UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Katedra i Zakład Podstaw Nauk Medycznych

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Predyspozycja do wystąpienia miażdżycy u dzieci i młodzieży z Trisomią 21.

Badania biochemiczne i metabolomiczne.

ROZPRAWA DOKTORSKA

Cykl publikacji powiązanych tematycznie

PROMOTOR:

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Wrocław, 2023

Pragnę złożyć najszczersze podziękowania mojej Promotorce, Pani dr n. med. Ewie Barg, za nieustające wsparcie na każdym etapie pracy nad moją rozprawą doktorską. Jej nieograniczona chęć dzielenia się wiedzą, ogromna wyrozumiałość oraz niezłomność w dążeniu do doskonałości były dla mnie nieocenioną motywacją od wielu lat. Jestem głęboko wdzięczna za otwarcie przede mną drzwi do świata nauki i stałe inspirowanie mnie do dalszego rozwoju.

Dziękuję także moim Rodzicom, Agnieszce i Maciejowi, którzy nigdy nie kwestionowali moich wyborów i zawsze stali u mojego boku. Dzięki Ich wsparciu, miłości i zaangażowaniu miałam szansę rozwijać się, zdobywać wiedzę i realizować swoje pasje. Bez Ich pomocy nie byłabym w miejscu, w którym jestem obecnie.

Ogromne podziękowania kieruję do mojego Męża, Edwina, który przez ostatnie 13 lat był niezachwianą skałą w moim życiu naukowym. Nigdy nie zwątpił w moje umiejętności, nawet kiedy sama byłam przekonana o ich braku. Dziękuję za cierpliwość i pomoc, również w najtrudniejszych momentach mojej drogi.

Nie mogę nie wspomnieć o moich ukochanych Kotach, Biszkopcie z Karmelem. Dziękuję za mruczenie łagodzące stres, bycie cierpliwą i zainteresowaną widownią podczas prób moich wystąpień i za towarzystwo w nocy, kiedy bez końca pisałam prace naukowe, a oni oferowali mi wsparcie swoją cichą, ale obecną postacią.

Spis treści

1. CYKL PRAC STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

1. **Hetman Marta**, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, DOI: https://doi.org/10.5114/pedm.2023.131162, **MNSiW 100.**

2. **Hetman Marta**, Placzkowska Sylwia, Barg Ewa: Comparative Analysis of Obesity Prevalence, Antioxidant and Oxidant Status in Children with Down Syndrome – A Sibling-Controlled Study, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, DOI: https://doi.org/10.5114/pedm.2023.131513, **MNSiW 100.**

3. **Hetman Marta**, Barg Ewa: Pediatric population with Down syndrome: obesity and the risk of cardiovascular disease and their assessment using omics techniques - review, Biomedicines, 2022, vol. 10, nr 12, art.3219 [14 s.], DOI:10.3390/biomedicines10123219, **MNSiW 100, IF(4,7).**

4. **Hetman Marta**, Moreira Helena, Barg Ewa: The best tool for the assessment of developmental disorders in children with down syndrome: comparison of standard and specialized growth charts - cross sectional study, Frontiers in Endocrinology, 2022, vol. 13, art.928151 [11 s.], DOI:10.3389/fendo.2022.928151, **MNSiW 100, IF(5,2).**

Sumaryczny IF: 9,9

Sumaryczna Punktacja Ministerialna: 400

2. WYKAZ SKRÓTÓW

ZD	**Zespół Downa, Trisomia 21 (ang. *Down Syndrome, Trisomy 21*)**
PWDS	**Osoby z Zespołem Downa (ang. *People with Down Syndrome*)**
CVD	**Choroby sercowo-naczyniowe (ang. *Cardiovascular diseases*)**
LDL	**Cholesterol o niskiej gęstości (ang. *Low density lipoprotein*)**
HDL	**Cholesterol o wysokiej gęstości (ang. *High density lipoprotein*)**
HCY	**Homocysteina (ang. *Homocysteine)***
Lp(a)	**Lipoproteina (a) (ang. *Lipoprotein(a)*)**
TAS	**Całkowity potencjał antyoksydacyjny (ang. *Total Antioxidant Status*)**
TOS	**Całkowity potencjał oksydacyjny (ang. *Total Oxidant Status*)**
OSI	**Współczynnik stresu oksydacyjnego (ang. *Oxidative Stress Index*)**
HOMA-IR	**Wskaźnik insulinooporności (ang. *homeostatic model assessment for insulin resistance*)**
BMI	**Wskaźnik masy ciała (ang. *Body Mass Index*)**
BMI SDS	**Standaryzowany wskaźnik masy ciała (ang. *Standardised Body Mass Index*)**
SG	**Grupa Badana (ang. *Study Group*)**
CG	**Grupa Kontrolna (ang. *Control Group*)**
BMI PC	**BMI wyrażone w percentylach (ang. *Body Mass Index Percentile*)**
TG	**Trójglicerydy (ang. *Triglyceride*)**
APO A1	**Apolipoproteina A1 (ang. *Apolipoprotein A1*)**
APO B	**Apolipoproteina B (ang. *Apolipoprotein B*)**

TCH **Cholesterol całkowity (ang. *Total Cholesterol*)**

TMI **Wskaźnik masy ciała Tri-Ponderal (ang. *Tri-Ponderal Mass Index*)**

SOD-1 **Dysmutaza ponadtlenkowa 1 (ang. *Superoxide Dismutase 1*)**

3. OMÓWIENIE ROZPRAWY DOKTORSKIEJ

3.1. Wstęp

We współczesnej medycynie coraz częściej dąży się do diagnozowania chorób przed wystąpieniem ich pierwszych objawów. W przeszłości uważano, że jedynie geny wpływają na utrzymanie homeostazy organizmu. Pomimo wieloletnich badań, poznania i sekwencjonowania genomu, wciąż nie jesteśmy w stanie rozpoznać licznych chorób oraz zaprojektować skutecznej terapii we wczesnym etapie. Obecnie uważa się, że ludzki organizm to niezwykle złożona struktura, z genomem jako fundamentem, a następnie kolejnymi warstwami jakimi jak transkrypty, proteom czy metabolom. W świetle tej wiedzy, metabolizm staje się kluczowym obszarem zainteresowania w różnych dziedzinach biologii. Rozpoznanie wczesnych czynników ryzyka wielu chorób, w tym układu krążenia i metabolicznych, ma kluczowe znaczenie dla wprowadzenia skutecznej profilaktyki już od najmłodszych lat.

Zespół Downa (ZD, Trisomia 21, T21) to najczęstsze zaburzenie chromosomalne o globalnej częstości występowania 1:1000-1100 urodzonych noworodków [1]. Dodatkowy chromosom 21, lub przynajmniej jego część, powoduje szereg zaburzeń klinicznych (wady serca, opóźnione wzrastanie, nieprawidłowości hematologiczne i endokrynologiczne, choroby autoimmunizacyjne, zaburzenia jelitowe, stomatognatyczne, wady wzroku i słuchu, obturacyjny bezdech senny). Dodatkowo osoby z ZD (PWDS) są narażone na zwiększone ryzyko chorób układu krążenia i niewydolność układu krążenia (najczęściej z powodu wady serca), niedorozwój płuc, hipotonię mięśniową, osteoporozę, zapalenie stawów oraz cukrzycę [2]. Nie istnieje specyficzny fenotyp ZD. Osoby z ZD mogą różnić się od siebie zarówno pod względem cech zewnętrznych i występowania przewlekłych chorób. Ten złożony stan powoduje, że PWDS powinny mieć zindywidualizowany proces diagnostyczno-terapeutyczny. Rozwój medycyny wpłynął na średnią długość życia osób z ZD, wydłużając do ponad 50 lat, co wiąże się z pojawieniem się nowych problemów zdrowotnych w tej populacji [3]. Pomimo znaczących postępów w badaniach nad ZD, wiele aspektów tego zespołu pozostaje niezbadanych, a różnorodność objawów i powiązań komplikuje proces diagnostyczny.

W latach 70-tych uważano ZD za zespół „wolny" od ryzyka wystąpienia zaburzeń lipidowych i ich konsekwencji, w tym chorób sercowo-naczyniowych (CVD) [4]. Należy wspomnieć jednak, że obecny styl życia oraz nawyki żywieniowe uległy znacznej modyfikacji, a długość życia jest znacznie dłuższa. Osoby z ZD z powodów genetycznych narażone są na zwiększony stres oksydacyjny, który nasila procesy degeneracyjne tkanek [5]. Dodatkowo zaburzenia hormonalne występujące w tej populacji są ściśle powiązane z regulacją procesów antyoksydacyjnych i metabolicznych. Wśród osób z ZD występuje także specyficzny problem, charakteryzujący się przyspieszonymi przemianami z powodu niedożywienia w początkowych okresach życia do występowania nadwagi w późniejszym wieku. PWDS wykazują tendencję do otyłości, będącej wynikiem dodatniego bilansu energetycznego. Dorosłe osoby z ZD mają dwa razy większe prawdopodobieństwo otyłości i niemal czterokrotnie większe prawdopodobieństwo ekstremalnej otyłości w porównaniu z dorosłymi bez ZD [6]. PWDS prowadzą mniej aktywny tryb życia niż ich rówieśnicy i, co gorsza, z wiekiem stają się mniej

aktywne. Pomimo niższego poziomu aktywności, spożywanie kalorii jest wyższe w tej grupie w porównaniu do rówieśników.

Rodzaj zaburzeń gospodarki lipidowej w ZD wciąż nie jest poznany, a dane literaturowe są nieliczne, niejednoznaczne i często sprzeczne. Z fizjologicznego punktu widzenia osoby z ZD są jednocześnie narażone, jak i chronione przed występowaniem zaburzeń lipidowych i ich konsekwencji. Trudność stanowi ustalenie, który aspekt jest przeważający. Zaburzone procesy metaboliczne przyczyniają się zarówno do występowania otyłości jak i szybciej ujawniających się procesów neurodegeneracyjnych, co powoduje gorszą jakość życia tych pacjentów.

Miażdżyca naczyń, przewlekła choroba zapalna, charakteryzująca się nagromadzeniem lipidów i materiałów włóknistych w ścianach tętnic, prowadzi do CVD– jednego z głównych problemów zdrowotnych współczesnego świata. Rozwój miażdżycy determinowany jest przez złożenie czynników genetycznych, ekspozycji środowiskowych oraz licznych procesów metabolicznych. Nadmierna ilość cholesterolu o niskiej gęstości (LDL), gromadząc się w ścianach tętnic, tworzy podstawę blaszki miażdżycowej. Natomiast cholesterol o wysokiej gęstości (HDL) wspiera usuwanie cholesterolu z blaszek miażdżycowych, zatem jego działanie jest protekcyjne. Podwyższony poziom homocysteiny (HCY), aminokwasu wytwarzanego w procesie metabolizmu metioniny, jest rozważany jako jeden z czynników ryzyka rozwoju CVD [7]. Wykazano, że nadmiar HCY może negatywnie wpływać na śródbłonek naczyniowy, prowadząc do jego uszkodzenia, a także promować stany prozapalne i prozakrzepowe [7]. W praktyce klinicznej stężenie HCY jest często mierzone jako jeden z wielu wskaźników ryzyka CVD. Lipoproteina(a) (Lp(a)), jest czynnikiem, którego wysoki poziom w organizmie może także zwiększać ryzyko rozwoju CVD [8]. Wysoki poziom Lp(a) jest często uwarunkowany genetycznie, co sprawia, że może być trudny do kontrolowania dietą lub trybem życia. Monitorowanie poziomu Lp(a) i włączenie go do oceny ogólnego ryzyka sercowo-naczyniowego stanowi istotny element strategii prewencyjnej.

Metabolomika, koncentrująca się na identyfikacji i ilościowym określeniu małych cząsteczek w próbkach biologicznych, stała się kluczowa w badaniach nad układem sercowo-naczyniowym [9]. Dzięki metabolomice możliwe jest uzyskanie bardziej klarownego obrazu procesów metabolicznych zachodzących na poziomie komórkowym. Konkretne metabolity mogą dostarczyć informacji na temat związku między procesami metabolicznymi u osób ZD a miażdżycą [10].

3.2. Cel badań i problemy badawcze

Celem badań przedstawionych w rozprawie doktorskiej jest ocena procesów metabolicznych i metabolomicznych u osób z ZD i ich wpływu na rozwój miażdżycy w populacji pediatrycznej.

Rozprawa doktorska koncentruje się na kilku kluczowych obszarach:

1. Dogłębna analiza wskaźników stresu oksydacyjnego: Całkowity Potencjał Antyoksydacyjny (TAS), Całkowity Potencjał Oksydacyjny (TOS), Współczynnik Stresu Oksydacyjnego (OSI), oraz ich korelacja z innymi wskaźnikami metabolicznymi, takimi jak Indeks Masy Ciała (BMI), Standaryzowany Współczynnik Masy Ciała (BMI

SDS), Wskaźnik Insulinoooporności (HOMA-IR) oraz stężenia glukozy i insuliny na czczo.
2. Porównanie tych parametrów między dziećmi i młodzieżą z ZD a ich rodzeństwem bez ZD, co pozwoli na analizę, czy obecność ZD wpływa na podwyższone ryzyko miażdżycy.
3. Ocena profili lipidowych i związanych z nimi metabolitów u osób z ZD.
4. Wyniki przedstawionych badań dążą do odpowiedzi na pytanie, czy osoby z ZD są bardziej narażone na rozwój miażdżycy i powiązane z nią choroby sercowo-naczyniowe.
5. Identyfikacja optymalnego narzędzia do oceny rozwoju (parametrów antropometrycznych) dzieci i młodzieży z ZD.

Poprzez skoncentrowanie się na tych obszarach, badanie dostarcza cennych informacji, które mogą przyczynić się do lepszej opieki nad pacjentami z Trisomią 21 oraz do opracowania nowych strategii prewencji i leczenia miażdżycy w tej szczególnej grupie populacyjnej.

