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ROZPRAWA DOKTORSKA

Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia

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The role of cannabidiol (CBD) in reduction of brain insulin resistance

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- **Charytoniuk T**, Sztolsztener K, Bielawiec P, Chabowski A, Konstantynowicz-Nowicka K, Harasim-Symbor E.: Cannabidiol Downregulates Myocardial de Novo Ceramide Synthesis Pathway in a Rat Model of High-Fat Diet-Induced Obesity. 2022, *International Journal of Molecular Sciences* **IF=6.208 MEiN=140**
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- **Charytoniuk T**, Harasim-Symbor E, Polak A, Drygalski K, Berk K, Chabowski A, Konstantynowicz-Nowicka K.: Influence of Resveratrol on Sphingolipid Metabolism in Hepatocellular Carcinoma Cells in Lipid Overload State. 2019, *Anti-Cancer Agents in Medicinal Chemistry* **IF= 2.049 MEiN=70**
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- **Charytoniuk T**, Małyszko M, Bączek J, Fiedorczuk P, Siedlaczek K, Małyszko J.: Progression to chronic kidney disease in patients undergoing nephrectomy for small renal masses: a price to pay for a therapeutic success? 2018, *Postgraduate Medicine* **IF=2.237 MEiN=30**
- **Charytoniuk T**, Drygalski K, Konstantynowicz-Nowicka K, Berk K, Chabowski A. Alternative treatment methods attenuate the development of NAFLD: A review of resveratrol molecular mechanisms and clinical trials. 2017, *Nutrition* **IF=3,734 MEiN=30**

Publikacje – współautor:

- Kołakowski A, Dziemitko S, Chmielecka A, Żywno H, Bzdęga W, **Charytoniuk T**, Chabowski A, Konstantynowicz-Nowicka K. Molecular Advances in MAFLD-A Link between Sphingolipids and Extracellular Matrix in Development and Progression to Fibrosis, 2022, *International Journal of Molecular Sciences* **IF=6.208 MEiN=140**
- Berk K, Konstantynowicz-Nowicka K, **Charytoniuk T**, Harasim-Symbor E, Chabowski A. Distinct Effects of Cannabidiol on Sphingolipid Metabolism in Subcutaneous and Visceral Adipose Tissues Derived from High-Fat-Diet-Fed Male Wistar Rats. 2022, *International Journal of Molecular Sciences* **IF=6.208 MEiN=140**
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- Berk K, Drygalski K, Harasim-Symbor E, **Charytoniuk T**, Iłowska N, Łukaszuk B, Chabowski A, Konstantynowicz-Nowicka K. The effect of enterolactone on liver lipid precursors of inflammation. 2019, *Life Sciences.* **IF=3.647 MEiN=70**
- Drygalski K, Berk K, **Charytoniuk T**, Iłowska N, Łukaszuk B, Chabowski A, Konstantynowicz-Nowicka K. Does the enterolactone (ENL) affect fatty acid transporters and lipid metabolism in liver? 2017, *Nutrition & Metabolism.* **IF=3.483 MEiN=35**

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- 2019 - 1 miejsce w sesji plakatowej; „Cannabidiol (CBD) as a new therapeutic approach for the brain sphingolipid accumulation." Charytoniuk Tomasz, Berk Klaudia, Konstantynowicz-Nowicka Karolina. Medical International Conference for Students "MEDICS". Bukareszt, Rumunia.
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- 2017 - 2 miejsce w sesji nauk podstawowych "The effect of enterolactone on intrahepatocellular concentration and apoptosis in HepG2 cells." Tomasz Charytoniuk. BGMU LXXI 2017. Mińsk, Białoruś.
- 2017 - "The preliminary assessment of the resveratrol influence on the spontaneous contractility of the non-pregnant uterus in human." Chomentowski Andrzej, Charytoniuk Tomasz, Drygalski Krzysztof. 4TH Lublin International Medical Congress for Students and Young Doctors, Lublin, Polska.
- 2016 - "Dexamethasone affects saturation index and intracellular lipid composition in primary rat hepatocytes in lipid overload." Drygalski Krzysztof, Charytoniuk Tomasz,

Iłowska Nicoletta, Berk Klaudia. 11th BIMC Bialystok International Medical Congress for Young Scienctists. Bialystok, Polska.

- 2016 - „The effect of dexamenthasone on intrahepatocellular sphingolipid concentration in the presence of lipid overload in HepG2 cells.” 3rd Lublin International Medical Congress for Students and Young Doctors. Lublin, Polska
- 2016 - „The end of staplers in bariatric surgery? Totally Robotic Staplerless Sleeve Gastrectomy - Animal Study.” Fiedorczuk Piotr, Charytoniuk Tomasz, Nobis Aleksander, Bączek Jan, Rogula Tomasz. 11th BIMC Bialystok International Medical Congress for Young Scientists, Bialystok, Polska.
- 2016 – „Share of collagen fiber in the structure of Lymph nodes of the European Bison Bison Bonasus (L.) - a preliminary study.” Charytoniuk Tomasz, Puchalski Mateusz, Czykier Elżbieta. 3RD Lublin International Medical Congress for Students and Young Doctors. Lublin, Polska.
- 2016 - “The effect of dexamenthasone on intrahepatocellular sphingolipid concentration in the presence of lipid overload in HepG2 cells.” Charytoniuk Tomasz. 3RD Lublin International Medical Congress for Students and Young Doctors. Lublin, Polska.
- 2016 - “Udział tkanki łącznej mięśniowej gładkiej w budowie histologicznej węzła chłonnego żubra Bison bonasus (L.) - badanie wstępne.” Puchalski Mateusz, Charytoniuk Tomasz, Czykier Elżbieta. Konferencja Postępy w Badaniach Biomedycznych. Warszawa, Polska.
- 2016 - „Can the Totally Robotic Stapler-less technique be an reliable alternative to the stapler method in the Sleeve Gastrostomy? - Animal Study.” Bączek Jan, Fiedorczuk Piotr, Charytoniuk Tomasz, Nobis Aleksander Tytus. VI International Students' Conference of Young Medical Researchers, Wroclaw, Polska.
- 2016 - „The influence of Dexamethasone on hepatic lipid transport.” Berk Klaudia, Iłowska Nicoletta, Charytoniuk Tomasz, Drygalski Krzysztof. VI International Students' Conference of Young Medical Researchers, Wroclaw, Polska.
- 2015 - „Opis anatomiczny węzłów chłonnych żubra Bison bonasus (L.) - badanie wstępne.” Czykier Elżbieta, Charytoniuk Tomasz. XXXII Zjazd Polskiego Towarzystwa Anatomicznego, Warszawa, Polska.
- 2015 - „Totally robotic stapler-less sleeve gastrectomy - a feasibility animal study.” Nobis Aleksander Tytus, Bączek Jan, Fiedorczuk Piotr, Charytoniuk Tomasz, Rogula

Tomasz, Wojskowicz Piotr. 2nd Lublin International Medical Congress for Students and Young Doctors, Lublin, Polska.

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- 2019 Wyróżnienie Dziekana Wydziału Lekarskiego Uniwersytetu Medycznego w Białymstoku
- 2019 Nagroda Prorektora ds. Nauki Uniwersytetu Medycznego w Białymstoku za wybitne osiągnięcia naukowe
- 2018 Stypendium Ministra Zdrowia za wybitne osiągnięcia
- 2016 Stypendium Ministra Zdrowia za wybitne osiągnięcia
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Staże zagraniczne

- 2018 Asklepios Klinik Barmbek – Asklepios Kliniken, Hamburg, Niemcy; Oddział Urologii
- 2017 Centre hospitalier de l'Université de Montréal, Montreal, Kanada; Oddział Chirurgii Naczyniowej, Oddział Neurochirurgii
- 2016 Chang Gung Memorial Hospital, Taipei/Linkou, Tajwan; Oddział Chirurgii Plastycznej
- 2015 Cleveland Clinic, Cleveland, OH, USA; Instytut Bariatrii i Metabolomiki
- 2015 Sygehus Sønderjylland, Aabenraa/Sønderborg, Dania; Oddział Endokrynologii

Kierownik projektów badawczych:

- „Rola kannabidiolu (CBD) w niwelowaniu stanu zapalnego mózgowia.”, Uniwersytet Medyczny w Białymstoku, nr projektu SUB/1/DN/21/003/1118, 2021r.
- „Rola kannabidiolu (CBD) w metabolizmie sfingolipidów mózgowia”, Uniwersytet Medyczny w Białymstoku, nr projektu SUB/1/DN/20/005/1118, 2020r.
- „Brain insulin resistance, sphingolipid metabolism and new possible therapeutic approach with cannabidiol (CBD).”; program „Strategy of Excellence - the University

of Research" Ministerstwa Nauki i Szkolnictwa Wyższego, nr projektu 0017/SDU/2018/18, 2019r.

- „Wpływ enterodiolu na zawartość sfingolipidów oraz rozwój insulinooporności w hepatocytach"; program „KNOW Krajowy Naukowy Ośrodek Wiodący" Ministerstwa Nauki i Szkolnictwa Wyższego, nr projektu 203/KNOW/16, 2016-2017r.

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Zestawienie publikacji

Rodzaj	**Liczba**	**IF**	**MEiN**
Prace oryginalne **włączone do rozprawy**	1	7.419	100
Prace przeglądowe **włączone do rozprawy**	2	12.131	280
Prace oryginalne niewłączone do rozprawy	7	27.479	555
Prace przeglądowe niewłączone do rozprawy	4	14.906	310
Streszczenia zjazdowe	16	-	-
Razem	**29**	**61.935**	**1245**

Rozprawa doktorska

Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia

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1. Artykuły stanowiące cykl prac włączonych do rozprawy doktorskiej

Nazwa czasopisma	Tytuł artykułu	IF	MEiN	Data publikacji	Rodzaj publikacji
Biomedicine & Pharmacotherapy	Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance.	7.419	100	13.08.2021	Praca oryginalna
International Journal of Molecular Sciences	Can Physical Activity Support the Endocannabinoid System in the Preventive and Therapeutic Approach to Neurological Disorders?	5.923	140	09.06.2020	Praca przeglądowa
International Journal of Molecular Sciences	The Endocannabinoid System and Physical Activity-A Robust Duo in the Novel Therapeutic Approach against Metabolic Disorders.	6.208	140	09.03.2022	Praca przeglądowa

2. Wykaz stosowanych skrótów i oznaczeń

2-AG - 2-arachidonyloglicerol

7TM GPCRs - 7-transbłonowe receptory sprzężone z białkiem G

AD - choroba Alzheimera

AEA - anandamid

Akt - kinaza białkowa B

ASAH1 – ceramidaza kwaśna

ASAH2 - ceramidaza obojętna

ATP - adenozyno-5′-trifosforan

BCA - kwas bicynchoninowy

BSA - surowicza albumina bydlęca

Ca^{2+} - jony wapnia

cAMP - cykliczny adenozyno-3′,5′-monofosforan

CB1R - receptor kannabinoidowy typu 1

CB2R - receptor kannabinoidowy typu 2

CBD – kannabidiol

CDK 5 - kinaza cyklino-zależna 5

CER - ceramid

eCBome - endokannabinoidom

ECS - układ endokannabinoidowy

FAAH - hydrolaza amidowa kwasów tłuszczowych

GAPDH - dehydrogenaza aldehydu 3-fosfoglicerynowego

GLC - chromatografia gazowa

GPCRs - receptory sprzężone z białkiem G

GPR55 - receptor sprzężony z białkiem G55

GSK-3α i -3β - kinaza syntazy glikogenu 3α i 3β

HFD - dieta bogatotłuszczowa

HPLC - wysokosprawna chromatografia cieczowa

HRP - peroksydaza chrzanowa

HTR1A - receptor serotoninowy 1A

IR – insulinooporność

IRec – receptor insulinowy

IRS-1 - substrat 1 receptora insuliny

JNK - N-końcowa kinaza białka C-Jun

LASS4 i LASS6 - syntaza ceramidu 4 i 6

LCFA - długołańcuchowe kwasy tłuszczowe

MAGL - lipaza monoglicerolowa

MAPK - kinaza białkowa aktywowana mitogenami

N-SMase – sfingomielinaza obojętna

NKT - nienasycone kwasy tłuszczowe

OUN - ośrodkowy układ nerwowy

p38-MAPK - kinaza białkowa p38 aktywowana mitogenami

pAkt - fosforylowana forma kinazy białkowej B

pGSK-3α i -3β – fosforylowana forma kinazy syntazy glikogenu 3α i -3β

PHCA - ceramidaza zasadowa

PKB - kinaza białkowa B

PPAR α, β, γ - receptory aktywowane przez proliferatory peroksysomów α, β, γ

pTau – fosforylowane białko tau związane z mikrotubulami

PVDF - polifluorek winylidenu

RIPA - bufor radioimmunoprecypitacyjny

S1P - sfingozyno-1-fosforan

SA1P - sfinganino-1-fosforan

SFA - sfinganina

SFO - sfingozyna

SM - sfingomielina

SPHK1 i SPHK 2 – kinaza sfingozyny 1, 2

SPTLC1 i SPTLC2 - palmitoilotransferaza serynowa 1 i 2

T2DM - cukrzyca typu 2

Tau – białko tau związane z mikrotubulami

TBST – sól fizjologiczna buforowana tris-em z Tween 20

THC – tetrahydrokannabinol

TLC - chromatografia cienkowarstwowa

TRPV1 - receptor waniloidowy przejściowego potencjału

VR1 - receptor waniloidowy 1

Δ8-THC - delta-8-tetrahydrokannabinol

Δ9-THC - delta-9-tetrahydrokannabinol

3. Wstęp

Częstość występowania chorób metabolicznych, m.in. cukrzycy typu 2 (T2DM), otyłości oraz chorób neurodegeneracyjnych, m.in. choroby Alzheimera (AD), zwiększyła się istotnie w ciągu ostatnich dwóch dekad, szczególnie w krajach z tzw. zachodnimi wzorcami żywieniowymi [1–3]. Powszechnie dostępne dane wskazują na znaczne rozpowszechnienie ww. jednostek chorobowych w krajach rozwiniętych, a wielu autorów jednoznacznie definiuje patologie te jako epidemie XXI wieku [4,5]. AD definiowana jest klinicznie jako choroba otępienna charakteryzująca się postępującym zanikiem zdolności intelektualnych i poznawczych, stopniową utratą pamięci oraz zmianami behawioralnymi prowadzącymi docelowo do niezdolności do samodzielnego funkcjonowania [6]. Wieloczynnikowe podłoże molekularne AD próbują tłumaczyć liczne hipotezy, spośród których najbardziej uznawaną jest teoria opisująca odkładanie się w obrębie komórek nerwowych depozytów β-amyloidu (Aβ) w postaci płytek starczych i hiperfosforylowanego białka tau w postaci splątków neurofibrylarnych [7]. Wielu autorów uważa, iż jednym z wspólnych, kluczowych czynników związanych z rozwojem ww. chorób metabolicznych i neurodegeneracyjnych jest insulinooporność (IR) [8,9]. Insulina pierwotnie definiowana jako główny regulator obwodowego stężenia glukozy, w ostatnich latach została uznana także za kluczowy czynnik biorący udział w procesach pamięciowych, poznawczych oraz neuroplastyczności [10–12]. Niektóre badania wykazały, że rozwój ogólnoustrojowej insulinooporności i osłabionego działania insuliny w obrębie tkanki mózgowej są istotnymi czynnikami ryzyka rozwoju chorób neurodegeneracyjnych m.in. choroby Alzheimera [13]. Co istotne, korelacja pomiędzy IR mózgu, otyłością i chorobami otępiennymi wykazywana była również w wielu badaniach klinicznych [14–17].

3.1 Insulinowy szlak przekaźnictwa sygnału w korze mózgowej

Insulina jest hormonem anabolicznym produkowanym i wydzielanym przez komórki β wysp trzustkowych. Powszechnie wiadomo, że jest ona szeroko zaangażowana nie tylko w metabolizm węglowodanowy, lecz również m.in. pośrednio w biosyntezę białek, nasilenie lipogenezy, czy procesy mitogenne [18–20]. Działanie tego hormonu odbywa się poprzez połączenie ze swoistym receptorem insulinowym (IRec), który znajduje się w obrębie błony komórkowej m.in. hepatocytów, adipocytów, lecz również w obrębie struktur ośrodkowego układu nerwowego (OUN) [19]. Badania wykazują, iż największą ekspresję IRec w OUN wykazuje kora mózgowa, podwzgórze, hipokamp, prążkowie, opuszka węchowa oraz móżdżek [21,22]. Receptor insulinowy, zbudowany z dwóch podjednostek α i dwóch

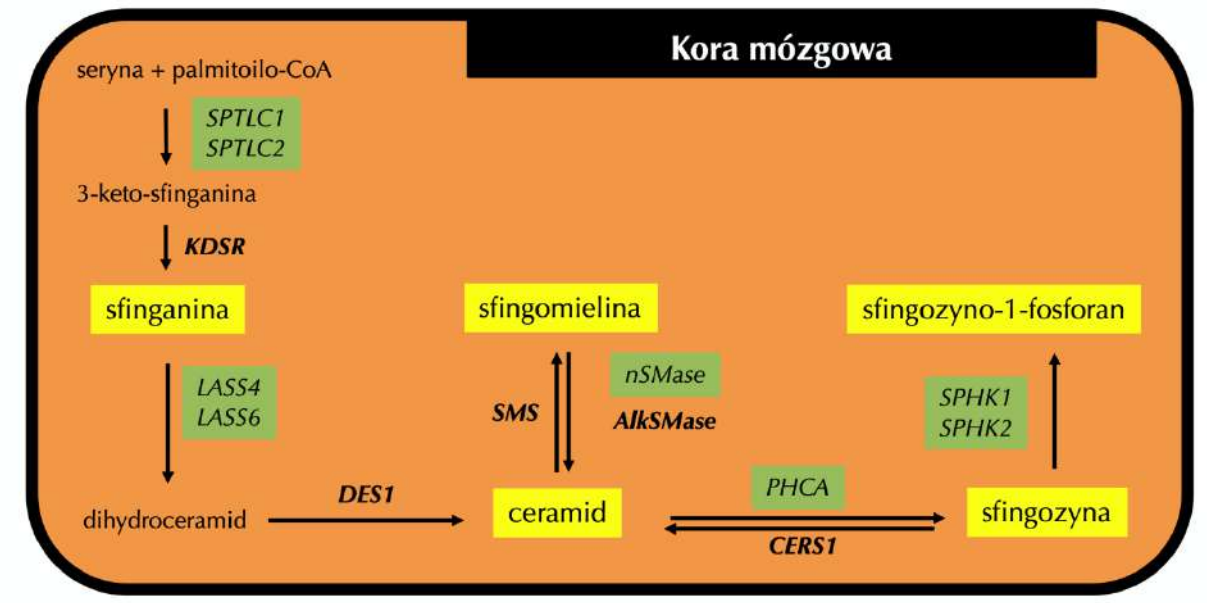
podjednostek β, należy do grupy receptorów o aktywności kinazy tyrozynowej. Insulinowe przekaźnictwo sygnału może odbywać się dwoma podstawowymi szlakami, które są wzajemnie ze sobą powiązane. Pierwszym jest szlak PI3K/Akt/GSK odpowiedzialny za większość efektów metabolicznych insuliny poprzez generowanie odpowiedzi fizjologicznej na ten hormon. Drugim jest natomiast szlak Raf/Ras/MEK/MAPK, znany również jako MAPK/ERK, który odpowiada za regulację ekspresji genów oraz, przy współudziale szlaku PI3K/Akt/GSK, bierze udział w procesach mitogennych i różnicowaniu komórek [23].

Insulina rozpoczyna swoje działanie komórkowe od związania z zewnątrzkomórkową podjednostką α IRec prowadząc tym samym do zmiany konfiguracji receptora insulinowego i autofosforylacji reszt tyrozynowych wewnątrzkomórkowej podjednostki β. Następnie, przy udziale adenozyno-5′-trifosforanu (ATP), dochodzi do fosforylacji reszt tyrozynowych, a tym samym aktywacji białek substratowych, do których należą m.in. substraty 1 i 2 receptora insuliny (IRS-1, -2) stanowiące kluczowe miejsca przekazywania sygnału w szlaku insulinowym. Co istotne, fosforylacja innych reszt aminokwasowych tj. serynowych i treoninowych, zarówno receptora insulinowego, jak i IRS, prowadzi do hamowania szlaku insulinowego [24]. Aktywowana forma IRS-1 doprowadza do pobudzenia szlaku 3-kinazy fosfatydyloinozytolu (PI3K), którego ufosforylowana forma katalizuje konwersję występującego w błonie plazmatycznej difosforanu fosfatydyloinozytolu (PIP2, phosphatidylinositol 4,5-bisphosphate) do trifosforanu fosfatydyloinozytolu (PIP3, phosphatidylinositol 3,4,5-trisphosphate). W następstwie dochodzi do połączenia PIP3 z kinazą-1 zależną od fosfatydyloinozytolu (PDK-1), do której substratów należy m.in. kinaza białkowa B, zwana również jako białko Akt (Akt/PKB). Ufosforylowana forma PKB prowadzi następnie do wzrostu fosforylacji m.in. kinazy syntazy glikogenu 3 α/β (GSK-3α/β) przy specyficznych resztach serynowych w pozycji 21 i 9 (Ser21 i Ser9) oraz spadku fosforylacji przy resztach tyrozynowych odpowiednio w pozycji 279 i 216 (Tyr279 i Tyr216) co prowadzi do inaktywacji GSK-3 α/β [25,26].

Wiele publikacji dowiodło, że insulinowy szlak przekaźnictwa sygnału PI3K/Akt/GSK poprzez udział w autofagii, neurogenezie, proliferacji i różnicowaniu neuronów odgrywa niezwykle istotną rolę w ośrodkowym układzie nerwowym [27,28]. Wieloetapowy, podlegający precyzyjnej regulacji insulinowy szlak przekaźnictwa sygnału może ulegać znacznym zaburzeniom, a czynnikami, które w niekorzystny sposób mogą z nim interferować są m.in. sfingolipidy.

3.2 Sfingolipidy i ich metabolizm w korze mózgowej

Powszechnie wiadomo, że dieta w stylu zachodnim, bogata w długołańcuchowe kwasy tłuszczowe (LCFA) powoduje nie tylko rozrost tkanki tłuszczowej, ale także ich nadmierny transport do komórek tkanek docelowych, które nie są przystosowane do akumulacji znacznej ilości lipidów tj. tkanki mięśniowej, wątroby, czy tkanki mózgowej [29,30]. Nadmierna zawartość LCFA zaburza szlaki ich utleniania i prowadzi do wzmożonej estryfikacji kwasów tłuszczowych do różnych frakcji lipidowych m.in. sfingolipidów [31]. Ta szeroko badana klasa lipidów, poza odgrywaniem istotnej roli strukturalnej w błonach komórkowych, bierze udział również w szlakach sygnalizacji komórkowej, a także we wzroście, różnicowaniu i regulacji apoptozy komórek [32]. Wśród wielu sfingolipidów do najbardziej bioaktywnych frakcji tej grupy lipidów należą m.in. ceramid (CER), sfingozyna (SFO), sfinganina (SFA) i sfingomielina (SM) [33]. W ciągu ostatniej dekady sfingolipidy były szeroko badane zarówno w kontekście metabolizmu lipidów, jak i glukozy, a szczególną uwagę skupiono na ceramidzie. Frakcja ta jest powszechnie uznawana za prekursor dla wielu innych sfingolipidów, m.in. sfingozyny, czy sfingozyno-1-fosforanu (S1P), stąd uważana jest za rdzeń i podstawowy element szlaku metabolizmu sfingolipidów. Możemy wyróżnić trzy podstawowe drogi syntezy CER (Ryc. 1).



Ryc. 1. Metabolizm sfingolipidów w korze mózgowej.

Pierwszą z nich jest szlak syntezy *de novo* ceramidu, możliwy dzięki kondensacji dwóch składowych tj. seryny i palmitoilo-CoA z wykorzystaniem palmitoilotransferazy serynowej (SPTLC) oraz szlak rozpadu sfingozyny przy udziale kinazy sfingozyny (SPHK), zwany również szlakiem ratunkowym (ang. *salvage pathway).* Trzeci szlak powstawania ceramidu stanowi hydroliza sfingomieliny przy udziale sfingomielinaz [34,35].

Rola ceramidu w procesach zachodzących w obrębie ośrodkowego układu nerwowego jest szeroko opisywana w literaturze. Poza niezwykle istotną funkcją strukturalną w obrębie neuronalnych błon komórkowych, m.in. tworzeniu błonowych tratw lipidowych i przekazywaniu sygnału zarówno pomiędzy, jak i w obrębie neuronów, ceramid jest również znacznie zaangażowany w molekularne podłoże neuropatologii. Rola tego sfingolipidu w formowaniu depozytów β-amyloidu, zaburzeniach mitochondrialnych, dysfunkcji autofagii i starzeniu komórkowym w istotny sposób przyczynia się do zwiększenia ryzyka rozwoju chorób neurodenegeracyjnych, w tym choroby Alzheimera [35–37]. Co więcej, zjawisko wzmożonego transportu dokomórkowego lipidów i w konsekwencji akumulacji wewnątrzkomórkowej sfingolipidów może doprowadzić do istotnych zaburzeń metabolizmu i klinicznie skutkuje rozwojem m.in. insulinooporności [38,39].

3.3 Zjawisko insulinooporności mózgowej

Oporność na insulinę określa się mianem stanu patologicznego, w którym tkanki docelowe m.in. tkanka tłuszczowa, mięśnie czy tkanka mózgowa charakteryzują się zmniejszoną wrażliwością na insulinę. W konsekwencji komórki β wysp trzustkowych produkują zwiększoną ilość insuliny, aby hormon ten mógł wywołać efekt biologiczny w tkankach docelowych [40]. Co istotne, w przeciwieństwie do mięśni szkieletowych i wątroby, gdzie poszczególne klasy lipidów, w tym sfingolipidy, ingerują w szlak sygnalizacji insuliny poprzez hamowanie stymulowanej insuliną fosforylacji Akt/PKB, w tkance mózgowej otyłych zwierząt z rozwiniętą insulinoopornością występuje zwiększona fosforylacja Akt/PKB, prowadząca do wzrostu fosforylacji GSK-3α/β w tyrozynie 279/216 co w konsekwencji podwyższa aktywność GSK-3. Zjawisko to wynika prawdopodobnie z tego, że insulina modyfikuje aktywność Akt poprzez różne substraty receptora insuliny w poszczególnych tkankach i docelowo prowadzi do osłabienia insulinowrażliwości, a tym samym stopniowego rozwoju insulinooporności [41,42].

