehydroepiandrosterone levels are associated with lower risk of type 2 diabetes: the Rotterdam Study.* Diabetologia, 2017. **60**(1): p. 98-106.
37. Lin, C.-J., et al., *Indoxyl Sulfate, Not P-Cresyl Sulfate, is Associated with Advanced Glycation end Products in Patients on Long-Term Hemodialysis.* Kidney and Blood Pressure Research, 2015. **40**(2): p. 121-129.
38. Sahin, K., et al., *Ingested capsaicinoids can prevent low-fat-high-carbohydrate diet and high-fat diet-induced obesity by regulating the NADPH oxidase and Nrf2 pathways.* Journal of inflammation research, 2017. **10**: p. 161-168.
39. Qin, B., R.A. Polansky Mm Fau - Anderson, and R.A. Anderson, *Cinnamon extract regulates plasma levels of adipose-derived factors and expression of multiple genes related to carbohydrate metabolism and lipogenesis in adipose tissue of fructose-fed rats.* (1439-4286 (Electronic)).
40. Shang, C., et al., *Beneficial effects of cinnamon and its extracts in the management of cardiovascular diseases and diabetes.* Food Funct, 2021. **12**(24): p. 12194-12220.

11. Authorship Contribution Statements (Information about nature of participation and author's contribution (in %) and statements from co-authors)

1. Hameed, Ahsan, Patrycja Mojsak, Angelika Buczynska, Hafiz Ansar Rasul Suleria, Adam Kretowski, and Michal Ciborowski. 2020. "Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM" *Journal of Clinical Medicine* 9, no. 7: 2257. https://doi.org/10.3390/jcm9072257

Author's name and surname	Nature of participation	Contribution in %
PhD student: Ahsan Hameed	Designed the study, conducted the search for literature, and wrote the entire manuscript	60
Patrycja Mojsak	Searched for the literature and edited the manuscript.	10
Angelika Buczynska	Literature search and interpretation of data in tabular form.	10
Hafiz Ansar Rasul Suleria	Reviewed and edited the manuscript.	5
Adam Kretowski	Provided the idea and introduced the topic for the manuscript.	5
Michal Ciborowski	Supervised the study thoroughly and revised and edited the manuscript.	10

2. Hameed, Ahsan, Mauro Galli, Edyta Adamska-Patruno, Adam Krętowski, and Michal Ciborowski. 2020. "Select Polyphenol-Rich Berry Consumption to Defer or Deter Diabetes and Diabetes-Related Complications" *Nutrients* 12, no. 9: 2538. https://doi.org/10.3390/nu12092538

Author's name and surname	Nature of participation	Contribution in %
PhD student: Ahsan Hameed	Designed the study, conducted the search for literature, and wrote the entire manuscript	65
Mauro Galli	Searched for the literature and edited the manuscript.	10
Edyta Adamska-Patruno	Supervised the study thoroughly and revised and edited the manuscript	10
Adam Kretowski	Provided the idea and introduced the topic for the manuscript.	5
Michal Ciborowski	Supervised the study thoroughly and revised and edited the manuscript	10

3. Hameed, Ahsan, Edyta Adamska-Patruno, Joanna Godzien, Przemyslaw Czajkowski, Urszula Miksza, Karolina Pietrowska, Joanna Fiedorczuk, Monika Moroz, Witold Bauer, Julia Sieminska, Maria Górska, Adam Jacek Krętowski, and Michal Ciborowski. 2022. "The Beneficial Effect of Cinnamon and Red Capsicum Intake on Postprandial Changes in Plasma Metabolites Evoked by a High-Carbohydrate Meal in Men with Overweight/Obesity" *Nutrients* 14, no. 20: 4305. https://doi.org/10.3390/nu14204305

Author‘s name and surname	Nature of participation	Contribution in %
PhD student: Ahsan Hameed	Designed the study, conducted the search for literature, and wrote the entire manuscript	52
Edyta Adamska-Patruno	Supervised the study thoroughly and revised and edited the manuscript	4
Joanna Godzien	Searched for the literature and edited the manuscript.	4
Przemyslaw Czajkowski	Performed selected investigations and analyses.	4
Urszula Miksza	Performed selected investigations and analyses.	4
Karolina Pietrowska	Performed selected investigations and analyses.	4
Joanna Fiedorczuk	Performed selected investigations and analyses.	4
Monika Moroz	Performed selected investigations and analyses.	4
Witold Bauer	Performed selected statistical analyses.	4
Julia Sieminska	Performed selected investigations and analyses.	4
Maria Górska	Performed selected investigations.	4
Adam Kretowski	Supervised the study and acquired funding.	4
Michal Ciborowski	Supervised the study thoroughly and revised and edited the manuscript.	4

I hereby declare that all co-authors agreed to use these articles in the current dissertation.