3.4. Materiał i metody

Projekt badawczy i uczestnicy: Cykl publikacji obejmuje trzy prace oryginalne oraz pracę przeglądową.

Praca przeglądowa (artykuł nr 2) porusza problem otyłości i ryzyka chorób sercowo-naczyniowych w populacji osób z ZD, wykorzystania technik omnicznych do ich oceny.

Trzy prace oryginalne (artykuł nr 1, nr 3 i nr 4): Pierwsza z nich to badanie oparte na metodologii ankietowej oraz pomiarach antropometrycznych obejmujące grupę 411 dzieci i młodzieży z ZD. Dwie kolejne prace analizują wyniki przeprowadzonych badań laboratoryjnych oraz analiz metabolomicznych, w których uczestniczyło 42 osób z ZD oraz 20 osób stanowiących rodzeństwo uczestników grupy badanej. Uczestnicy byli rekrutowani z różnych regionów Polski. Badanie fizykalne, pomiary antropometryczne oraz pobranie materiału biologicznego (krew oraz mocz) przeprowadzono w Klinice Pediatrycznej we Wrocławiu.

Pomiary antropometryczne: Podczas badań mierzono masę i wysokość ciała uczestników. Pomiary wykonywano z dokładnością do 0,1 kg dla masy oraz do 0,1 cm dla wysokości ciała.

Kwestionariusze internetowe: Zebrano szczegółowe informacje dotyczące rodziców dzieci z ZD, okresu okołoporodowego, chorób współistniejących i leczenia L-tyroksyną. Dodatkowo przeprowadzono ankietę on-line składającą się z 4 pytań dotyczących korzystania z siatek centylowych w gabinetach lekarskich, na którą odpowiedziało 281 rodziców.

Metody laboratoryjne: Badania laboratoryjne wykonano w Diagnostycznym Laboratorium Naukowo-Dydaktycznym Wydziału Farmaceutycznego Uniwersytetu Medycznego im. Piastów Śląskich we Wrocławiu. Analizowano TAS, TOS, glukozę na czczo i insulinę, cholesterol całkowity (TCH), LDL, HDL, trójglicerydy (TG), apolipoproteinę A1 (APO A1),

apolipoproteinę B (APO B), Lp(a), HCY. Wszystkie badania laboratoryjne były przeprowadzane w próbkach biologicznych pobranych od badanych pacjentów na czczo.

Wskaźniki wyliczone: Bazując na uzyskanych wynikach pomiarów antropometrycznych i badań laboratoryjnych dokonano obliczeń matematycznych.

Wyliczono Indeks masy ciała (BMI) według wzoru 1, Wskaźnik masy ciała Tri-Ponderal (TMI) według wzoru 2, OSI według wzoru 3 oraz Wskaźnik APOB/APOA1 według wzoru 4:

$$\boldsymbol{BMI} = \frac{masa\ ciała\ [kg]}{wysokość\ ciała^2\ [m^2]} \text{ (wzór 1)}$$

$$\boldsymbol{TMI} = \frac{masa\ ciała\ [kg]}{wysokość\ ciała^3\ [m^3]} \text{ (wzór 2)}$$

$$\boldsymbol{OSI} = \frac{TOS\ [\frac{mmol}{l}]}{TAS\ [\frac{mmol}{l}]} \text{ (wzór 3)}$$

$$\frac{\boldsymbol{APOB}}{\boldsymbol{APOA1}}\ \boldsymbol{Wskaźnik} = \frac{APOB\ [\frac{mg}{dl}]}{APOA1\ [\frac{mg}{dl}]} \text{ (wzór 4)}$$

Standaryzowany Wskaźnik Masy ciała (BMI SDS) wyliczono z użyciem Kalkulatora LMSgrowth (dodatek do programu Excel), zgodnie ze wzorem 5:

$$\boldsymbol{BMI\ SDS} = \frac{\boldsymbol{BMI\ value - BMI\ value\ for\ 50th\ percentile}}{\frac{1}{2}\boldsymbol{BMI\ value(BMI\ value\ for\ 50th\ percentile - BMI\ vaule\ for\ 3rd\ percentile)}} \text{ (wzór 5)}$$

Analiza danych: Analizę danych przeprowadzono przy użyciu oprogramowania Statistica v. 13.

Testy metabolomiczne: Badania metabolomiczne przeprowadzono w Katedrze Biochemii, Biologii Molekularnej i Biotechnologii, Wydziału Chemii, Politechniki Wrocławskiej. Wszystkie Próbki surowicy były przechowywane przed analizą w temperaturze -80°C w Katedrze i Zakładzie Podstaw Nauk Medycznych.

Analiza 1H NMR: Standardowe badania 1H NMR przeprowadzono na spektrometrze Bruker AVANCE II 600.58 MHz. Wszystkie spektra przetworzono wstępnie w systemie Matlab. Przeprowadzono analizę wielowymiarową i jednowymiarową w celu oceny uzyskanych wyników.

3.5. Podsumowanie wyników

Artykuł nr 1.

Hetman M, Moreira H, Barg E.
The best tool for the assessment of developmental disorders in children with down syndrome: comparison of standard and specialized growth charts - cross-sectional study. Front Endocrinol. 2022;13(928151):1-11. doi:10.3389/fendo.2022.928151.
IF = 5.2; Punkty MEiN = 100

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1. **Dane Dotyczące Rodziców:** U 69% ojców stwierdzono nadwagę, z czego u 31% dodatkowo otyłość. Średnia wartość BMI ojców to 27,02. U 42% matek stwierdzono nadwagę, z czego u 26% dodatkowo otyłość. Średnia wartość BMI matek to 24,89. Nie wykazano istotnych statystycznie korelacji pomiędzy aktualną masą ciała dziecka a aktualną masa ciała rodziców.
2. **Masa urodzeniowa dziecka:** Przeanalizowano dane dotyczące masy urodzeniowej 266 dzieci z ZD. 59 dzieci zakwalifikowano do grupy wcześniaków (urodzone ≤36 tygodnia ciąży). W grupie wcześniaków średnia masa urodzeniowa wynosiła 2872g. W grupie dzieci urodzonych o czasie średnia masa urodzeniowa wynosiła 2967,96g. Stwierdzono istotną statystycznie dodatnią korelację pomiędzy masą urodzeniową dziecka a jego aktualną masą ciała (r=0,1526).
3. **Karmienie piersią:** Przeanalizowano dane dotyczące karmienia piersią 109 dzieci z ZD. 85 dzieci (78%; 18 dzieci urodzonych ≤ 36hbd) było karmionych piersią. Maksymalny czas karmienia piersią wynosił 40 miesięcy; minimalny 0,5 miesiąca (mediana 9 miesięcy; średnia 11,06 miesiąca). Do najczęstszych przyczyn z powodu których dzieci nie były karmione piersią należały: brak odruchu ssania u dziecka i/lub brak laktacji u matki. Nie stwierdzono istotnych statystycznie korelacji pomiędzy karmieniem piersią a masą ciała, wysokością ciała i BMI dziecka.
4. **Terapia L-tyroksyną:** Przeanalizowano dane dotyczące przyjmowania L-tyroksyny wśród 265 osób z ZD. 65% z nich (169) przyjmowało L-tyroksynę. Nie wykazano istotnej statystycznie korelacji pomiędzy przyjmowaniem L-tyroksyny a aktualną masą ciała dziecka oraz BMI. Analizując wysokość ciała dzieci, wykazano mniejszy rozrzut wartości percentylowych wśród dzieci przyjmujących L-tyroksynę. Oznacza to, że porównując grupę dzieci przyjmujących L- tyroksynę i dzieci nieprzyjmujących L- tyroksyny, dzieci przyjmujące lek częściej mieściły się w szerokim zakresie normy (pomiędzy 3 a 97 percentylem).
5. **Choroby współistniejące:** Przeanalizowano dane dotyczące chorób współistniejących wśród 265 osób z ZD. Do najczęstszych chorób współistniejących należały: niedoczynność tarczycy (52,98%); wady wzroku (41,79%); i wady serca (23,88%).
6. **Siatki centylowe:** Przeanalizowano odsetek wyników znajdujących się poza granicami normy używając siatek centylowych standardowych vs wyspecjalizowanych dla ZD w trzech kategoriach: masa ciała, wysokość ciała i BMI. Jako normę przyjęto zakres 3- 97percentyl: masa ciała: 30% vs 11%; wysokość ciała: 38% vs 27%; BMI: 28% vs 21%.

- **Masę ciała** analizowano w trzech kategoriach: prawidłowa masa ciała, niedobór masy ciała, nadmierna masa ciała. Dane nanoszono na dwa rodzaje siatek centylowych: standardową oraz wystandaryzowaną dla ZD, porównując wyniki według schematu: (% osób zakwalifikowanych do tej samej kategorii z użyciem siatki standardowej vs % osób zakwalifikowanych do danej kategorii z użyciem siatki centylowej wystandaryzowanej dla ZD):
  - Prawidłowa masa ciała (70% vs. 89%; **p<0,02**);
  - Niedobór masy ciała (27% vs. 5%; **p<0,0001**);
  - Nadmierna masa ciała (3% vs. 6%; p>0,05).
- Wysokość ciała analizowano w trzech kategoriach: prawidłowa wysokość, niedobór wysokości oraz nadmierna wysokość ciała. Dane nanoszono na dwa rodzaje siatek centylowych: standardową oraz wystandaryzowaną dla ZD:
  - Prawidłowa wysokość ciała: (61% vs. 73%; p>0,05);
  - Niedobór wysokości ciała: (4% vs. 4%);
  - Nadmierna wysokość ciała (35% vs. 23%; **p<0,005**).
- BMI analizowano w trzech kategoriach: prawidłowe BMI, niedowaga, otyłość. Dane nanoszono na dwa rodzaje siatek centylowych: standardową oraz wystandaryzowaną dla ZD:
  - Prawidłowe BMI: (72% vs. 79; p>0,05);
  - Niedowaga: (21% vs. 19%; p>0,05);
  - Otyłość: (7% vs. 2%; **p<0,005**).

7. **Ankieta internetowa:** na pytania zawarte w ankiecie internetowej dotyczącej siatek centylowych odpowiedziało 281 rodziców osób z DS. Poniżej przedstawiono pytania zawarte w ankiecie oraz wyniki:
   - **Pytanie 1:** Czy słyszałaś/eś kiedykolwiek o istnieniu siatek centylowych wystandaryzowanych dla dzieci z ZD?
     - Tak (73,3%)
     - Nie (26,7%)
   - **Pytanie 2:** Który typ siatek centylowych (standardowa czy wystandaryzowana dla dzieci z ZD) jest częściej używany przez lekarzy?
     - Standardowa (81,8%)
     - Wystandaryzowana (13,2%)
   - **Pytanie 3:** Czy któryś z lekarzy Twojego dziecka zastosował wystandaryzowaną siatkę centylową co najmniej 1 raz?
     - Tak (28,2%)
     - Nie (71,8%)
   - **Pytanie 4:** Czy dla Ciebie, jako rodzica, siatki wystandaryzowane są istotne?
     - Tak (93,2%)
     - Nie (6,8%)

Artykuł nr 2.

Hetman M, Barg E.
Pediatric population with Down syndrome: obesity and the risk of cardiovascular disease and their assessment using omics techniques - review. Biomedicines. 2022;10(12):3219-3232. doi:10.3390/biomedicines10123219.
IF = 4.7; Punkty MEiN = 100

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1. **Miażdżyca i CVD:** Analiza wyników innych autorów wykazała wysokie ryzyko wczesnego rozwoju miażdżycy u osób z hiperhomocysteinemią, hipermetioninemią i homocystynurią. Zidentyfikowano fenylalaninę jako biomarker CVD. Sugerowany jest także wpływ znanych czynników ryzyka CVD u PWDS, takich jak cukrzyca, otyłość, nadciśnienie i zaburzenia lipidowe. Wyniki przeprowadzanych innych badań wykazują, że PWDS umierają częściej w porównaniu z ogólną populacją w młodszym wieku z powodu chorób serca. Udokumentowano także, że choroba niedokrwienna serca jest rzadką przyczyną zgonu wśród PWSD. Wskazano również obecność niekorzystnych profili lipidowych u dzieci i młodzieży z ZD w porównaniu z ich rówieśnikami.
2. **Otyłość:** Dorosłe osoby z ZD są bardziej narażone na otyłość w porównaniu z osobami bez ZD. Wpływają na to takie czynniki jak: niższe wydatki energetyczne, niezdrowa dieta (bogata w przetworzoną żywność; z dużą zawartością tłuszczów nasyconych oraz węglowodanów prostych) oraz niewielka aktywność fizyczna. Przy niższej aktywności w porównaniu do rówieśników, przyjmują większą ilość kalorii w pożywieniu. Otyłość staje się szczególnie widoczna w okresie dojrzewania, ale największy jej wzrost obserwuje się między 2. a 6. rokiem życia.
3. **Stres Oksydacyjny:** PWDS wykazują podwyższony poziom stresu oksydacyjnego. Zaobserwowano obniżone stężenie glutationu w krwi oraz nadmierną ekspresję genu dysmutazy ponadtlenkowej (*SOD-1*).
4. **Zaburzenia Metaboliczne i Endokrynologiczne:** Dzieci z ZD mają wyższe prawdopodobieństwo, niż ogólna populacja, rozwoju zaburzeń endokrynologicznych i metabolicznych takich jak: dysfunkcja tarczycy, cukrzyca, niska wysokość ciała, niedobór witaminy D i otyłość.
5. **Badania Metabolomiczne:** Opisano liczne zaburzenia w koncentracji metabolitów wśród PWDS. Należą do nich: zwiększone poziomy fenyloalaniny i tyrozyny w surowicy krwi; niższe poziomy histydyny, lizyny, tyrozyny, fenyloalaniny, leucyny, izoleucyny i tryptofanu w osoczu; zwiększone stężenia leucyny, izoleucyny, cysteiny i fenyloalaniny w osoczu; obniżone stężenie seryny w osoczu; zwiększone stężenie lizyny w osoczu; podwyższone stężenia metabolitów związanych z cyklem metyzacji w osoczu: cysteina, cystationina, cholina i dimetyloglicyna; zwiększone stężenia S- adenozynohomocysteiny i S-adenozylometioniny.