Co ciekawe, Chaurasia i Summers zasugerowali, że to właśnie ceramid gromadzący się w nadmiarze wewnątrzkomórkowo można uznać za kluczowy czynnik prowadzący do rozwoju insulinooporności w różnych tkankach [43]. Ponadto Brozinick i in. wykazali *in vivo* na

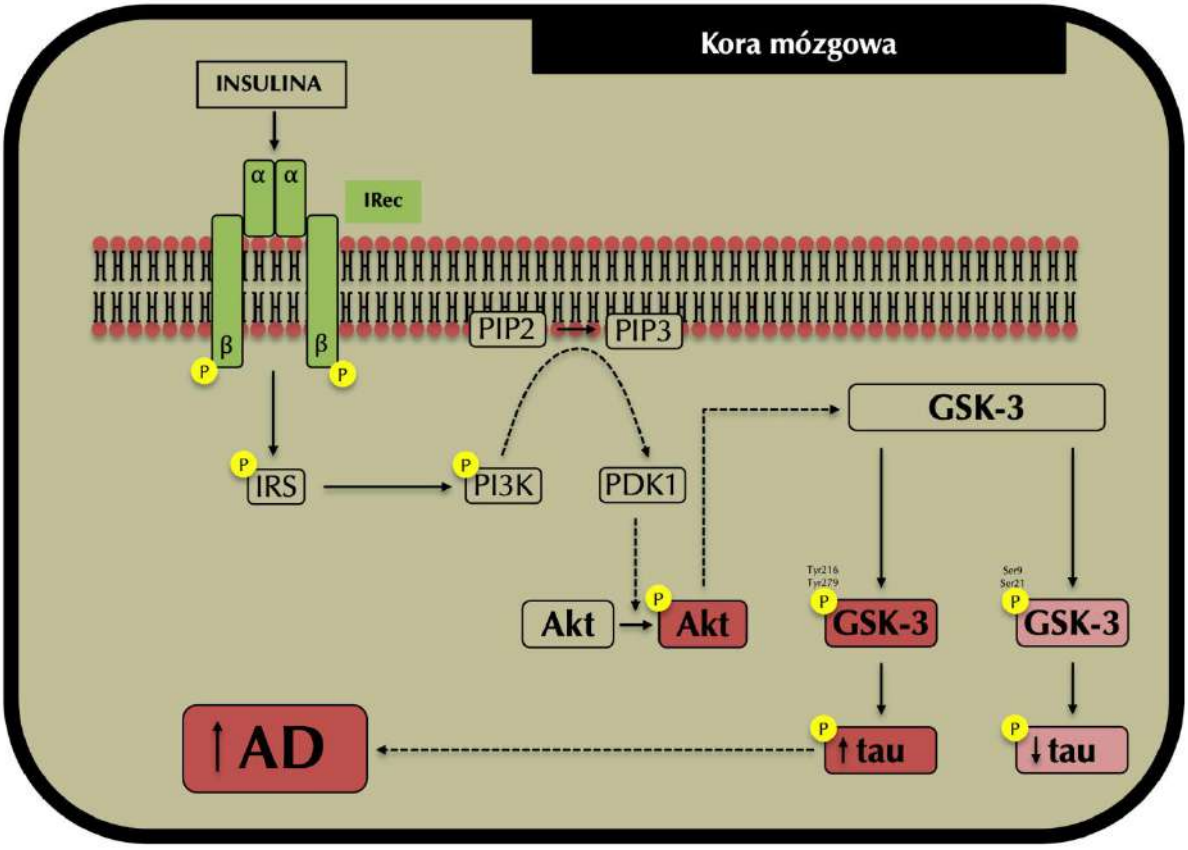
makakach królewskich, że sfingolipidy, głównie ceramid, ingerując w szlak sygnałowy insuliny, mogą przyczyniać się do zmniejszenia wrażliwości na insulinę i rozwoju ogólnoustrojowej insulinooporności [44]. Niektóre badania sugerowały również zdefiniowanie ceramidu, jako podstawowego biomarkera patologii metabolicznych, w tym insulinooporności [44,45]. Co istotne, Dekker i in. wykazali, że hamowanie enzymów biorących udział w syntezie *de novo* sfingolipidów, m.in. palmitoilotransferazy serynowej 1 (SPTLC1), spowodowało poprawę wrażliwości na insulinę i zmniejszenie lipogenezy w zwierzęcym modelu otyłości [51]. Ponadto, liczne dane wykazały, że zaburzenia szlaku sfingolipidowego mogą przyczyniać się do istotnych zmian szlaków sygnałowych w komórce, w tym insulinowego szlaku przekaźnictwa sygnału, a tym samym także rozwoju chorób neurodegeneracyjnych [46,47]. Dlatego też wielu autorów uważa, iż poszczególne elementy metabolizmu sfingolipidów mogą stanowić nowe punkty uchwytu do leczenia chorób zarówno metabolicznych, jak i neurodegeneracyjnych.

3.4 Rola insuliny i zaburzeń insulinowego szlaku przekaźnictwa sygnału w rozwoju chorób neurodegeneracyjnych

Insulina wydzielana przez komórki β wysp trzustkowych przedostaje się do mózgu poprzez barierę krew-mózg (poza częściami podwzgórza, które są wyłączone) na zasadzie transportu przezbłonowego opartego na transporcie czynnym zachodzącym przy udziale nośników białkowych, a proces ten zachodzi niezależnie od receptora insulinowego [48]. Badania wykazały, iż stosunek stężenia insuliny w płynie mózgowo-rdzeniowym do stężenia insuliny w osoczu ulega zmniejszeniu w ogólnoustrojowej insulinooporności, czy otyłości, ale również jest on obniżony u pacjentów z chorobami neurodegeneracyjnymi, w tym AD. Uważa się zatem, iż zmniejszony transport przez barierę krew-mózg, a tym samym zmniejszenie stężenia insuliny w obrębie ośrodkowego układu nerwowego może przyczyniać się do zaburzeń insulinowego szlaku przekaźnictwa sygnału w obrębie OUN, a tym samym rozwoju insulinooporności mózgowia i chorób neurozwyrodnieniowych [21,49].

Dotychczas wielu autorów wykazało istotną korelację pomiędzy rozwojem insulinooporności a występowaniem chorób neurodegeneracyjnych, m.in. choroby Alzheimera. Hiperfosforylacja białka tau oraz zwiększone depozyty β-amyloidu, stanowiące dwie podstawowe hipotezy rozwoju choroby Alzheimera, były szczególnie często analizowane w kontekście zaburzeń szlaku sygnałowego insuliny w obrębie tkanki mózgowej [50]. Niektóre badania wskazywały również na istotne znaczenie dysfunkcji mitochondriów, czy zaburzenia metabolizmu lipidów pod postacią wewnątrzkomórkowego wzrostu zawartości

poszczególnych frakcji lipidowych (diacylogliceroli, triacylogliceroli) i zwiększonego utleniania lipidów w wyniku nasilonego stresu oksydacyjnego związanego z insulinoopornością czy też zaburzenia procesu beta-oksydacji lipidów [11,51,52]. Ze wszystkich elementów insulinowego szlaku przekaźnictwa sygnału PI3K/Akt/GSK szczególną rolę w rozwoju neuropatologii przypisywano GSK-3α/β. Badania *in vitro* i *in vivo* wykazały, że zwiększona fosforylacja w Tyr 279/216 GSK-3α/β i zahamowana fosforylacja w Ser 21/9 GSK-3α/β prowadzi do zwiększonej aktywności enzymatycznej GSK, co w następstwie doprowadza do hiperfosforylacji białka tau, którego nadmierna agregacja upośledza neurogenezę i procesy pamięciowe oraz ma właściwości prozapalne (Ryc. 2) [53–57].



Ryc. 2. Zaburzenia w insulinowym szlaku przekaźnictwa sygnału prowadzące do rozwoju choroby Alzheimera (AD).

W regulacji szlaku sygnałowego insuliny PI3K/Akt/GSK i szlaku sfingolipidów w ośrodkowym układzie nerwowym bierze udział również wysiłek fizyczny. Badania przeprowadzone na modelu zwierzęcym wykazały, że wysiłek fizyczny jest jedną z istotnych metod przeciwdziałania i leczenia nie tylko otyłości czy cukrzycy typu 2, ale również chorób

neurodegeneracyjnych, ponieważ może prowadzić do hamowania hiperfosforylacji białka tau i zwiększenia insulinowrażliwości w obrębie kory mózgowej szczurów karmionych dietą bogatotłuszczową [58]. Układem biologicznym, którego składowe podlegają również znacznym zmianom pod wpływem wysiłku fizycznego czy diety bogatotłuszczowej jest układ endokannabinoidowy (ECS) [59].

3.5 Układ endokannabinoidowy i endokannabinoidom

Układ endokannabinoidowy (ECS), jako istotny regulator homeostazy organizmu, bierze udział w licznych procesach fizjologicznych. Wiele badań pokazuje również, że dysregulacja tego endogennego systemu sygnałowego może brać udział w rozwoju licznych chorób metabolicznych tj. otyłości, czy cukrzycy typu 2, zaś regulując aktywność czy też zawartość jego komponentów np. poprzez wysiłek fizyczny można wywołać efekty terapeutyczne [60–63]. Układ ten obejmuje (1) receptory kannabinoidowe typu 1 i 2 (CB1R, CB2R), (2) endogenne ligandy tych receptorów, znane jako endokannabinoidy (głównie anandamid – AEA oraz 2-arachidonilоglicerol - 2-AG) oraz (3) enzymy zaangażowane w ich biosyntezę, wychwyt zwrotny oraz degradację endokannabinoidów tj. hydrolaza amidów kwasów tłuszczowych (FAAH) i lipaza monoglicerolowa (MAGL) [64].

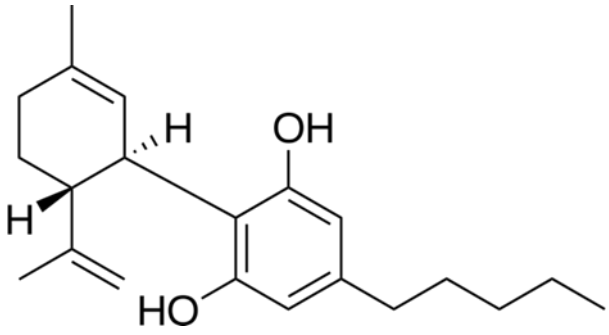
Receptory kannabinoidowe typu 1 i typu 2 należą do rodziny 7-transbłonowych receptorów sprzężonych z białkiem G (7TM GPCRs), a zatem ich aktywacja poprzez konkretny ligand prowadzi do zahamowania cyklazy adenylowej i zmniejszenia stężenia cyklicznego adenozyno-3′,5′-monofosforanu (cAMP). W rezultacie cAMP nasila aktywność kinazy białkowej aktywowanej mitogenami p42/p44 (MAPK) i N-końcowej kinazy C-Jun (JNK), które aktywują różne jądrowe czynniki transkrypcyjne, a tym samym zmieniają metabolizm komórkowy. Co więcej, receptory kannabinoidowe obecne w zakończeniach presynaptycznych neuronów aktywują kanały potasowe i hamują wapniowe, co skutkuje hamowaniem uwalniania innych neuroprzekaźników (tzw. mechanizm „odwrotnego przekaźnictwa”) [65–67]. Co istotne, w przeciwieństwie do klasycznych neurotransmiterów, endokannabinoidy nie są magazynowane w obrębie pęcherzyków synaptycznych, lecz są syntetyzowane w obrębie błony komórkowej neuronu postsynaptycznego jedynie w odpowiedzi na podwyższone wewnątrzkomórkowe stężenie jonów wapnia (Ca^{2+}) [68,69]. Receptor kannabinoidowy typu 1 znajduje się głównie w strukturach mózgu, zwłaszcza w obrębie kory nowej, hipokampu, jąder podstawnych i pnia mózgu, lecz jego obecność stwierdzono również w mięśniach szkieletowych, płucach, jądrach, przewodzie pokarmowym, wątrobie, trzustce oraz tkance tłuszczowej. Z kolei receptor kannabinoidowy

typu 2 zlokalizowany jest głównie na komórkach układu odpornościowego (makrofagach, limfocytach B, limfocytach T), krwiotwórczych komórkach macierzystych i progenitorowych obecnych w szpiku kostnym, a także w grasicy i węzłach chłonnych, dlatego też aktywacja CB2R może wywoływać liczne efekty immunomodulujące [67,70–72].

Wraz z rozwojem wiedzy na temat ECS, niektórzy badacze zdefiniowali rozszerzony układ endokannabinoidowy jako endokannabinoidom (eCBome). Jest on opisywany jako złożony lipidowy układ sygnałowy składający się z ponad 100 pochodnych kwasów tłuszczowych i ich receptorów, a także enzymów. eCBome poza podstawowymi elementami wchodzącymi w skład układu endokannabinoidowego zawiera również znaczną liczbę różnych receptorów błonowych i jądrowych, m.in. PPAR, receptor waniloidowy przejściowego potencjału 1 (TRPV1), różne receptory sprzężone z białkiem G (GPCRs), ich endogenne ligandy oraz wiele enzymów odgrywających ważną rolę w endokannabinoidowych szlakach sygnałowych [65,73].

3.6 Kannabidiol – terapeutyk przyszłości w leczeniu insulinooporności i chorób neurodegeneracyjnych

Na podstawie licznych badań okazuje się, że układ endokannabinoidowy, endokannabinoidom i poszczególne fitokannabinoidy, m.in. kannabidiol (CBD), mogą w przyszłości stać się idealnym narzędziem do poszukiwania nowych metod terapeutycznych zarówno chorób metabolicznych, jak i neurodegeneracyjnych.



Ryc 3. Struktura chemiczna kannabidiolu (CBD).

Konopie siewne (*Cannabis sativa* L.) znajdują szerokie zastosowanie terapeutycznie od tysięcy lat i chociaż słyną głównie z tetrahydrokannabinolu (THC), to jest on tylko jednym z wielu fitokannabinoidów syntetyzowanych przez te rośliny [65]. Kannabidiol jest związkiem pochodzącym z *Cannabis sativa*, lecz jest on pozbawiony komponenty psychoaktywnej [66]. Badania wykazują, że jest on niezwykle obiecującym fitokannabinoidem ze względu na

doskonały profil bezpieczeństwa i wiele stwierdzonych potencjalnych efektów terapeutycznych, m.in. w leczeniu schorzeń neurologicznych. Co ciekawe, kannabidiol ma bardzo niskie powinowactwo do receptorów kannabinoidowych. Współdziała jednak z innymi złożonymi układami sygnałowymi zaliczanymi obecnie do endokannabinoidomu [67,68]. Poszczególne prace wykazały, że mechanizmy działania CBD mogą być powiązane z licznymi celami molekularnymi, m.in. z receptorami sprzężonymi z białkiem G, receptorami serotoninowymi, adenozynowymi, opioidowymi, czy receptorami aktywowanymi przez proliferatory peroksysomów γ (PPARγ). CBD może również prowadzić do hamowania FAAH, co docelowo powoduje wzrostu stężenia anandamidu w surowicy. Uważa się, że różnorodne cele molekularne kannabidiolu są w znacznym stopniu skorelowane z jego istotnym wpływem na wiele różnych szlaków sygnałowych [69]. Liczne badania *in vitro* jednoznacznie wykazały, że CBD prezentuje działanie antyapoptotyczne, antyzapalne i antyoksydacyjne jednocześnie prowadząc do hamowania procesów neurotoksycznych [74–76]. Co więcej, wyniki badań na liniach komórkowych znalazły również potwierdzenie na licznych modelach zwierzęcych z jednoczesnym stwierdzeniem znacznej poprawy parametrów neurofizjologicznych m.in. pamięci przestrzennej, zdolności poznawczych i deficytów poznania społecznego po ekspozycji na kannabidiol [74,77–79]. Badanie prowadzone przez Libro i in. *in vitro* na mezenchymalnych komórkach macierzystych wykazało, że CBD może prowadzić do hamowania ekspresji GSK-3β, jednego z głównych elementów patogenezy choroby Alzheimera, poprzez intensyfikację sygnalizacji PI3K/Akt z jednoczesną regulacją w dół genów kodujących białka zaangażowane w tworzenie depozytów β-amyloidu i fosforylację białka tau [80]. Co więcej, badanie Ozaity i in. wykazało *in vivo*, że jeden z podstawowych fitokannabinoidów, tj. THC, może wykazywać właściwości neuroprotekcyjne również poprzez aktywację szlaku PI3K/Akt oraz hamowanie aktywności GSK-3β w mózgu myszy CD-1 [81]. Niektórzy badacze wykazują również, że kannabidiol może znaleźć szerokie zastosowanie w leczeniu poszczególnych chorób metabolicznych m.in. cukrzycy typu 2, czy powikłań cukrzycowych [82]. Zapobieganie zarówno chorobom metabolicznym i neurodegeneracyjnym jest niezwykle alarmującym, ogólnoświatowym problemem, gdyż liczba osób cierpiących na te choroby rośnie rokrocznie we wszystkich populacjach świata, a insulinooporność, cukrzycę typu 2 i chorobę Alzheimera można określić mianem współczesnych epidemii, które będą wymagały zastosowania innowatorskich terapeutyków.

Szczegółowe powiązanie chorób neurologicznych z układem endokannabinoidowym zostało przedstawione w pracy przeglądowej wchodzącej w skład rozprawy doktorskiej:
Can Physical Activity Support the Endocannabinoid System in the Preventive and Therapeutic Approach to Neurological Disorders?
Charytoniuk Tomasz, Zywno H, Konstantynowicz-Nowicka K, Berk K, Bzdega W, Chabowski A.: 2020, *International Journal of Molecular Sciences*

Szczegółowe powiązanie chorób metabolicznych z układem endokannabinoidowym zostało przedstawione w pracy przeglądowej wchodzącej w skład rozprawy doktorskiej:
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Charytoniuk Tomasz, Zywno H, Berk K, Bzdega W, Kolakowski A, Chabowski A, Konstantynowicz-Nowicka K.: 2022, *International Journal of Molecular Sciences*

4. Cel pracy

Rozpowszechnienie otyłości i powiązanych z nią insulinooporności oraz chorób neurodegeneracyjnych istotnie zwiększyło się w ciągu ostatnich dekad na całym świecie wśród różnych grup społecznych i etnicznych, stając się ogromnym obciążeniem zarówno dla systemów ochrony zdrowia, jak i ekonomii wielu krajów. Jednym z głównych mechanizmów prowadzących do rozwoju insulinooporności jest nadmierna, przekraczająca zdolności oksydacyjne komórek podaż kwasów tłuszczowych, które są następnie estryfikowane do różnych frakcji lipidowych m.in. sfingolipidów. Nadmierna wewnątrzkomórkowa akumulacja tej grupy lipidów prowadzi do istotnych zaburzeń w szlaku sygnałowym insuliny czego efektem jest rozwój nie tylko insulinooporności mięśni szkieletowych, ale również mózgowia co ma swoje konsekwencje, nie tylko w rozwoju chorób metabolicznych, ale także neurodegeneracyjnych, w tym także w rozwoju choroby Alzheimera. Pomimo, iż choroby te są bardzo rozpowszechnione we współczesnym świecie, wciąż brakuje skutecznych i bezpiecznych środków terapeutycznych. Badania wykazują, że fitokannabinoidy mogą stanowić niezwykle obiecujący rodzaj terapii, która potencjalnie może przyczynić się do niwelowania insulinooporności z jednoczesnym ograniczeniem jej niepomyślnych skutków zarówno w kontekście chorób metabolicznych, jak i neurodegeneracyjnych. Dlatego też zasadnym wydawało się określenie, czy kannabidiol, jeden z głównych fitokannabinoidów, poprzez zmiany w metabolizmie sfingolipidów tkanki mózgowej, wywoła korzystny wpływ na insulinooporność. Szczegółowe cele zrealizowanego projektu obejmowały:

1. Ocenę wpływu kannabidiolu na zmiany zawartości poszczególnych frakcji sfingolipidów tj. sfingozyny, sfinganiny, ceramidu, sfingozyno-1-fosforanu, sfinaganino-1-fosforanu oraz sfingomieliny
2. Ocenę wpływu kannabidiolu na zmiany ekspresji i fosforylacji białek ze szlaku sygnałowego insuliny w korze mózgowej szczurów karmionych dietą bogatotłuszczową
3. Ocenę zależności pomiędzy zmianami ilości sfingolipidów a stopniem fosforylacji białek ze szlaku sygnałowego insuliny w korze mózgowej szczurów karmionych dietą bogatotłuszczową
4. Ocena wpływu kannabidiolu na stopień fosforylacji białka tau, zaangażowanego w rozwój chorób neurozwyrodnieniowych, w korze mózgowej szczurów karmionych dietą bogatotłuszczową
5. Ocena wpływu kannabidiolu na wybrane elementy endokannabinoidomu w korze mózgowej szczurów karmionych dieta bogatotłuszczową

5. Materiały i metody

Szczegółowe omówienie materiałów i metod znajduje się w pracy oryginalnej, wchodzącej w skład rozprawy doktorskiej:

Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance.

Charytoniuk Tomasz, Sztolsztener Klaudia, Harasim-Symbor Ewa, Berk Klaudia, Chabowski Adrian, Konstantynowicz-Nowicka Karolina.:. 2021, *Biomedicine & Pharmacotherapy.*

5.1 Model doświadczalny

Badania zostały przeprowadzone na samcach szczurów szczepu Wistar o masie początkowej 60-100 gramów. Zgoda na niniejsze badania została wydana przez lokalną Komisję Etyczną ds. Doświadczeń na Zwierzętach w Olsztynie (nr zgody – 71/2018). Podczas trwania eksperymentu zwierzęta były przetrzymywane w pomieszczeniu o regulowanym cyklu dobowym (12 godzin dzień, 12 godzin noc), temperaturze (22°C ± 2) i wilgotności powietrza (35-60%), miały również zapewniony stały dostęp do wybranej paszy i wody. Po 7 dniach aklimatyzacji zwierzęta zostały losowo rozdzielone do czterech grup doświadczalnych, w każdej grupie znajdowało się po 10 osobników:

I) **grupa kontrolna, Control** była karmiona standardową paszą dla gryzoni zawierającą 60% węglowodanów, 20% tłuszczu i 20% białka (Labofeed B, „Morawski", Kcynia, Polska) oraz przez ostatnie 2 tygodnie eksperymentu otrzymywała dootrzewnowe iniekcje rozpuszczalnika substancji czynnej składającego się z etanolu/Tween20/0,9%NaCl w stosunku 1:1:8 oraz dawce 1 ml/kg masy ciała na dzień,

II) **grupa HFD**, w której insulinooporność była indukowana poprzez podawanie diety bogatotłuszczowej (HFD) zawierającej 60% tłuszczu, 20% węglowodanów i 20% białka (Research Diets, New Brunswick, NJ, USA) oraz otrzymywała przez ostatnie 2 tygodnie eksperymentu dootrzewnowe iniekcje rozpuszczalnika substancji czynnej składającego się z etanolu/Tween20/0,9%NaCl w stosunku 1:1:8 oraz dawce 1 ml/kg masy ciała na dzień,

III) **grupa CBD** była karmiona standardową paszą dla gryzoni oraz otrzymywała dootrzewnowe iniekcje CBD (10 mg/kg masy ciała/dzień) raz dziennie o tej samej porze przez ostatnie 14 dni (6 i 7 tydzień) eksperymentu,

IV) **grupa HFD+CBD**, w której insulinooporność była indukowana poprzez podawanie diety bogatotłuszczowej (HFD) oraz otrzymywała dootrzewnowe iniekcje CBD (10 mg/kg masy ciała/dzień) raz dziennie o tej samej porze przez ostatnie 14 dni (6 i 7 tydzień) eksperymentu.

W powyższych warunkach zwierzęta ze wszystkich grup były hodowane łącznie przez 7 tygodni. Pod koniec eksperymentu szczury usypiane były poprzez wziewne podanie izofluranu, a następnie od uśpionych zwierząt zostały pobrane próbki kory mózgowej. Fragmenty przedczołowej kory mózgowej zostały wyizolowane, a następnie zamrożone w ciekłym azocie (-196°C) bezpośrednio po pobraniu i przechowywane w temperaturze -80°C do dalszej analizy.

Droga podania CBD, tj. droga dootrzewnowa, oraz dawka zostały wybrane na podstawie analizy danych literaturowych, które wykazały, że iniekcje dootrzewnowe nie powodowały powstawania psychoaktywnych pochodnych CBD, takich jak Δ9-THC i Δ8-THC, które były obecne po podaniu doustnym, a dawka 10mg/kg masy ciała była efektywna [83].

Szczegółowe badania objęły ocenę:

- stopnia ekspresji enzymów i receptorów endokannabinoidomu tj. **receptora kannabinoidowego typu I** (CB1R), **hydrolazy amidów kwasów tłuszczowych 1** (FAAH1), **receptora sprzężonego z białkiem G55** (GPR55), **receptora waniloidowego** 1 (VR1), **receptorów aktywowanych przez proliferatory peroksysomów α, β, γ** (PPAR α, β, γ), **receptora serotoninowego 1A** (HTR1A) metodą Western Blot;
- całkowitej ekspresji białek i ich fosforylowanych form zaangażowanych w szlak sygnałowy insuliny tj. **substratu 1 receptora insuliny i jego fosforylowanej formy** (IRS-1, pIRS-1 (S302)), **kinazy białkowej B i jej fosforylowanych form** (Akt, pAkt (S473), pAkt (Thr305), pAkt (Thr308), pAkt (S472, S473, S474**)), kinazy syntazy glikogenu 3α i jej fosforylowanych form** (GSK-3α, pGSK-3α (Ser21), pGSK-3α (Tyr279)), **kinazy syntazy glikogenu 3α i jej fosforylowanych form** (GSK-3 β, pGSK-3β (Ser9), pGSK-3β (Tyr216) metodą Western Blot;

- tkankowego stężenia poszczególnych sfingolipidów tj. **sfingozyny** (SFO), **sfingozyno-1-fosforanu** (S1P), **sfinganiny** (SFA), **sfinganino-1-fosforanu** (SA1P) i **ceramidu** (CER) metodą wysokosprawnej chromatografii cieczowej (HPLC);
- tkankowego stężenia **sfingomieliny** (SM) metodą chromatografii gazowej (GLC)
- całkowitej ekspresji poszczególnych enzymów zaangażowanych w metabolizm sfingolipidów tj. **palmitoilotransferazy serynowej 1 i 2** (SPTLC1, SPTLC2), **syntazy ceramidu 4 i 6** (LASS4, LASS6), **ceramidazy kwaśnej** (ASAH1), **ceramidazy obojętnej** (ASAH2), **ceramidazy zasadowej** (PHCA), **sfingomielinazy obojętnej** (N-SMase) metodą Western Blot;
- całkowitej ekspresji **białka Tau** (ang. *tubulin associated unit*) i jego fosforylowanych form zaangażowanych w choroby neurozwyrodnieniowe tj. pTau (S202), pTau (S396), pTau (S404), pTau (S416) metodą Western Blot;

5.2 Analiza całkowitej ekspresji wybranych białek metodą Western blot

Próbki kory mózgowej zostały zhomogenizowane przy użyciu homogenizatora ręcznego w temperaturze 4°C w buforze lizującym RIPA (radioimmunoprecipitation assay buffer) zawierającym inhibitory fosfataz i proteaz (Roche, Szwajcaria), a następnie próbki z lizatem były odwirowywane przy 12000 obr./min przez 20 min w 4°C. W uzyskanym supernatancie oznaczono całkowite stężenie białka wykorzystując kwas bicynchoninowy (BCA). Następnie przeprowadzono elektroforetyczny rozdział białek w 10% żelu poliakrylamidowym (Criterion TGX Stain-Free Precast Gel, Bio-Rad, USA), w którym po ekspozycji na światło ultrafioletowe (UV) tryptofan obecny w próbkach białek tworzy fluorescencyjny produkt poddawany transferowi na błony nitrocelulozowe lub błony z polifluorku winylidenu (PVDF). Następnie fluorescencyjne białko całkowite obecne na błonie jest wykrywane za pomocą urządzenia do obrazowania *charge-coupled device* (CCD) i oświetlenia UV. W celu zablokowania niespecyficznych wiązań i tym samym zapobiegnięciu niespecyficznemu łączeniu przeciwciał membrany inkubowano w 5% roztworze surowiczej albuminy bydlęcej (bovine serum albumin, BSA) lub w 5% roztworze odtłuszczonego mleka w proszku rozpuszczonych w buforze TBST (Tris-Buffered Saline with Tween 20) i poddano całonocnej inkubacji z odpowiednimi przeciwciałami I-rzędowymi. Po inkubacji i 3-krotnym przepłukaniu membran buforem TBST, zostały one poddane godzinnej inkubacji z odpowiednim II-rzędowym przeciwciałem anty-IgG znakowanym peroksydazą chrzanową (HRP). W celu zobrazowania na membranie miejsc wiązania znakowanych przeciwciał

wykorzystano chemiluminescencyjny substrat (Clarity Western ECL Substrate, Bio-Rad, Hercules, CA, USA), a następnie otrzymane sygnały odczytano densytometrycznie w systemie do wizualizacji ChemiDoc (Image Laboratory Software, Bio-Rad, Warszawa, Polska). Uzyskany obraz badanego białka i białka całkowitego był nakładany na siebie w systemie ImageLab w celu normalizacji ekspresji białka.