Signature

Patrycja Mojsak

name and last name of the author

01.12.2022

date, place

Medical University of Bialystok

affiliation/name of the university

Statement

I confirm that in the article:

Hameed, Ahsan, Patrycja Mojsak, Angelika Buczynska, Hafiz Ansar Rasul Suleria, Adam Kretowski, and Michal Ciborowski. 2020. "Altered Metabolome of Lipids and Amino Acids Species: A Source of Early Signature Biomarkers of T2DM" *Journal of Clinical Medicine* 9, no. 7: 2257 https://doi.org/10.3390/jcm9072257 which is a part of doctoral dissertation of Ahsan Hameed, my contribution included literature search and assistance in data collection.

I agree to use this publication by Ashan Hameed, in the procedure for awarding the doctoral degree in the field of medical sciences and health sciences in the discipline of medical sciences.

Patrycja Mojsek

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Angelika Buczyńska

.....
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.....Angelika Buczyńska.....
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name and last name of the author

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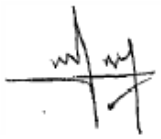

…… ……
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Edyta Adamska-Patruno

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01.12.2022

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Edyta Adamska-Patruno

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I agree to use this publication by **AHSAN HAMEED** in the procedure for awarding the doctoral degree in the field of medical sciences and health sciences in the discipline of medical sciences.

Joanna Godzień

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Przemysław Czajkowski
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Karoline Pietrowska

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Pietrowska Karolina

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Julia Sieminska
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Julia Sieminska
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Witold Bauer
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01.12.2022, Białystok
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.....
signature

Mauro Galli

name and last name of the author

15.12.2022

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BTI, A-STAR

affiliation/name of the university

Statement

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signature

Maria Górska

.....
name and last name of the author

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Adam Kretowski

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name and last name of the author

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Ciborowski

Signature

Michal Ciborowski — 07.12.2022

name and last name of the author — *date, place*

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Adam Kretowski

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.....

signature

12. Consent from the Bioethics Committee

UNIWERSYTET MEDYCZNY
w Białymstoku
KOMISJA BIOETYCZNA
15-089 Białystok, ul. Jana Kilińskiego 1

Uchwała nr: R-I-002/35/2009 29-01-2009r.

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP / Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** /na prowadzenie tematu badawczego: „Analiza genetycznych uwarunkowań odpowiedzi metabolicznej na dietę o różnej zawartości węglowodanów, białek i tłuszczy. Poszukiwanie genetycznych markerów do indywidualizacji żywienia pacjentów z otyłością i cukrzycą typu 2" przez prof. dr hab. Marię Górską wraz z zespołem badawczym.

Przewodnicząca Komisji

prof. dr hab. Elżbieta Hassmann-Poznańska

...YTET MEDYCZNY
... Białymstoku
...JA BIOETYCZNA
...stok, ul. Jana Kilińskiego 1

Białystok, 28-06-2018

Uchwała nr: R-I-002/314/2018

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Opracowanie preparatu o właściwościach wpływających na poprawę parametrów metabolicznych u osób z otyłością i zaburzeniami gospodarki węglowodanowej" przez dr n. med. Edytę Adamską-Patruno wraz z zespołem badawczym z UMB.

Przewodnicząca Komisji Bioetycznej UMB

prof. dr hab. Otylia Kowal-Bielecka

Białystok, 31-01-2019

UNIWERSYTET MEDYCZNY
w Białymstoku
KOMISJA BIOETYCZNA
15-089 Białystok, ul. Jana Kilińskiego 1

Decision number: R-I-002/95/2019

The Bioethics Committee of Medical University of Bialystok, after getting acquainted with the research project in accordance with the principles of GCP/Guidelines for Good Clinical Practice/ **approves** the research project entitled :„Intervention of newly designed nutraceutical from cinnamon and capsicum on serum metabolome of obese and type 2 diabetic patients" to be conducted by Ahsan Hameed and study team from MUB.

Chair of the Bioethics Committee

Prof. Otylia Kowal-Bielecka