Artykuł nr 3.

Hetman M, Placzkowska S, Barg E.
Comparative Analysis of Obesity Prevalence, Antioxidant and Oxidant Status in Children with Down Syndrome - A Sibling-Controlled Study. Pediatr Endocrinol Diabetes Metab. 2023;29(3).
Punkty MEiN = 100

Wyniki badań: SG - study group, osoby z ZD; CG - control group, grupa kontrolna

1. **BMI:** Nie wykazano istotnej statystycznie różnicy pomiędzy SG i CG (22,20 vs 20,20 kg/m2; p=0,2005);
2. **BMI SDS:** Nie wykazano istotnej statystycznie różnicy pomiędzy SG i CG (0,79 vs - 1,19; p=0,1264);
3. **BMI PC:** Nie wykazano istotnej statystycznie różnicy pomiędzy SG i CG (70,56 vs 60,97; p=0,1282).
   Analizując wartości percentyli w SG stwierdzono niedowagę u 2 osób (4,76%), prawidłowe wartości u 21 osób (50%), nadwagę u 11 osób (29,19%) oraz otyłość u 8 osób (19,05%). W CG, stwierdzono niedowagę u 1 osoby (5%), prawidłowe wartości u 15 osób (75%), nadwagę u 3 osób (15%) oraz otyłość u jednej osoby (5%).
4. **TAS:** Wykazano istotną statystycznie różnicę między SG a CG (1,92 vs 1,79 mmol/l; p=0015). Jedynie w CG stwierdzono istotną statystycznie dodatnią korelację pomiędzy BMI i TAS (r=0,4459).
5. **TOS:** Wykazano istotnie statystyczną różnicę między SG a CG (51,52 vs 33,05 mmol/l; p=0,0147). W SG stwierdzono istotną statystycznie ujemną korelację pomiędzy BMI a TOS (r=-0,3957).
6. **OSI:** Wykazano istotnie statystyczną różnicę między SG a CG (2475,02 vs 1949,75; p=0,0388). W SG stwierdzono istotną statystycznie ujemną korelację pomiędzy BMI a OSI (r=-0,3925).
7. **Glukoza na czczo:** Nie wykazano istotnie statystycznej różnicy między SG a CG (p=0,9327). W przypadku CG, ale nie w SG, stwierdzono istotną statystycznie ujemną korelację pomiędzy BMI a stężeniem glukozy na czczo (r=-0,4887).
8. **Insulina na czczo:** Nie wykazano istotnie statystycznej różnicy stężeń między SG a CG (p=0,9459).
9. Zarówno w SG, jak i CG nie stwierdzono istotnych statystycznie korelacji pomiędzy glikemią na czczo a TAS, TOS, OSI; jak również pomiędzy insuliną na czczo a TAS, TOS, OSI.
10. **HOMA-IR:** Nie wykazano istotnie statycznej różnicy między SG a CG (p=0,6930). W SG twierdzono istotną statystycznie dodatnią korelację pomiędzy HOMA-IR i BMI SDS (r=0,4349), HOMA-IR i BMI PC (r=0,4368) oraz ujemną HOMA-IR i TOS (r=- 0,4605), OSI (r =-0,3692).

Istotne statystycznie korelacje w grupie SG

Dodatnie:

- BMI z OSI: r=0,3926
- BMI SDS z HOMA-IR: r=0,4349
- BMI PC z:
    - insuliną: r=0,3581
    - OSI: r=0,3526
    - HOMA-IR: r=0,4368

Ujemne:

- BMI z TOS: r=-0,3957
- BMI SDS z:
    - TOS: r=-0,4136
    - OSI: r=-0,3947
- BMI PC z TOS: r=-0,3570
- TOS z HOMA-IR: r=-0,4605
- OSI z HOMA-IR: r=-0,3692

Istotnie statystyczne korelacje w grupie CG

Dodatnie:

- BMI z TAS: r=0,4459
- Glukoza z insuliną: r=0,5468

Ujemne:

- BMI z glukozą: r=-0,4887

Artykuł nr 4.

Hetman M, Mielko K, Placzkowska S, Bodetko A, Młynarz P, Barg E.
Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies. Pediatr Endocrinol Diabetes Metab. 2023;29(3).
Punkty MEiN = 100

Wyniki badań: SG - study group, osoby z ZD; CG - control group, grupa kontrolna

1. **Badania metabolomiczne:** Analizując materiał biologiczny zidentyfikowano łącznie 31 metabolitów (3-hydroksymaślan, octany, aceton, alanina, cholina, kreatynina, etanol, mrówczan, glukoza, glutamina, glicyna, histydyna, izomaślan, izoleucyna, mleczan, leucyna, lizyna, metionina, metyloamina, oksypurynol, fenyloalanina, prolina, piroglutaminian, pirogronian, bursztynian, treonina, tryptofan, tyrozyna, walina, ksantyna, metanol). Porównano SG (39 próbek surowicy) z CG (20 próbek surowicy). Wykazano istotną statystycznie ($p<0,05$) różnicę między SG a CG (**octany, cholina, kreatynina, mrówczan, glutamina, histydyna, lizyna, prolina, piroglutaminian, treonina, tyrozyna i ksantyna**). Dodatkowo zastosowana procedura Benjaminiego-Hochberga spowodowała wykluczenie **choliny, tyrozyny, treoniny i histydyny** (różnice nieistotne statystycznie).
2. **TMI:** Wykazano istotnie statystyczną różnicę między SG a CG (16,22 vs 13,67 kg/m^3; p= 0,0200);
3. **HDL:** Wykazano istotnie statystyczną różnicę między SG a CG (46,88 vs 59,19 mg/dl; p=0,0001);
4. **TG:** Wykazano granicznie istotną statystycznie różnicę między SG a CG (87,02 vs 63,34 mg/dl; p=0,0588);
5. **LDL:** Wykazano istotną statystycznie różnicę między SG a CG (104 vs 89,78 mg/dl; p=0,0331);
6. **APO A1:** Wykazano istotną statystycznie różnicę między SG a CG (119,64 vs 158 mg/dl; p=0,000005);
7. **TCH:** Nie wykazano istotnej statystycznie różnicy między SG a CG (p=0,3747);
8. **APO B:** Nie wykazano istotnej statystycznie różnicy między SG a CG (p=0,1612);
9. **HCY:** Nie wykazano istotnej statystycznie różnicy między SG a CG (p=0,5320);
10. **Lp(a):** Nie wykazano istotnej statystycznie różnicy między SG a CG (p=0,3466);
11. **APOB/APOA1:** Nie wykazano istotnej statystycznie różnicy między SG a CG (p=0,3466).

Istotnie statystyczne korelacje występujące w obu grupach

Dodatnie korelacje:

- Wiek z masą ciała, wysokością ciała, i BMI;
- Masa ciała z wysokością ciała;
- TCH z TG.

Wartości współczynników korelacji (SG vs CG):

- Wiek z masą ciała r=0,8136 vs r=0,6812;
- Wiek z wysokością ciała: r=0,7702 vs r=0,7840;
- Wiek z BMI: r=0,6346 vs r=0,5701;
- Masa ciała z wysokością ciała: r=0,8649 vs CG r=0,9440;
- TCH z TG: r=0,4511 vs r=0,4445.

Istotne statystycznie korelacje występujące wyłącznie w grupie SG

Dodatnie:

- Masa ciała z homocysteiną (r=0,3422) oraz z Lp(a) (r=0,4036);
- BMI SDS z homocysteiną (r=0,3103);
- TCH z Lp(a) (r=0,4036);
- TG z LDL (r=0,4158) oraz z APO B (r=0,4307);
- LDL z Lp(a) (r=0,4480);
- APO B z Lp(a) (r=0,4840);
- Współczynnik APOB/APOA1 z TCH (r=0,5318).

Ujemne:

- BMI SDS z APO A1 (r=-0,3901);
- BMI PC z APO A1 (r=-0,3552);
- Współczynnik APOB/APOA1 z HDL (r=-0,6457).

Istotnie statystycznie korelacje występujące wyłącznie w CG

Dodatnie:

- Wiek z HDL (r=0,4963); z TCH (r=0,5197); z LDL (r=0,4845); z APO B (r=0,6922);
- Masa ciała z APO B (r=0,5246);
- Wysokość ciała z APO B (r=0,5701);
- BMI z HDL (r=0,4776);
- TG z APO A1 (r=0,4978);
- Współczynnik APOB/APOA1 z Lp(a) (r=0,5837).

Ujemne:

- Nie wykazano.

Podsumowanie wyników przedstawionych prac:

1. **Narzędzia diagnostyczne:** Nie zaobserwowano jednolitego, kompleksowego narzędzia do oceny zaburzeń rozwojowych w ZD. Stwierdzono różnice między wynikami uzyskanymi przy użyciu standardowych siatek centylowych i wystandaryzowanych dla ZD. Konieczne jest monitorowanie rozwoju u PWDS, uwzględniając zarówno standardowe, jak i wyspecjalizowane narzędzia. Mimo dostępu do specjalistycznych wykresów wzrostu dla dzieci z ZD, takie narzędzia nie są rutynowo stosowane w codziennej praktyce medycznej.
2. **Masa ciała:** U dzieci i młodzież z ZD występuje znacznie częściej problem z nadwagą/ otyłością w porównaniu do rówieśników bez ZD, nawet pomimo podobnych warunków rodzinnych.
3. **Stres oksydacyjny:** PWDS wykazują podwyższone wartości wskaźników stresu oksydacyjnego, co może przyczyniać się do zwiększonego ryzyka miażdżycy. Wyższe wartości stresu oksydacyjnego wskazują na specyfikę metaboliczną ZD, która może wpływać negatywnie na stan naczyń krwionośnych.
4. **Profil lipidowy:** Zaburzenia lipidowe, takie jak obniżony poziom HDL oraz podwyższony poziom LDL, są wyraźne zaznaczone u PWDS. Dodatkowo osoby PWDS wykazują zaburzenia w stężeniach APO A1 i APO B, co może wskazywać na zwiększone ryzyko miażdżycy i CVD.
5. **Inne zaburzenia:** Osoby z ZD wykazują wyższe wartości współczynnika HOMA-IR w porównaniu do grupy kontrolnej, pomimo braku istotnych różnic w stężeniu glukozy i insuliny na czczo. Powinno to stanowić wskazówkę do monitorowania u osób z ZD.
6. **Metabolomika:** Analiza metabolomiczna wykazała specyficzne nieprawidłowości metaboliczne w SG, które mogą predysponować do powikłań sercowo-naczyniowych, takich jak miażdżyca. Niejednoznaczne profile metabolitów, zwłaszcza w kontekście lipoprotein i biomarkerów stresu oksydacyjnego, podkreślają złożoność interakcji metabolicznych w ZD. Wyspecjalizowane badania metabolomiczne mogą dostarczyć nowych informacji o mechanizmach przyczyniających się do zwiększonego ryzyka miażdżycy w tej grupie pacjentów. Może to wpłynąć na lepsze rozumienie choroby i rozwój spersonalizowanej strategii leczenia dla PWDS. W świetle tych wyników, metabolomika stanowi kluczowe narzędzie w przyszłych badaniach nad oceną ryzyka sercowo-naczyniowego, pozwalając na identyfikację potencjalnych markerów oraz ścieżek metabolicznych związanych z patogenezą miażdżycy.

3.6. Etyka

Protokół badania został zaaprobowany przez Komitet Bioetyczny Uniwersytetu Medycznego we Wrocławiu (**KB 674/2020**). Badanie przeprowadzono przestrzegając zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej Światowego Stowarzyszenia Lekarzy przyjętą przez 18 Zgromadzenie Ogólne Światowego Stowarzyszenia Lekarzy (WMA), w Helsinkach w czerwcu 1964 r., a zmienionej przez 64 Zgromadzenie Ogólne WMA, w Brazylii w październiku 2013 r. Badania zostały przeprowadzone z zachowaniem pełnej anonimowości uzyskanych danych.

3.7. Wnioski

1. Istnieje potrzeba nie tylko stworzenia jednolitego, kompleksowego narzędzia diagnostycznego, ale również zbudowania multidyscyplinarnego zespołu specjalistów (w tym dietetyków, psychologów, endokrynologów), który będzie w stanie holistycznie podchodzić do opieki nad osobami z ZD.
2. Regularne monitorowanie za pomocą narzędzi zarówno standardowych, jak i wyspecjalizowanych, powinno stać się normą w praktyce medycznej.
3. Osoby z ZD mają wyższe ryzyko nadwagi i otyłości, co wskazuje na konieczność wdrożenia zindywidualizowanych programów dietetycznych oraz promocji zdrowego stylu życia wśród tej grupy i ich rodzinach.
4. Podwyższone wartości wskaźników stresu oksydacyjnego u osób z ZD sugerują, że istnieje w tej populacji większe ryzyko wystąpienia miażdżycy i innych chorób sercowo-naczyniowych.
5. Wyraźnie zaznaczone zaburzenia lipidowe u osób z ZD (w tym obniżony poziom HDL i podwyższony poziom LDL) sugerują, że kontrola profilu lipidowego powinna być istotnym elementem opieki medycznej nad tą grupą pacjentów.
6. U pacjentów z ZD istnieje potencjalne ryzyko rozwinięcia insulinooporności, a wyższe wartości HOMA-IR sugerują konieczność monitorowania parametrów gospodarki węglowodanowej.
7. Analiza metabolomiczna podkreśla złożoność interakcji metabolicznych w ZD i wskazuje na konieczność dalszych badań w celu lepszego zrozumienia ryzyka sercowo-naczyniowego w tej grupie.
8. Metabolomika może stanowić kluczowe narzędzie do identyfikacji markerów ryzyka i zrozumienia ścieżek metabolicznych związanych z patogenezą miażdżycy, otwierając drogę do rozwoju spersonalizowanej strategii profilaktyki i leczenia.
9. Przeprowadzone badania wskazują na konieczność przybliżenia występujących zaburzeń zarówno opiekunom pacjentów z ZD jak i osobom zajmującymi się tą grupą.