5.3 Analiza stężenia sfingolipidów w korze mózgowej

Do pomiaru stężenia poszczególnych sfingolipidów tj. sfingozyny, sfingozyno-1-fosforanu, sfinganiny, sfinganino-1-fosforanu i ceramidu zastosowano metodę wysokosprawnej chromatografii cieczowej. Sfingolipidy zostały wyekstrahowane z zsonifikowanych próbek kory mózgowej przy użyciu roztworu metanolu z kwasem solnym w obecności standardów wewnętrznych (C17-sfingozyny i C17-sfingozyno-1-fosforanu). Ilość sfingozyno-1-fosforanu i sfinganino-1-fosforanu oznaczono metodą pośrednią po defosforylacji odpowiednio sfingozyny i sfinganiny z użyciem fosfatazy alkalicznej (ALP, Sigma Aldrich, Saint Louis, MO, USA). Niewielką ilość wyekstrahowanych lipidów przeniesiono do nowej probówki zawierającej N-palmitoilo-D-erytro-sfingozynę (zasada C17) jako standard wewnętrzny, a następnie poddano hydrolizie alkalicznej w celu konwersji ceramidu do sfingozyny. Sfingozyna, sfinganina i defosforylowane zasady sfingoidowe pochodzące z ceramidu zostały poddane procesowi derywatyzacji i następnie przekształcone do ich o-ftalaldehydowych pochodnych i następnie zanalizowane za pomocą systemu HPLC (*Varian ProStar, Agilent Technologies, Santa Clara USA*) wyposażonego w detektor fluoroscencyjny i kolumnę w układzie faz odwróconych C18. Stężenie sfingolipidów zostało przedstawione w pikomolach na miligram tkanki.

5.4 Analiza stężenia sfingomieliny w korze mózgowej

W celu oceny stężeń sfingomieliny (SM) lipidy były ekstrahowane przy pomocy mieszaniny roztworów chloroform/metanol w stosunku objętościowym 2:1 zgodnie z metodą Folcha [84]. Sfingomielina została oddzielona od innych wyekstrahowanych fosfolipidów za pomocą chromatografii cienkowarstwowej (TLC). Następnie poszczególne frakcje kwasów tłuszczowych poddano transmetylacji w 14% metanolowym roztworze trójfluorku boru, a następnie rozpuszczono w heksanie. Zawartość poszczególnych estrów metylowych kwasów tłuszczowych (FAME, fatty acid methyl esters) została oznaczona zgodnie z czasami retencji odpowiedniego standardu metodą chromatografii gazowej (GLC; chromatograf gazowy

Helwett-Packard 5890 Series II wyposażony w kolumnę kapilarną Hewlett-Packard-INNOWax oraz detektor płomieniowo-jonizujący, Agilent Technologies, Santa Clara, CA, USA). Całkowite stężenie SM została zmierzona jako suma poszczególnych frakcji kwasów tłuszczowych i wyrażona w pikomolach na miligram tkanki.

5.5 Analiza statystyczna

Wszystkie dane przedstawiono jako wartość średnią ± odchylenie standardowe (SD). W analizie statystycznej zastosowano test Shapiro-Wilka oraz test Bartletta celem zapewnienia rozkładu normalnego i jednorodności wyników. Różnice statystyczne pomiędzy badanymi grupami analizowano za pomocą dwuczynnikowej analizy wariancji (two-way ANOVA), a następnie odpowiedniego testu post hoc (test Tukeya i test t-Studenta). W analizie wykorzystano oprogramowanie GraphPad Prism 5 (La Jolla, CA, USA). Za poziom istotności statystycznej uznano $p < 0,05$.

6. Wyniki

Szczegółowe omówienie uzyskanych wyników znajduje się w pracy oryginalnej, wchodzącej w skład rozprawy doktorskiej:

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W opisie wyników wykorzystano odniesienia do figur zamieszczonych w wyżej wymienionej publikacji.

6.1 Wpływ kannabidiolu na stężenie sfingolipidów

Stężenie sfingozyny (SFO) w homogenatach kory mózgowej istotnie wzrosło w przypadku grupy karmionej dietą bogatotłuszczową (+29,2%, $p < 0,01$, ryc. 1A) i grupy, która miała podawany kannabidiol (+62,7%, $p < 0,01$, ryc. 1A) w porównaniu do grupy kontrolnej. Ponadto w eksperymencie zaobserwowano znaczne zmniejszenie zawartości SFO w grupie HFD + CBD (-31,6%, $p < 0,001$, ryc. 1A) w porównaniu z grupą HFD. Podobnie poziom sfinganiny (SFA) wzrósł wyraźnie w grupach HFD (+20,4%, $p < 0,05$, Ryc. 1B) i CBD (+50,2%, $p < 0,01$, Ryc. 1B) w porównaniu z grupą kontrolną. Zauważyliśmy istotne zmniejszenie stężenia sfinganiny jedynie w grupie HFD + CBD (- 28,2%, $p < 0,01$, Ryc. 1B) w porównaniu z grupą karmioną dietą bogatotłuszczową. Podanie CBD istotnie zmniejszyło zawartość ceramidu w korze mózgowej u szczurów z grupy HFD (-14,3%, $p < 0,05$, Ryc. 1C). Co więcej, zaobserwowaliśmy istotny wzrost CER w grupie HFD (+18,9%, $p < 0,05$, Ryc. 1C) w stosunku do grupy kontrolnej. U szczurów z grupy CBD wykazaliśmy istotnie zwiększoną zawartość sfingozyno-1-fosforanu w stosunku do grupy kontrolnej, jak również w porównaniu z grupą szczurów karmionych dietą bogatotłuszczową (odpowiednio +65,2%, +53,9%; $p < 0,05$, ryc. 1D). We wszystkich grupach doświadczalnych zmiana zawartości SA1P nie osiągnęła istotności statystycznej (ryc. 1E). W przypadku sfingomieliny w porównaniu z homogenatami mózgu uzyskanymi od szczurów z grupy kontrolnej i karmionych dietą bogatotłuszczową, zaobserwowaliśmy znaczny wzrost zawartości tego

sfingolipidu w grupie CBD (odpowiednio +13,4%, +17,1%; p < 0,05, ryc. 1F) i HFD + CBD (odpowiednio +9,9%, p < 0,05; +13,5%, p < 0,01, rys. 1F).

6.2 Wpływ kannabidiolu na ekspresję enzymów szlaku syntezy *de novo* ceramidu oraz enzymów wspólnych dla szlaku *de novo* i ratunkowego

W korze mózgowej szczurów zaobserwowaliśmy istotne zmiany w ekspresji palmitoilotransferazy serynowej 1 i 2. Odnotowaliśmy istotny spadek ekspresji SPTLC1 w grupie CBD (-24,4%, p < 0,01, Ryc. 2A) oraz w grupie CBD+HFD (-29,5%, p<0,05, Ryc. 2 A) w porównaniu z grupą kontrolną i w grupie CBD (-13,8%, p < 0,05, ryc. 2A) w porównaniu z grupą HFD. Podanie dootrzewnowe CBD u szczurów karmionych dietą bogatotłuszczową spowodowało zmniejszenie ekspresji SPTLC2 w porównaniu z grupą kontrolną i HFD (odpowiednio – 28,7%, p < 0,01; – 18,6%, p < 0,05, ryc. 2B). Co więcej, dieta bogatotłuszczowa z iniekcjami CBD spowodowała znaczne zmniejszenie ekspresji syntazy ceramidowej 6 (LASS6) (−40,0%, p < 0,05, ryc. 2C) w porównaniu z grupą kontrolną oraz (-22,0%, p < 0,01, ryc. 2C) grupą HFD. Zaobserwowaliśmy znaczne zahamowanie ekspresji syntazy ceramidowej 4 (LASS4) w grupie CBD i HFD+CBD w porównaniu z grupą kontrolną (CBD: -21,9%, HFD + CBD: − 24,4%, p < 0,05, Ryc. 2D) i grupą HFD (CBD: -23,9%,HFD+CBD:- 26,4%,p<0,05,Rys.2D).

6.3 Wpływ kannabidiolu na ekspresję enzymów zaangażowanych w metabolizm ceramidu

We wszystkich badanych grupach nie wykazaliśmy istotności statystycznej w zmianach ekspresji ceramidazy kwasowej (ASAH1) (ryc. 3A). Zaobserwowaliśmy trend w kierunku zmniejszenia ekspresji ceramidazy neutralnej (ASAH2) (CBD vs HFD: p = 0,1357, Ryc. 3B) i ceramidazy zasadowej (PHCA) (HFD vs kontrola: p = 0,1924, CBD vs kontrola: p = 0,0717, ryc. 3C), jednak również nie osiągnęliśmy istotności statystycznej. Co istotne, iniekcje CBD znacznie zmniejszyły ekspresję neutralnej sfingomielinazy (N-SMazy) w porównaniu z grupą kontrolną (-38,6%, p < 0,05, ryc. 3D). Ponadto, w porównaniu ze szczurami, którym podawano dietę HFD, zaobserwowaliśmy istotny wzrost ekspresji N-SMazy w grupie HFD + CBD (+99,0%, p < 0,05, Ryc. 3D). Ekspresja kinazy sfingozyny 1 (SPHK1) była obniżona w grupie CBD w porównaniu z grupą kontrolną (-35,9%, p < 0,05, Ryc. 3E) oraz w grupie CBD+HFD w porównaniu z grupą kontrolną i HFD (- 53,5%, − 44,2%, p < 0,01, Ryc. 3E). Zaobserwowaliśmy również znaczące zmniejszenie ekspresji kinazy sfingozyny 2 (SPHK2) po podaniu CBD w grupie HFD (-37,7%, p < 0,05, ryc. 3F) w porównaniu z grupą kontrolną i (-18,6%, p < 0,05, rys. 3F) grupą HFD.

6.4 Wpływ kannabidiolu na ekspresję białek zaangażowanych w szlak sygnałowy insuliny

W korze mózgowej szczurów karmionych dietą bogatotłuszczową wykazaliśmy znaczny wzrost współczynnika fosforylacji białek bezpośrednio zaangażowanych w szlak sygnałowy insuliny, wyrażony w stosunku formy fosforylowanej kinazy białkowej B tj. pAkt (Thr305) do całkowitej Akt (+362,4%, $p < 0,001$, Rys. 4A), współczynnika pAkt(Thr308):Akt (+52,6%, $p<0,05$, Rys. 4B), współczynnika pAkt(S472,S473,S474):Akt (+140,8%, $p < 0,001$, Rys. 4C) oraz formy fosforylowanej kinazy syntazy glikogenu-3α i 3β tj. pGSK-3α(Tyr279) do GSK-3α/β (+25,0%, $p < 0,05$, Ryc. 4G) i współczynnika pGSK-3β(Tyr216):GSK-3α/ β (+71,1%, $p < 0,001$, Ryc. 4I) w porównaniu ze szczurami z grupy kontrolnej. Z kolei podawanie HFD spowodowało wyraźne zmniejszenie współczynnika fosforylacji pGSK-3α(Ser21):GSK-3α /β (- 37,6%, $p < 0,001$, ryc. 4F, vs grupa kontrolna) i współczynnika pGSK-3β(Ser9):GSK-3α/β (- 80,8%, $p < 0,001$, ryc. 4B, vs grupa kontrolna). W warunkach diety bogatotłuszczowej wstrzyknięcie CBD spowodowało znaczny spadek współczynnika pGSK-3α(Tyr279):GSK-3α/β (-22,0% i -37,0%, $p < 0,001$, ryc. 4G, vs grupa kontrolna i HFD) oraz współczynnika pGSK-3β(Tyr216):GSK-3α/β (−32,8%, $p < 0,05$, ryc. 4I, vs grupa HFD). Co więcej, w grupie HFD+CBD zauważyliśmy znaczny wzrost współczynnika fosforylacji Akt(Thr305) (+204,6%, $p < 0,01$, Ryc. 4A), Akt(S472,S473,S474) (+158,7% , $p < 0,01$, Ryc. 4C) w porównaniu do grupy kontrolnej i wzrost współczynnika fosforylacji GSK-3α(Ser21) (+38,2%, $p < 0,001$, Ryc. 4F), GSK-3β (Ser9) (+481,6% , $p < 0,001$, Fig. 4H) w porównaniu do grupy HFD. Jednocześnie ekspozycja na CBD powodowała znaczne zmniejszenie współczynnika fosforylacji Akt(Thr305) (- 70,3%, $p < 0,001$, Rys. 4A), Akt(S472,S473,S474) (- 34,1%, $p < 0,05$, ryc. 4C), Akt(S473) (- 36,0%, $p < 0,01$, ryc. 4D), GSK-3α (Tyr279) (- 23,5%, $p < 0,05$, ryc. 4G) i GSK-3β (Tyr216) (-28,1%, $p < 0,05$, ryc. 4I) w porównaniu do grupy HFD. CBD również spowodował zwiększenie współczynnika pAkt(S472,S473,S474):Akt (+58,7%, $p < 0,05$, ryc. 4C, w porównaniu z grupą kontrolną), pGSK-3α(Ser21):GSK-3α/β (+25,2%, $p < 0,05$, Ryc. 4F, vs. grupa HFD) i zwiększenie współczynnika pGSK-3β(Ser9):GSK-3α/β (+288,3%, $p < 0,001$, Ryc. 4H, vs. grupa HFD). W grupie otrzymującej CBD w porównaniu ze szczurami z grupy kontrolnej zauważyliśmy znaczny spadek współczynnika fosforylacji pAkt(S473):Akt (−38,4%, $p < 0,01$, ryc. 4D). Co ciekawe, poziom zmian współczynnika formy fosforylowanej substratu receptora insuliny-1

tj. pIRS-1 (S302) do IRS-1 nie osiągnął istotności statystycznej we wszystkich badanych grupach (ryc. 4E).

6.5 Wpływ kannabidiolu na ekspresję białka Tau

Karmienie szczurów dietą bogatotłuszczową spowodowało znaczny wzrost współczynnika fosforylacji wyrażony w stosunku formy fosforylowanej białka Tau, tj. pTau(S202) (+23,9%, $p < 0,001$, ryc. 5A), pTau(S396) (+17,6%, $p < 0,0001$, ryc. 4B), pTau(S404) (+20,0%, $p < 0,0001$, ryc. 5C) i pTau(S416) (+23,5%, $p < 0,05$, ryc. 5D) do formy nieufosforylowanej białka Tau. Po podaniu CBD szczury karmione dietą bogatotłuszczową wykazały znaczne zmniejszenie współczynnika fosforylacji pTau(S202):Tau (-22,8%, $p < 0,001$, Ryc. 4A), pTau (S396):Tau (- 13,5%, $p < 0,05$, Ryc. 4B) i pTau(S416):Tau (-16,5%, $p < 0,05$, Ryc. 4D) w porównaniu z grupą kontrolną. Fosforylacja pTau(S396) wzrosła w grupie CBD w stosunku do grupy kontrolnej oraz HFD (odpowiednio +41,0% i +19,9%; $p < 0,01$, Rys. 5B). Podawanie CBD znacząco zmniejszyło fosforylację pTau(S416) u szczurów z grupy kontrolnej (-18,4%, $p < 0,05$, ryc. 5D, w porównaniu z grupą HFD).

6.6 Wpływ kannabidiolu na ekspresję wybranych składowych endokannabinoidomu kory mózgowej

We wszystkich grupach zmiana ekspresji receptora CB1 i receptora VR1 nie osiągnęła istotności statystycznej (ryc. 6A i B). Podawanie diety bogatotłuszczowej spowodowało znaczny spadek ekspresji PPARα i PPARγ (odpowiednio − 37,8% i − 26,3%, $p < 0,05$, ryc. 6F i H) oraz znaczny wzrost ekspresji 5-HTR1A (+59,0%, $p < 0,05$, Fig. 6C). Co ciekawe, zauważyliśmy znaczne zmniejszenie ekspresji 5-HTR1A u szczurów, którym podawano CBD karmionych dietą standardową (CBD: − 36,3%, $p < 0,05$; ryc. 6C) oraz karmionych dietą bogatotłuszczową (HFD + CBD: − 58,3%, $p < 0,001$; ryc. 6C) w porównaniu z grupą kontrolną oraz (CBD: - 59,9%, $p < 0,01$; HFD + CBD: - 73,8%, $p < 0,001$; Ryc. 6C) grupą HFD. Po podaniu CBD ekspresja PPARγ zmniejszyła się u szczurów z grupy kontrolnej (-23,7%, $p < 0,05$, ryc. 6H, w porównaniu z grupą kontrolną) i wzrosła w trakcie karmienia dietą bogatotłuszczową (+30,3%, $p < 0,05$, ryc. 6H, w porównaniu z grupą HFD). Co więcej, ekspresja PPARα wzrosła w grupie CBD (+33,0%, $p < 0,05$, Rys. 6F) w porównaniu z grupą HFD. W porównaniu ze szczurami z grupy kontrolnej, zaobserwowaliśmy zmniejszoną ekspresję receptora GPR55 w grupie HFD + CBD (-30,4%, $p < 0,05$, ryc. 6D). Zmiana ekspresji FAAH1 i receptora PPARβ nie osiągnęła istotności statystycznej we wszystkich badanych grupach (ryc. 6E i G).

7. Wnioski

Z przeprowadzonych badań wynika, iż:

1. Podanie kannabidiolu w zwierzęcym modelu insulinooporności indukowanej dietą bogatotłuszczową, prowadzi do obniżenia zawartości ceramidu, sfingozyny i sfinganiny poprzez hamowanie zarówno enzymów szlaku syntezy *de novo* ceramidu, jak i enzymów ze szlaku ratunkowego (ang. salvage pathway) w korze mózgowej szczurów.
2. Zastosowanie kannabidiolu w wyżej wymienionych warunkach powoduje inaktywację kinazy syntazy glikogenu 3 α/β poprzez obniżenie współczynnika fosforylacji GSK-3 przy specyficznych resztach tyrozynowych odpowiednio w pozycji 279 i 216 (Tyr279 i Tyr216) oraz wzrost współczynnika fosforylacji przy specyficznych resztach serynowych w pozycji 21 i 9 (Ser21 i Ser9), a tym samym skutkuje zmniejszeniem stopnia insulinooporności kory mózgowej.
3. Równocześnie wyżej wspomniane zmniejszenie stopnia insulinooporności kory mózgowej było powiązane ze spadkiem akumulacji sfingolipidów.
4. Zastosowanie kannabidiolu w warunkach reżimu diety bogatotłuszczowej spowodowało także wzrost ekspresji PPARγ i zmniejszenie fosforylacji białka tau, co w dalszej konsekwencji, prawdopodobnie doprowadza do zmniejszenia produkcji i odkładania depozytów β-amyloidu, a tym samym ukazuje potencjalne wykorzystanie kannabidiolu w terapii chorób neurodegeneracyjnych.
5. Dodatkowo podanie kannabidiolu spowodowało znaczny spadek ekspresji receptora serotoninowego 1A (HTR1A), który jest pośrednio zaangażowany w rozwój stresu oksydacyjnego, utratę neuronów i pogorszenie pamięci w zwierzęcych modelach choroby Alzheimera. Mimo tego, iż rola HTR1A w insulinooporności mózgowia nie została jeszcze dobrze poznana, powiązanie CBD z receptorem HTR1A może stanowić kolejny istotny element poszukiwań nowych metod leczenia chorób neurodegeneracyjnych.

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Cannabidiol – A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance

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ABSTRACT

Obesity-related insulin resistance (IR) and attenuated brain insulin signaling are significant risk factors for neurodegenerative disorders, e.g., Alzheimer's disease. IR and type 2 diabetes correlate with an increased concentration of sphingolipids, a class of lipids that play an essential structural role in cellular membranes and cell signaling pathways. Cannabidiol (CBD) is a nonpsychoactive constituent of *Cannabis sativa* plant that interacts with the endocannabinoidome. Despite known positive effects of CBD on improvement in diabetes and its aftermath, e.g., anti-inflammatory and anti-oxidant effects, there are no studies evaluating the effect of phytocannabinoids on the brain insulin resistance and sphingolipid metabolism. Our experiment was carried out on Wistar rats that received a high-fat diet and/or intraperitoneal CBD injections. In our study, we indicated inhibition of de novo synthesis and salvage pathways, which resulted in significant changes in the concentration of sphingolipids, e.g., ceramide and sphingomyelin. Furthermore, we observed reduced brain IR and decreased tau protein phosphorylation what might be protective against neuropathologies development. We believe that our research will concern a new possible therapeutic approach with *Cannabis* -plant derived compounds and within a few years, cannabinoids would be considered as prominent substances for targeting both metabolic and neurodegenerative pathologies.

1. Introduction

Nowadays, metabolic pathologies, e.g., obesity or type 2 diabetes mellitus (T2DM) are some of the major medical concerns that widely occur among different populations, especially those with a predominance of the Western dietary pattern [1,2]. Consequently, obesity and its associated disorders constitute a serious threat to global health and might be regarded as a worldwide epidemic. Insulin resistance (IR), a condition with impaired insulin response in both peripheral tissues (e.g., liver or skeletal muscles) and the central nervous system, including the brain, is a primary pathology leading to the development of metabolic disorders [3]. It is also known that obesity-related systemic insulin resistance, its aftermath, e.g., oxidative stress and inflammation, as well as attenuated brain insulin signaling, are common features and significant risk factors for the occurrence of diabetic neuropathy, dementia or Alzheimer's disease (AD) [4,5]. A significant correlation between metabolic disorders such as IR or T2DM and neuropathologies, e.g., dementia with cognitive impairment, was widely demonstrated [6–8]. Insulin resistance and T2DM correlate with an increased tissue concentration of sphingolipids, a class of lipids that besides playing an essential structural role in cellular membranes, are also known to be significantly involved in cell signaling pathways or cell-to-cell both recognition and interaction [9,10]. Moreover, some studies (both in vitro and in vivo), as well as clinical trials demonstrated that disturbances in sphingolipid pathway might contribute not only to the development of insulin resistance, but also neurodegenerative disorders by changes in tau protein phosphorylation [11]. Furthermore, modulations in sphingolipid metabolism might constitute a novel possible therapeutic approach for those pathological conditions that widely affect the central nervous system [12,13]. The endocannabinoid system (ECS) is a crucial factor in maintaining energy homeostasis, subsequently participating in numerous metabolic and neurodegenerative pathologies [14,15]. Interestingly, some researchers defined an expanded endocannabinoid system as an endocannabinoidome

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(eCBome) – a complex lipid signaling system composed of more than 100 fatty acid-derived mediators and their receptors, as well as its anabolic and catabolic enzymes of more than 50 proteins [16]. The expanded endocannabinoid pathway includes G-protein-coupled receptors, known as cannabinoid receptors 1 and 2 (CB1, CB2) and the endogenous agonists of these receptors, known as endocannabinoids, mainly anandamide (AEA) and 2- arachidonoylglycerol (2-AG) [17]. It is known that both CB1 and CB2 receptors are diffusely distributed throughout the organism, i.e., in the central nervous system, gastrointestinal tract or cardiovascular system [18,19]. Moreover, other receptors such as vanilloid receptor subtype 1 (VR1), 5-hydroxytryptamine receptor 1A (5-HTR1A), peroxisome proliferator-activated nuclear receptors (PPARs) and G-protein-coupled receptor 55 (GPR55) are part of the endocannabinoidome [20]. Considering the molecules that are widely known to interact with the eCBome, one of the most distinguishable is a nonpsychoactive constituent of *Cannabis sativa* plant – cannabidiol (CBD). Despite the presented in literature positive effects of cannabinoids such as CBD on improvement in diabetes and its aftermath, e.g., anti-inflammatory, anti-oxidant, and tissue-protective effects, no studies evaluate the effect of phytocannabinoids on the brain insulin resistance [21,22]. Moreover, none of the research presented a correlation between cannabidiol and sphingolipid metabolism in the brain tissue. Thus, our research will introduce a comprehensive assessment of cannabidiol action on insulin resistance development and sphingolipid metabolism in the cerebral cortex, what would present a base for novel possible treatment methods of both metabolic and neurodegenerative pathologies.

2. Materials and methods

2.1. Animal model

The experiment was carried out on four weeks male Wistar rats weighing initially 60–100 g. During the experiment, the animals were maintained at 22 ± 2 °C, relative humidity of 35–60% and on a 12/12-h light/dark cycle with libitum access to food and water. The experiment was conducted after 5 days of acclimatization. Next, all the animals were randomly divided into experimental groups consisting of 10 animals each. The first group (control) received certified standard chow containing 60% carbohydrate, 20% fat and 20% protein. The second high-fat diet (HFD) group was fed with chow composed of 60% fat, 20% carbohydrate, 20% protein. All the animals from a particular group were maintained at the above-described conditions for 7 weeks. In both groups, half of the animals was receiving a solvent of an active substance, i.e. ethanol/Tween20/0.9NaCl (1:1:8 v/w, 1 ml/kg of body weight/day for the last 2 weeks of the experiment). Moreover, the rest of animals in both groups were administered intraperitoneally CBD injections (10 mg/kg of body weight/day) once a day at the same time for the last 14 days (6th–7th week of the experiment). The route of CBD administration was based on studies conducted by Merrick et al. who showed that intraperitoneal injections did not cause formation of psychoactive CBD derivatives such as Δ9-THC and Δ8-THC which were present after oral administration [23]. Moreover, according to studies conducted on mice suffering from diabetes, which showed that treatment with higher doses of cannabidiol (20 mg/kg of body weigh/day) exerted the same or comparable effects as 10 mg of CBD in diabetic heart muscle, the dose of 10 mg/kg of body weight/day CBD was chosen [24]. At the end of the experiment, rats were anesthetized with inhalation of isoflurane. The cerebral cortex was isolated, and the tissue was quickly frozen (clamped with aluminum tongs pre-cooled in liquid nitrogen) and kept at − 80 °C until further analysis. In this study, all the animal procedures were complied with the standard ethical guidelines and approved by the Animal Ethics Committee of the Medical University of Bialystok (approval number - 71/2018).

2.2. Sphingolipids determination

The high performance liquid chromatography method was used to measure the concentration of sphingosine, sphingosine-1-phosphate, sphinganine, sphinganine-1-phosphate and ceramide. This procedure was described in details by Baranowski et al. [25]. In brief, in the presence of C17-sphingosine as a standard, lipids were extracted from ultrasonicated samples of the cerebral cortex. An obtained lipid extract was transferred to a fresh tube containing N-palmitoyl-D-erythro-sphingosine (C17 base) and afterwards, was alkaline hydrolyzed to deacylate ceramide to sphingosine. Released from CER free SFO and SFA were converted to their o-phthalaldehyde derivatives and then analyzed by HPLC system (Varian ProStar, Agilent Technologies) with a fluorescence detector and C18 reversed-phase column. The sphingolipids contents were presented in picomoles per milligram of tissue.

2.3. Sphingomyelin determination

In brief, according to the Folch method lipids were extracted from, homogenized by ultrasonication, samples using a chloroform:methanol solution (2:1, vol/vol) [26]. In order to obtain sphingomyelin fraction, extracted lipids were separated with the use of thin-layer chromatography (TLC) [27]. Next, the particular fatty acids fraction was methylated by solution of boron fluoride and 14% methanol. Gas liquid chromatography – GLC method (Hewlett-Packard 5890 Series II gas chromatograph, containing a capillary column and flame ionization detector, HP-INNOWax) was used to detect and quantify sphingomyelin fraction content in relation to appropriate retention times of standards. The concentration of sphingomyelin was measured as the sum of individual fatty acids in the specified fraction. The sphingomyelin content was presented in nanomoles per gram of tissue.

2.4. Proteins determination

Before the immunoblotting procedure, the cerebral cortex was homogenized in radioimmunoprecipitation assay (RIPA) buffer at 4 °C with protease and phosphatase inhibitors. Then, the homogenates were centrifuged, and the obtained supernatants were used for the analysis. Total protein concentration was estimated with bicinchoninic acid (BCA) assay.

All the samples were separated using 10% Critetion TGX Stain – Free Precast Gel (Bio Rad, Poland) electrophoresis and transferred onto nitrocellulose or polyvinylidene fluoride (PVDF) membranes. Then, the membranes were blocked with 5% BSA or 5% not-fat dry milk and immunoblotted with primary antibodies. Afterwards, the blots were incubated with secondary antibodies conjugated to horseradish peroxidase. Obtained signals were quantified densitometrically using a ChemiDoc visualization system (BioRad) and were detected and analyzed with ImageLab software (BioRad), where the image of total protein and protein of interest overlapped in order to receive normalized proteins expression and the control was set at 100%.