Podsumowanie wniosków:

Na podstawie analizy lipidogramu, wskaźników stresu oksydacyjnego, nadwagi i otyłości oraz badań metabolomicznych, można stwierdzić, że osoby z ZD są w znaczącym stopniu narażone na ryzyko rozwoju miażdżycy i powiązanych z nią chorób sercowo-naczyniowych.

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4. ARTYKUŁ PIERWSZY

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 05 August 2022
DOI 10.3389/fendo.2022.928151

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Pediatric Endocrinology,
a section of the journal
Frontiers in Endocrinology

RECEIVED 25 April 2022
ACCEPTED 06 July 2022
PUBLISHED 05 August 2022

CITATION
Hetman M, Moreira H and Barg E
(2022) The best tool for the
assessment of developmental
disorders in children with down
syndrome: comparison of standard
and specialized growth charts - cross
sectional study.
Front. Endocrinol. 13:928151.
doi: 10.3389/fendo.2022.928151

The best tool for the assessment of developmental disorders in children with down syndrome: comparison of standard and specialized growth charts - cross sectional study

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Down Syndrome (DS) is a chromosomal abnormality associated with a spectrum of cognitive and physical disabilities. Children with DS are exposed to both lower and excess body weight and follow distinct growth-curve patterns that deviate significantly from those of children without chromosomal defects. Anthropometric parameters are assessed in the pediatric population with the use of growth charts. The study is based on data from 411 children and adults with DS from Poland. Detailed information concerning children and online survey results were also analyzed. Centiles and standard deviation scores (SDS) of obtained anthropometric parameters were aligned with the data using the LMS method. The study aims to identify which type of growth chart (standard vs specialized) is a leading tool for earlier detection of developmental disorders in DS. The results obtained in the two types of growth charts differed. The advantage of the specialized growth charts over the standard ones cannot be unequivocally determined. Only the combination of both tools allows to detect the development disorders early in the broadest possible way.

KEYWORDS

Down syndrome, growth charts, childhood malnutrition, childhood disability, obesity

Introduction

Down Syndrome (DS, also referred to as trisomy 21) is one of the most common chromosomal abnormalities among live-born neonates and is associated with a spectrum of cognitive and physical disabilities, such as congenital heart disease, hypothyroidism, gastrointestinal disorders, and obstructive sleep apnea (1). The occurrence of DS in 95%

of cases is related to meiotic non-disjunction causing trisomy of chromosome 21. The other types of trisomy are Robertsonian translocation and mosaic type (2). DS occurs in every 700-1000 live births (3) and its prevalence estimates between 6.1 to 13.1 per 10 000 people (4). It is predicted that 94.4% of children with DS born in 2000 will survive up to 2020, 90.8% up to 2030, and 76.3% up to 2050 (4). Early identification of developmental disorders can improve the quality of life in the future. To extend lifespan and improve the quality of life, the development of children with DS should be controlled with the use of optimal and appropriate tools.

The most often used parameters to evaluate the child's growth are anthropometric data such as body height and body weight. Body weight, as a single measure, is not sufficient to assess the nutrition of a given individual, therefore in this study nutritional status is analyzed with Body Mass Index (BMI) - a statistical index used to estimate the body fat content. It is worth remembering that this method is not ideal, but it may be the first step in assessing excess body fat. In the pediatric population, the proper assessment of BMI should be conducted on BMI growth charts.

During the first two years of life, children with DS are characterized by reduced body weight (5, 6), which may result from suction/swallowing disorders associated with muscle hypotonia and dysfunctions in the oral motor system (7). In underweight children the weight for the height it's a good measurement tool for controlling them. After the second year of life, the occurrence of overweight and obesity in children with DS is more frequent than in the general population (the prevalence of obesity at the level of 30-50%) (8–10), thereby increased BMI is common in DS (11).

Statural growth, as an indicator of development, often represents a child's health status. The growth retardation of children with DS commences prenatally (12). Morris et al. (6) demonstrated that for gestations up to 38 weeks the median birth weight of newborns with DS is similar to that of babies without DS, however, after 38 weeks their median birth weight rises slower than in unaffected babies. Other researchers also indicate a decreased birth weight in children with DS (13). After birth, the growth velocity is most reduced between 6 months and 3 years of age (14). Short stature is a phenotype of DS and can be influenced by genetic components and other factors, such as comorbidities. Styles et al. (15) compared developmental patterns in terms of body weight, height, and head circumference in children with DS compared to children without DS. Appreciable skewness was noted for body weight, which indicates the difference in the initial weight of children with DS compared to those without DS (15).

Growth charts constitute crucial tools used to assess the growth and nutritional status of children. Currently, various growth charts have been developed and adapted to racial and ethnic backgrounds or a given disease that may interfere with the proper development of a child. The most commonly used DS-specialized growth charts in the US are based on work done in 1988 (14). A great number of countries have constructed DS-specialized charts (5, 16, 17-19). Since these specialized growth charts were developed, concerns have been raised regarding their usefulness. Children with DS follow distinct growth-curve patterns that deviate significantly from those of children without chromosomal defects, therefore the use of specialized growth charts appears to be a superior method in development evaluation. The study aims to identify which type of growth chart (standard vs. specialized) is a leading tool for the earlier detection of developmental disorders in DS.

Material and methods

Design and participants

A cross-sectional study design was based on data from 411 people with DS: 386 (94%) children and 25 (6%) adults; 188 (46%) girls and 223 (54%) boys, aged 0.17 months – 36.72 years (median: 4.85) from Poland recruited from general pediatric practices and parents' interest groups. Inclusion criteria were patients with a diagnosis of DS. There were no exclusion criteria. The study was conducted in the years 2020-2021 in Wroclaw (Poland) as a part of the doctoral dissertation carried out at the Wroclaw Medical University. The ethical approval on the research protocol and consent form was obtained from the Bioethics Committee, Wroclaw Medical University (approval number KB 674/2020). The study was carried out in accordance with the Declaration of Helsinki. Administrative approvals were obtained from each institute to access the participants' data. Written informed consent was obtained from the parents of the participants prior to data collection and anthropometric measurements.

Data collection

The data were derived using two approaches between January 2020 - June 2021: by retrospectively examining medical records (20%) available at health clinics (additionally an online/telephone interview with parents or guardians was conducted to confirm the data and by obtaining the consent of data usage) and by actively recruiting participants (80%). Active recruitment and examination of retrospective medical records were conducted among children from all over Poland. All parents were invited to an online survey as an additional part of the study. After written informed consent was obtained, actively recruited children underwent an anthropometric examination in a pediatric clinic in Wroclaw (Poland) with collecting the anthropometric parameters such as body height and weight. For telephone calls, written consent was obtained by sending the consent form online. Then, the parent was asked to return the signed consent by post (original document) or online

(scan of the document). Body Mass Index (BMI) was calculated using the formula: weight/height^2 (kg/m^2). Trained personnel (consisted of 3 people: two doctors (including authors) and a nurse) obtained measurements following standardized techniques (20), discussed prior to the research initiation. The design of the study (Collection Data part) is represented in Figure 1.

Specific data and online questionnaire

Specific data were collected from 200-300 participants depending on the parameter. Detailed information concerning parents of children with DS (Table 1), the perinatal period (Table 2), comorbidities (Table 3), and L-thyroxine treatment were obtained. In addition, an online 4-questions questionnaire concerning the topic of growth charts and their usage in medical offices was conducted (Table 4). Two hundred eighty-one parents (including those taking part in the main part of the study) answered the survey.

The ranges for assessing the parents' BMI: underweight [15.0, 18.5); healthy weight [18.5, 25.0); overweight [25.0, 30.0), and obesity ≥30The ranges for assessing the birth weight: high birth weight - greater than 4200g; normal weight - 2500g–4200g; low birth weight - less than 2500g; very low birth weight - less than 1500g; and extremely low birth weight less than 1000g.

Anthropometric measurements

Body weight: body weight (kg) was measured using the same electronic digital scales model (OMRON BF-515) with light clothes and barefoot for older children and without clothing or diapers for infants and toddlers (to the nearest 0.1kg for children >3 years and 0.05kg for children <3 years). Body height: length (to nearest 0.1 cm) was measured on an infant length board (SECA 234) for infants and toddlers unable to stand unsupported (in the supine position). For all others, height (to nearest 0.1 cm) was measured with a stadiometer (SECA 264). The trained personnel controlled the correct body posture of the child during the measurement: straight back, both feet on the ground, back of the body pressed against the wall. The same devices were used for all measurements, without changing the conditions. Birthdate information were extracted from the family or children's questionnaire. Body weight, body height,

TABLE 1 Parents' basic characteristics.

		Mother	Father
Age during pregnancy [years]	Average	32.41 ± 5.91	33.92 ± 3.43
	Median	32	33
	Min.	17	20
	Max.	54	58
Body weight [kg]	Average	68.40 ± 12.87	87.27 ± 13.75
	Median	65	86
	Min.	43	57
	Max.	110	130
Body height [cm]	Average	165.64 ± 5.73	179.59 ± 6.91
	Median	165	180
	Min.	147	159
	Max.	178	198
BMI [kg/m^2]	Average	24.89 ± 4.5	27.02 ± 3.77
	Median	24.17	26.87
	Min.	17.92	18.93
	Max.	39.40	37.55

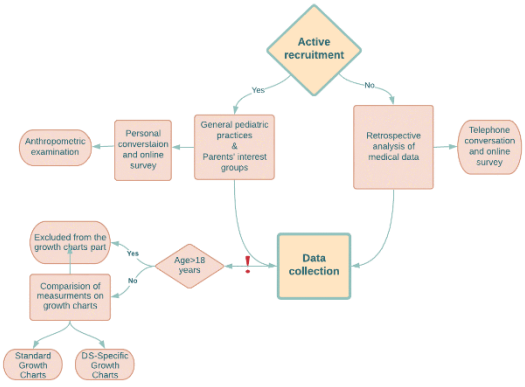

FIGURE 1
The design of the study (Data Collection).

TABLE 2 Characteristics of postpartum parameters in the group of breastfed-children (85 children; 18 babies were born ≤36hbd).

	Min.	Max.	Median	Average
Birth weight [g]	1490	3970	2800	2840 ± 600
Birth length [cm]	41	59	51	51.29 ± 0.03
Week of pregnancy	31	42	38	37.55 ± 0.00
Apgar score 1'	0	10	9	8.25 ± 0.00

TABLE 3 The prevalence of selected common comorbidities in children with DS reported by parents.

Comorbidities	N	[%]
Hypothyroidism	142	52.98
Vision defects	112	41.79
Cardiac defects	64	23.88
Hearing problems	59	22.01
Immunodeficiency	34	12.69
Others	25	9.33
Lipid disorders	19	7.08
Autoimmune diseases	18	6.71
Malignancy	4	1.49
Hyperthyroidism	3	1.11
Hypertension	2	0.74

*N- the amount of participants (DS) with chosen comorbidity.

and BMI were expressed in the standard deviation score (SDS) value using the LMSgrowth Calculator (21)- a Microsoft Excel add-in. Centiles and SDS were fitted into the data using the LMS method (22). The LMS method summarizes the changing distribution of weight, height, head circumference, and BMI according to age by three curves representing the median (M), coefficient of variation (S), and skewness (L), the latter expressed as a Box-Cox power. The method assumes that the data in each age group can be rendered normally and distributed by applying a suitable power transformation (23). SDS indicates how many standard deviations an observation is above or below the mean independently of age and sex, which is a useful way of putting data from different sources onto the same scale (Equation (1)).

With the use of this statistics tool, it is possible to analyze the variability of the observed parameter over a certain period in a group of patients, especially those of developmental age.

$$x\ \ SDS = \frac{x\ \ value - x\ \ value\ \ for\ \ 50th\ \ centile}{\frac{1}{2}x(x\ \ value\ \ for\ \ 50th\ \ centile - x\ \ value\ \ for\ \ 3rd\ \ centile)} \quad (1)$$

$$x - height/weight/BMI \quad (1)$$

Growth charts and data analysis

To standardize the data, the British (24) growth charts included in the LMSgrowth Calculator were used for the calculations and taken as reference for the population of children without DS (population growth charts). DS-specific growth charts were used as the reference point for the population of children with DS (15). Three ranges were assumed (in percentile (PC)): <3rd, 3rd-97th, and> 97th, where 3rd-97th means a wide range of the norm (Table 6). However, it should be remembered that values >90th PC should be considered as overweight and that further calculations are related to obesity (>97th PC). Data from people over the age of eighteen (6%) were not considered in the comparison of growth charts. However, their parents were included in the online questionnaire part, mainly referring to their earlier experiences.

Statistical analysis

The data were processed using Statistica v. 13.3. The data were checked for normality using the Shapiro-Wilk test. Non-parametric statistical tests were applied. The Mann-Whitney U test was used for non-parametric data. Spearman's rank correlation (r) was performed to investigate the specific data (such as perinatal period, parents' physical status, L-thyroxine treatment, all affecting the current body height, body weight, and BMI of the child). The chi-squared test was used for data distribution. Descriptive statistics are presented as median/mean ± SD/ percentages. P-values <0.05 were considered significant developmental disorders in DS.

TABLE 4 Internet survey (questions with answers).

No.	Question	Answers	Results [%]
1	Have you ever heard about specialized growth charts for children with DS?	Yes	73.3
		No	26.7
2	Which type of charts is more often used by clinicians?	Standard	81.8
		Specific	13.2
3	Have any of the clinicians used specialized charts at least once?	Yes	28.2
		No	71.8
4	Are specialized growth charts important for you as a parent?	Yes	93.2
		No	6.8

10.3389/fendo.2022.928151

Results

The study is based on data from 411 people with DS. Two hundred and fifty-five people from the study group have simple meiotic non-disjunction trisomy of chromosome 21; 12 are mosaic type; 10 have Robertsonian translocation. In the remaining cases of the questions about the type of mutation, the parents did not provide an answer, did not know the answer to the question, or never tested the child for a given mutation. The mean birth weight was 2898.02 ± 513g (median 2800 g); average birth length 0.52m ± 0.04m (median 0,51). The average age of delivery (weeks) was 37.7 ± 2.17 weeks.

Parents data

Table 1 presents the parents' basic characteristics. Fathers: One hundred thirty-six fathers (69%) have a BMI ≥25 (overweight) of which 31% corresponding to a BMI ≥30 (obesity). The average BMI value is27.02 ± 3.77. Mothers: eighty-four mothers (42%) have a BMI ≥25 (overweight) of which 26% corresponding to a BMI ≥30 (obesity). The average BMI value is 24.89 ± 4.58. There is no correlation between the current weight of the child and the parents' weight.

Child's birth weight

Data on birth weight were collected from 266 children. Fifty-nine babies were born ≤36hbb and were treated as premature babies. Among preterm babies, the mean birth weight was 2872.96 ± 484g (median 2800.00g; min.1490g; max. 3970). Among full-term babies (≥39hbd), the mean birth weight was 2967.97 ± 444g (median 2800g; minimum 2004g; maximum 4500g). Taking into account the entire group of 266 children, the mean birth weight was 2898.02 ± 513g (median 2800 g; min. 1490 g; max. 4500g). A positive correlation (low correlation) (rs = 0.152555) is found between the baby's birth weight and their current body weight.

Breastfeeding

Out of 109 children whose parents answered the question about breastfeeding, 85 (78%; girls: 36; 18 babies were born ≤ 36hbd) were breastfed. Max. duration of breastfeeding: 40 months; min. 0.5 months (median 9 months; average 11.06 ± 0.52months). Among those who were breastfed, 39 pregnancies were completed by natural childbirth. There were complications during childbirth in 15 cases. The median duration of pregnancy was 38 weeks (average 37.55 ± 0.00). The most common reasons for not breastfeeding were: lack of suckling reflex in the child and/or lack of lactation in the mother. There was no correlation between breastfeeding and body weight, body height, and BMI of the child. The median Apgar score among children fed breast milk after birth was 9 (average 8.25 ± 0.00). Table 3 presents basic characteristics of postnatal parameters in the group that was breastfed.

L-thyroxine therapy

There were 265 responses related to L-thyroxine therapy (L-thyroxine was taken by 169 children, 64%). There is no correlation between L-thyroxine intake and body weight or BMI. However, a statistically significant difference was identified for the body height growth charts readings. Smaller spread of values concerned children taking L-thyroxine. This means that when comparing the group of children taking the L-thyroxine and those not taking L-thyroxine, the children taking the medicine were within the wide normal range (3rd PC- 97th PC) more often.

Comorbidities

The data relating to selected comorbidities (Table 3) were collected from 198 participants. The three most common comorbidities in the study group are hypothyroidism affecting 52.98%; vision defects affecting 41.79%; and cardiac defects affecting 23.88%.

Growth charts

Table 5 shows percentiles and corresponding values of the SDS and their interpretation in relation to anthropometric parameters. A graphic representation of Table 6 is shown in Figures 2–4. Comparing the results obtained on two types of growth charts (standard vs. specialized): body weight - results outside the norm 30% vs. 11%; body height - 39% vs. 27%; and BMI - 28% vs. 21%. More results beyond the norm (under 3rd PC and above 97th PC) were obtained using standard growth charts.

Body weight

Using standard growth charts to assess body weight in a child with DS instead of the specific charts, 19 percentage points (p.p.) fewer children were considered in the range of normal body weight (70% vs. 89%, $p<0.02$), 21 p.p. more children had body weight deficiency (27% vs. 6%, $p<0.0001$); 2 p.p. fewer children had excess body weight (3% vs. 5%) (Figure 2). The statistically significant difference was observed in groups with weight deficiency and normal body weight.

TABLE 5 Percentiles and corresponding values of the standard deviation score and their interpretation in relation to anthropometric parameters.

Percentile (PC)	Standard deviation score (SDS)	Body weight	Body height	BMI
<3rd.	<-1.88	Underweight	Growth deficiency	Underweight
3rd.-10th	≥-1.88,<-1.66	Normal	Normal	Normal
10th.-90th	≥-1.66, ≤1.66	Normal	Normal	Normal
90th.-97th	>1.66, ≤1.88	Overweight	Normal	Overweight
>97th	>1.88	Obesity	High growth	Obesity

Body height

Using standard growth charts to assess body height in a child with DS instead of the specialized growth charts, 12 p.p. fewer (61% vs. 73%) children can be included in the normal range; 12 p.p. more (35% vs. 23%; p<0.005) children is above 97th PC (Figure 3). There is no difference in groups <3rd PC (4% vs. 4%) The statistically significant difference was observed in group with body height >97th PC.

Body mass index

Using standard growth charts to assess BMI in a child with DS instead of the specialized ones, 7 p.p. fewer children were considered in the range of normal BMI (72% vs. 79%), 5 p.p. more children had BMI <3rd PC (7% vs. 2%); and 2 p.p. more children had BMI >97th PC (21% vs. 79% p<0.005) (Figure 4). The statistically significant difference was observed only in group with BMI>97th PC.

Online survey

Two hundred and eighty-one people (parents of DS people) answered the questions from the Internet survey. The survey deals with the topic of growth charts and their application in clinical practice by doctors. The condition for completing the survey was answering all the questions. The results of the online survey are shown in Table 4.

Discussion

In this study we compared the assessment of children and adolescents' development in terms of body weight, body height, and BMI, using growth charts for the standard population and the sub-population of people with DS. The main objective was to identify which type of growth chart is the best tool for earlier detection of developmental disorders in DS. The results obtained on the two types of growth charts differed. Due to numerous comorbidities, disease phenotype, and social conditions, people with DS can be considered as a vulnerable population that requires systematic monitoring of their health status. Advances in medical care and increased access to knowledge have improved the health and well-being of individuals with DS. Currently, the illusion created in society is that the number of people with DS is decreasing. However, children with DS, one of the most common chromosomal abnormalities, will continue to be born, and with the current medical knowledge their lives may be longer, better and healthier. Monitoring of the child's health by doctors and parents should be performed with the use of appropriate assessment tools, of which the simplest and most common are growth charts. The challenge is to choose the kind of growth charts for the assessment of a given parameter so that the obtained results have a real impact on clinical decisions. The original hypothesis assumed that DS-specialized growth charts are chief tools in the comprehensive assessment (body weight, body height, BMI) of the developmental disorders in a child with DS. The obtained results, combined with clinical knowledge and experience, appear to contradict this hypothesis.

Body weight and BMI

Monitoring the child's development from the earliest stages of life with the use of optimal tools gives a chance to improve their quality of life in the future. Early health intervention can

TABLE 6 Distribution of data (body height, body weight, BMI) in both types of growth charts- standard and specialized growth charts.

		Body weight		Body height		BMI	
PC	Classification	StandardN (%)	DSN (%)	StandardN (%)	DSN (%)	StandardN (%)	DSN (%)
<3rd.	Under	108 (27)	23 (5)	141 (35)	90 (23)	83 (21)	76 (19)
3rd.-97th	Norm	280 (70)	350 (89)	246 (61)	288 (73)	290 (72)	306 (79)
>97th	Over	13 (3)	19 (6)	14 (4)	14 (4)	28 (7)	7 (2)

*PC- percentile, DS- Down Syndrome specialized growth charts, N- amount of individuals.

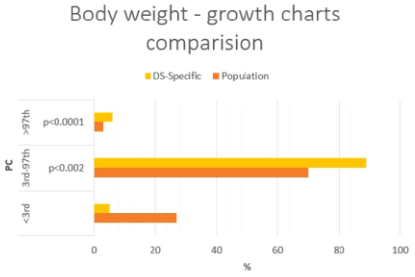

FIGURE 2
Percentage comparison of the number of individuals qualified for the given body weight categories on the standard growth charts and DS-specialized growth charts.

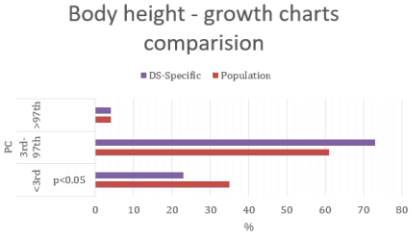

FIGURE 4
Percentage comparison of the number of individuals qualified for the given BMI categories on the standard growth charts and DS-specialized growth charts.

meaningfully affect adulthood. The problem of weight disorders among people with DS is very complex and challenging, concerning mainly the rapid transformation between undernutrition in the first period of life and excessive weight gain in later years. Therefore, depending on the age, this population is exposed to both deficiency and excess body weight and all the associated health consequences. As mentioned earlier, children with DS are characterized by a lower birth weight than children without chromosomal abnormalities (5, 6). However, in adolescence and adulthood, due to numerous comorbidities and the characteristics of the syndrome itself, people with DS are exposed to excessive body weight. Systematic nutritional evaluations since the day the baby is born throughout later years of life is essential. A higher obesity rate compared to the general population is observed among adolescents and adults with DS (25), therefore prevention and early treatment are principal aspects. DS has traditionally been considered as an "atheromafree" condition (26), however, the recent studies appear to contradict this thesis (27, 28). BMI, as based on body height and body weight, is a superior indicator of body nutrition. Both BMI and body weight are assessed using growth charts to detect body weight disorders. Hatch-Stein et al. (17) observed that for individuals with DS, the 85th percentile on standard growth charts is a better indicator of excess adiposity than the 85th percentile on the DS-specific BMI growth charts and claimed that standard charts should be the preferred method for early identification of obesity in children with DS. The results obtained in our study appear to confirm this. The percentage of out-of-normal results was analyzed, yielding a higher results percentage for body weight and BMI when using standard growth charts instead of the specialized ones. The use of specialized growth charts can deceptively reassure parents and lulls doctors into a false sense of a child's security. Since DS from adolescence is predisposed to excess body weight and has an increased risk of cardiovascular disease, the assessment of their BMI should be more rigorous, hence we recommend using standard growth charts. The percentage of children with DS with excess body weight is increased by genetic predisposition, low physical activity, and a high-calorie diet. Poor knowledge of healthy foods has been described in children and adolescents with DS (29). Increasing physical activity should be carried out wisely. Due to the cardiological burden in the group of people with DS, it may be necessary to assess the body's efficiency and consult a cardiologist before increasing physical activity. Introducing a balanced diet and regular meals should not be neglected. All the above-mentioned activities should be carried out with comprehensive care of i.e. the physician, nutritionists, psychologist, or trainer. A tremendous role in the whole process is played by parents and guardians. The whole family should be characterized by proper nutrition and activity patterns. Our study indicates that parents also face the problem of being overweight and obesity, which is particularly illustrated by the high BMI of the fathers (however, there is no correlation

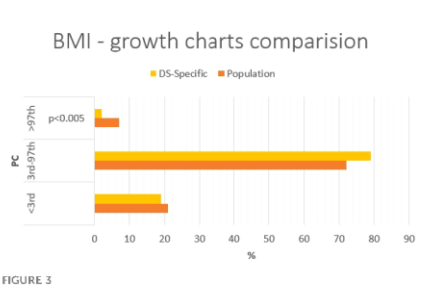

FIGURE 3
Percentage comparison of the number of individuals qualified for the given body height categories on the standard growth charts and DS-specialized growth charts.

between the current weight of the child and the parents' weight.). Fortunately, the awareness of parents who are trying to limit flour products and products with high sugar and saturated fat content in their children's diet is growing. Nevertheless, this challenging task is becoming very difficult to implement as the child grows older. However, it is not only the excess body weight that is a problem. In the study group, 1/5 of children were born with low birth weight. Low body weight in the first stage of a child's development may result from both maternal and child factors. On the side of maternal factors, there are, among others, problems with lactation, a lack of willingness to breastfeed, stress, and being overwhelmed by a new life situation related to childbirth (30–32). Breastfeeding in DS children is possible and preferred. A chance for its success can be obtained with the appropriate support of the family and competent health professionals. Frequent feeding problems in DS are the lack or very weak suckling reflex, prematurity, and defects in the digestive tract (33). Heart defects, which cause great and quick fatigue in newborns, contribute to the weak sucking reflex. Similar problems were confirmed in specific data collected in our study. Feeding difficulties, slow weight gain, and its deficiency may result in a slow and significantly impeded psychomotor development of a child. Introducing new products to a child's diet should take into account not only the type of product but also its texture (e.g., small pieces, mousses). Reduced feeding abilities with the increased risk of dysphagia and aspiration are predominant in the first years of life (32). If a child with DS is found to undergo weight loss and/or slow weight gain referring the child for a video-fluoroscopic swallow assessment and the diagnosis of contributing diseases (e.g., heart defects, celiac disease, gastrointestinal defects (Hirschsprung's disease, duodenal atresia, and others)) should be considered (32). Very important is the detection of disorders associated with both in deficiency and excess body weight and the approach of steps designed to fix these disorders.