The antibodies were used to detect the expression of sphingolipid pathway enzymes: SPTLC1 (Abcam, UK), SPTLC2 (Santa Cruz Biotechnology, USA), SPHK1, SPHK2, LASS4 (Sigma Aldrich, GER), LASS6 (Abcam), PHCA (Sigma Aldrich), ASAH1, ASAH2, N-SMase (Santa Cruz Biotechnology), endocannabinoidome enzyme and receptors: CB1, FAAH1, GPR55 (Abcam), VR1, PPARα, PPARβ, PPARγ (Santa Cruz Biotechnology), 5-HTR1A (Thermo Fisher Scientific, USA), proteins and their phosphorylated forms directly involved in insulin signaling pathway: IRS-1, pIRS-1 (S302), Akt, pAkt (S473) (Cell Signaling), pAkt (Thr305), pAkt (Thr308) (Santa Cruz Biotechnology), pAkt (S472, S473, S474) (Abcam), GSK-3α/β, pGSK-3β (Ser9) (Thermo Fisher Scientific), pGSK-3α (Ser21) (Cell Signaling), pGSK-3α (Tyr279), pGSK-3β (Tyr216) (Santa Cruz Biotechnology) as well as phosphorylation state of proteins involved in neurodegenerative diseases: Tau, pTau (S202), pTau (S396), pTau (S404), pTau (S416) (Cell Signaling).

2.5. Data analysis

The data from the experiment are presented as the mean ± SD. The statistical software GraphPad Prism 5 was used in our analysis. The normality of the data distribution and homogeneity of the variance were examined using Shapiro-Wilk and Bartlett's tests, respectively. Statistical differences were determined based on one-way ANOVA followed by an appropriate post-hoc test (Tukey, pairwise student's *t*-test). $P < 0.05$ was considered statistically significant in all the cases.

3. Results

3.1. Effects of CBD treatment on the cerebral cortex sphingolipids and sphingomyelin (SM) contents

The concentration of sphingosine (SFO) in the cerebral cortex homogenates increased significantly in high-fat feeding (+29.2%, $p < 0.01$, Fig. 1A) and CBD-treated (+62.7%, $p < 0.01$, Fig. 1A) groups in comparison to the standard fed chow group. Moreover, we observed a considerable reduction in the SFO content in high-fat diet (HFD) + CBD (−31.6%, $p < 0.001$, Fig. 1A) compared with the HFD group. Similarly, the level of sphinganine (SFA) rose markedly in the HFD (+20.4%, $p < 0.05$, Fig. 1B) and CBD-treated (+50.2%, $p < 0.01$, Fig. 1B) groups in comparison with the control group. We noticed a relevant diminishment in sphinganine concentration only in HFD + CBD (−28.2%, $p < 0.01$, Fig. 1B) compared with the high-fat diet group. Treatment with CBD significantly decreased ceramide (CER) in HFD rats (−14.3%, $p < 0.05$, Fig. 1C). We observed a significant increase in CER in HFD group (+18.9%, $p < 0.05$, Fig. 1C). In rats injected with CBD, we revealed a considerably elevated content of sphingosine-1-phosphate (S1P) in relation to the control group, as well as compared to the high-fat feeding group (+65.2%, +53.9%, respectively; $p < 0.05$, Fig. 1D). In all the experimental groups, content of sphinganine-1-phosphate (SA1P) remained unchanged (Fig. 1E). Compared to the brain homogenates obtained from the control and high-fat feeding rats, we observed a notable increase in the sphingomyelin content after CBD treatment (+13.4%, +17.1%, respectively; $p < 0.05$, Fig. 1F) and HFD + CBD (+9.9%, $p < 0.05$; +13.5%, $p < 0.01$, respectively, Fig. 1F).

3.2. Effects of CBD treatment on the ceramide de novo synthesis pathway

In the cerebral cortex homogenates, we observed a substantial changes in the expression of serine palmitoyltransferase 1 (SPTLC1) and 2 (SPTLC2). There was a significant decrease in SPTLC1 after CBD injection (−24.4%, $p < 0.01$, Fig. 2A) and during high-fat feeding with CBD (−29.5%, $p < 0.05$, Fig. 2A) compared to the control and after CBD injection (−13.8%, $p < 0.05$, Fig. 2A) compared to the HFD group. The CBD injection in HFD group provoked a reduction in SPTLC2 expression compared to the control and HFD groups (−28.7%, $p < 0.01$; −18.6%, $p < 0.05$, respectively, Fig. 2B). What is more, the high-fat feeding with the exposure to the CBD resulted in a considerable reduction in ceramide synthase 6 (LASS6) expression (−40.0%, $p < 0.05$, Fig. 2C) in comparison with the standard chow fed and (−22.0%, $p < 0.01$, Fig. 2C) HFD groups. We noticed a substantial decrease in CBD alone and CBD with HFD groups in the expression of ceramide synthase 4 (LASS4) (CBD: −21.9%, HFD + CBD: −24.4%, $p < 0.05$, Fig. 2D) compared to the control and (CBD: −23.9%, HFD + CBD: −26.4%, $p < 0.05$, Fig. 2D) rich in fat diet groups.

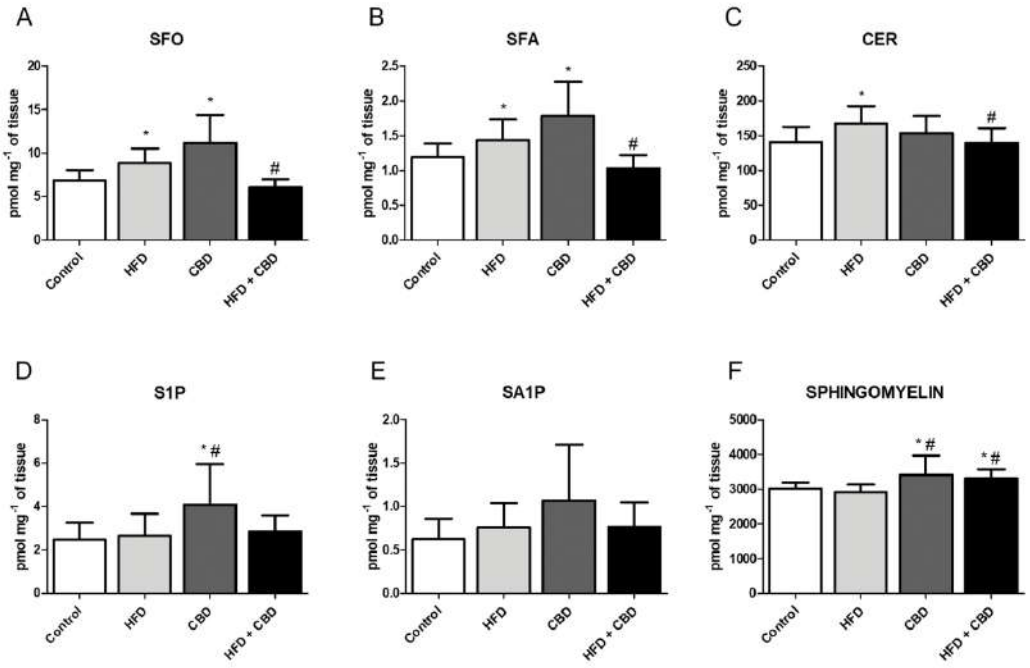

Fig. 1. Content of sphingosine (SFO, A), sphinganine (SFA, B), ceramide (CER, C), sphingosine-1-phosphate (S1P, D), sphinganine-1-phosphate (SA1P, E) and sphingomyelin (F) in the cerebral cortex homogenates. Total sphingolipids and sphingomyelin content was measured by high performance liquid chromatography (HPLC) or gas liquid chromatography (GLC) methods, respectively. The results are presented as mean ± SD. *$p < 0.05$ significant difference vs. control group, #$p < 0.05$ significant difference vs. high-fat feeding group; HFD – high-fat diet, CBD – cannabidiol, HFD + CBD – high-fat diet + cannabidiol.

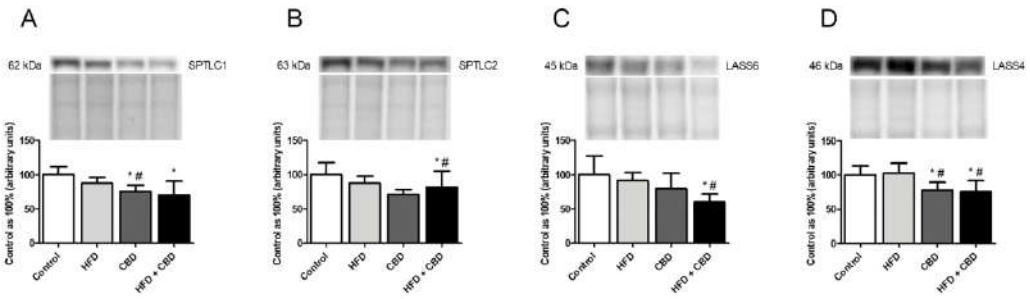

Fig. 2. The expression of proteins involved in the ceramide de novo synthesis pathway, i.e., serine palmitoyltransferase 1 (SPTLC1, A), serine palmitoyltransferase 2 (SPTLC2, B), ceramide synthase 6 (LASS6, C) and ceramide synthase 4 (LASS4, D) in the cerebral cortex homogenates. The expression of proteins was measured by Western blot method. The results are presented as mean ± SD. $*p < 0.05$ significant difference vs. control group, $\#p < 0.05$ significant difference vs. high-fat feeding group; HFD – high-fat diet, CBD – cannabidiol, HFD + CBD – high-fat diet + cannabidiol.

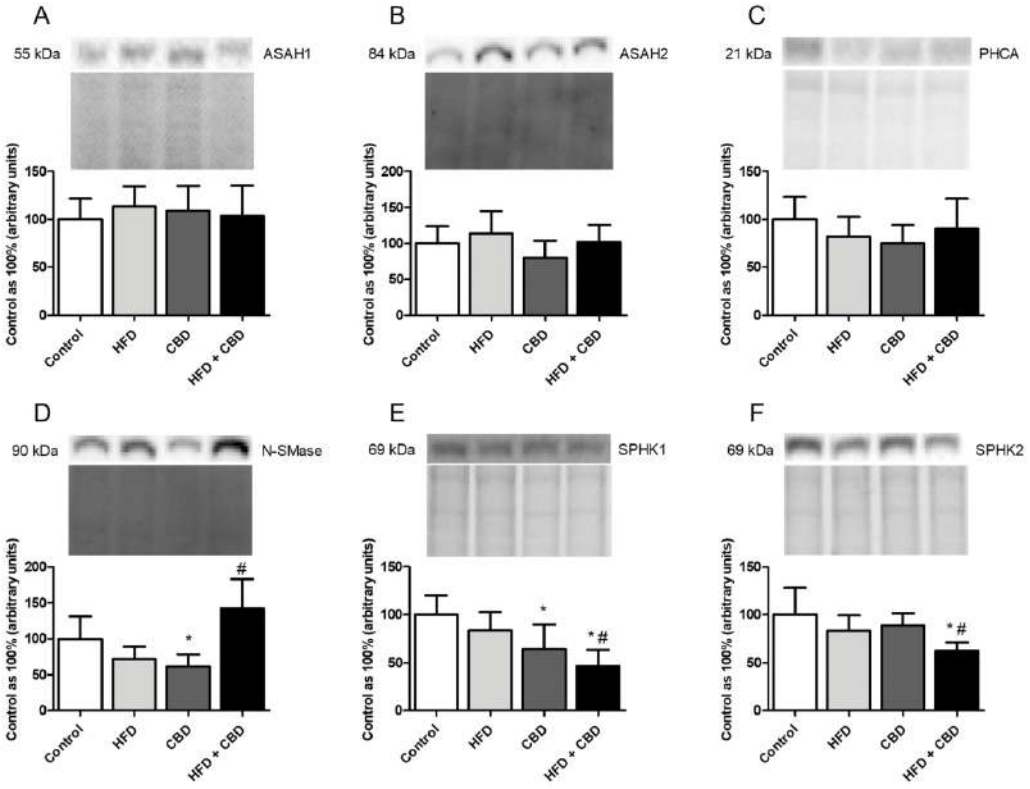

Fig. 3. The expression of proteins involved in sphingolipid metabolism pathway, i.e., acid ceramidase (ASAH1, A), neutral ceramidase (ASAH2, B), alkaline ceramidase (PHCA, C) and neutral sphingomyelinase (N-SMase, D), sphingosine kinase 1 (SPHK1, E) and sphingosine kinase 2 (SPHK2, F) in the cerebral cortex homogenates. The expression of proteins was measured by Western blot method. The results are presented as mean ± SD. $*p < 0.05$ significant difference vs. control group, $\#p < 0.05$ significant difference vs. high-fat feeding group; HFD – high-fat diet, CBD – cannabidiol, HFD + CBD – high-fat diet + cannabidiol.

3.3. Effects of CBD treatment on the enzymes from sphingolipid metabolism pathway

In all the examined groups, we did not show a significant changes in the expression of acid ceramidase (ASAH1) (Fig. 3A). We observed a trend towards decrease in the expression of neutral ceramidase (ASAH2) (CBD vs. HFD: $p = 0.1357$, Fig. 3B) and alkaline ceramidase (PHCA) (HFD vs. control: $p = 0.1924$, CBD vs. control: $p = 0.0717$, Fig. 3C). Injection with CBD reduced the expression of neutral sphingomyelinase (N-SMase) markedly compared to the control rats (−38.6%, $p < 0.05$, Fig. 3D). Moreover, compared to the rats administered with HFD, we observed a significant increase in N-SMase expression in HFD + CBD group (+99.0%, $p < 0.05$, Fig. 3D). The expression of sphingosine kinase 1 (SPHK1) was decreased in CBD group compared to the control (−35.9%, $p < 0.05$, Fig. 3E) and in CBD combined with HFD group in comparison with the control and HFD groups (−53.5%, −44.2%, respectively; $p < 0.01$, Fig. 3E). However, there was a notable reduction in the expression of sphingosine kinase 2 (SPHK2) after CBD injection in high-fat feeding group (−37.7%, $p < 0.05$, Fig. 3F) compared with the control and (−18.6%, $p < 0.05$, Fig. 3F) HFD groups.

3.4. Effects of CBD treatment on the insulin signaling pathway

In the cerebral cortex homogenates, rats fed a high-fat diet revealed a significant increase in the phosphorylation state of proteins directly involved in insulin signaling pathway, i.e., protein kinase B (pAkt (Thr305):Akt ratio (+362.4%, $p < 0.001$, Fig. 4A), pAkt(Thr308):Akt ratio (+52.6%, $p < 0.05$, Fig. 4B), pAkt(S472,S473,S474):Akt ratio (+140.8%, $p < 0.001$, Fig. 4C)), glycogen synthase kinase – 3α/β (pGSK-3α(Tyr279):GSK-3α/β ratio (+25.0%, $p < 0.05$, Fig. 4G), pGSK-3β(Tyr216):GSK-3α/β ratio (+71.1%, $p < 0.001$, Fig. 4I) compared to the appropriate control rats. On the other hand, HFD administration resulted in a pronounced reduction in inactivating phosphorylation ratio of the pGSK-3α(Ser21):GSK-3α/β (−37.6%, $p < 0.001$, Fig. 4F, vs. control group) and pGSK-3β(Ser9):GSK-3α/β ratio (−80.8%, $p < 0.001$, Fig. 4B, vs. control group). In lipid overload condition, CBD injection induced a substantial decrease in the pGSK-3α(Tyr279):GSK-3α/β ratio (−22.0% and −37.0%, $p < 0.001$, Fig. 4G, vs. control and HFD groups, respectively) and pGSK-3β(Tyr216):GSK-3α/β ratio (−32.8%, $p < 0.05$, Fig. 4I, vs. HFD group). What is more, in HFD group treatment with cannabidiol caused a substantial increase in the phosphorylation ratio of the Akt(Thr305) (+204.6%, $p < 0.01$, Fig. 4A), Akt(S472,S473,S474) (+158.7%, $p < 0.01$, Fig. 4C) from that in the appropriate control group and ratio of the GSK-3α(Ser21) (+38.2%, $p < 0.001$, Fig. 4F), GSK-3β (Ser9) (+481.6%, $p < 0.001$, Fig. 4H) from that in the appropriate HFD group. Concomitantly, the exposure to the CBD alone resulted in a considerable reduction in the phosphorylated ratio of the Akt(Thr305) (−70.3%, $p < 0.001$, Fig. 4A), Akt(S472,S473,S474) (−34.1%, $p < 0.05$, Fig. 4C), Akt(S473) (−36.0%, $p < 0.01$, Fig. 4D), GSK-3α (Tyr279) (−23.5%, $p < 0.05$, Fig. 4G) and GSK-3β(Tyr216) (−28.1%, $p < 0.05$, Fig. 4I) compared to the rich in fat diet group. CBD alone also provoked an elevation of the pAkt(S472,S473,S474):Akt ratio (+58.7%, $p < 0.05$, Fig. 4C, vs. control group), pGSK-3α(Ser21):GSK-3α/β ratio (+25.2%, $p < 0.05$, Fig. 4F, vs. HFD group) and pGSK-3β(Ser9):GSK-3α/β ratio (+288.3%, $p < 0.001$, Fig. 4H, vs. HFD group). In comparison with rats fed a standard diet, we noticed a substantial decrease in the phosphorylation ratio of Akt(S473) (−38.4%, $p < 0.01$, Fig. 4D). Moreover, the phosphorylated insulin receptor substrate – 1(S302):insulin receptor substrate – 1 ratio (pIRS-1(S302):IRS-1 ratio) remained unchanged in all of the examined groups (Fig. 4E).

3.5. Effects of CBD treatment on the Tau phosphorylation state

Lipid overload condition resulted in a significant increase in the phosphorylation of Tau protein, i.e., Tau(S202) (+23.9%, $p < 0.001$, Fig. 5A), Tau(S396) (+17.6%, $p < 0.0001$, Fig. 4B), Tau(S404) (+20.0%, $p < 0.0001$, Fig. 5C) and Tau(S416) (+23.5%, $p < 0.05$, Fig. 5D). After CBD injection, rats fed a HFD revealed a substantial reduction in pTau(S202):Tau ratio (−22.8%, $p < 0.001$, Fig. 4A), pTau (S396):Tau ratio (−13.5%, $p < 0.05$, Fig. 4B) and pTau(S416):Tau ratio (−16.5%, $p < 0.05$, Fig. 4D) compared to the control group. In addition, the phosphorylation of Tau(S396) rose markedly in the CBD-treated group in relation to the control as well as HFD groups (+41.0% and + 19.9%, respectively; $p < 0.01$, Fig. 5B). Treatment with CBD significantly decreased Tau(S416) phosphorylation in rats fed standard chow (−18.4%, $p < 0.05$, Fig. 5D, vs. HFD group).

3.6. Effects of CBD treatment on the endocannabinoidome components

In all the experimental groups, the expression of cannabinoid receptor type 1 (CB1) and vanilloid receptor subtype 1 (VR1) remained unchanged (Fig. 6A and B). The high-fat feeding resulted in a substantial decrease in peroxisome proliferator-activated receptors α and γ (PPARα and PPARγ) expression (−37.8% and −26.3%, $p < 0.05$, Fig. 6F and H, respectively) and a substantial rise in 5-hydroxytryptamine receptor 1A (5-HTR1A) expression (+59.0%, $p < 0.05$, Fig. 6C). Furthermore, we noticed a substantial reduction in the expression of 5-HTR1A in rats injected with CBD (CBD: −36.3%, $p < 0.05$; HFD + CBD: −58.3%, $p < 0.001$; Fig. 6C) compared to the control and (CBD: −59.9%, $p < 0.01$; HFD + CBD: −73.8%, $p < 0.001$; Fig. 6C) high-fat feeding groups. After CBD injection, PPARγ expression was diminished in rats fed a standard diet (−23.7%, $p < 0.05$, Fig. 6H, vs. control group) and increased during high-fat feeding (+30.3%, $p < 0.05$, Fig. 6H, vs. HFD group). Additionally, the expression of PPARα was increased in CBD group (+33.0%, $p < 0.05$, Fig. 6F) compared to the HFD group. Compared to the brain homogenates obtained from the control rats, we observed a noticeably reduced expression of the G-protein-coupled receptor 55 (GPR55) in HFD combined with CBD group (−30.4%, $p < 0.05$, Fig. 6D). What is more, the expression of fatty acid amide hydrolase 1 (FAAH1) and peroxisome proliferator-activated receptor β (PPARβ) showed no significant changes in all the examined groups (Fig. 6E and G).

4. Discussion

Cannabis sativa plant has been used therapeutically for thousands of years worldwide. Although it is primarily known for tetrahydrocannabinol (THC), this is just one of the dozens of cannabinoids produced by this herbaceous plant. Over the last decade, phytocannabinoids were considered as prominent compounds that might be applied with beneficial effects in the treatment of various metabolic, as well as neurodegenerative disorders. One of the most common representatives of this plant-derived group and one of the principal constituents of *Cannabis sativa* is cannabidiol – a compound lacking the psychotropic effects typical for THC and with promising effects on a multitude of various cell functioning aspects [28–30]. However, even though numerous studies demonstrated the effects of phytocannabinoids on the metabolism of brain structures, e.g., cerebral cortex, hypothalamus, there is a lack of data concerning the effect of cannabinoids on brain concentration of sphingolipids, its metabolism, as well as brain insulin resistance. Thus, our research was undertaken in order to assess, whether cannabidiol affects the concentration of sphingolipids and insulin signaling pathway in the cerebral cortex of obese Wistar rats. Despite a very limited amount of data, some studies indicated that phytocannabinoids, as well as other agonists of CB1 or CB2 receptors, might affect sphingolipid metabolism in different tissues. However, these studies concerned the correlation between ceramides and cell apoptosis, not insulin signaling pathway, as well as were not performed on brain tissue [31,32]. The Western pattern diet, greatly rich in harmful saturated fatty acids, enhances uptake and storage of various lipid classes including sphingolipids and their essential fractions – ceramide, sphingosine, sphinganine and sphingomyelin. Interestingly, some researchers indicated a broad correlation between

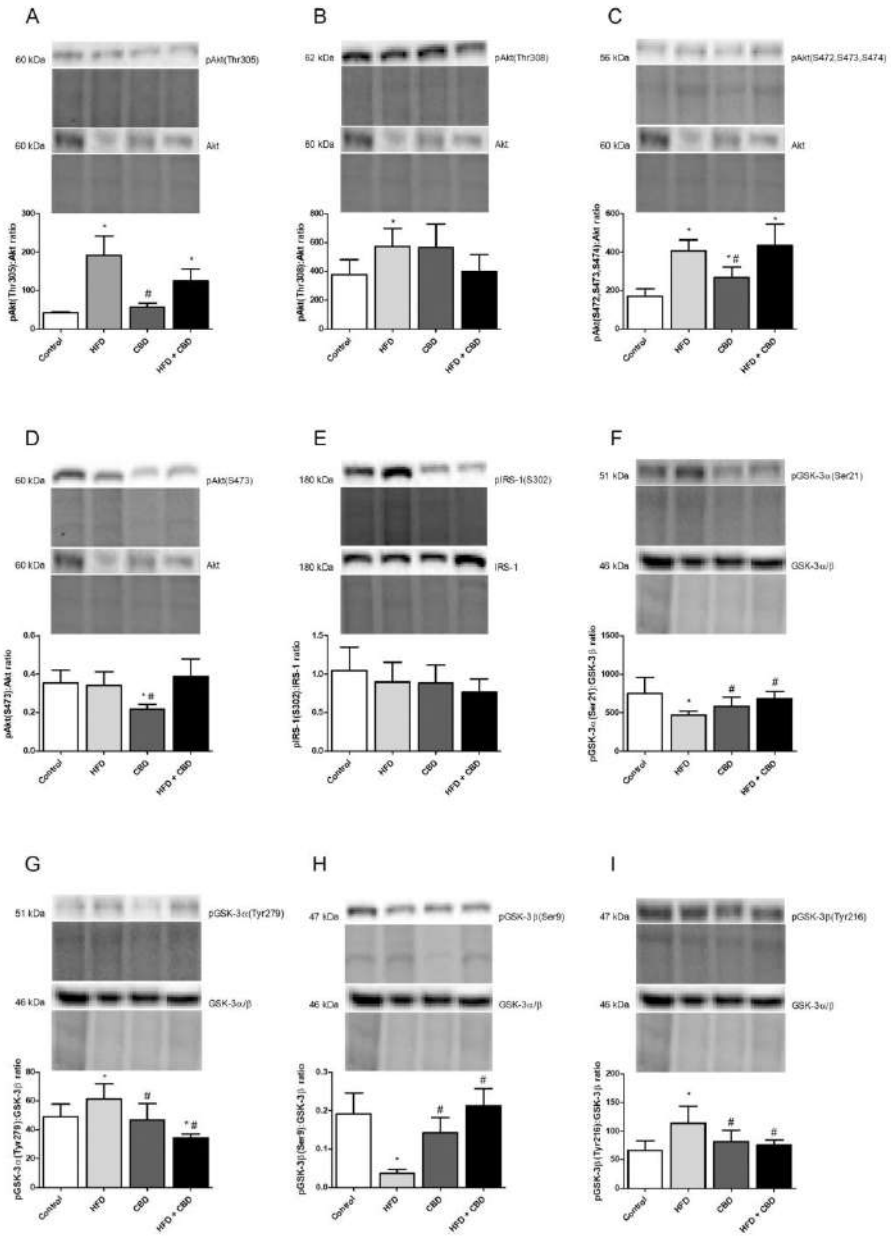

Fig. 4. The phosphorylation ratio of the protein kinase B (pAkt(Thr305):Akt ratio, A; pAkt(Thr308):Akt ratio, B; pAkt(S472,S473,S474):Akt ratio, C; pAkt(S473):Akt ratio, D), insulin receptor substrate – 1 (pIRS-1(S302):IRS-1 ratio, E) and glycogen synthase kinase – 3α/β (pGSK-3α(Ser21):GSK-3α/β ratio, F; pGSK-3α(Tyr279):GSK-3α/β ratio, G; pGSK-3β(Ser9):GSK-3α/β ratio, H; pGSK-3β(Tyr216):GSK-3α/β ratio, I) in the cerebral cortex homogenates. The phosphorylation ratio was calculated based on proteins expression measured by Western blot method. The results are presented as mean ± SD. $*p < 0.05$ significant difference vs. control group, $\#p < 0.05$ significant difference vs. high-fat feeding group; HFD – high-fat diet, CBD – cannabidiol, HFD + CBD – high-fat diet + cannabidiol.