Body height

People with DS are characterized by different patterns of growth compared to children without DS. The greatest impairment can be observed between 6 months and 3 years of age and in the puberty period, when they reach their final height (at age 15-16 years) (34). Furthermore, a shorter and earlier puberty spike related to the earlier achievement of the target height (girls: average of 9.5 years old, boys: average of 11 years old) is observed (35, 36). The assessment of body height using standard growth charts may be unfavorable as short stature is the phenotype of DS. However, the final growth of children with DS depends both on the characteristics of trisomy 21 and on the genetic potential transmitted by parents. The administration of growth hormone (GH) therapy in children with DS is associated with numerous controversies. Palloti et al. (37) observed in attempts of 3-year GH treatment an average improvement in the final height (boys by 5.16 cm; girls by 7.35cm). On the contrary, the other study shows that early treatment with GH does not affect the improvement of final height, but has a positive effect on psychomotor development and increases head circumference (38). In GH therapy a very problematic issue concerns the high risk of cancer, especially in the presence of the Philadelphia chromosome. Administration of GH could increase this risk of proliferative processes, consequently, the legitimacy of its administration should be considered. DS-specific growth charts used to assess a child's body heigh may provide valuable data to parents resulting in perceiving their child within the normal range. This can avoid unnecessary deliberations on the supply of growth hormone, the action of which, as presented above, may also have negative consequences. Additionally, there are risks of diseases that may result in delaying the rate of growth and achieving final growth, such as celiac disease or hypothyroidism. DS-specific charts were created based on data from people with Down syndrome, the presence of child measurements below the lower limit of normal is a signal for medical intervention, hence we recommend using population growth charts to assess the body height in DS.

L-thyroxine therapy

Many reports suggest that L-thyroxine therapy in the first years of life (also in children without diagnosed hypothyroidism) may result in better psychomotor development, support the child's physical therapy, and reduce thyroid immunization (39, 40). Thyroid diseases are one of the most common comorbidities among the population of people with DS (41). Most people at various stages of their lives are at risk of developing hypothyroidism. Among our study group, hypothyroidism was the most common accompanying disease in DS. In the study group, children taking L-thyroxine were within the normal range more often. On this basis, it can be concluded that the supply of L-thyroxine may support the proper growth of children with DS (40).

Comorbidities

Children with DS suffer from many comorbidities that may have nutritional implications and consequences. At the same time, thyroid disease is one of the most common accompanying diseases in DS. Obesity, as a civilization disease, very frequently affects people with DS. Obesity is also known to be associated with type 2 diabetes, cardiovascular disease, metabolic syndrome and some types of neoplastic processes. Complications of obesity

and related diseases can cause and intensify neurodegenerative processes (42).

Parental outcomes

It is well known that the older age of the mother is associated with Down's syndrome in children (43). According to the data (2011–2015), the average age at birth of the first child was 25.5 years for men and 23.1 years for women (44). In the study population the average age of mothers and fathers was: 32.41 ± 5.91 and 33.92 ± 3.43 years (Table 1). It is worth remembering that, paradoxically, taking into account the entire population, more children with DS are born to young mothers as they are of reproductive age. This can of course change over time as more and more women choose to have offspring later in life.

Online survey

The results of the online survey appear to be disturbing. So far, no growth charts adapted to the population of individuals with DS have been officially developed in Poland. Nevertheless, access to many other DS-specialized growth charts, including those developed by the CDC (5), is quick, simple and common. Despite that, as the survey results indicate, specific growth charts are not used in everyday medical practice in Poland although parents are aware of their existence. There can be many reasons for that state. First, many physicians are unaware of the existence of specialized growth charts. Secondly, in everyday practice, it is easier and more efficient to use standard growth charts. What's more, standard growth charts are available in every child's health booklet (parents should have it with them at every medical visit), which makes it effortless and faster to apply the child's data on the charts. Additionally, many doctors do not believe that it is necessary to use specialized charts to assess the development of children with DS. The usage of DS-specialized growth charts has certain consequences mainly regarding the previously mentioned issue of BMI assessment and the apparent dormancy of the parent's vigilance, while activities related to reducing the child's weight should already be taken. The optimal solution seems to be the assessment of a child on both types of growth charts to fully control their development at every stage. Fortunately, many parents are printing specialized growth charts and having them with them or pasting them into a child's health book. However, the results obtained only on specialized growth charts (body weight, BMI in some cases) may cause the parent to perceive the body weight as healthy even though it may require early intervention. Nevertheless, DS-specialized growth charts should be implemented into pediatric departments as an important and additional tool to properly assess the development of children and adolescents with DS. When extending the scope of research on this topic it would be worth expanding the research group to include people from the medical community.

Advantages and limitations of the current study

This study should be interpreted in light of its limitations. First, the data we obtained from medical records (20%) could be the results of measurements performed without the use of standardized techniques, and this could lead to measurement errors. Part of the study population came from a pediatric endocrinological health clinic, which means that they are treated for endocrine reasons. At the same time, most children with Down's syndrome are burdened with comorbidities, so it would be difficult to single out a group for example without heart defects and thyroid problems. A sample of children attending a medical office was used, not a random sample from the target population. Our research, in order to be more valuable, could also be expended by measurement of the head circumference. As the standard growth charts, the 1990 British growth charts were used. This choice was dictated by the high detail of the mentioned growth charts and their coherence with the LMSgrowth Microsoft Excel add-in used. This study also has some strengths. First, the sample size is large. Second, the data for youth aged 4 months–36 years covers almost the full range of development. Thanks to medical data collected from parental groups our research group includes children from all over Poland, not just from one region. The gathered group of over 400 children covers the spectrum of children with many DS-typical diseases.

Conclusions

There is no single comprehensive tool for the assessment of the developmental disorders in DS. The differences between the results obtained using standard growth charts and specialized ones were identified, however, they are ambiguous in the clinical meaning. It is both type of growth charts that are capable of detecting development disorders early in the broadest possible way. The findings of our study can be valuable for healthcare professionals, parents, and guardians in drawing attention to the need for complex monitoring of developmental disorders in people with DS. Accurate assessment of anthropometric indicators of the development may enable to improve the quality of life and to extend the period of a healthy lifespan.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Bioethics Committee, Wroclaw Medical University (approval number KB 674/2020). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MH: term, Conceptualization, Investigation, Resources, Data Curation, Writing - Original Draft, Project administration, Visualization. HM: Validation, Formal analysis, Data Curation, EB: Methodology, Resources, Writing - Review and Editing, Supervision, Project administration. All authors contributed to the article and approved the submitted version.

Funding

This research received no external funding. However the publication will be financed by the Wroclaw Medical University, including subsidy funds (Wroclaw Medical University; SUBK.D130.22.055) for the project "Children and young adults with Down Syndrome- metabolomics".

Acknowledgments

Our greatest thanks go to the children and their families who participated in this study, the parents' interest groups and organizations that assisted in reaching those families. We would also like to thank Dr. Chapman and Dr. Madera of King's College Hospital who were extremely helpful at the very beginning of our research in guiding us to electronic data on standardized growth charts for children with Down's syndrome. The provided data has been used only for scientific purposes.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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5. ARTYKUŁ DRUGI

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Review

Pediatric Population with Down Syndrome: Obesity and the Risk of Cardiovascular Disease and Their Assessment Using Omics Techniques—Review

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Abstract: People with Down syndrome (PWDS) are more at risk for developing obesity, oxidative stress disorders, metabolic disorders, and lipid and carbohydrate profile disorders than the general population. The presence of an additional copy of genes on chromosome 21 (i.e., the superoxide dismutase 1 gene (SOD1) and gene coding for the cystathionine β-synthase (CBS) enzyme) raises the risk for cardiovascular disease (CVD). As a result of disorders in metabolic processes and biochemical pathways, theoretically protective factors (low homocysteine level, high SOD1 level) do not fulfil their original functions. Overexpression of the CBS gene leads to the accumulation of homocysteine—a CVD risk factor. An excessive amount of protective SOD1, in the case of a lack of compensatory increase in the activity of catalase and peroxidase, leads to intensifying free radical processes. The occurrence of metabolic disorders and the amplified effect of oxidative stress carries higher risk of exposure of people with DS to CVD. At present, classic predispositions are known, but it is necessary to identify early risk factors in order to be able to employ CVD and obesity prophylaxis. Detailed determination of the metabolic and lipid profile may provide insight into the molecular mechanisms underlying CVD.

Keywords: Down syndrome; obesity; metabolomics; lipidomics; cardiovascular disease

Citation: Hetman, M.; Barg, E. Pediatric Population with Down Syndrome: Obesity and the Risk of Cardiovascular Disease and Their Assessment Using Omics Techniques—Review. *Biomedicines* **2022**, *10*, 3219. https://doi.org/10.3390/biomedicines10123219

Academic Editor: Estefania Nuñez

Received: 21 November 2022
Accepted: 9 December 2022
Published: 12 December 2022

1. Introduction

Diagnosing a disease before its first symptoms appear poses a great challenge in medicine. In the past, it was believed that only genes affected the maintenance of homeostasis and health in the body. After many years of research and genome sequencing, we are still unable to diagnose numerous diseases or to design an effective therapy at an early stage. It was assumed that the organism is a complex structure, at the base of which is the genome, and its subsequent levels being transcripts, proteome and metabolome [1]. Metabolism plays a key role in all areas of biology, which is why more and more of those areas are being studied from its perspective. Many adult conditions, such as cardiovascular and metabolic disease, originate during childhood, therefore the knowledge of risk factors for these diseases will allow for the implementation of an early and effective prophylaxis.

Down syndrome (DS, trisomy 21, T21) is the most common chromosome abnormality (caused by trisomy of the whole or a part of chromosome 21) with a worldwide incidence rate of 1:1000–1100 in newborns [2]. The extra chromosome 21, or at least a portion of it, results in a constellation of clinical features (cardiac defects, delayed growth, hematology and endocrine abnormalities, autoimmune diseases, intestinal, stomatognathic disturbances, vision and hearing defects and obstructive sleep apnea, and others) [3,4]. Additionally, people with DS (PWDS) are at increased risk for cardiovascular diseases (CVD) (mitral valve prolapse, endocarditis, atherosclerosis (AS) and congestive heart failure [5]), pulmonary hypoplasia, muscle hypotonia, osteoporosis, arthritis, osteoarthritis, and diabetes mellitus [6,7]. There is no specific DS phenotype. Individuals may differ from each other

both in terms of external features and chronic diseases. Such a complex condition contributes to the demand for profound medical care. Advancements in medicine have led to a marked improvement in life expectancy of PWDS, with the estimated median age of survival approaching 60 years [8]. Obesity-related diseases (CVD, cancer, type II diabetes, and others) have received more attention [9]. The problem of body weight disorders among PWDS is complex and challenging, concerning mainly the rapid transformation between undernutrition in the first period of life and excessive weight gain in later years. For the purposes of this review, however, we will focus on the problems of excess body weight in children and adolescents with DS. The appearance of new disease entities in this population is a challenge for health practitioners. Due to the burden of many conditions, PWDS should be monitored from an early age with the constraints associated with their health status.

2. Omics Techniques: Metabolomics and Lipidomics

Omics techniques are a rapidly evolving field of molecular sciences. Metabolomics, a relatively young branch of omics science, is an interdisciplinary approach that encompasses biology, chemistry, and bioinformatics. The metabolomic examination should enable the detection of abnormalities in the patient's health at an early stage of the development of clinical symptoms or even before their manifestation [10]. The advantage of metabolomics research is its low invasiveness, thanks to the use of mainly readily available body fluids, i.e., blood serum, plasma, saliva, urine, and tissues after prior preparation. It provides a modern bioanalytical tool to define perturbations in metabolic pathways and enables the detection of predictive biomarkers. Thus, metabolomics can contribute to more efficient diagnosis, treatment, and disease prevention. The definition of metabolomics officially appears in the literature in 1999 as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" [11]. Metabolomics is based on the qualitative and quantitative study of small-molecule (<1.5 kDa) compounds that are intermediates and products of metabolism (lipids, amino acids, short peptides, nucleic acids, sugars, alcohols, or organic acids) and reflects in endogenous metabolism and exogenous sources such as, among others, diet or physical activity. Metabolites are involved in all biochemical reactions (any process that occurs in the body is reflected in the metabolome) and measuring them can potentially evaluate the state of the organism. There are two approaches in metabolomics: untargeted and targeted. The untargeted approach makes it possible to identify metabolic new biomarkers; the targeted approach identifies and quantifies a limited number of known metabolites. Both the presence and absence of specific metabolites can be the source of information about possible disorders in the patient's health and draw attention to a medical problem.

Lipid homeostasis is essential for maintaining full health; therefore its evaluation is of fundamental importance. Any abnormalities in lipid metabolism play an important role in many diseases, including metabolic syndrome, diabetes, CVD, lipodystrophies, neurological/neurodegenerative disorders, and central nervous system damage [12]. Most cases of CVD are difficult to associate with well-known risk factors. Many patients, despite having optimal blood lipid levels, are exposed to CVD [13]. Therefore, it is important to search for new biomarkers that will enable the very early diagnosis and effective prevention of CVD. Single biomarkers in cardiology are very effective in confirming the occurrence of an acute event. However, it is very difficult to precisely estimate the risk of atherosclerotic disease at an early stage. So far, researchers have relied on well-established risk factors, such as smoking, hypertension, dyslipidemia, and diabetes as risk factors of developing CVD [12,14–16]. Detailed determination of the metabolic profile may provide insight into the molecular mechanisms underlying AS [13,17–20].

2.1. Metabolomics

Metabolomics is a very promising tool for investigating human health, however, the analysis of the metabolome is challenging for many reasons, among others, different analytic approaches and the lack of standardization. The techniques used in metabolomics are

magnetic resonance (NMR) spectrometers, mass spectrometers (MS), gas-chromatography (GC), liquid chromatography (LC) systems, ion mobility systems (IMS), capillary electrophoresis (CE) systems, integrated liquid chromatography-mass spectrometry (LC-MS), integrated capillary electrophoresis-mass spectrometry (CE-MS), integrated ion mobility spectrometry-mass spectrometry (IMS-MS) and gas chromatography-mass spectrometry (GC-MS) [21].

As a novel technique, metabolomics can provide insight into obesity and the risk of cardio-metabolic complications, and be used to uncover pathways underlying diet–disease associations. The heart, being a metabolically active organ [22], and its diseases constitute one of the main targets of the omics' techniques of today. The results of metabolomics studies help to clarify the pathophysiology of many diseases, optimize treatment, and distinguish specific diagnostic biomarkers, which is especially important in the case of asymptomatic diseases [23]. In 1991, the first biomarkers of coronary heart disease using NMR spectroscopy [24] were discovered. The introduction of metabolomics to epidemiological research is of great importance for the understanding of pathophysiology and the discovery of new biomarkers for the early prevention and detection of AS and CVD [25]. In 2017, the American Heart Association published a statement on potential health and CVD effects of metabolomics and its current challenges in clinical practice [26].

Understanding the pathogenesis of childhood obesity with the help of molecular studies is one of the major challenges of current medicine. Metabolomic studies on obesity and comorbidities are conducted in adults on a large scale. Unfortunately, little research has been completed on groups of children and adolescents [27]. Hellmuth et al. combined metabolomics data from four large European cohorts finding a strong positive association of sphingomyelin (SM) 32:2 (molecular species containing myristic acid and sphingadienine) and lyso-phosphatidylcholine (LPC) 14:0 with BMI z-score (this metabolite was found to have a positive association with BMI adults [28]) and no association of non-esterified fatty acid (NEFA) 16:1 with BMI z-score [29]. An LPC 14: 0 was considered a predictor of obesity at 6 years of age (study of serum in 6-month-old infants) [30]. The rate of FA 14:0 was also elevated in phospholipids (study among obese 15-year-old children) [31]. The authors [29] additionally concluded that the concentration of lipids with 14:0 (exception of NEFA 14:0) is seemingly higher in children with high BMI and may subsequently be used more often for the synthesis of SM. In addition, the 14:0 synthesis can be enhanced by a high-calorie diet and high glycemic load of food. SM 32:2 may be a potential biochemical marker for the combined effect of genetic predisposition, high dietary intake of total energy, glycemic load, and linoleic acid [29]. Atherosclerosis, the major cause of CVD, is often attributed to lifestyle factors [32]. A high risk of the early development of AS has been proved in people with hyperhomocysteinemia, hypermethioninemia, and homocystinuria [33]. Wurtz et al. identified phenylalanine and various fatty acids as biomarkers for CVD [34]. Biomarkers related to insulin resistance and energy metabolism have also already been identified [35]. A consistent metabolic profile of childhood obesity was observed including amino acids (particularly branched chain and aromatic), carnitines, lipids, and steroids [36,37].

2.2. *Lipidomics*

Lipidomics, a discipline belonging to metabolomics, is described as the quantitative characteristic of the complete lipid complex [17]. The subject of research in this subdiscipline is lipids, i.e., a functional unit characterizing the molecular lipid image of a biological sample under study. However, thanks to lipidomics, it is possible to quantify various lipid molecules (acylglycerols, sterols, sphingolipids, and others) [17,19]. Lipidomic evaluation allows for a picture of lipid concentrations, for example, the total plasma lipidomics of the tested total plasma shows a detailed and much more complete picture of lipid metabolism and possible abnormalities of lipid metabolism—as opposed to studies of isolated lipoproteins [12]. Lipidomics had identified ceramides and sphingolipids as potential mediators of cellular dysfunction.

Lipidomics study the structural, signaling and metabolic functions of lipid compounds. Due to the large variety of lipid types in cells/tissues, the lack of homogeneity and their frequent biochemical modifications, detailed characterization may be a difficult task [38]. For these reasons, data on lipidomics rarely appear in the scientific literature when compared with other "omics" technologies. Thus, a sufficient amount of data on the relationship between lipid metabolites with CVD are still lacking [39]. Lipids are presented as CVD risk factors only in major classes and not as individual molecular entities in diabetes [40].

The main challenge of lipidomics is the demonstration of new risk factors and the early detection of the risk of atherogenesis at the clinical level. It seems necessary to identify early risk factors in order to undertake CVD prophylaxis.

3. Down Syndrome

3.1. Cardiovascular Disease

In 1977, Murdoch et al. found a complete absence of atherosclerosis in five posthumously examined PWDS [41]. In addition, Pueschel et al. confirmed the lack of significant differences in the level of total cholesterol (TC), low-density lipoprotein (LDL), apolipoprotein B (apoB), and apoB/apolipoprotein A (apoA) between the examined group of PWDS and the control group [42]. As a result of conducted research in the 1970′s, it was thought that people with DS were no more at risk of atherosclerosis than the general population. However, the lifestyle and eating habits differed significantly from those present, and life expectancy was much shorter. Recently, there have been scientific studies that may suggest that significant and known risk factors for CVD and AS have been observed among people with DS: diabetes [43,44], obesity [43,45–47] and hypertension [44], and lipid disorders [48,49]. At the same time, it has been shown that PWDS have a lower incidence of AS [41,44,50–56]. Additionally, lower BPs at rest may have a protective role against the development of atherosclerosis in PWDS [53,57].

Interestingly, Landes et al. showed that PWDS were more likely to die at younger ages from heart diseases compared with the general population [58]. The study of Hill et al., Day et al., and Hermon et al. showed an increased risk of death for PWDS due to CVD in comparison with the general population [59–61]. However, Torr et al. analyzed morbidity and mortality among PWDS and indicated ischemic heart disease to be a minor cause of death [62]. Adelekan et al. found that children with DS have less favorable lipid profiles than their siblings [8]. Sheela et al. showed that youth with DS had more atherogenic lipid and lipoprotein particle profiles, including higher LDL-C levels, compared with those without DS [49]. Buonouomo et al. found high levels of TC, LDL-C, and TG and low HDL-C in individuals aged 2–9 years old with DS [62]. This study group with DS also had a higher prevalence of prediabetes and an increased amount of visceral fat [49]. In general, the increased LDL-C level in youth with DS reveals a greater risk of atherosclerosis. Adults with DS also have a high risk of stroke, driven largely by high cardioembolic risk [44].

Lipoprotein(a) (Lp(a)) seems to be involved in the pathogenesis of CVD [63]. Krzesińska et al. compared lipid parameters, protein composition, antioxidative properties of HDL, and Lp(a) levels in adolescents with DS and healthy individuals [64], and showed unfavorable lipid profiles in conjunction with significantly higher Lp(a) levels and quality changes in HDL particles in adolescents with DS. Serum Lp(a) levels are relatively stable over a lifetime [65], therefore a once-in-a-lifetime Lp(a) measurement could help identify those at increased risk of CVD [66]. Data appearing in the literature seem to be contradictory. Most, however, argue for the need to refute the belief that DS is a disease free from atherosclerosis. In this situation, it is advisable to extend the research on atherosclerosis risk factors and predisposition to related diseases in people with DS with the use of omics techniques.

3.2. Excessive Body Weight and Physical Activity

The literature repeatedly reports that DS children are predisposed to obesity [67–72], abnormal or excessive fat accumulation caused by a positive energy balance, which has

been associated with a negative impact on health [73]. Adults with DS are twice as likely to be obese and nearly four times more likely to be extremely obese in comparison with adults without DS [74]. The literature describes potential causes of obesity tendency among children and adolescents with DS: decreased energy expenditure at rest; increased leptin levels; untreated hypothyroidism; unhealthy diet; and low physical activity [70,75]. Additionally, children and adolescents with DS show less physical activity than their peers without DS [76–78], although the tested level of physical activity in adolescents without DS turned out to be insufficient in 80% of them [79]. What is worse, PWDS tend to become less active as they become older, with higher rates of obesity in girls [80–84]. Although the activity level among children with DS was lower, the caloric intake was higher in this group [75]. The greatest acceleration in obesity occurs between the ages of 2–6 years [84]. In the teenage period, when PWDS gain more independence and the ability to choose the type and amount of food (with the predominance of processed products with excessive amount of salt and sugar), obesity begins to be most visible. Yahia et al. pointed out that prepubertal obese-DS displayed excess body adiposity with pronounced central fat distribution, atherogenic lipid profile, and higher insulin resistance compared with matched obese-control [85,86]. Wernio et al. pointed out that overweight children with DS were characterized by higher levels of triglycerides, atherogenic index of plasma, and apoA2 and apoE levels [87]. Obesity also contributes to the worsening of obstructive sleep apnea symptoms and the burden of congenital heart disease [70,88,89]. With age, it becomes more and more difficult to persuade teenagers to play sports regularly. However, the DONUT STUDY showed that, despite potential difficulties in the pursuit of a correct diet and inadequate approach to physical activity, children with DS could achieve results that are substantially the same as those of non-DS children [90]. Moreover, some children and adolescents with DS are limited by reduced respiratory efficiency and congenital heart diseases [81]. An additional obstacle to increasing physical activity among PWDS is the COVID-19 epidemic that has been present for over 2 years. Amatori et al. showed a negative impact of COVID-19: decreased physical activity and increased sedentary behaviors [91]. It is worth remembering that the patterns of proper nutrition should function throughout a household. Stefanowicz-Bielska et al. proved that in families of overweight and obese children with DS, other members had nutritional disorders more frequently [92]. Caregivers and siblings should be equally involved in shaping healthy habits and lifestyle. Different levels of intellectual disability can also make it difficult to make correct food choices. Hence the repeated emphasis on the importance of the role of the family as a promoter of a healthy lifestyle. Roccatello et al. analyzed meals of choice of the people with DS finding bread, pasta and sweets as their favorite go-to foods [92]. The least-liked food was vegetables. Fruit juices and ready-to-drink tea were the main sources of simple sugars [92], which can contribute to liver steatosis and hypertension (the impact of fructose) [75]. Introducing healthy eating habits may be fundamental to sustaining good health. Jobling et al. conducted an intervention study (education program) [93]. The program was successful in convincing people with DS to reduce their consumption of sweets but the researchers' actions did not change other unhealthy eating habits [93]. However, Naczk et al. enrolled adolescents with DS in a thirty-three weeks swimming program that resulted in decreases in body mass, body fat, and BMI [94]. Because regular physical activity is recommended to reduce the risk of developing health conditions such as heart disease, cancer, type 2 diabetes, high blood pressure, osteoporosis, and obesity [95,96], sports programs of this type play a very important role in acquired heart-disease prevention. As children and adolescents with DS are predisposed to overweight and obesity, and also tend to be physically inactive, they are at a significant risk of mortality and many serious diseases. Me et al. have shown that breastfeeding may be a protective factor for obesity and high body fat in children [97]. In 2022, a systematic review of DS and breastfeeding was conducted: around 50–23.3% of the children with DS were never breastfed and rates of breastfeeding in infants with DS were lower than those in controls in three studies [98].

3.3. Oxidative Stress

PWDS have been identified as having high oxidative stress (the imbalance between free radical production and the prooxidative state within the cell determines a biological state [99]), which is connected with the risk of the development of AS, neurodegeneration, cell ageing, cancer, and immunological disorders [100,101]. Oxidative stress, which damages blood vessel tissues, also plays a role in the pathogenesis of AS. In oxidatively damaged tissues, the development of AS is facilitated [102]. Endothelial cell function may be impaired in PWDS despite their protection against AS [103]. Furthermore, high oxidative stress has been related to elevated insulin resistance, poor insulin sensitivity, and hypertension [104]. It has been shown that T21 is associated with pro-oxidant status and increased susceptibility to oxidative damage [105–107]. T21 of the chromosome increases the representation and expression of Cu/Zn superoxide dismutase (SOD1), the gene which is located on the distal segment of chromosome 21 (21q22.1) [108]. It has been shown that PWDS have an increased SOD1 activity by as much as 150% compared with people without DS [109]. SOD-1 is the main enzyme in the antioxidant defense system. Under physiological conditions SOD-1, together with catalase and peroxidases, protects the body against the harmful effects of very reactive free oxygen radicals which are a potential threat to cellular structures. Radicals and reactive oxygen species (ROS) are formed during normal cellular metabolism, however, in the conditions of their increased formation or disturbances in the activity of antioxidant enzymes, free radical damage occurs. It is believed that excessive SOD-1 activity is responsible for the increased formation of hydrogen peroxide and it heightens the risk of oxidative stress (prolonged higher SOD activity may lead to glutathione depletion, deficiencies in catalase and peroxidases' activity). SOD-1 catalyzes the conversion of the peroxide anion to hydrogen peroxide, which leads to the continuous production of two major reactive oxygen species in oxygen cells in the mitochondria [109]. In the pathogenesis of AS, ROS are responsible for the formation of oxidatively modified LDLs (oxyLDL), which are pro-atherogenic substances [110–112]. The biological effects of ROS are controlled by a wide spectrum of antioxidant defense mechanisms such as the action of vitamins E and C, uric acid, glutathione, and antioxidant enzymes. The reduced concentration of glutathione in the blood along with the overexpression of the SOD-1 gene in PWDS additionally contributes to elevated exposure to the negative effects of oxidative stress. The increased activity of SOD-1 as an antioxidant enzyme could explain the protective role in preventing atherosclerotic lesions. In the situation of disturbed antioxidant balance, as is the case in DS, due to the lack of compensatory higher activity of catalase and peroxidase, free radical processes are intensified. It is known that high SOD-1 activity means a disturbed balance of the antioxidant system: the peroxidation processes of lipid peroxides, participating in the formation of atherosclerotic plaques, dominate. In people who experience increased oxidative stress, biologically important molecules such as lipids, proteins, or nucleic acids are oxidized, which significantly affects the incorrect function of both individual organs and the entire body. Therefore, it seems that PWDS will be additionally exposed [111]. Lipid peroxidation (LPO) in free radical reactions is the process of oxidation of unsaturated fatty acids or lipids in which peroxides of these compounds are formed. They are an important link in the atherosclerotic process [112]. They modify physical properties of cell membranes and inhibit the activity of membrane enzymes and transport proteins. There is also a link between an aerobic modification of LDL-C and inflammatory activity of macrophages through the induction of macrophage cyclooxygenase 2 expression by LPO products [110]. Chronic oxidative stress leads to the intensification of degenerative processes and premature aging of tissues. With age, numerous hormonal and metabolic disorders appear and worsen, which is the leading problem in older children and adolescents with DS who require constant and targeted medical care. An earlier description of DS as a "non-atherosclerotic model" could be justified, inter alia, by increased activity of the defense enzyme SOD-1 and the altered metabolism of homocysteine. The results of the current research seem to contradict this assumption. In this situation, it is advisable to extend the research workshop on atherosclerosis risk factors and predisposition to other

diseases in people with DS to include metabolomics research. Wernio et al. pointed out that fat mass, fat mass/height2 index, and visceral fat mass in children with DS correlated with advanced oxidative protein product level [87]. Figure 1 shows the processes described above.