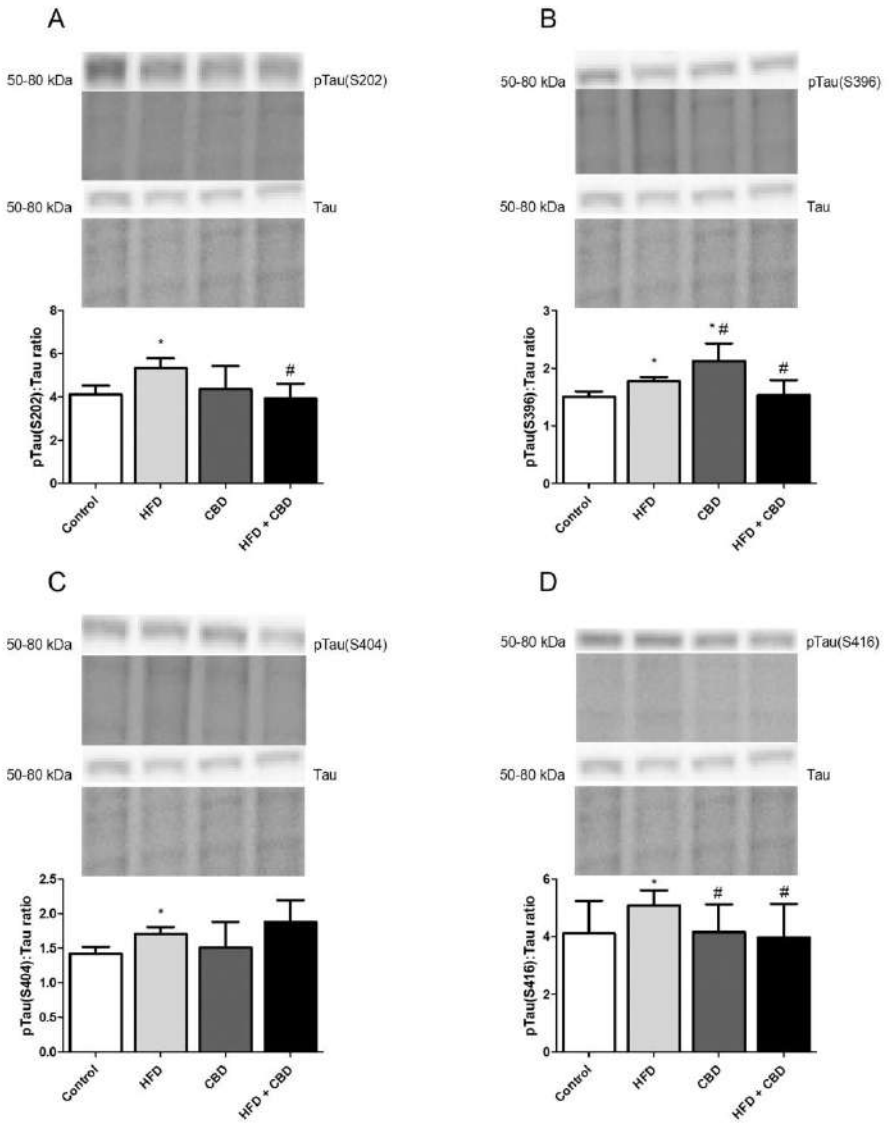

Fig. 5. The phosphorylation ratio of Tau protein, i.e., pTau(S202):Tau ratio (A), pTau(S396):Tau ratio (B), pTau(S404):Tau ratio (C) and pTau(S416):Tau ratio (D) in the cerebral cortex homogenates. The phosphorylation ratio was calculated based on proteins expression measured by Western blot method. The results are presented as mean ± SD. $*p < 0.05$ significant difference vs. control group, $\#p < 0.05$ significant difference vs. high-fat feeding group; HFD – high-fat diet, CBD – cannabidiol, HFD + CBD – high-fat diet + cannabidiol.

sphingolipid metabolism and neurodegenerative pathologies [12]. Our analysis conducted on Wistar rats brain demonstrated notable changes in concentration of all four sphingolipids mentioned above in the group of rats after exposure to CBD and HFD. However, we did not observe any significant differences in concentrations of two other sphingolipids – sphingosine-1-phosphate and sphinganine-1-phosphate after administration of cannabidiol in rats being exposed to a high-fat diet. Recently, sphingolipids were extensively investigated in numerous aspects of both glucose and lipid metabolism, whereas the substantial focus in the studies was placed on ceramide. This sphingolipid fraction is widely known to be a precursor for other sphingolipids, e.g., sphingosine or sphingomyelin, hence it is considered as a major element and a core of the sphingolipid metabolism pathway. We may distinguish two fundamental ways of CER synthesis – de novo ceramide synthesis route, due to

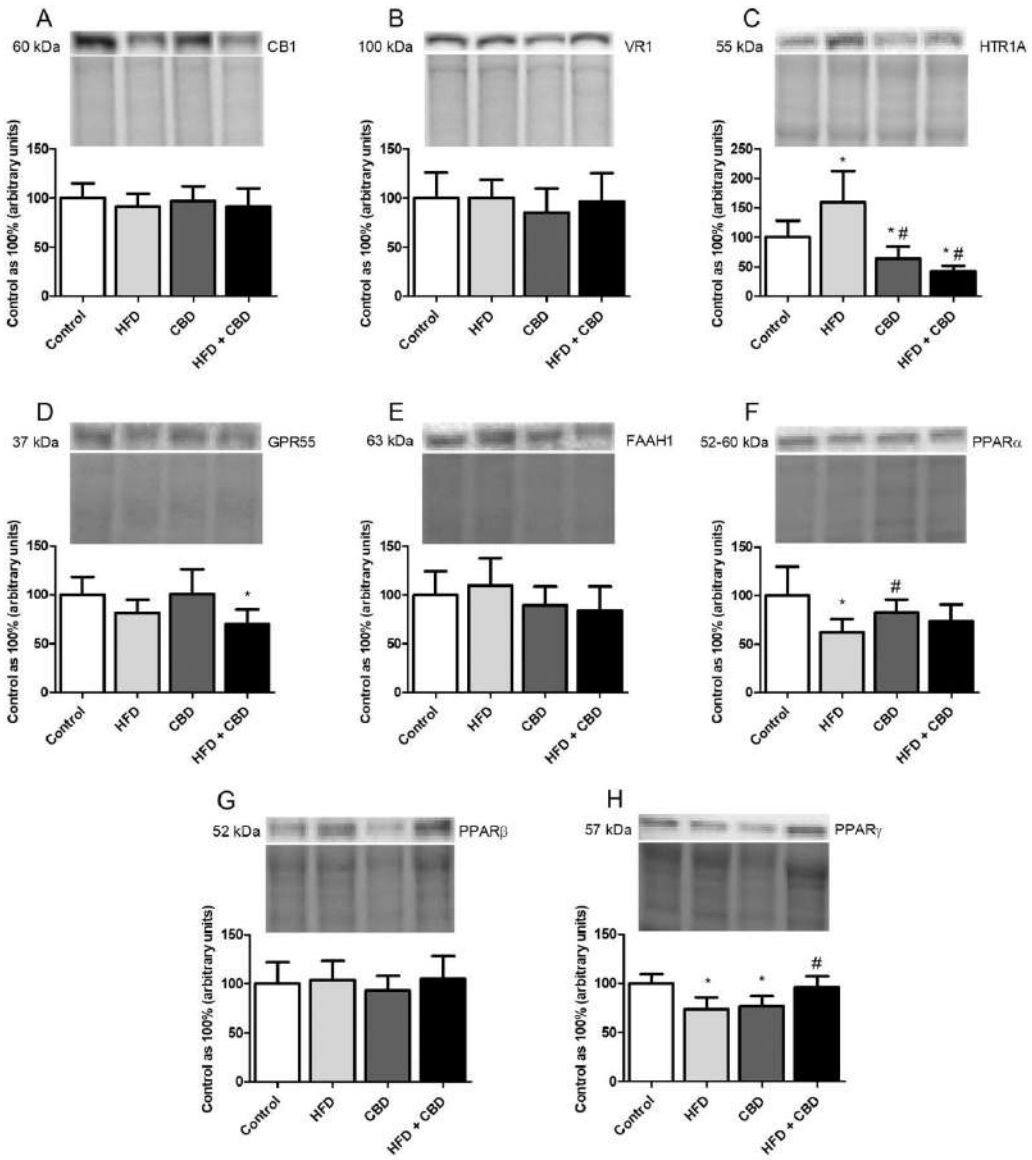

Fig. 6. The expression of proteins from endocannabinoidome, i.e., cannabinoid receptor type 1 (CB1, A), vanilloid receptor subtype 1 (VR1, B), 5-hydroxytryptamine receptor 1A (HTR1A, C), G-protein-coupled receptor 55 (GPR55, D), fatty acid amide hydrolase 1 (FAAH1, E) and peroxisome proliferator-activated receptors (PPARα, F; PPARβ, G; PPARγ, H) in the cerebral cortex homogenates. The expression of proteins was measured by Western blot method. The results are presented as mean ± SD. $*p < 0.05$ significant difference vs. control group, $\#p < 0.05$ significant difference vs. high-fat feeding group; HFD – high-fat diet, CBD – cannabidiol, HFD + CBD – high-fat diet + cannabidiol.

condensation of two components – serine and palmitoyl-CoA, and a second pathway – a sphingosine breakdown, also called the salvage pathway [33]. Although our analysis indicated a significant decrease in ceramide concentration after exposure to CBD and the high-fat diet, a trend towards an increase in ceramide concentration was observed after injections in the CBD alone group. Our findings are in accordance with the results of Gustafsson et al., who indicated on Mantle Cell Lymphoma cells that methanandamide, an analogue of anandamide that is an agonist of both CB receptors, increased mRNA levels of ceramide synthases 3 and 6, led to the enhanced de novo ceramide synthesis pathway

and, subsequently, elevated its concentration [34]. We may assume that cannabidiol led to a decrease in ceramide concentration only under conditions of elevated fatty acids availability in a rodent model of obesity. On the other hand, although observed in ceramide content trend, the expression of enzymes from de novo synthesis pathway was significantly decreased after CBD treatment, which may indicate that under normal conditions, CBD is able to reduce ceramide levels as well. Additionally, we observed a notable decrease in the expression of enzymes involved in de novo synthesis pathway namely serine palmitoyltransferase 2, as well as ceramide synthase 4 and 6 in rats after HFD together with CBD treatment. Moreover, our data indicated that a high-fat diet with cannabidiol led to lower expression of proteins from the salvage pathway. Interestingly, observed changes in the expression of enzymes are in accordance with decreased concentration of two sphingolipid fractions (SFO and SFA), which are precursors of ceramide – SFA in de novo synthesis pathway and SFO in salvage pathway. Our findings unequivocally indicated that CBD in the high availability of fatty acids led to an inhibition of two possible routes of ceramides synthesis. In turn, considering the effect of cannabidiol on sphingomyelin hydrolysis, the third possible pathway of ceramide formation, we found a significant increase in the concentration of SM after treatment with CBD alone, as well as CBD and the high-fat diet. Our data are in contrast with studies conducted by Burstein et al., who evaluated the effect of cannabidiol on sphingomyelin concentration in fibroblasts and indicated a considerable decrease in this sphingolipid content after exposure to CBD [35]. It is known that alterations in SM brain concentration play an essential role in the pathogenesis of neurodegenerative disorders. However, the exact role and unequivocal pattern of sphingomyelin action are not yet determined [36]. He et al. indicated in the postmortem brain tissues of Alzheimer's disease patients a reduction in SM concentration and elevation of ceramide [37]. In contrast, Lu et al. demonstrated that SM synthase 1 (SMS-1) knockout in the hippocampus of amyloid precursor protein/ presenilin-1 (APP/PS1) transgenic mice might lead to the attenuation of Alzheimer-like pathology [38]. Considering the enzymes involved in sphingomyelin metabolism, our research showed that neutral sphingomyelinase (N-SMase), which hydrolyzes SM to ceramide, is slightly lower in cannabidiol alone and notably higher in CBD and HFD groups. Interestingly, Filippov et al. demonstrated that the gene expression of N-SMase, as well as A-SMase (acid sphingomyelinase), might be overexpressed in the human AD brain specimens [39]. Furthermore, Dinkins et al. indicated that genetic N-SMase2 deficiency in an AD mouse model improves memory and learning functions [40]. It is noteworthy that some researchers place hope on targeted inhibition of N-SMase, which may be clinically beneficial in the treatment of neurodegenerative disorders [41]. On the contrary, some studies indicated that N-SMase, widely present in the hippocampus, modulates the postsynaptic function, which is significantly impaired in neuropathologies [42]. This might provide a conclusion that cannabidiol, which led to a higher expression of N-SMase, under conditions of higher availability of fatty acids (i.e., model of obesity with insulin resistance), might improve the postsynaptic function. As there is no uniform view on SM metabolism, its concentration and enzymes, in Alzheimer disease, it is not possible to ascertain if cannabidiol via its enhancement of sphingomyelin concentration and neutral sphingomyelinase expression might contribute to the impairment or augmentation of neuropathologies. This issue needs profound clarification and further studies on AD models.

Various groups of lipids, including sphingolipids, greatly interfere with the insulin-signaling pathway via inhibition of insulin-stimulated protein kinase B (Akt/PKB) phosphorylation in skeletal muscles and the liver, which constitutes an intermediary process for the anabolic metabolism. Consequently, it is known that the suppression of Akt/PKB phosphorylation contributes to inhibited inactivating phosphorylation of glycogen synthase kinase 3 α/β in Ser 21/Ser 9 respectively, which leads to the impairment in insulin sensitivity (IS) [43]. Interestingly, some studies considered inhibition of GSK-3 as a promising target in the treatment of type 2 diabetes [44]. However, unlike in muscles and the liver, phosphorylation of Akt/PKB in the brain of insulin resistant obese mice was increased followed by increased activity of GSK-3 [45]. Considering changes in the expression of insulin signaling pathway proteins, we observed a significant increase in phosphorylated protein kinase B/protein kinase B ratio (pAkt/Akt) in Thr 305 and 308 as well as Ser 472, 473 and 474 phosphorylation sites after HFD, which is in accordance with scientific literature [45]. The CBD treatment in HFD group showed only a trend toward a decrease in Thr 305 and 308 phosphorylation sites of pAkt/Akt ratio, but it did not reach the level of significance. In order to definitively assess insulin signaling pathway, we measured changes in phosphorylation of glycogen synthase kinase-3 α and β, which is able to affect phosphorylation of tau protein, important in neuropathologies development [46]. In the HFD group, we noticed a significant increase in the phosphorylated glycogen synthase kinase-3 α/β /glycogen synthase kinase-3 α/β ratio (pGSK-3/GSK-3) at Tyr 279 and Tyr 216 and a notable decrease in phosphorylation at Ser 21 and Ser 9. CBD injections in the high-fat diet group reversed phosphorylation at all the four sites in comparison with the HFD group. Thus, it might be said that cannabidiol inactivated GSK-3 in HFD group and led to augmented insulin sensitivity, and subsequently reduced the development of brain insulin resistance. The phenomenon of reduced insulin resistance in our study might have been developed indirectly via interference of cannabidiol with sphingolipid metabolism, under conditions of the high-fat diet. However, we are aware that the main limitation of our study is lack of clear link between CBD treatment, sphingolipid accumulation and brain insulin resistance attenuation. Thus, in future perspective it is important to conduct experiments with selective inhibitors of ceramide de novo pathway such as Myriocin in order to confirm our findings. It is known that the excessive storage of sphingolipids is associated with various disturbances in metabolism and might result in the development of insulin resistance [47,48]. Based on our research, it might be said that cannabidiol via lower sphingolipid accumulation may reduce brain insulin resistance. Interestingly, Chaurasia and Summers suggested that ceramide, among other sphingolipids, may be considered as a crucial factor that contributes to the development of insulin resistance in different tissues [49]. Moreover, Brozinick et al. indicated in vivo that sphingolipids, mainly ceramide, widely affect insulin sensitivity and, therefore, may be considered as a biomarker of systemic insulin resistance [50]. Most importantly, Dekker et al. demonstrated that the inhibition of enzymes involved in sphingolipids synthesis, e.g., SPTLC1 resulted in improved insulin sensitivity and reduced lipogenesis in an animal model of obesity [51]. Therefore, our findings might provide a conclusion that cannabidiol under conditions of high-fat availability led to an improvement in insulin sensitivity and, subsequently, impaired the development of insulin resistance in a rodent model of obesity. These results may be a base for the novel potential use of cannabidiol in the treatment of metabolic disorders, including insulin resistance.

The eCBome is considered to be involved in the control of energy homeostasis and, therefore, might modify the development of metabolic as well as neurodegenerative pathologies [16,52]. Early studies examining CBD targets showed a lack of its affinity for CB1 and CB2 receptors [53]. However, recent reports explained that CBD is a negative allosteric modulator of CB1 receptors [54]. Thus, in order to elucidate the effect of CBD on endocannabinoid receptors, we assessed the expression of CB1 receptor but we did not observe significant changes. Another way how CBD may target the endocannabinoid system is inhibition of anandamide degradation through fatty acid amide hydrolase 1 enzyme [55]. However, in our study the expression of FAAH1, the enzyme responsible for the endocannabinoid degradation, also remained unchanged. Thus, in order to elucidate which one from multiple molecular CBD targets was affected in our study, we also examined GPR55, 5-HTR1A and VR1. Cannabidiol is known to have a deep affinity to G-protein-coupled receptor 55 – a novel cannabinoid receptor that might be found primarily in the cerebellum, frontal cortex and hypothalamus [56,57].

Interestingly, Lipina et al. indicated in vivo that GPR55 deficiency in mice might increase adiposity and impair insulin signaling in skeletal muscles, adipose tissue, and the liver [58]. Although our findings indicated a reduced expression of GPR55 after treatment with CBD in the high-fat diet group in comparison to the control, we did not observe any significant changes in the expression of vanilloid receptor subtype 1. Another important CBD target is peroxisome proliferator-activated nuclear receptor, especially PPARγ isoform, for which CBD works as an agonist [59]. In our study we observed a significant increase in the expression of PPARγ after CBD treatment in HFD group compared to HFD alone group. It seems to be very important because activation of PPARγ reduces inflammation through impairment in NF-κB pathway and prevents neuropathology development or progression [60]. Studies conducted by Scuderi et al. revealed, similarly to our studies, that CBD in neurons activated PPARγ receptors. Moreover, they showed that this activation resulted in decreased amyloid precursor protein levels, which caused reduced amyloid beta deposition in these cells [61]. We suspect that observed in our studies diminishment in tau phosphorylation after CBD treatment in HFD group, induced by inactivation of GSK, may be the result of PPARγ receptors activation.

Furthermore, our data indicated a substantial decrease in the expression of 5-hydroxytryptamine receptor 1A in both CBD alone and CBD with high-fat diet groups. In vivo study conducted by Afshar et al. indicated that 5-HTR1A antagonists might reduce oxidative stress and neuronal loss in a Wistar rat model of Alzheimer's disease and may prevent its progression [62]. Moreover, it is known that antagonism of the 5-hydroxytryptamine receptor 1A ameliorates memory retention and certain types of learning [63]. Thus, it might be speculated that cannabidiol might attenuate the development of neuropathologies via its interference with 5-HTR1A receptors. This might constitute another essential target of novel treatments methods for neurodegenerative disorders. However, the exact role of 5-HTR1A in brain insulin resistance is still not well known. There is indirect evidence that indicates that activation of 5-HTR1A receptors may regulate MAPK and Akt signaling pathways in the mammalian brain [64]. The changes in Akt phosphorylation results in modulation of GSK-3 activity, which is a constitutively active kinase, inactivated by phosphorylation at serine 9 or 21 and activated by phosphorylation at tyrosine 216 or 279 [65]. The activated GSK-3 promotes tau protein hyperphosphorylation which may cause increased production of amyloid β and finally leads to cognitive dysfunction [66]. In our study, we observed inactivation of GSK-3 and, as a result decreased phosphorylation of tau protein after CBD administration in HFD group, which showed that this cannabinoid may be effective in treatment of the neurodegenerative diseases. Moreover, we may speculate that the interaction of CBD with this receptor may be a possible mechanism through which this phytocannabinoid affected the sphingolipid metabolism and accumulation in the cerebral cortex.

5. Conclusion

In conclusion, our study demonstrated a wide range of cannabidiol action on sphingolipid metabolism in insulin resistant brain tissue. The main routes changed by this phytocannabinoid, under condition of high-fat diet, were ceramide de novo synthesis and salvage pathways. We indicated innovatively that CBD might be considered as an essential factor that leads to the reduction of brain insulin resistance and tau protein phosphorylation, two main factors predisposing to the neuropathologies occurrence. The novelty and originality of this project are compounded by the fact that preventing metabolic and neurodegenerative pathologies is a very alarming issue since the number of individuals suffering from those diseases increases annually among different populations. Thus, we believe that our research will concern a new possible therapeutic approach with a *Cannabis*-plant derived compounds and within a few years, those substances would be considered as prominent compounds for targeting both metabolic and neurodegenerative pathologies.

CRediT authorship contribution statement

Tomasz Charytoniuk: Conceptualization, Writing – original draft, Project administration. **Klaudia Sztolsztener:** Methodology, Software, Writing – original draft. **Ewa Harasim-Symbor:** Methodology, Supervision. **Klaudia Berk:** Software, Writing – review & editing. **Adrian Chabowski:** Writing – review & editing, Supervision, Funding acquisition. **Karolina Konstantynowicz-Nowicka:** Conceptualization, Writing – original draft, Project administration.

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Conflict of interest statement

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.112057.

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9. Publikacja nr 2

International Journal of *Molecular Sciences*

MDPI

Review

Can Physical Activity Support the Endocannabinoid System in the Preventive and Therapeutic Approach to Neurological Disorders?

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Abstract: The worldwide prevalence of neurological and neurodegenerative disorders, such as depression or Alzheimer's disease, has spread extensively throughout the last decades, becoming an enormous health issue. Numerous data indicate a distinct correlation between the altered endocannabinoid signaling and different aspects of brain physiology, such as memory or neurogenesis. Moreover, the endocannabinoid system is widely regarded as a crucial factor in the development of neuropathologies. Thus, targeting those disorders via synthetic cannabinoids, as well as phytocannabinoids, becomes a widespread research issue. Over the last decade, the endocannabinoid system has been extensively studied for its correlation with physical activity. Recent data showed that physical activity correlates with elevated endocannabinoid serum concentrations and increased cannabinoid receptor type 1 (CB1R) expression in the brain, which results in positive neurological effects including antidepressant effect, ameliorated memory, neuroplasticity development, and reduced neuroinflammation. However, none of the prior reviews presented a comprehensive correlation between physical activity, the endocannabinoid system, and neuropathologies. Thus, our review provides a current state of knowledge of the endocannabinoid system, its action in physical activity, as well as neuropathologies and a possible correlation between all those fields. We believe that this might contribute to finding a new preventive and therapeutic approach to both neurological and neurodegenerative disorders.

Keywords: endocannabinoid system; ECS; cannabinoids; phytocannabinoids; physical activity; neurological disorders; neurodegenerative disorders

1. Introduction

The worldwide prevalence of neurological and neurodegenerative disorders has spread extensively in the past two decades among various social groups, races, and ethnicities, becoming a common morbidity and an enormous burden [1–3]. Chronic pain, Parkinson's disease (PD), depression, and anxiety are a tremendous reality with unfavorable prognosis for many individuals. In 2016, the global number of patients who suffered from dementia and Alzheimer's disease (AD) was about 43.8 million. It increased from 20.2 million in 1990 and will almost double every 20 years, reaching 74.7 million in 2030 and 131.5 million in 2050 [4,5]. Data from numerous studies indicate a substantial correlation between the endocannabinoid system (ECS), a key regulator of energy homeostasis, and the development of neurophysiological processes, such as modulation of neurogenesis, synaptic plasticity, as well as emotions and memory. It was widely demonstrated that the ECS, cannabinoids, and its receptors correlate not only with umpteen physiological aspects, but also are associated with the

development of certain neuropathologies, such as pain, depression, as well as neurodegenerative disorders [6,7]. Furthermore, our review will also present a current state of knowledge on the correlation between the endocannabinoid system and physical activity (PA). Although present times are much overwhelmed with a vast amount of research that provides indisputable evidence of the beneficial effects of exercises on neurological pathologies, the exact biological systems and their mechanisms involved in this process are still questionable and widely discussed. Over the last decade, the endocannabinoid system was extensively studied for its correlation with physical activity, which was recognized as a factor that notably modifies this essential biological system and its related molecular pathways [8,9]. Some recent studies demonstrated that plasma levels of endocannabinoids are notably higher after physical activity and might be associated with the long-term beneficial effects on neurophysiology, namely, mood, appetite, mental health, memory, as well as cognitive processes [10–12]. Interestingly, some studies indicate that physical activity significantly increases the expression of cannabinoid receptor CB1 (CB1R), a cannabinoid receptor that widely occurs in the striatum, and endocannabinoids, such as anandamide, which both might be correlated with the attenuation of neurological pathologies [10,13]. Several studies revealed that physical activity is associated, at least partially, with its positive results on, for example, metabolic diseases via normalization of the ECS. Furthermore, none of the ECS reviews present a comprehensive correlation between physical activity, its effect on the endocannabinoid system, and subsequently on neurological disorders. Thus, our review significantly contributes to this area of research as it presents many multifaceted aspects of the endocannabinoid system, including its action in physical activity, neurological and neurodegenerative disorders, and a possible correlation between all these fields.

2. An Overview of the Endocannabinoid System—From Endo to Phytocannabinoids

The endocannabinoid system is defined as a widespread biological lipid system that plays an essential modulatory role in the endocrine, immune, and brain tissue [14,15]. The endocannabinoid pathway includes elementarily G protein-coupled receptors, known as cannabinoid receptors CB1 and CB2 (CB2R), and the endogenous agonists of these receptors, known as endocannabinoids, principally anandamide (AEA, *N*-arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG) [16]. It is known that CB1R is predominantly found in the brain (cerebral cortex), whereas CB2R is principally expressed in a number of immune system cells [17]. Considering endocannabinoids, those molecules are synthesized from omega-3 (docosahexaenoic acid, DHA and eicosapentaenoic acid, EPA) or omega-6 (arachidonic acid, AA) long-chain polyunsaturated fatty acids (LC-PUFAs) [18]. Anandamide is a molecule generated from *N*-arachidonoyl phosphatidylethanolamine (NAPE), an AEA membrane precursor, whereas the fatty-acid amide hydrolase (FAAH) catalyzes the hydrolysis of AEA to arachidonic acid and ethanolamine [19]. Although anandamide is a ligand for CB1R, it might interact with non-classic cannabinoid receptors, such as transient receptor potential vanilloid type 1 (TRPV1) or peroxisome proliferator-activated receptors α and γ (PPARα and PPARγ) [20,21]. On the contrary, 2-arachidonoylglycerol, a ligand for both CB1R and CB2R is mainly formed from the degradation of diacylglycerols (DAGs) by diacylglycerol lipases α and β (DAGLα and DAGLβ) and subsequently hydrolyzed by the monoacylglycerol lipase (MAGL) to AA and glycerol [14]. It is worth mentioning that some researchers define an expanded endocannabinoid system as an endocannabinoidome (eCBome)—a complex lipid signaling system composed of more than 100 fatty acid-derived mediators and their receptors, as well as the anabolic and catabolic enzymes of more than 50 proteins. Thus, a vast number of studies reported that eCBome is deeply involved in the control of energy metabolism and its disturbances that may lead to the development of numerous metabolic pathologies [22,23]. Although endocannabinoids constitute a large group of cannabinoids, we may also distinguish two other classes—synthetic cannabinoids and phytocannabinoids—as essential modulators of the endocannabinoid system.

Phytocannabinoids—Compounds with Dualistic Nature

Cannabis sativa L. has been known for both its healing and psychoactive properties for thousands of years. The first reports of pharmacological usage of cannabis came from ancient China, around ~3000 B.C.E., where it was used in the treatment of such conditions as gout, constipation, or rheumatism. In the Medieval Era, *C. sativa* was widely used in the treatment of pain, inflammation, vomiting, and fever. Although the antiepileptic attributes of cannabis were described in the 19th century by Irish physician William O'Shaughnessy, its first use in this pathology was in the Islamic world 100 years prior [24]. Currently, *C. sativa* is the most popular illicit drug in the world, used by approximately 4% of the global population. Still, it is an object of interest to many researchers around the world due to its large potential as a therapeutic agent [25,26]. Phytocannabinoids are a different group of more than 90 terpenophenolic derivatives produced by *C. sativa*. These compounds originate mostly from non-enzymatic reactions of decarboxylation, oxidation, and isomerization of the cannabinoid precursors. In the biosynthesis of phytocannabinoids that occur in *C. sativa* only three enzymes have an essential significance: cannabinoid acid synthase (CBDA), cannabichromenic acid synthase (CBCA), and tetrahydrocannabinolic acid synthase (THCA). These enzymes are responsible for the conversion of a primary phytocannabinoid precursor—cannabigerolic acid (CBGA)—into final products [27,28]. The most abundant component of *C. sativa* is tetrahydrocannabinol (THC), which comprises ~17% of the total phytocannabinoid content and is represented by different isomers, including the most well-known—Δ9-THC. It was proven that Δ9-THC has anticonvulsant, neuroprotective, and anti-inflammatory effects, but its psychoactive and addictive properties cause limitations in its clinical usage. Other phytocannabinoids that may be found in the plant are, for example, cannabidiol (CBD) and cannabinol (CBN) [29–31]. The main chemotypes of cannabis preparations are divided into three types: THC predominant (Type I); mixed THC and CBD (Type II, THC and CBD are mixed in 1:1 ratio); and CBD predominant (Type III) [24]. CBD, CBN, and other compounds from this group do not express such psychoactive effects as Δ9-THC [32]. CBD is a much more interesting phytocannabinoid due to its excellent safety profile, and many reported therapeutic effects, especially in the treatment of neurological conditions. Cannabidiol has a very low affinity to cannabinoid receptors. However, it interacts with other complex signaling systems [25,31]. The recent data showed that mechanisms of CBD action might be associated with many molecular targets, including orphan G protein-coupled receptors, serotonin, adenosine, opioid or PPARγ receptors, as well as transient receptor potential (TRP), glycine or sodium channels. CBD also inhibits FAAH which leads to elevation of AEA concentration in serum. The variety of molecular targets for CBD may be correlated with its influence on many different signaling pathways [33]. Furthermore, CBD interacts with cytochrome P450 isoenzymes which may lead to the altered metabolism of other drugs [34].

3. Physical Activity and Its Correlation with the Endocannabinoid System and Neurophysiology

3.1. PA and the Endocannabinoid System

Physical activity can be defined as repetitive and planned muscle movements that result in energy disbursement. Some researchers broadly portray PA as an essential, cost-saving, and effective factor in terms of prevention, treatment, and management of numerous pathologies [35]. Although the positive effects of physical exercise in the pathophysiology of various diseases are widely known, the molecular mechanisms are still widely discussed. However, one of the most probable and examined mechanisms that is changed during PA is the endocannabinoid system. Physical activity was presented as a significant factor that might lead to the activation of the endocannabinoid-signaling pathway, and a clear mutual correlation between them was indicated in several studies. Based on various research, it is worth noting that PA was demonstrated to modulate the ECS in different ways. Several studies conducted on both animal- and human-based models described significant alterations in blood levels of cannabinoid receptors agonists (i.e., AEA and 2-AG) after exercise. In addition to the endocannabinoids mentioned above, OEA and PEA, the analogous endocannabinoid (eCB) compounds that do not act directly on cannabinoid receptors, were also significantly altered during exercise. Sparling et al. were

the first to describe the correlation between acute exercise and higher AEA and 2-AG levels in the blood in human-based models. The elevation of AEA levels may be associated with its acting on peripheral sensory fibers and pain relief as well as the occurrence of "runner's high" in many regions of the brain, especially in the right anterior lobe and left caudate nucleus [36]. Moreover, the increase in AEA concentration is supposed to be triggered by higher cortisol secretion during acute exercise performance [37,38]. Interestingly, a study by Fuss et al. indicated that exercise-mediated runner's high might occur due to the interference between physical activity and peripheral CB1 and CB2 receptors, as well as activation of CB1 receptors on forebrain GABAergic (γ-aminobutyric acid) neurons [39]. A large number of studies indicated that moderate and acute physical activity resulted in increased levels of serum concentrations of AEA, OEA, PEA, and 2-AG. Recently, Brellenthin et al. indicated that both AEA and 2-AG circulating levels are significantly higher after exercise, but the increase in AEA is more substantial in the prescribed (approx. 70%–75% max. activity), in comparison to preferred (i.e., self-selected), aerobic exercise [40]. A recent in vivo study conducted by Thompson et al. on mice demonstrated that voluntary physical activity significantly affected circulating endocannabinoid levels differently depending on recent activity and genetic background in comparison to high runner mice (acute PA), which had significantly lower AEA levels. Interestingly, the same study revealed differences in AEA and 2-AG levels between the sexes: males tended to have increased 2-AG levels, whereas AEA levels were higher in females [41]. Recently, Stensson et al. conducted a study on women with fibromyalgia; they indicated that a 15-week person-centered resistance exercise program led to a significant increase in AEA and 2-AG concentration, and therefore might increase muscle strength and provide some neurological alterations, such as analgesia or antidepressant effects [42]. On the contrary, some studies report the constant levels of 2-AG concentration in response to moderate or acute exercises performed by humans [43,44]. Moreover, lower circulating levels of 2-AG after both moderate and preferred physical activity were also demonstrated in some recent research conducted on women with major depressive disorders [11]. Perhaps, alterations in the circulating 2-AG levels depend on the type of physical activity, its intensity, and duration, as well as possible comorbidities. However, further research is necessary to clarify this issue. It is commonly known that physical activity may also affect the expression of cannabinoid receptors, both CB1R and CB2R. Some studies indicated that chronic exercises might be correlated with the upregulation of CB1R expression and density in mice, most notably in the hippocampus [45,46]. Interestingly, a recent study by Crombie et al. revealed that isometric handgrip exercise for three minutes led to significant alterations in the ECS; not only in higher blood circulating levels of AEA, 2-AG, OEA, and PEA, but also increased expression of cannabinoid receptor type 1, which resulted in significant analgesic effects [47]. It is broadly known that the endocannabinoid system and its signaling pathway might be remarkably involved in the dopamine neurotransmission in synapses at midbrain and striatal sites [48]. Furthermore, the activation of cannabinoid receptor type 1 in GABAergic neurons of the human ventral tegmental area (VTA) in the midbrain may result in disinhibition of VTA dopamine release, involved in reward-directed processes that occur during physical activity (mainly voluntary). Thus, this demonstrated a significant and promising correlation between the expression of CB1R, GABA, and dopamine [44,49]. Interestingly, Merill et al. using an animal model indicated that ventral tegmental area GABAergic and DAergic cells are able to produce various eCBs, and therefore might be involved in the alterations in the neuronal activity or plasticity in adaptive reward processing or addiction [50]. These studies might be considered, at least partially, as a way to answer the firm doubts concerning the molecular effects of physical activity on the ECS and higher motivation via the reward system. It is worth mentioning, in terms of physical activity, that stimulation of the CB1R at the nerve terminals of neuromuscular junction might lead to the inhibition of acetylcholine (ACh) release and Ca^{2+} flux that causes decreased muscular tension [35]. Interestingly, there is a lack of findings focused on the interaction between physical activity and phytocannabinoids (e.g., CBD or CBN). Few studies present the action of CBD in muscle recovery by reducing inflammation in the tissue and alleviating pain. However, it underlines the potential usage of phytocannabinoids in the rehabilitation and restoration process after severe

physical activity [17,51]. The area of correlation between physical activity and the endocannabinoid system is still unexplored and needs fulfillment by further studies. In addition, novel research should examine the exact mechanism involved in the effect of PA on endocannabinoid signaling, as well as investigate conditions such as the PA type, exercise duration, intensity, age, and sex, which are the most effective in inducing ECS changes.

3.2. PA and Neurophysiology—Interference with the Endocannabinoid System

The involvement of physical activity in the neurophysiology components, including mood, pain, cognition, and neurogenesis, is indisputable, as indicated in a vast number of studies. Nevertheless, the mutual relationship between physical activity, the endocannabinoid system, and human neurophysiology remains not well discovered and needs proper fulfillment. The molecular alterations in the endocannabinoid signaling triggered by physical activity mentioned above may directly correlate with systemic effects. Starting with the influence on mood, various types of exercise, both acute and chronic, and ending with resistant and aerobic training, all activate the endocannabinoid signaling and result in significant mood improvements, antidepressant effect, reduced anger, and tension, as well as increased vigor and motivation [35,40,44,52]. Euphoric and analgesic phenomena widely described by athletes that occur during a forced and prolonged physical activity called "runner's high" are the result of the activity of endorphins, monoamines, and endocannabinoids and their influence on the reward system in the brain [36,53]. Therefore, the "runner's high" may lead to heavy exercise addiction probably, due to endogenous opioids release, which may be augmented by the ECS [54,55]. On the other hand, the endocannabinoid system is actively involved in the "runner's high" associated with exercise-induced hypoalgesia. However, the mechanism of this phenomenon is not yet fully understood. Most of the data describe possible interaction between the ECS and the endogenous opioid system in reducing pain sensitivity associated with physical activity. Studies conducted on healthy individuals revealed that short-time isometric exercise produced a significant analgesic effect, which was associated with increased serum concentrations of AEA, 2-AG, and β-endorphins. Moreover, transiently increased pain thresholds in exercising limbs were observed [47,56,57]. On the contrary, a recent study performed by Hughes et al. showed expanded β-endorphin concentrations, whereas 2-AG remained unchanged during forced resistant exercises [58]. The body of evidence suggests that interplay between PA and ECS may influence cognition processes such as memory and learning and may be associated with the development of adult neurogenesis in some regions of the brain. The activation of the HPA (hypothalamic–pituitary–adrenal) axis during stressful situations, which physical activity admittedly is, leads to augmented endocannabinoid synthesis in the peripheral blood and, subsequently, increased activation of postsynaptic β-adrenoceptors which facilitate memory consolidation especially during emotional events [49,59]. Furthermore, a recent article by Wang et al. showed that CB1R signaling in glutamatergic neurons, enhanced by treadmill running, played an essential role in memory and learning improvement and resulted in increased synthesis of neurotrophins and spine density of the hippocampal neurons in mice [60]. Many studies indicate a significant influence of PA and ECS on neurogenesis. Physical activity constitutes a significant factor that leads to enhanced synthesis of BDNF (brain-derived neurotrophic factor), which is a crucial player in the modulation of neurogenesis in the dentate gyrus of hippocampus and subventricular zone. The increased levels of BDNF correlated with expanded AEA and 2-AG levels and CB1R expression within neural progenitor cells. These findings indicate a clear interaction between BDNF and the ECS; what results is the overall promotion of proliferation, regeneration, and viability of neurons [61–63]. Interestingly, Heyman et al. showed that intense and prolonged physical activity resulted in enhanced synthesis of BDNF among male cyclists, probably due to elevated circulating levels of endocannabinoids (i.e., AEA among male cyclists). These findings correlate with neuroplasticity development and antidepressant effect induced by exercise [37]. In summary, the activation of the endocannabinoid system through various types of physical activity may provide a promising influence on neurophysiology aspects. The popularity of physical activity as a cheap, plausible, and effective method of maintaining a healthy lifestyle and

prophylaxis of an enormous number of disorders, including neurological, is increasing tremendously worldwide. Therefore, we believe that more data focused on positive outcomes of different types of exercise may even increase awareness among people and, at least partially, contribute to decreased prevalence of neurological and neurodegenerative conditions. The large number of studies conducted both on animal- and human-based models shows that targeting the ECS by physical activity may provide promising results in the treatment of neurological conditions; they have been gathered and examined and are presented in Table 1.

Table 1. A summary of studies analyzing the correlation between physical activity, the ECS, and possible positive outcomes on brain physiology and various neurological disorders.

Subjects	Performed Activity	Main Outcomes	Reference
Healthy men runners (n = 8), cyclist (n = 8), controls (n = 8)	Running on a treadmill/cycling on an ergometer for 45 min (HR_{max} = 70%–80%)	ECS alterations: ↑AEA Brain physiology and neurological alterations: Anxiolytic and analgesic effect, sense of well-being → "runner's high"	[36]
Well trained male cyclist (n = 11)	Moderate cycling on an ergometer for 60 min (55% W_{max}) followed by intense cycling for 30 min (75% W_{max})	ECS alterations: ↑AEA, PEA, OEA Brain physiology and neurological alterations: Increased BDNF and cortisol levels, antidepressant and reward effect, possible promotion of neuroplasticity	[37]
Women with fibromyalgia (n = 37), controls (n = 33)	15-week person-centered resistance exercise program	ECS alterations: ↑AEA, 2-AG Brain physiology and neurological alterations: Antidepressant and analgesic effect, increased muscle strength	[42]
Patients with PTSD (n = 12), controls (n = 24)	Low/moderate 10 min warm-up (HR_{max} = 40%–60%) followed by 30 min of moderate walking or running on a treadmill (HR_{max} = 70%–75%).	ECS alterations: ↑AEA, 2-AG, OEA Brain physiology and neurological alterations: Antidepressant effect, analgesic effect, reduced stress, fatigue, confusion, anger, and anxiety	[64]
Patients with episodic migraine (n = 30), controls (n = 28)	12 week aerobic exercise program—40 min of walking/running on a treadmill 3 times per week	ECS alterations: ↓AEA Brain physiology and neurological alterations: Amelioration of migraine headaches, reduced frequency of migraine attacks	[65]
Women with MDD (n = 17)	30 min of moderate cycling followed by 30 min of preferred exercise	ECS alterations: ↑AEA, OEA, ↓2-AG Brain physiology and neurological alterations: Minimal antidepressant effect	[11]
Patients with relapsing-remitting MS (n = 30)	2 weeks of therapeutic exercise program—1 h of aerobic exercise followed by 1 h of swimming in the pool.	ECS alterations: Different polymorphisms in *CNR1* gene lead to various responses on physical therapy associated with altered CB1R density in motor cortex. Brain physiology and neurological alterations: ↑ cortical plasticity and response to physiotherapy	[66]
Healthy men (n = 29) and women (n = 29)	Isometric handgrip exercise for 3 min (MVC = 25%)	ECS alterations: ↑AEA, 2-AG, OEA, PEA ↑CB1R Brain physiology and neurological alterations: Significant analgesic effect; ECS interplays with endogenous opioid release → "exercise-induced antinociception"	[47]
Healthy women (n = 9)	1 day—30 min of dancing 2 day—30 min of cycling on ergometer	ECS alterations: ↑OEA (only while dancing) Brain physiology and neurological alterations: Reduced appetite, decreased negative emotions, "runner's high"	[10]

Table 1. *Cont.*

Subjects	Performed Activity	Main Outcomes	Reference
Cannabis users (n = 37), controls (n = 42)	Treadmill running	ECS alterations: not described Brain physiology and neurological alterations: Improved psychomotor speed, visual memory, sequencing ability among cannabis users → possible interplay between cannabinoids and physical activity	[67]
Male Sprague-Dawley rats (n = 40)	Wheel running	ECS alterations: ↑AEA, CB1R Brain physiology and neurological alterations: Increased progenitor cell proliferation within dentate gyrus, promotion of neurogenesis	[61]
Male Wistar rats treated with LPS (animal model presenting signs of neuroinflammation)	Forced treadmill running for 8 weeks 5 times per week. MWT performed.	ECS alterations: ↑2-AG, CB1R Brain physiology and neurological alterations: Improved memory and cognitive function, reduced inflammatory effect ↓COX-2	[68]
Male Swiss mice (n = 72)	5 min of treadmill running for 3 days	ECS alterations: ↑CB1R Brain physiology and neurological alterations: Increased spatial memory ↑BDNF	[46]
Male Swiss mice	High-intensity swimming exercise (HISE)	ECS alterations: ↑AEA, CB1R Brain physiology and neurological alterations: Significant analgesic effect → "exercise-induced antinociception", reduced inflammation	[69]

S2-AG: 2-arachidonoylglycerol, AEA: anandamide, BDNF: brain derived neurotrophic factor, CB1R: cannabinoid receptor type 1, ECS: endocannabinoid system, HR_{max}: maximum heart rate, MDD: major depressive disorder, HISE: high-intensity swimming exercise, MS: multiple sclerosis, MVC: maximum ventilatory capacity, MWT: maze water test, OEA: *N*-oleoylethanolamine, PEA: palmitoylethanolamide, W_{max}: maximal trial power output, LPS: lipopolysaccharide. ↑—increase, ↓—decrease, →—further step.

4. The Endocannabinoid System and Its Correlation with Neuropathologies

The endocannabinoid system constitutes a crucial player in the development of various neuropathological states, including depression, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and epilepsy. These conditions probably may also be caused by dysregulations that occur in the endocannabinoid signaling pathway. The background of these disturbances seems to be very complex and includes altered cannabinoid receptors signaling and expression as well as fluctuations in endocannabinoid concentrations in serum [70,71].

4.1. Depression and Anxiety

Significant alterations in cannabinoid receptors expression occur in depression and anxiety. The genetic overexpression of the CB2R resulted in decreased depressive-like behavior, whereas CB1R deficiency was correlated with the development of depressive symptoms in rodents. In human studies, patients with depression had lower levels of AEA and 2-AG in serum [72–74]. Studies conducted on the brains of patients with depression who committed suicide showed an increased density of CB1R in the prefrontal cortex. The density of CB2R remained unchanged [75]. This evidence suggests that the ECS hypoactivity may result in the development of depression and depression-like states. Certain polymorphisms in the CB1R coding gene—*CNR1*—seem to be associated with susceptibility to depression and its treatment-resistance development. In turn, the knockout of the CB1R in mice resulted in anxiety-like behavior [72,76,77]. Moreover, a study conducted by Kong et al. revealed that certain polymorphisms in *CNR2* coding CB2R also correlated with increased susceptibility to depression development among patients [78]. Furthermore, overexpression of CB1R was detected in anxiety-related brain areas such as the amygdala, hippocampus, and striatum among posttraumatic

stress disorder (PTSD) diagnosed patients [75]. Additionally, decreased levels of AEA and 2-AG were described in the blood of patients with PTSD [79].

4.2. Alzheimer's Disease (AD)

In the animal models that expressed a mutant form of amyloid precursor protein (APP), including Tg2576 transgenic mice and APP/PS1 mice, significant alterations in the ECS were observed. Most of the studies showed the downregulation and impaired signaling within CB1R in microglial cells of the hippocampus and prefrontal cortex of transgenic mice [80–82]. In turn, the upregulation of CB2R that occurred in mice models may be associated with neuroprotective and anti-inflammatory properties resulting from CB2R activation [70,81]. The higher serum levels of 2-AG were associated with hippocampal degradation induced by amyloid-β peptide [70,80]. Altmura et al. revealed that the elevated levels of 2-AG might be associated not only with neuroprotective mechanisms but also with amelioration in cerebral circulation [83]. Furthermore, overexpression of FAAH was revealed in the brains of people with AD and may correlate with exacerbated inflammatory processes [80,84,85]. The results of the clinical trials or post-mortem studies also showed elevated levels of the endocannabinoids and higher expression of CB2R. However, the expression changes in CB1R remained inconsistent. Some studies showed no alterations in the expression and density of CB1R. In contrast, a significant decrease in the expression of this receptor in the brain cortex of AD patients was found. Thus, these contradictory data should be clarified by future research [81,84].

4.3. Parkinson's Disease (PD)

In Parkinson's disease, considerable changes within the ECS were observed and well described in studies conducted on animal models as well as shown in the post-mortem brains of PD patients. These changes included both hyper- and hypoactivity of CB1R signaling, increased levels of 2-AG and AEA, and altered expression of CB1R and CB2R in the basal ganglia of people with PD and transgenic mice including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mouse models and lipopolysaccharide (LPS) rat models [85–87]. A recent study conducted on brain samples of patients with PD revealed a significant decrease in MAGL expression [88]. Ceccerini et al. showed that individuals with PD-related cognitive impairment had decreased expression of CB1R, especially in the midcingulate and superior frontal gyrus [89]. Furthermore, this receptor is supposed to be involved in motor disturbances that occur in PD. The administration of CB1R antagonists, such as rimonabant, resulted in ameliorated dyskinesia and motor impairment in experimental models (i.e., 6-hydroxydopamine (6-OHDA) or MPTP-lesioned rats) [90]. The upregulation of CB2R may be associated with the anti-inflammatory process and reduction of the degradation of dopaminergic neurons, whereas the downregulation of these receptors results in exacerbation of these processes [80,85].

4.4. Multiple Sclerosis (MS)

Studies conducted on mice with experimental autoimmune encephalomyelitis (EAE)—a preclinical model of MS—showed that complex alterations in CB1R and CB2R signaling occur. The activation of these receptors was correlated with neuroprotective and anti-inflammatory effects [86]. The CB1R and CB2R knockout mice showed enhanced inflammation and reduced neurodegeneration [91]. In turn, the concentration of AEA was significantly increased, whereas, 2-AG remained unchanged or either increased in mice with EAE [48,87]. Human studies revealed significant elevation of serum AEA, OEA, and PEA concentrations. As in the animal data, 2-AG remained unchanged. Furthermore, the levels of anandamide were increased in the cerebrospinal fluid (CSF), brain, and peripheral tissues of MS patients. The mRNA of both CB1R and CB2R was increased among patients with primary-progressive MS which might suggest possible compensating mechanisms [92–94]. Moreover, an increased expression and activity of FAAH were detected [95]. These findings show that the ECS is involved in multiple sclerosis pathogenesis and targeting the ECS may be a promising treatment method for relapsing or acute multiple sclerosis.

4.5. Epilepsy

Interestingly, there is a lack of data that describes the involvement of the ECS in epilepsy pathogenesis. In most preclinical studies, the injection of kainic acid or pentylenetetrazole, as well as an electric shock, were used to induce an acute seizure attack in animal models. In the studies conducted on animals, overexpression of CB1R, and elevated concentrations of 2-AG in blood after induction of seizures were described [96,97]. A significant decrease in expression of CB1R and DAGL-α was revealed in the human epileptic hippocampus. Moreover, the concentration of AEA in the cerebrospinal fluid of epileptic patients was also decreased. All these changes were associated with impaired GABA signaling in the brain [96,98]. Nowadays, the usage of cannabinoid-derived compounds in the treatment of epilepsy, especially the drug-resistant kind, is becoming more common worldwide. The majority of studies showed notable relieving effects after cannabis treatment on epilepsy management, mainly drug-resistant ones [99]. Jessberger et al. described a possible correlation between seizure-generated new granular cells and the promotion of neurogenesis in the adult brain, particularly in the hippocampus in what may be a compensating mechanism of "brain recovering" after an injury caused by seizure [100].

5. The Triad—Physical Activity, the Endocannabinoid System, and a Novel Therapeutic Approach to Neurological Pathologies—How Might All These Be Linked?

The summary of the correlation between physical activity, its effect on the endocannabinoid system, and subsequently neuropathologies is presented in Figure 1.

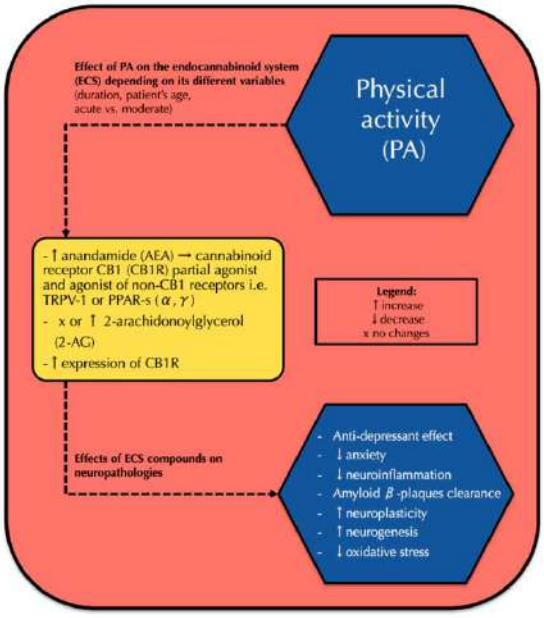

Figure 1. The effect of physical activity on the endocannabinoid system components and, subsequently, its possible impact on the attenuation of neuropathologies. PA: physical activity, ECS: endocannabinoid system, AEA: anandamide, CB1R: cannabinoid receptor type 1, TRPV-1: transient receptor potential vanilloid type 1, PPAR: peroxisome proliferator-activated receptors α, 2-AG: 2-arachidonoylglycerol.

There is no doubt that the involvement of the ECS in the pathogenesis of both neurological and neurodegenerative disorders is crucial. The targeting of many ECS components, including cannabinoid receptors, endocannabinoids, or enzymes responsible for their degradation by both natural and synthetic agents, may be a promising and effective treatment method for these conditions and accompanying symptoms. However, despite some studies, there is still a number of undiscovered areas in this field. Furthermore, a broad correlation between the endocannabinoid system and physical exercises, both acute and chronic, was indicated in a number of both animal- and human-based models [8,35]. Understanding the effects of physical activity on the ECS may contribute towards considering exercise as an alternative approach to the clinical management of neuropathologies. Although there is a limited amount of evidence demonstrating positive ECS-related effects of physical activity on the attenuation of, for example, obesity and type 2 diabetes mellitus (T2DM), the involvement of physical activity in the treatment of neurological diseases by affecting the endocannabinoid signaling is not yet well discovered [38,101,102]. Interestingly, studies conducted on animal models after one weak treadmill running revealed ameliorated spatial memory results in a common memory test with an increased CB1R expression in the hippocampus [46]. Furthermore, the activation of the ECS by physical activity is widely considered to suppress inflammation and oxidative stress in neuronal tissue by affecting cannabinoid receptors signaling and modulating the function of lymphocytes [35,44]. Thus, targeting the ECS by physical activity might provide auspicious treatment results of various neurological disorders. We believe that this uncharted territory with excellent research potential may constitute a field that will be broadly explored within the next few years. Moreover, physical activity will be considered, at least partially, as a novel therapeutic approach to the attenuation of neuropathologies.

6. Conclusions

In conclusion, our review comprehensively summarizes substantial studies that describe precisely the endocannabinoid system from its molecular structure and compounds to its correlation with physical activity. Moreover, we profoundly demonstrate the ECS action in various neurological and neurodegenerative pathologies, as well as point out mechanisms that might be investigated in further studies. The development and worldwide prevalence of neuropathologies are very alarming, and their prevention has become a substantial issue. Therefore, it is essential to find new therapeutic targets, which may probably contribute to a reduction in the annual increase of individuals suffering from these diseases among all of the global populations. Consequently, researchers have great hope for the endocannabinoid system, its elements, pathways, and ligands, and consider the ECS as a possible preventive and therapeutic approach for both neurological and neurodegenerative pathologies, which might be correlated, at least partially, with physical activity.

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Abbreviations

2-AG	2-arachidonoylglycerol
6-OHDA	oxidopamine
AA	arachidonic acid
ACEA	arachidonyl-2-chloroethylamide
ACEA	arachidonyl-2-chloroethylamide
ACh	acetylcholine
AD	Alzheimer's disease

AEA	anandamide, N-arachidonoylethanolamine
Aβ	amyloid-β
BACE1	β-secretase 1
BDNF	brain-derived neurotrophic factor
CB1R	cannabinoid receptor type 1
CB2R	cannabinoid receptor type 2
CBCA	cannabichromenic acid synthase
CBD	cannabidiol
CBDA	cannabinoid acid synthase
CBGA	cannabigerolic acid
CBN	cannabinol
CSF	cerebrospinal fluid
DA	dopamine
DAG	diacylglycerol
DAGLα	diacylglycerol lipase α
DAGLβ	diacylglycerol lipase β
DHA	docosahexaenoic acid
EAE	autoimmune encephalomyelitis
eCB	endocannabinoid
eCBome	endocannabinoidome
ECS	endocannabinoid system
EHA	eicosapentaenoic acid
EPM	elevated plus maze test
FAAH	fatty-acid amide hydrolase
FST	forced swimming test
GABAHPA	γ-aminobutyric acidhypothalamic–pituitary–adrenal
HISE	high-intensity swimming exercise
HR_{max}	maximum heart rate
LC-PUFAs	long-chain polyunsaturated fatty acids
LPS	lipopolysaccharide
MAGL	monoacylglycerol lipase
MDD	major depressive disorder
MPTP	1-methyl-4-phenyl-l,2,3,6-tetrahydropyridine
MS	multiple sclerosis
NAPE	*N*-arachidonoyl phosphatidylethanolamine
OEA	*N*-oleoylethanolamine
PA	physical activity
PD	Parkinson's disease
PEA	palmitoylethanolamide
PPARα	peroxisome proliferator-activated receptors α
PPARγ	peroxisome proliferator-activated receptors γ
T2DM	type 2 diabetes mellitus
THCA	tetrahydrocannabinolic acid synthase
TRP	transient receptor potential
TRPV1	transient receptor potential vanilloid type 1
VTA	ventral tegmental area
Δ9-THC	Δ9-tetrahydrocannabinol
MVC	maximum ventilatory capacity
W_{max}	maximal trial power output

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10. Publikacja nr 3

International Journal of *Molecular Sciences*

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Review

The Endocannabinoid System and Physical Activity—A Robust Duo in the Novel Therapeutic Approach against Metabolic Disorders

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Abstract: Rapidly increasing worldwide prevalence of obesity and related pathologies encompassing coronary heart disease, hypertension, metabolic syndrome, or type 2 diabetes constitute serious threats to global health and are associated with a significantly elevated risk of premature death. Considering the enormous burden of these pathologies, novel therapeutic and preventive patterns are indispensable. Dysregulation of one of the most complex biological systems in the human body namely, the endocannabinoid system (ECS) may result in metabolic imbalance and development of insulin resistance, type 2 diabetes, or non-alcoholic fatty liver disease. Furthermore, many studies showed that physical exercises, depending on their type, intensity, and frequency, exert various alterations within the ECS. Emerging evidence suggests that targeting the ECS via physical activity may produce robust beneficial effects on the course of metabolic pathologies. However, the data showing a direct correlation between the ECS and physical activity in the aspect of metabolic health are very scarce. Therefore, the aim of this review was to provide the most up-to-date state of knowledge about the interplay between the ECS activity and physical exercises in the novel therapeutic and preventive approach toward metabolic pathologies. We believe that this paper, at least in part, will fulfill the existing gap in knowledge and encourage researchers to further explore this very complex yet interesting link between the ECS, its action in physical activity, and subsequent positive outcomes for metabolic health.

Keywords: endocannabinoid system; physical activity; obesity; cannabinoid receptor; anandamide; 2-arachidonylglycerol

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1. Introduction

Nowadays, metabolic disorders, e.g., obesity, metabolic syndrome, or type 2 diabetes mellitus (T2DM) are some of the major medical concerns that occur among different populations, especially those with a predominance of the Western dietary pattern [1,2]. It is estimated that about 2 billion people across the world are overweight or obese. Consequently, obesity and its associated pathologies constitute serious threats to global health and are widely regarded as a worldwide epidemic, thereby posing as one of the leading causes of death in well-developed countries [3]. Various and complex molecular mechanisms are involved in the pathogenesis of these disorders, including insulin resistance, chronic inflammation, defective metabolism within adipose tissue, excessive oxidative stress, and alterations in the endocannabinoid system (ECS) [4,5]. The ECS constitutes a versatile and complex regulator of human energy homeostasis, which is involved in metabolic health functions, for instance, appetite, food intake, energy expenditure, metabolism of carbohydrates and lipids, likewise hedonic rewards such as palatability [6,7]. Recent studies

indicate that dysregulation in the components of ECS is linked with the metabolic imbalance and, subsequently, the development of obesity and related disorders. Interestingly, the ECS is widely known for its interplay with physical activity (PA) [7,8]. Although the correlation between the ECS and PA was described in the literature, underlying molecular alterations remain unknown and deserve to be reviewed more extensively. A body of evidence suggests that targeting the ECS signaling via exercise may result in significant beneficial outcomes either in the supportive treatment or prophylaxis of metabolic disorders [9,10]. Therefore, considering the scarcity of studies showing this relationship, this review aimed to provide the most up to date state of knowledge about the interplay between the ECS and PA in the novel therapeutic and preventive approach towards metabolic pathologies, such as obesity, insulin resistance, type 2 diabetes mellitus, and non-alcoholic fatty liver disease (NAFLD).

2. The Endocannabinoid System—A Brief Overview of the Key Functions and Elements

The ECS constitutes one of the most complex biological systems in the human body, creating a milieu responsible for the plethora of essential functions, including maintaining metabolic and cardiovascular homeostasis, mediating immune responses, and modulating the signaling within reward systems, as well as playing a relevant role in the brain physiology components, such as mood, cognition, and neurogenesis [11–13]. Furthermore, the ECS is involved in fertility, reproduction, and pregnancy [14]. In general, the ECS comprises (1) cannabinoid receptors (CBRs), (2) endocannabinoids (eCBs)—lipid ligands of cannabinoid receptors originating from omega-3 and omega-6 polyunsaturated fatty acids, among which anandamide (N-arachidonoyl-ethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) received the most attention and were best described in the literature, (3) enzymes involved in bioformation and degradation of eCBs [15,16]. Cannabinoid receptors type 1 and type 2 (CB1R and CB2R) belong to the family of 7-transmembrane G-protein-coupled receptors (GPCRs) (Gi/o); thus, their activation, by suitable ligand, leads to the inhibition of adenyl cyclase and decreases in the concentration of cyclic adenosine monophosphate (cAMP). As a result, cAMP intensifies the activity of p42/p44 mitogen-activated protein kinase (MAPK) and Jun N-terminal kinase (JNK) which activate divergent nuclear transcription factors and alter the cellular metabolism. Additionally, cannabinoid receptors activate potassium and inhibit calcium channels in the presynaptic end of neurons that results in the overall inhibition of neurotransmitter release [17–19]. The distribution of CB1R is mainly clustered within brain structures, especially the neocortex, hippocampus, basal ganglia, and brain stem; likewise, its presence was confirmed in the skeletal muscles, lungs, testes, gastrointestinal tract, liver, pancreas, and adipose tissue. In turn, CB2R is mainly expressed on the immune cells (macrophages, T lymphocytes, B lymphocytes), hematopoietic stem, and progenitor cells present in bone marrow, as well as in the thymus and lymph nodes; thereby its activation triggers wide-range of immunomodulatory effects (Figure 1) [19–22]. Endocannabinoids are synthesized on demand in response to the elevated intracellular Ca^{2+} concentration [18]. 2-AG, which is synthesized from diacylglycerol (DAG), acts as a full agonist with a moderate affinity of cannabinoid receptors, whereas is hydrolyzed by monoacylglycerol lipase (MAGL) [23,24]. In turn, AEA constitutes a partial agonist of cannabinoid receptors synthesized from N-arachidonoyl phosphatidylethanolamine (NAPE) coordinated by N-acyl-phosphatidylethanolamine phospholipase D-like esterase (NAPE-PLD) and is degraded to arachidonic acid (AA) by the activity of fatty acid amid hydrolase (FAAH) [15,18]. Next to the well-known eCBs, analogous "endocannabinoid-like" lipid ligands were described namely oleoylethanolamine (OEA) and palmitoylethanolamide (PEA). Although OEA and PEA do not bind typical cannabinoid receptors, they possess a high affinity toward peroxisome proliferator-activated receptor alpha (PPARα) and orphan GPCRs. These compounds play an important role in hypoxia-induced intestinal permeability and suppression of the inflammation within the gastrointestinal tract, which highlights their potential importance in the aspect of cancer research [25,26]. Recently some

researchers provided the term "endocannabinoidome" (eCBome), which refers to the broad and expanded signaling system that, apart from the aforementioned elements, contains a large number of different membrane and nuclear receptors, i.e., PPARs, transient receptor potential vanniloid 1 ion channel (TRPV1), various GPCRs, their endogenous ligands, and dozens of enzymes playing important roles in endocannabinoid signaling. Endocannabinoidome may constitute a promising target in the therapy of various pathologies in which the components of ECS seem to be involved; therefore, further research on the emerging new metabolic implications of eCBome remains in high demand [16,17,27,28].

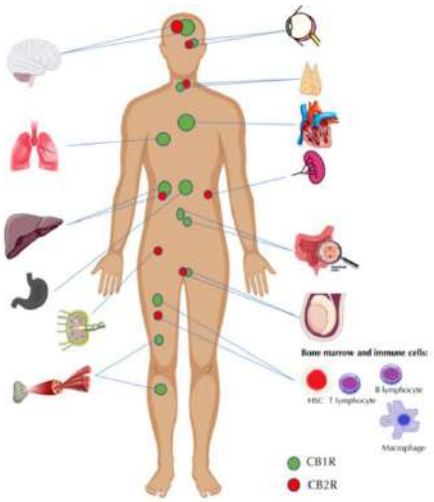

Figure 1. The distribution of cannabinoid receptors within the human body. CB1R—cannabinoid receptor type 1, CB2R—cannabinoid receptor type 2, HSC—hematopoietic stem cell (some graphic was acquired from vecteezy.com, accessed date: 1 November 2021).

3. The Endocannabinoid System and Metabolic Pathologies

Many researchers indicated that ECS might be widely involved in the regulation of energy balance and feeding behavior through both central and peripheral mechanisms [27]. Central mechanisms include hedonic energy regulation in the nucleus accumbens (NAc) and the ventral tegmental area that results in a higher motivation via the reward system. Moreover, another essential mechanism that leads to an increased appetite and higher food intake is homeostatic regulation in the hypothalamus, a major site of ECS action. Conversely, in the peripheral levels of action, ECS is widely associated with reduced expression of adiponectin, a protein hormone involved in suppressing glucose production in the liver and enhancing fatty acid oxidation in skeletal muscles. Another mechanism of ECS action is decreased AMPK activity (5'AMP-activated protein kinase), which is involved in insulin sensitivity, and its inhibition regulates lipogenesis and fatty acids or cholesterol synthesis in both the liver and adipose tissue [28]. Interestingly, the concentration of anandamide might be decreased via leptin action (an appetite-reducing adipose tissue-origin hormone that plays an essential role in food intake, body weight control, and metabolism), which enhances FAAH expression and leads to a lower concentration of anandamide in adipose tissue [29,30]. It is commonly known that low circulating levels of the leptin, as well as leptin resistance, are widely correlated with a higher risk of obesity and its aftermath [31,32]. Interestingly, a recent study conducted by Tam et al. indicated that a peripheral inhibition of CB1R in mice with diet-induced obesity (DIO) leads to an increase in the leptin sensitivity, which results in hypophagia via the reactivation of melanocortin

signaling in the arcuate nucleus (ARC) in the hypothalamus [33]. Some studies reported that anandamide may lead to increased liver lipogenesis via the CB1R [34,35]. Another study by Karaliota et al. demonstrated that AEA may act as a PPAR-γ agonist and subsequently leads to the development of ECS-related adipogenesis in primary rat preadipocytes isolated from epididymal adipose tissue of male Wistar rats [36]. Numerous studies have indicated that endocannabinoids widely affect proliferation, as well as differentiation of adipocytes [33,37,38]. Amidst various components of endocannabinoidome, researchers point out the relevance of the link between the ECS and ion channel TRPV1 in ensuring undisturbed energy homeostasis. It is highly expressed within the central nervous system as well as peripheral tissues, including stomach and adipose tissue. TRPV1 is a non-selective cation channel activated by capsaicin, high temperature, and acidosis that belong to the transient receptor potential protein superfamily [39]. Moreover, among various stimuli which interact with TRPV1, N-acyldopamines, leukotriene B4 and N-acylethanoloamines (e.g., AEA) may be distinguished as TRPV1 agonists. The effect of endocannabinoids on TRPV1 may be exerted through direct activation on this channel or indirectly through activation of the CB1R that activates the phospholipase C-PKC pathway. However, endocannabinoids may also activate CB1R, which leads to the inhibition of the adenylate cyclase-PKA pathway, resulting in diminished TRPV1 activity. Those correlations are still unclear and, at least in part, depend on the concentration of AEA [37,40]. The metabolic functions of TRPV1 include its influence on glucose metabolism and lipid oxidation, regulation of the appetite (via interaction with appetite-regulating hormones e.g., ghrelin and leptin) as well as activation of thermogenesis [40]. Numerous studies highlighted that impaired signaling within TRPV1 may contribute to the development of various metabolic disorders, including obesity and T2DM [38,41]. Therefore, the complex interactions between the ECS and TRPV1 are still a subject of discussion and should be clarified in future studies.

3.1. Obesity and Non-Alcoholic Fatty Liver Disease (NAFLD)

A substantial correlation between metabolic disturbances and endocannabinoid system overactivation was unequivocally demonstrated in both in vivo animal studies and clinical trials [42,43]. So far, a vast number of studies have linked obesity with higher circulating levels of endocannabinoids [44,45]. It is known that obesity and higher food intake might lead to an overactivation of ECS by increased cannabinoid receptor activity. Moreover, this might become a vicious circle—obesity-related ECS activation might contribute to an intensified lipogenesis, higher food intake, and consequently lead to further fat storage. Increased fat accumulation in adipose tissue might be associated with activation of CB1R since it was indicated that those receptors might increase the activity of lipoprotein lipase, a key enzyme regulating triglyceride hydrolysis to free fatty acids, which are subsequently transported and deposited in the liver [46]. Thus, inhibition of CB receptors seems to be relevant.

Interestingly, rimonabant was the first CB1R antagonist approved for the treatment of obesity since clinical trials revealed that it might promote weight loss in obese patients. However, due to a number of serious psychiatric adverse effects, e.g., anxiety or depression, the clinical use of rimonabant was discontinued, and the drug was withdrawn from the worldwide market [47]. A human-based study by Bennetzen et al. demonstrated that levels of 2-arachidonoylglycerol were significantly reduced in the abdominal adipose tissue, whereas weight loss led to a normalization of this endocannabinoid. Furthermore, the same study indicated that the low expression of CB1 in abdominal adipose tissue was normalized after weight loss, whereas in gluteal adipose tissue the CB1 expression was decreased after weight loss. Thus, this research, together with other related studies, presented substantial results that ECS is widely dysregulated among individuals suffering from obesity [48,49] Moreover, it indicates that not only CB receptor expression is affected by obesity but also levels of ECs. Indeed, some researchers reported that in obese patients, both AEA and 2-AG levels are increased, and elevated plasma concentrations of endocannabinoids (ECs) might be correlated with obesity aftermath, e.g., cardiovascular diseases, as well as non-alcoholic

fatty liver disease (NAFLD) [50–52]. Moreover, increased concentrations of endocannabinoids observed in small intestinal mucosa and plasma of high-fat diet-fed obese mice inhibited may change TRPV1 activity, which is involved in energy homeostasis [53]. However, because of various interactions of this channel with other systems, the precise role of TRPV1 in regulating of food intake and energy expenditure needs further investigation. All these outcomes highlight those alterations in dysregulated ECS signaling are very complex and should be investigated in depth.

Non-alcoholic fatty liver disease is an obesity-related metabolic pathology induced by enhanced triacylglycerols accumulation leading to hepatic steatosis, excluding alcohol abuse as a pathogenic factor [54]. Numerous studies have indicated that NAFLD occurrence is widely correlated with the dysfunction of the endocannabinoid system because lipid accumulation in the liver might be mediated via alterations in ECS [55,56]. It is known that the basal hepatic expression of cannabinoid receptors is indistinct, with low levels of both CB1R and CB2R. However, an increased expression of the cannabinoid receptors was proven in NAFLD. Moreover, CB1R was shown as a key mediator of insulin resistance development, increased liver lipogenesis, and steatosis in both animal and human studies, whereas CB2R might be considered as a promoter of inflammatory processes underlying the pathogenesis of liver steatosis [57]. It is known that blockage of cannabinoid receptor type 1 may result in attenuation of hepatic oxidative stress, as well as the impairment of inflammatory response by inhibiting the production of pro-inflammatory cytokines [58,59] Furthermore, Irungbam et al. recently demonstrated in both in vitro (AML12 liver cell line) and in vivo studies on mice with a global CB1R receptor knockout that lower CB1R signaling reduced liver steatosis via downregulation of perilipin 2—the protein that is involved in the suppression of lipolysis and lipid accumulation [60]. Shi et al. conducted in vitro studies in which application of CB1R antagonist in HepG2 cells resulted in a significant decrease in factors widely involved in lipogenesis, i.e., sterol regulatory element-binding protein (SREBP1c), carbohydrate responsive element-binding protein (ChREBP), liver X receptors (LXRs), acetyl-CoA carboxylase (ACC1) and fatty acid synthase (FAS) [61]. Moreover, Deveaux et al. conducted a study on mice which indicated that the inactivation of cannabinoid receptor type 2 reduced steatosis, liver triacylglycerol concentration, and obesity-associated inflammation caused by a high-fat diet [62]. Interestingly, CB1R was defined as a pro-fibrogenic activator, while cannabinoid receptor type 2 was proposed to have anti-fibrogenic properties in the liver [35,63]. Thus, ECs and their receptors might be considered as a possible and promising target for the treatment of liver steatosis and its metabolic aftermath.

3.2. *Insulin Resistance and Type 2 Diabetes*

Insulin resistance (IR), defined as a condition with impaired insulin action and response in tissues, is widely considered to be one of the most fundamental factors associated with the development of many metabolic disorders, e.g., type 2 diabetes mellitus or metabolic syndrome [64,65]. A vast number of studies has provided evidence indicating that activation of peripheral CB1R located in insulin-dependent tissues, i.e., liver, adipose tissue, or skeletal muscles, is widely associated with the development of dyslipidemia, insulin resistance, and type 2 diabetes [66]. Additionally, plenty of studies reported that CB1R blockage leads to increased insulin sensitivity and, subsequently, improves both glucose and lipid metabolism [67,68]. Considering the pathophysiological basis of IR development, Liu et al. demonstrated that it is correlated with activation of CB1R that inhibits insulin signaling via endoplasmic reticulum stress-dependent suppression of enzymes involved in the insulin signaling pathway, e.g., PKB/Akt [69]. Interestingly, further Liu et al. studies provided notable results that blockage of CB1R in obese mice improved glycemic control through the hepatic Sirt1/mTORC2/Akt pathway, as well as increased fatty acid β-oxidation via LKB1/AMPK signaling route [70]. A study conducted by Jourdan et al. demonstrated that intravenous administration of β-D-glucan-encapsulated siRNA to knock down CB1R gene expression, improved insulin sensitivity in Kupfer cells of mice with

obesity. Thus, it might be said that the CB1R played a significant role in the development of liver insulin resistance via Kupffer cell-related inflammatory mechanisms [71]. Considering the CB2R, principally expressed in immune tissues, several in vitro and in vivo studies demonstrated CB2R-mediated anti-inflammatory effects of endocannabinoids, i.e., AEA and 2-AG, as well as synthetic CB2R agonists in models of various metabolic diseases, e.g., obesity [72–74]. Furthermore, Verty et al. reported that activation of CB2R reduced the inflammatory response and promoted anti-obesity effects by reducing body weight, while not changing mood-related behaviors [75]. Although plenty of studies support the claim that endocannabinoids are anti-inflammatory mediators, a limited amount of evidence demonstrated the pro-inflammatory action of endocannabinoids via CB2R activation [76,77]. Moreover, Turcotte et al. showed that most of the pro-inflammatory effects involve 2-AG, but not AEA [78].

Considering the correlation between the endocannabinoid system and oxidative stress, a study conducted by Mendizabal-Zubiaga et al. showed that the mitochondrial activation of CB1R in muscle cells might be correlated with the mitochondrial regulation of oxidative activity. Thus, this might be highly associated with the development of insulin resistance, since mitochondrial dysfunction is correlated with a loss of muscular oxidative capacity in response to a high intake of fatty acids, and therefore may be involved in the development of metabolic disorders [79,80]. Notably, Dipanjan et al. demonstrated that activation of the CB1R is involved in gluconeogenesis via direct activation of an endoplasmic reticulum (ER) membrane-localized stress-dependent liver-specific transcription factor called CREBH—cyclic AMP-responsive element-binding protein H, which is considered to be one of the key mediators in both glucose and lipid metabolism [81,82]. Thus, it may be said that there is a molecular association between the activation of the endocannabinoid system and levels of circulating glucose.

An endocannabinoid-related study conducted by Jourdan et al. on the Zucker diabetic rats indicated that the loss of pancreatic beta cell functions and its amount is associated with the release of pro-inflammatory cytokines, i.e., IL-1β and IL-18. Those molecules, acting as paracrine substances that induce beta-cell apoptosis, are released from infiltrating M1 macrophages as a result of macrophages-origin CB1R signaling that participates in selective activation of the Nlrp3-ASC inflammasome [83]. Moreover, a recent study by Kim et al. conducted on pancreatic beta-cell lines MIN6 and βTC6 incubated with synthetic CB1R agonist WIN55,212-2 showed a decreased expression of anti-apoptotic protein Bcl-2 and cell cycle regulator cyclin D2, together with caspase-3-dependent apoptosis afterward [84]. Thus, these two studies unequivocally determine the effect of CB1R activation in beta-cell apoptosis and therefore insulin signaling deficiency and the development of type 2 diabetes. It is commonly known that insulin resistance, as well as T2DM, are widely associated with an increased tissue concentration of sphingolipids—a class of lipids whose fractions are also involved in cellular growth, differentiation, and regulation of apoptosis in addition to playing an essential role as structural molecules in cellular membranes [85,86]. Cinar et al. linked the action of the endocannabinoid system and insulin resistance development together with excessive sphingolipids deposition. It was indicated in a study conducted on C57Bl6/J mice with high-fat diet-induced obesity (DIO) that the factor, which is widely involved in the development of hepatic IR, is increased de novo ceramides synthesis, which is mediated by endoplasmic reticulum (ER) stress-dependent activation of CB1R [87]. Thus, it may be assumed that changes in CB receptors and other components of the ECS may be associated with a favorable prognosis not only of obesity and NAFLD but also IR and type 2 diabetes.

4. The Endocannabinoid System and Physical Activity

Physical activity is broadly portrayed as one of the most popular, potent, cost-friendly, and pleasant ways to maintain a prolonged and healthy life. It constitutes a major pillar in the treatment of metabolic disorders next to the proper diet, pharmacotherapy, chemoprevention, and in the most severe cases, bariatric interventions [88]. What is more, it affects

human mental health, leading to mood, perception, concentration, and creativity improvement as well as producing a very interesting phenomenon called "runner's high" [89]. Despite widely known positive effects in the therapy of various diseases, the exact molecular mechanisms induced by PA are still a subject of discussion. Interestingly, many studies outlined the influence of different types of physical activity on endocannabinoid signaling. These results, however, seem to be partially unclear and confusing; therefore, they deserve to be gathered and described comprehensively. The study conducted by Sparling et al. was arguably the first showing that moderate exercises led to the increased level of AEA but not 2-AG in the sera of healthy subjects after 50 min of treadmill running or cycling on a stationary bike at the level of 70–80% of maximum heart rate [90]. Other studies also indicated that PA, especially at medium intensity, led to the increased blood level of AEA, whereas 2-AG remained unchanged [91–94]. Heyman et al. revealed the link between significantly increased plasma AEA, OEA, and PEA levels during intense exercise followed by 15 min of recovery and elevated serum brain-derived neurotrophic factor (BDNF) levels, thereby indicating an important role of the ECS in the production of neuroplastic and antidepressant effects triggered by PA. A simultaneous increase in OEA and PEA along with AEA probably comes from the fact that these compounds share similar synthesis pathways, whereas 2-AG formation seems to be regulated by different metabolic routes [95]. Conversely, the study conducted by Cedernaes et al. showed elevated blood 2-AG and OEA levels after acute ergometer exercises, while AEA remained stable [96]. The possible reason for this discrepancy may lie in the fasted status of the participants before activity [97]. Brellenthin et al. observed that both AEA and 2-AG are increased after aerobic exercises in the blood of healthy individuals, but the increase in AEA was more substantial in the prescribed by the investigator activity in comparison with self-selected ones, based on personal preferences [98]. Thompson et al. in their study on mice showed that males after exercise tended to have more increased 2-AG serum levels, whereas AEA content was higher in female subjects. These results raised an interesting concern that alterations in the circulating eCBs induced by PA may be at least partially, sex-dependent; however, more data is needed to clarify this issue [99]. It may be suspected that various changes in the levels of eCBs may be a result of different type and time of exercise, but they all exerted changes in the nervous system. Furthermore, Crombie et al. showed that acute isometric activity led to the elevated plasma levels of both AEA and 2-AG as well as OEA and PEA, which resulted in overall analgesic effects [100]. On the other hand, most animal studies pointed out that aerobic activity, i.e., swimming or treadmill running, was associated with increased expression and density of both CB1R and CB2R within the central nervous system, namely the hippocampus, striatum, and spinal cord, that were associated with positive neurological outcomes, including improved cognition, analgesia, and reduced neuroinflammation [101–103]. Nevertheless, there is evidence, that PA may lead to the downregulated expression of cannabinoid receptors in the aforementioned structures which suggests that further investigation is required to elucidate these discrepancies [101,104]. Physical activity, depending on such factors as type, frequency, intensity, and duration leads to the (I) activation of the ECS signaling, (II) significantly affects circulating blood levels of the endocannabinoids, mainly AEA and 2-AG, as well as (III) change in expression of the cannabinoid receptors (CB1R and CB2R). Despite the fact, that molecular interaction between the ECS and PA has been well described in the literature, it is still difficult to point out clear, unequivocal changes within endocannabinoid signaling induced by various types of exercise; thus, this topic needs further investigation in future studies.

5. The Triad—Physical Activity, the Endocannabinoid System, and the Novel Therapeutic Approach to Metabolic Disorders—How All These Components May Be Linked?

The summary of the correlation between physical activity, its effect on the ECS, and subsequent metabolic pathologies is presented in the Figure 2.

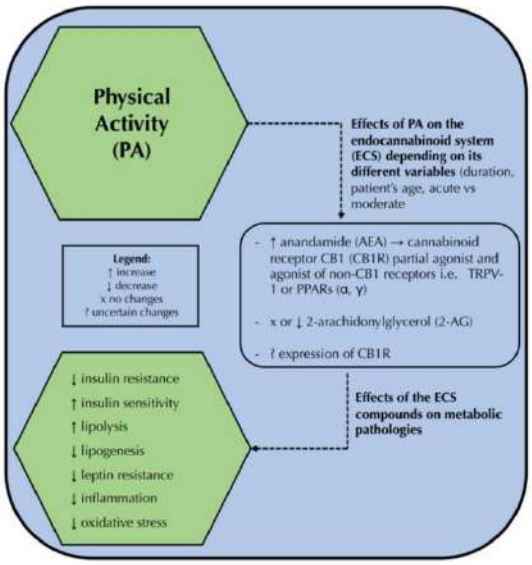

Figure 2. The effect of physical activity on the endocannabinoid system components and, subsequently, its possible impact on the attenuation of metabolic pathologies. PA: physical activity, ECS: endocannabinoid system, AEA: anandamide, CB1R: cannabinoid receptor type 1, ECS: the endocannabinoid system, TRPV-1: transient receptor potential vanilloid 1 ion channel, PPAR: peroxisome proliferator-activated receptors α, 2-AG: 2-arachidonoylglycerol.

There is no doubt that the involvement of the ECS in the pathogenesis of metabolic pathologies is crucial [105]. Therefore, targeting the elements of ECS, including receptors, endocannabinoids, and enzymes, by physical exercises and a healthy diet may exert robust beneficial outcomes in either prevention or supportive treatment of metabolic disorders [9]. To date, only a few studies conducted on humans (Table 1) and animal models, namely Wistar rats and C57B1/6J mice (Table 2), showed the positive influence of chronic and acute PA (e.g., treadmill running, swimming, and aerobic exercises) or combined with the appropriate caloric restriction on the endocannabinoid signaling and subsequently, auspicious course of the metabolic syndrome, for instance, body weight loss, decreased waist circumference and visceral adipose tissue percentage, improved insulin signaling, or enhanced lipid profile. Additionally, it is noteworthy that physical exercises and accompanying increased concentration of eCBs significantly improved mood, sense of well-being, and increased motivation through reward systems that may constitute important factors for maintaining patient cooperation with a physician, thereby providing efficient patient compliance, which is crucial during therapy targeting metabolic diseases. These effects probably arise from the fact that CB1R shares similar localization in the midbrain with dopamine receptors and may enhance the activity of dopaminergic neurons in ventral tegmentum and substantia nigra [106]. Interestingly, observed decreased blood eCBs levels after PA among obese individuals are opposite to those described in the studies involving healthy patients, which, as was mentioned above, indicated increased concentrations of AEA and/or 2-AG [91–94,98,99]. A similar concern deals with the altered expression of cannabinoid receptors. Indeed, a lot of experts suggested that PA in healthy animals was correlated with upregulated CB1R expression within brain structures which, on the contrary, was lowered in the brain and subcutaneous/visceral adipose tissue of obese mice. Neither animal nor human studies revealed consistent outcomes concerning the expression of genes encoding both cannabinoid receptors and enzymes involved in

eCBs metabolism like FAAH, DAGL, MAGL, and NAPE-PLD (Tables 1 and 2). However, Schönke et al., using the online tool MetaMEx, found downregulated skeletal muscle expression of DAGLβ as well as catabolic enzymes, namely MAGL and FAAH, in healthy individuals and patients with metabolic syndrome who underwent chronic aerobic or resistant exercises [107]. It is noteworthy that in vivo studies conducted by Gamelin et al. revealed that obese Wistar rats after a 12-week running period showed an increased expression of TRPV1 and CB1R both in the hippocampus or subcutaneous adipose tissue, which suggests potentially enhanced signaling within this ion channel and may result in the favorable course of obesity and other metabolic conditions [10,108]. In conclusion, all discrepancies and concerns related to ECS signaling suggest unclear and paradoxical involvement of the ECS in both physiological and impaired energy balance and might indicate possible compensation mechanisms in response to the significantly diminished metabolic homeostasis. It strengthens the evidence that the ECS itself constitutes a complex molecular apparatus that may be involved in producing beneficial therapeutic effects and may act as a culprit due to confirmed dysregulation in metabolic diseases [107]. A mutual correlation between the ECS and PA should be discussed more in-depth in the future considering the significant deficit of the studies in this area.

Table 1. A summary of the clinical studies showing the correlation between physical activity, alterations in the endocannabinoid system, and subsequent beneficial therapeutic effects in metabolic pathologies.

Subjects	Performed Activity	Main Outcomes	Reference
Viscerally obese men (n = 49)	1-year lifestyle modification program including regular physical activity and healthy diet	**ECS:** ↓ plasma AEA and 2-AG **Metabolic effects:** ↓ body weight and waist circumference ↓ visceral adipose tissue ↑ HDL_3_C	[109]
Overweight or obese women (n = 30)	20 weeks of moderate (HR_{max} = 45–50%) or intense aerobic exercises (HR_{max} = 70–75%) combined with caloric restriction	**ECS:** ↑ *cb1r* gluteal adipose tissue gene expression ↓ *faah* abdominal adipose tissue gene expression **Metabolic effects:** ↓ body weight and waist circumference ↓ glucose ↓ insulin	[110]
Obese women (n = 77)	6 days of normal, daytime physical activity and 6 days of moderate–vigorous physical activity	**ECS:** ↓ plasma 2-AG ↑ plasma AEA and OEA (only for moderate–vigorous physical activity) **Metabolic effects:** ↓ BMI and waist circumference (only for moderate–vigorous physical activity)	[111]

2-AG—2-arachidonylglycerol, AEA—anandamide, BMI—body mass index, *cb1r*—cannabinoid type 1 receptor gene, ECS—endocannabinoid system, *faah*—fatty acid amide hydrolase gene, HDL_3-C—high density lipoprotein—cholesterol, HR_{max}—maximum heart rate, OEA—oleoylethanolamide, ↑—increase, ↓—decrease.

Table 2. A summary of the animal studies showing the correlation between physical activity, alterations in the endocannabinoid system, and subsequent beneficial therapeutic effects in metabolic pathologies.

Subjects	Performed Activity	Main Outcomes	Reference
Male Wistar rats fed with HFD	1 h of swimming, 3 times a week for 6 months	**ECS:** ↓ expression of CB1R in VAT and SAT ↑ expression of PPARδ in VAT **Metabolic effects:** ↓ body weight ↓ visceral adipose tissue percentage ↓ blood pressure	[112]
C57Bl/6J male mice fed with HFD	1 h of treadmill running, 6 times a week for 6 weeks	**ECS:** ↓ plasma AEA and 2-AG ↓ CB1R and CB2R expression in the brain ↓ CB2R expression in the epididymal fat ↓ MAGL, DAGL- α and β, FAAH, and NAPE-PLD expression in the brain and epididymal fat **Metabolic effects:** ↓ body weight ↓ body fat percentage ↓ LDL-C ↓ TG ↑ HDL-C	[113]
Male Wistar rats fed with HFD	1 h of treadmill running, 5 times a week for 12 weeks (70–80% MAV)	**ECS:** no effect on AEA and 2-AG in SAT and VAT ↑ DAGL-α and FAAH expression in SAT ↑ *cb1r* and *trpv1* gene expression in SAT **Metabolic effects:** ↓ body weight ↓ fasting plasma glucose	[10]
Male Wistar rats fed with HFD	1 h of treadmill running 5 times a week for 12 weeks (70–80% MAV)	**ECS:** ↑ *cb1r* and *trpv1* gene expression in hippocampus ↑ DAGL-α expression in hippocampus ↑ *faah* gene expression in hippocampus ↓ *napepld* gene expression in hippocampus **Metabolic effects:**↓ body weight ↓ fasting plasma glucose	[108]

2-AG—2-arachidonylglycerol, AEA—anandamide, *cb1r*—cannabinoid receptor type 1 gene, CB1R—cannabinoid receptor type 1, CB2R—cannabinoid receptor type 2, DAGL α and β—diacylglycerol lipase α and β, ECS—the endocannabinoid system, *faah*—fatty acid amide hydrolase gene, FAAH—fatty acid amide hydrolase, HDL-C—high-density lipoprotein cholesterol, HFD—high-fat diet, LDL-C—low-density lipoprotein cholesterol, MAGL—monoacyl glycerol lipase, MAV—maximal aerobic velocity, NAPE-PLD—N-Acyl phosphatidylethanolamine phospholipase D, *napepld*—N-acyl phosphatidylethanolamine phospholipase D gene, PPARδ—peroxisome proliferator activation receptor δ, SAT—subcutaneous adipose tissue, TG—triglyceride, *trpv1*—transient receptor potential vanniloid 1 ion channel gene, VAT—visceral adipose tissue, ↑—increase, ↓—decrease.

6. Conclusions

To the best of our knowledge, this is the first review directly and comprehensively discussing the uncharted link between physical activity and its influence on the endocannabinoid signaling in the aspect of beneficial effects in the management of metabolic disorders. Considering the very alarming worldwide prevalence of these diseases as well as the unexplored potential of the topic, we believe that this paper, at least in part, will encourage researchers toward investigating this interesting, yet very complicated interplay. ECS and physical activity constitute robust and valuable therapeutic and preventive approaches that may significantly contribute to the decreased socioeconomic burden and the reduced annual number of patients suffering from obesity and other metabolic disorders. The future investigation should primarily encompass further discovery of the link between

physical activity, alterations within endocannabinoid signaling and subsequently improved metabolic status of overweight, obese, and diabetic individuals.

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11. Streszczenie w języku polskim

Współcześnie choroby metaboliczne, m.in. otyłość i cukrzyca typu 2 (T2DM), należą do najczęstszych jednostek klinicznych, które powszechnie występują w różnych populacjach. Otyłość, jak i powiązane z nią inne zaburzenia metaboliczne stanowią poważne zagrożenie zdrowotne na całym świecie i mogą być uważane za ogólnoświatową epidemię. Insulinooporność (IR) definiowana jako stan nieprawidłowego funkcjonowania szlaku sygnałowego insuliny zarówno w tkankach obwodowych, m.in. wątrobie, czy mięśniach szkieletowych, jak i ośrodkowym układzie nerwowym, m.in. w korze mózgu, jest jednym z głównych czynników prowadzących do rozwoju chorób metabolicznych. Po raz pierwszy zdefiniowana jako główny regulator obwodowego stężenia glukozy, insulina została również uznana za czynnik pełniący istotną rolę w przewodnictwie synaptycznym, regulowaniu procesów pamięciowych i procesach poznawczych.

Choroba Alzheimera stanowi najczęstszy typ otępienia powodujący jego ok. 60-70% przypadków. W 2020 roku liczba pacjentów cierpiących na choroby otępienne na całym świecie wynosiła około 55 milionów. Rozwój ogólnoustrojowej oporności na insulinę i osłabienie szlaku sygnałowego insuliny w mózgu są wspólnymi cechami choroby Alzheimera (AD), T2DM i otyłości. W ostatnich latach, wykazano istotną korelację pomiędzy rozwojem insulinooporności mózgowia, cukrzycy typu 2 i otępienia w licznych badaniach podstawowych, a także w próbach klinicznych.

Insulinooporność i T2DM są istotnie powiązane z nadmierną zawartością sfingolipidów, klasy lipidów, która poza istotną funkcją strukturalną w obrębie błon komórkowych, jest również zaangażowana w szlaki sygnalizacji wewnątrzkomórkowej. Niektóre badania wykazały, że zaburzenia szlaku sfingolipidowego mogą przyczyniać się do rozwoju nie tylko insulinooporności, ale także chorób neurodegeneracyjnych poprzez zmiany w fosforylacji białka tau. Uważa się, że zmiany w metabolizmie sfingolipidów mogą stanowić unikalną strategię leczenia zarówno zaburzeń metabolicznych, jak i neurodegeneracyjnych.

Dane z licznych badań wskazują również na istotną korelację pomiędzy układem endokannabinoidowym, kluczowym regulatorem homeostazy energetycznej, a rozwojem patologii metabolicznych m.in. otyłości, cukrzycy typu 2 (T2DM). Szlak endokannabinoidowy obejmuje głównie receptory sprzężone z białkiem G, znane jako receptory kannabinoidowe typu 1 i 2 (CB1R i CB2R) oraz endogennych agonistów tych receptorów, znanych jako endokannabinoidy, głównie anandamid (AEA) i 2-arachidonoiloglicerol (2-AG). Wiadomo, że CB1R znajduje się głównie w obrębie mózgowia, podczas gdy ekspresja CB2R zachodzi głównie w komórkach układu odpornościowego.

Dotychczas wiele badań wykazało, że kannabidiol (CBD), niepsychoaktywny związek pochodzenia roślinnego i jednocześnie rdzeń tego projektu, wykazuje istotne właściwości neuroprotekcyjne.

W celu oceny wpływu kannabidiolu na metabolizm sfingolipidów, insulinooporność i jej następstwa, badania przeprowadzono na samcach szczurów rasy Wistar. Otyłość i insulinooporność wywołano poprzez karmienie zwierząt dietą bogatotłuszczową (HFD) przez 7 tygodni. Szczury podzielono i losowo przydzielono do czterech grup – (1) grupa kontrolna karmiona standardową paszą dla gryzoni, (2) grupa HFD karmiona dietą bogatotłuszczową, (3) grupa CBD z dootrzewnowymi iniekcjami kannabidiolu karmiona standardową paszą dla gryzoni, (4) grupa HFD+CBD karmiona dietą bogatotłuszczową z dootrzewnowymi iniekcjami kannabidiolu. W badaniu zastosowano różne techniki analityczne, m.in. Western Blot, wysokosprawną chromatografię cieczową i chromatografię gazową.

Badanie wykazało, że kannabidiol w zwierzęcym modelu insulinooporności, indukowanej dietą bogatotłuszczową, prowadzi do znacznych zmian w zawartości głównych sfingolipidów, takich jak ceramid, sfingozyna, sfinganina i sfingomielina w obrębie kory mózgowej szczurów. Fitokannabinoid ten w istotny sposób zmodyfikował główne szlaki sfingolipidpowe w warunkach diety bogatotłuszczowej, tj. syntezy *de novo* ceramidów oraz szlak ratunkowy. Ponadto wykazano, że CBD można uznać za istotny czynnik prowadzący do zmniejszenia insulinooporności kory mózgowej, a także fosforylacji białka tau, tj. dwóch istotnych czynników predysponujących do rozwoju chorób neurodegeneracyjnych, w tym choroby Alzheimera.

Przeprowadzone badania przedstawiły szczegółową ocenę działania kannabidiolu na różne elementy metabolizmu tkanki mózgowej. Oryginalność tego projektu potęguje fakt, że zapobieganie zarówno chorobom metabolicznym, jak i neurodegeneracyjnym jest niezwykle istotną kwestią, gdyż liczba pacjentów cierpiących na te choroby rośnie z roku na rok w różnych populacjach. Jak dotąd nieliczne publikacje wykazały wpływ fitokannabinoidów na metabolizm glukozy i lipidów w obrębie tkanki mózgowej. Dlatego wydaje się, że badania te będą stanowiły podstawę do stworzenia nowego podejścia terapeutycznego ze związkami pochodzącymi z konopi indyjskich, a być może w kolejnych latach substancje te będą uważane za istotne związki w kontekście wspomagania leczenia chorób zarówno metabolicznych, jak i neurodegeneracyjnych.

12. Streszczenie w języku angielskim

Nowadays, metabolic pathologies, e.g., obesity or type 2 diabetes mellitus are one of the major medical concerns that widely occur among different populations. Consequently, obesity and its associated disorders constitute a serious threat to global health and might be considered as a worldwide epidemic. Insulin resistance, a condition with impaired insulin action and response in both peripheral tissues, e.g. liver or skeletal muscles, and central nervous system, e.g. brain cortex, is one of the major factors leading to the development of metabolic disorders. First defined as a main regulator of peripheral glucose concentration, insulin also was recognized as a key factor in synaptic transmission, memory and other cognitive processes.

Alzheimer's disease (AD) is a chronic neurodegenerative disease that is the most common type of dementia causing 60–70% of dementia cases. In 2020, the global number of patients who suffered from dementia was about 55 million. The development of systemic insulin resistance and attenuated brain insulin signaling are common features of AD, T2DM and obesity. A substantial correlation between brain IR, type 2 diabetes mellitus and dementia with cognitive impairment was widely demonstrated in numerous basic studies, including clinical trials.

Insulin resistance and T2DM are associated with higher tissue concentration of sphingolipids, a class of lipids that, in addition to having an important structural function in cellular membranes, are also known to be widely involved in intracellular signaling pathways. Moreover, certain research, both in vitro and in vivo, as well as clinical trials showed that disturbances in the sphingolipid pathway may contribute to the development of not only insulin resistance, but also neurodegenerative disorders via alterations in the phosphorylation of tau protein. It is believed that changes in metabolism of sphingolipids may provide a unique treatment strategy for both metabolic and neurological disorders.

Data from numerous studies indicated a substantial correlation between the endocannabinoid system (ECS), a key regulator of energy homeostasis, and the development of metabolic pathologies e.g. obesity, type 2 diabetes mellitus (T2DM). The endocannabinoid pathway includes elementarily G-protein-coupled receptors, known as cannabinoid receptor type 1 and 2 (CB1R and CB2R) and the endogenous agonists of these receptors, known as endocannabinoids, mainly anandamide (AEA) and 2-arachidonoylglycerol (2-AG). It is known that the CB1 receptor is predominantly found in the brain, whereas CB2 principally expressed on the cells of the immune system.

Cannabidiol (CBD), a non-psychoactive *Cannabis* plant-derived compound and a core of this project, was widely found to show neuroprotective properties.

In order to assess the outcomes of cannabidiol effect on sphingolipids metabolism, insulin resistance, and its aftermath, this project was carried out on male Wistar rats. An obesity and insulin resistance in this animal model was induced by feeding animals with a high fat diet (HFD) for a period of 7 weeks. The rats were divided and randomly assigned to four groups – (1) Control group fed with a standard chow for rodents, (2) HFD group fed with a high fat diet, (3) CBD group treated with cannabidiol with standard chow feeding (4) HFD+CBD group fed with high fat diet feeding. In this project different analytical techniques, including Western Blot, high performance liquid chromatography, and gas liquid chromatography, were used.

This study demonstrated that cannabidiol in an animal model of high-fat diet-induced obesity leads to significant changes in the content of the major sphingolipids such as ceramide, sphingosine, sphinganin and sphingomyelin in the brain cortex of insulin resistant Wistar rats. The main routes affected by this phytocannabinoid, under condition of high-fat diet, were ceramide *de novo* synthesis and the salvage pathway. Moreover, we indicated innovatively that CBD might be considered as an essential factor that leads to the reduction of brain insulin resistance, as well as tau protein phosphorylation, two essential factors predisposing to the occurrence of neuropathologies, e.g. Alzheimer's disease.

The proposed research introduced a comprehensive assessment of cannabidiol action on various aspects of brain metabolism. The novelty and originality of this project are compounded by the fact that preventing metabolic and neurodegenerative pathologies is a very alarming issue since the number of individuals suffering from those diseases increases annually among different populations. So far, extremely little amount of data demonstrated the effects of phytocannabinoids on brain both glucose and lipid metabolism. Thus, we believe that our research will concern a new possible therapeutic approach with a Cannabis-plant derived compounds and within a few years, those substances will be considered as prominent compounds for targeting both metabolic and neurodegenerative pathologies.

13. Oświadczenia współautorów

Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance.		
Autor	**Udział w przygotowaniu publikacji**	**Udział (%)**
Tomasz Charytoniuk	Opracowanie koncepcji pracy, współuczestnictwo w wykonywaniu analiz, zarządzanie projektem, zebranie piśmiennictwa, pisanie manuskryptu	70%
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Ewa Harasim-Symbor	Współuczestnictwo w wykonywaniu analiz, ocena merytoryczna i korekta manuskryptu	5%
Klaudia Berk	Współuczestnictwo w wykonywaniu analiz, pisanie manuskryptu	5%
Adrian Chabowski	Opracowanie koncepcji pracy, ocena merytoryczna i korekta manuskryptu	5%
Karolina Konstantynowicz-Nowicka	Opracowanie koncepcji pracy, zarządzanie projektem, ocena merytoryczna i korekta manuskryptu	10%

Can Physical Activity Support the Endocannabinoid System in the Preventive and Therapeutic Approach to Neurological Disorders?		
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Klaudia Berk	Zebranie piśmiennictwa	1%
Wiktor Bzdęga	Zebranie piśmiennictwa	5%
Adrian Kołakowski	Zebranie piśmiennictwa, zarządzanie projektem	20%
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Oświadczam, iż mój udział w przygotowaniu publikacji: **"Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance."** autorów: Charytoniuk Tomasz, Sztolsztener Klaudia, Harasim-Symbor Ewa, Berk Klaudia, Chabowski Adrian, Konstantynowicz-Nowicka Karolina opublikowanej w *Biomedicine & Pharmacotherapy* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 70% i polegał na opracowaniu koncepcji pracy, współuczestnictwie w wykonywaniu analiz, zarządzaniu projektem, zbieraniu piśmiennictwa i pisaniu manuskryptu.

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

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OŚWIADCZENIE

Oświadczam, iż mój udział w przygotowaniu publikacji: **"Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance."** autorów: Charytoniuk Tomasz, Sztolsztener Klaudia, Harasim-Symbor Ewa, Berk Klaudia, Chabowski Adrian, Konstantynowicz-Nowicka Karolina opublikowanej w *Biomedicine & Pharmacotherapy* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 5% i polegał na współuczestnictwie w wykonywaniu analiz i pisaniu manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Białystok, 7 listopada 2022r.

Prof. dr hab. Adrian Chabowski
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

OŚWIADCZENIE

Oświadczam, iż mój udział w przygotowaniu publikacji: **"Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance."** autorów: Charytoniuk Tomasz, Sztolsztener Klaudia, Harasim-Symbor Ewa, Berk Klaudia, Chabowski Adrian, Konstantynowicz-Nowicka Karolina opublikowanej w *Biomedicine & Pharmacotherapy* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 5% i polegał na opracowaniu koncepcji pracy, ocenie merytorycznej i korekcie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

KIEROWNIK
Zakładu Fizjologii
hab. Adrian Chabowski

/

Białystok, 7 listopada 2022r.

Dr n. med. Karolina Konstantynowicz-Nowicka
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

OŚWIADCZENIE

Oświadczam, iż mój udział w przygotowaniu publikacji: **"Cannabidiol - A phytocannabinoid that widely affects sphingolipid metabolism under conditions of brain insulin resistance."** autorów: Charytoniuk Tomasz, Sztolsztener Klaudia, Harasim-Symbor Ewa, Berk Klaudia, Chabowski Adrian, Konstantynowicz-Nowicka Karolina opublikowanej w *Biomedicine & Pharmacotherapy* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 10% i polegał na opracowaniu koncepcji pracy, zarządzaniu projektem, ocenie merytorycznej i korekcie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Konstantynowicz-Nowicka Karolina

Białystok, 7 listopada 2022r.

Lek. Tomasz Charytoniuk
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

Oświadczam, iż mój udział w przygotowaniu publikacji: **„Can Physical Activity Support the Endocannabinoid System in the Preventive and Therapeutic Approach to Neurological Disorders?"** autorów: Charytoniuk Tomasz, Żywno Hubert, Konstantynowicz-Nowicka Karolina, Berk Klaudia, Bzdęga Wiktor, Chabowski Adrian, opublikowanej w *International Journal of Molecular Sciences* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 70% i polegał na opracowaniu koncepcji pracy, zebraniu piśmiennictwa, pisaniu manuskryptu i zarządzaniu projektem.

Białystok, 7 listopada 2022r.

Hubert Żywno
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

H. Żywno

Białystok, 7 listopada 2022r.

Dr n. med. Karolina Konstantynowicz-Nowicka
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Konstantynowicz-Nowicka Karolina

Białystok, 7 listopada 2022r.

Lek. Klaudia Berk
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

OŚWIADCZENIE

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Białystok, 7 listopada 2022r.

Wiktor Bzdęga
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

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Wiktor Bzdęga

Białystok, 7 listopada 2022r.

Prof. dr hab. Adrian Chabowski
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
Ul. Kilińskiego 1
15-089 Białystok

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

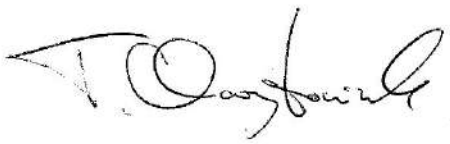
KIEROWNIK
Zakładu Fizjologii
prof. dr hab. Adrian Chabowski

Białystok, 7 listopada 2022r.

Lek. Tomasz Charytoniuk
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

Oświadczam, iż mój udział w przygotowaniu publikacji: **"The Endocannabinoid System and Physical Activity-A Robust Duo in the Novel Therapeutic Approach against Metabolic Disorders."** autorów: Charytoniuk Tomasz, Żywno Hubert, Berk Klaudia, Bzdęga Wiktor, Kołakowski Adrian, Chabowski Adrian, Konstantynowicz-Nowicka Karolina opublikowanej w *International Journal of Molecular Sciences* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 36% i polegał na opracowaniu koncepcji pracy, zebraniu piśmiennictwa, pisaniu manuskryptu i zarządzaniu projektem.



Białystok, 7 listopada 2022r.

Hubert Żywno
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

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Hubert Żywno

Białystok, 7 listopada 2022r.

Lek. Klaudia Berk
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

K Berk
Klaudia Katarzyna Berk
lekarz
3707575

Białystok, 7 listopada 2022r.

Wiktor Bzdęga
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Wiktor Bzdęga

Białystok, 7 listopada 2022r.

Adrian Kołakowski
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Adrian Kołakowski

Białystok, 7 listopada 2022r.

Prof. dr hab. Adrian Chabowski
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

Oświadczam, iż mój udział w przygotowaniu publikacji: **"The Endocannabinoid System and Physical Activity-A Robust Duo in the Novel Therapeutic Approach against Metabolic Disorders."** autorów: Charytoniuk Tomasz, Żywno Hubert, Berk Klaudia, Bzdęga Wiktor, Kołakowski Adrian, Chabowski Adrian, Konstantynowicz-Nowicka Karolina opublikowanej w *International Journal of Molecular Sciences* wchodzącej w skład rozprawy doktorskiej: **„Rola kannabidiolu (CBD) w niwelowaniu insulinooporności mózgowia"** wynosił 1% i polegał na ocenie merytorycznej i korekcie manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

KIEROWNIK
Zakładu Fizjologii
prof. dr hab. Adrian Chabowski

Białystok, 7 listopada 2022r.

Dr n. med. Karolina Konstantynowicz-Nowicka
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku

OŚWIADCZENIE

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. Tomasza Charytoniuka publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

Konstantynowicz-Nowicka Karolina

14. Uchwała komisji etycznej

UCHWAŁA NR 71/2018

z dnia 25.09.2018 r.

Lokalnej Komisji Etycznej do spraw doświadczeń na zwierzętach w Olsztynie

§ 1

Na podstawie art. 48 ust. 1 pkt. 1 / art. 48 ust. 1 pkt. 2[1] ustawy z dnia 15 stycznia 2015r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266), zwanej dalej „ustawą" po rozpatrzeniu wniosku pt.: **„Ocena roli kannabidiolu w regulacji ekspresji białkowych transporterów kwasów tłuszczowych (FAT/CD36, FABPpm i FATP1,4,6) w mięśniu sercowym szczurów z insulinoopornością indukowaną dietą bogatotłuszczową"** z dnia 18.09.2018 r., złożonego przez **Uniwersytet Medyczny w Białymstoku, Wydział Lekarski z Oddziałem Stomatologii i Oddziałem Nauczania w Języku Angielskim (0022)**, adres ul. Kilińskiego 1, 15-089 Białystok, zaplanowanego przez **Karolina Konstantynowicz-Nowicka**[2], przy udziale[3] (nie dotyczy) Lokalna Komisja Etyczna:

WYRAŻA ZGODĘ[4]

Na przeprowadzenie doświadczeń na zwierzętach w zakresie wniosku **72/2018**.

§ 2

W wyniku rozpatrzenia wniosku o którym mowa w § , Lokalna Komisja Etyczna ustaliła, że:

1. Wniosek należy przypisać do kategorii: **badania podstawowe (A), sercowo-naczyniowy układ krążenia i limfy.**
2. Najwyższy stopień dotkliwości proponowanych procedur to: **łagodna**.
3. Doświadczenia będą przeprowadzane na gatunkach lub grupach gatunków: **144 szt., Szczur wędrowny (*Rattus norvegicus*); stado niekrewniacze; Wistar Cmdb:Wi, wiek – 4 tygodnie, masa ciała – 60-100g.**
4. Doświadczenia będą przeprowadzane przez: **Konstantynowicz-Nowicka Karolina, Harasim-Symbor Ewa, Koźluk Anna, Zabrocka Grażyna, Agnieszka Popielska, Aneta Czeladko, Małgorzata Mackiewicz, Katarzyna Podłaszczyk, Anna Maraszkiewicz, Julia Szewczyk, Elżbieta Emilia Łapińska, Ewelina Białous**.
5. Doświadczenie będzie przeprowadzane w terminie[5] **od 15.10.2018 do 31.12.2020**.
6. Doświadczenie będzie przeprowadzone w ośrodku[6]: **Uniwersytet Medyczny w Białymstoku, Centrum Medycyny Doświadczalnej (0099).**
7. Doświadczenie będzie przeprowadzone poza ośrodkiem w: **nie dotyczy**
8. Użyte do procedur zwierzęta dzikie zostaną odłowione przez ....., w sposób: nie dotyczy

---

[1] Niewłaściwy zapis usunąć
[2] imię i nazwisko osoby, która zaplanowała i jest odpowiedzialna za przeprowadzenie doświadczenia
[3] Wypełnić w przypadku dopuszczenia do postępowania organizacji społecznej.
[4] Niewłaściwy zapis usunąć
[5] Nie dłużej niż 5 lat
[6] Podać jeśli jest to inny ośrodek niż użytkownik

9. Doświadczenie **nie zostanie**[7] poddane ocenie retrospektywnej.

§ 3

Uzasadnienie: Po dokonaniu oceny wniosku zgodnie z art. 47 ust. 1 i 2 ustawy z dnia 15 stycznia 2015 r. o ochronie zwierząt wykorzystywanych do celów naukowych lub edukacyjnych (Dz. U. poz. 266) Lokalna Komisja Etyczna w Olsztynie stwierdza, że projekt nie budzi zastrzeżeń pod względem celowości jego wykonania, liczby użytych zwierząt oraz zasadności i klasyfikacji procedur objętych wnioskiem i wyraża zgodę na przeprowadzenie doświadczenia. Osobą odpowiedzialną za przeprowadzenie badań zgodnie z procedurami opisanymi we wniosku jest **Karolina Konstantynowicz-Nowicka.**

§ 4

Integralną część niniejszej uchwały stanowi kopia wniosku, o którym mowa w § 1.

UNIWERSYTET WARMIŃSKO-MAZURSKI
w Olsztynie
LOKALNA KOMISJA ETYCZNA
do Spraw Doświadczeń na Zwierzętach
10-719 Olsztyn, ul. Oczapowskiego 13/4

(Pieczęć lokalnej komisji etycznej)

Podpis przewodniczącego komisji

PRZEWODNICZĄCY
Lokalnej Komisji Etycznej
do Spraw Doświadczeń na Zwierzętach
prof. dr hab. Jerzy Juśkiewicz

Pouczenie:

Zgodnie z art. 33 ust. 3 i art. 40 ustawy w zw. z art. 127 § 1 i 2 oraz 129 § 2 ustawy z dnia z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (Dz. U. 2017, poz. 1257 – t.j.; dalej KPA) od uchwały Lokalnej Komisji Etycznej strona może wnieść, za jej pośrednictwem, odwołanie do Krajowej Komisji Etycznej do Spraw Doświadczeń na Zwierzętach w terminie 14 od dnia doręczenia uchwały.

Na podstawie art. 127a KPA w trakcie biegu terminu do wniesienia odwołania strona może zrzec się prawa do jego wniesienia, co należy uczynić wobec Lokalnej Komisji Etycznej, która wydała uchwałę. Z dniem doręczenia Lokalnej Komisji Etycznej oświadczenia o zrzeczeniu się prawa do wniesienia odwołania przez ostatnią ze stron postępowania, decyzja staje się ostateczna i prawomocna.

Otrzymuje:

1) Użytkownik,
2) Organizacja społeczna dopuszczona do udziału w postępowaniu (jeśli dotyczy)
3) a/a
   Użytkownik kopie przekazuje: Osoba planująca doświadczenie; Zespół ds. dobrostanu.

[7] Niewłaściwy zapis usunąć

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