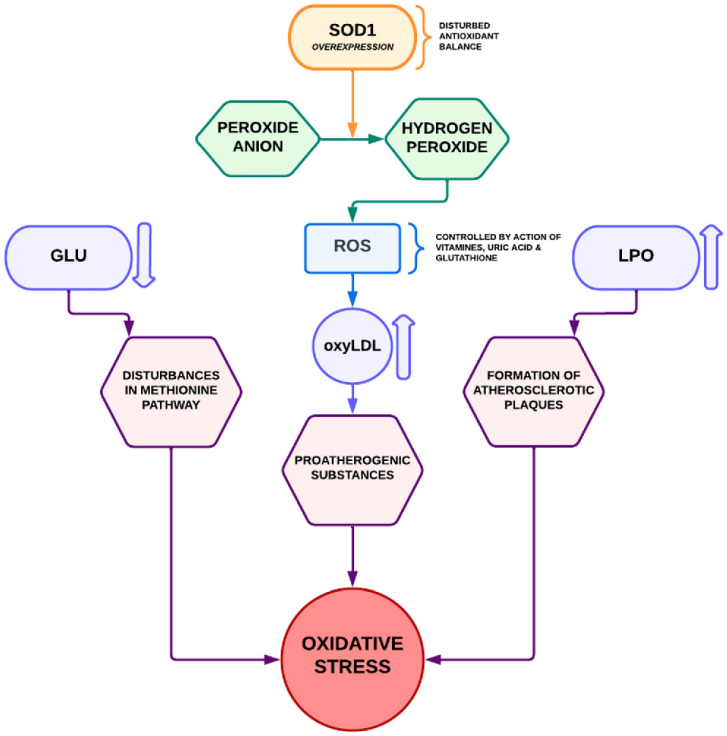

Figure 1. Oxidative stress processes in people with Down syndrome. SOD1-Superoxide dismutase; GLU-glutathione; ROS-reactive oxygen species; LPO-lipid peroxidation; oxyLDL-oxidatively modified LDLs.

3.4. Metabolic and Endocrinological Disorders

The gene coding for the enzyme β-cystathionine synthase (CBS) is located on chromosome 21. This enzyme is responsible for converting homocysteine (Hcy) and serine into cystathionine in the methionine metabolic pathway. Hcy, being a by-product of methionine metabolism, must be converted back to methionine (by re-methylation or by conversion to cysteine). In this process, the important part is played by folic acid and vitamins B (B6 and B12). Three copies of the CBS enzyme genes cause its overexpression; thus, people with DS have a reduced level of homocysteine, which should result in a reduced risk of AS. The reduced concentration of homocysteine also means a lower concentration of methionine, deficiency of tetrahydro folic acid (THF) (the so-called THF trap), and the participation of B vitamins in the methionine pathways. Additionally, the low concentration of homocysteine results in DNA hypermethylation. The disruption of the methionine metabolism pathways is caused by a number of unfavorable metabolic disorders that can be detected using metabolomics studies. Low availability of vitamin B (B6, B12, and folic acid) leads to impaired re-methylation of homocysteine to methionine and thus accumulation of homocysteine [113]. Recent data indicate that homocysteine accumulates in states of increased

oxidative stress associated with immune activation [113]. To better understand the above processes, simplified diagrams have been prepared: Figure 2 shows the correct metabolic pathways; Figure 3 shows the disorders occurring in DS.

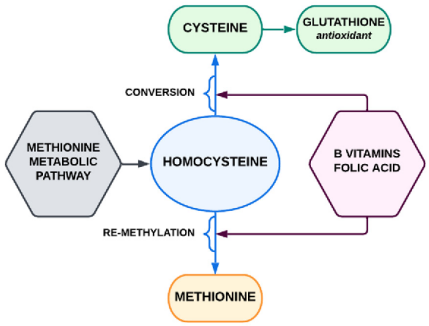

Figure 2. Methionine metabolic pathway (simplified).

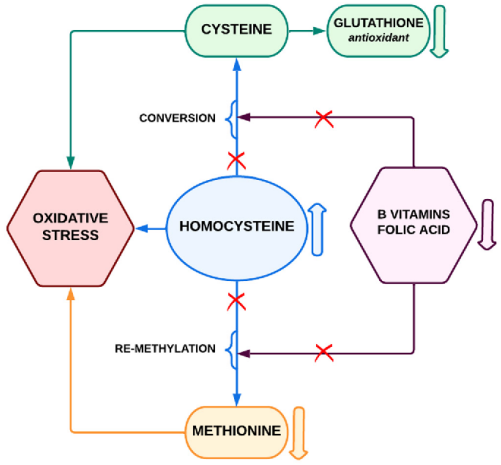

Figure 3. Methionine metabolic pathway (simplified)–pathway disorders in people with Down syndrome.

Children with DS have a higher likelihood of developing endocrine and metabolic disorders such as thyroid dysfunction, diabetes mellitus, short stature, vitamin D deficiency, and obesity than the general population [44,89,114–118]. Thyroid dysfunction is the most common endocrine abnormality in DS children: it is about 38 times more common in individuals with DS than in other people [119,120]. Thyroid hormones are involved in the regulation of carbo–lipid metabolism. They are related to oxidative stress by stimulating cellular metabolism and influencing antioxidant mechanisms as well as regulating oxygen consumption and producing free radicals [111,121]. It is estimated that the incidence of thyroid gland disorders in people with DS increases with age [122]. Aslam et al. demonstrated that at younger ages the incidence of diabetes in patients with DS is four times higher than that of control patients. Peak mean BMI is higher and established earlier in DS, contributing to T2DM risk [123]. The prevalence of type 2 diabetes mellitus in children with DS ranged between 0% and 3.6% [117]. Wernio et al. pointed out that in children with

DS fat mass, fat mass/height^2 index, and visceral fat mass correlated with thiobarbituric acid reactive-substances and advanced oxidative protein product-levels [87].

3.5. Metabolomics in Down Syndrome

To date, numerous disturbances in the concentration of metabolites in DS have been described, such as: increased levels of phenylalanine and tyrosine in blood serum [124]; lower plasma levels of free histidine, lysine, tyrosine, phenylalanine, leucine, isoleucine, and tryptophan [125]; increased plasma concentrations of leucine, isoleucine, cysteine, and phenylalanine [126]; decreased plasma concentration of serine [127]; increased plasma lysine concentration [127]; elevated concentrations of metabolites related to the methylation cycle such as cysteine, cystathionine, choline, and dimethylglycine [125]; and increased concentrations of S-adenosylhomocysteine and S-adenosylmethionine [125]. Little data exist on the use of metabolomics among PWZD [125,126]. At the same time, there are no data on the use of lipidomics in DS. Orozco et al. analyzed metabolomics of 31 PWZD and observed alterations to methylation metabolism, carnitine/O-acetyl carnitine, dimethyl sulfone, and myo-inositol in children with DS [128]. Obeid et al. reported similar findings in methylation pathway metabolites and found elevated blood cystathionine, cysteine, betaine, choline, and N,N-dimethylglycine in children and young adults with DS [125]. Caracausi et al. analyzed plasma and urine of children with DS and revealed DS/normal ratio in plasma being 1.23 (pyruvate), 1.47 (succinate), 1.39 (fumarate), 1.33 (lactate), and 1.4 (formate) [126]. As most of the altered concentrations were consistent with the 3:2 gene dosage model, there is a possibility that the mentioned changes are caused by the presence of three copies of chromosome 21 [126]. As a result of the use of different methods of omics techniques, as well as the differences in metabolites among children and adults, it is very difficult at the present stage to compare the results obtained in the studies mentioned in the review. However, it is very important to perform metabolomic and lipidomic tests in children with DS in order to be able to compare and analyze the data.

4. Summary and Conclusions

Trisomy of 21 chromosome affects the cardiovascular system in anatomical and physiological ways. Numerous hormonal and metabolic disorders are a leading problem in children and adolescents with DS. Those disorders aggravate with age and require constant targeted medical care. As a result of disorders in metabolic processes and biochemical pathways, theoretically protective factors (low homocysteine level, high SOD1 level) do not fulfil their original functions. The results of the current research seem to contradict the assumption that PWDS are not at risk of developing cardiovascular disease. At present, some classic predispositions are known but CVD prophylaxis requires identifying early risk factors. In such case, it is advisable to extend the research of omics techniques on atherosclerosis risk factors and predisposition to include related diseases in people with DS.

Author Contributions: Conceptualization, E.B. and M.H.; methodology, M.H.; software, M.H.; validation, E.B. and M.H.; formal analysis, E.B. and M.H.; investigation, M.H.; resources, E.B. and M.H.; data curation, M.H.; writing—original draft preparation, M.H.; writing—review and editing, E.B. and M.H.; visualization, M.H.; supervision, E.B.; project administration, E.B. and M.H.; funding acquisition, E.B. and M.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. However, the publication will be financed by the Wroclaw Medical University, including subsidy funds (Wroclaw Medical University; SUBK.D130.22.055) for the project "Children and young adults with Down Syndrome-metabolomics".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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6. ARTYKUŁ TRZECI

Pediatric Endocrinology Diabetes and Metabolism
Endokrynologia, Diabetologia i Choroby Przemiany Materii Wieku Rozwojowego

Original paper | Praca oryginalna
Pediatr Endocrinol Diabetes Metab 2023; 29
DOI: https://doi.org/10.5114/pedm.2023.131513

Comparative analysis of obesity prevalence, antioxidant and oxidant status in children with Down syndrome – a sibling-controlled study

Analiza porównawcza występowania otyłości, statusu antyoksydacyjnego i oksydacyjnego wśród dzieci z zespołem Downa – badanie z udziałem rodzeństwa jako grupy kontrolnej

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Abstract

Introduction: Down syndrome (DS), a common genetic disorder, leads to various physical, cognitive, and developmental challenges. The supplementary copy of chromosome 21 introduces an abundance of genes, which potentially can influence metabolic irregularities. The aim of the study is to conduct a comprehensive comparative assessment of oxidative stress indicators (TAS, TOS, OSI), BMI, fast-ing glucose, and insulin levels, HOMA-IR among children and adolescents with DS in contrast to their non-DS siblings.

Material and methods: and the control group (CG) comprised 20 individuals, siblings of SG (mean age 15.92 years). Anthropometric measurements were conducted. TAS, TOS, fasting glucose, and insulin were assessed. BMI, BMI SDS, OSI and HOMA-IR were calculated.

SG vs. CG: BMI – overweight (29,19% vs. 15%), obese (19,05% vs. 5%); TAS (1.92 mmol/l vs. 1.79 mmol/l [$p = 0.0015$]); TOS (51.52 mmol/l vs. 33.05 mmol/l [$p = 0.014$]); OSI (2475.02 vs. 1949,75 ($p = 0.038$); no significant differences in fasting glucose, insulin and HOMA-IR. Statistically significant correlations in SG: TOS and BMI, BMI SDS, HOMA-IR; OSI and BMI, BMI SDS, HOMA-IR; HOMA-IR and BMI SDS; fasting insulin and BMI PC; in CG: TAS and BMI; fasting glucose and fasting insulin.

The research results indicate differences in metabolic processes between the group of individuals with DS compared to the CG, despite shared environmental conditions. The presence of an additional copy of chromosome 21 may contribute to the occurrence of metabolic disorders. These findings emphasize the need for further research that will lead to a better understanding of these relationships and contribute to the development of effective therapeutic strategies.

Key words: Down syndrome, oxidative stress, body mass index, glucose, insulin.

Introduction

Trisomy 21 – Down syndrome (DS), is a genetic disorder that stems from the presence of all or part of an additional copy of chromosome 21. Down syndrome represents one of the most prevalent chromosomal abnormalities, leading to a wide array of physical, cognitive, and developmental complications. The observable traits and conditions associated with DS are incredibly diverse, ranging from distinct facial features and heart defects to varying degrees of intellectual disability. Over the years, a substantial body of research has underscored the intricate link between DS and oxidative stress [1–4]. The supplementary copy of chromosome 21 introduces an abundance of genes, which potentially can influence metabolic irregularities. Oxidative stress arises from an imbalance in the body's cellular environment, characterized by an excess of pro-oxidants (measured as total oxidant status – TOS) compared to antioxidants (total antioxidant status – TAS). To gain a comprehensive understanding of oxidative stress, it is crucial to consider the oxidative stress index (OSI), a measure that represents the balance between the total antioxidant capacity and total oxidant status of the body. It is the ratio of TOS to TAS, and it provides a powerful tool for assessing the overall oxidative stress status. In individuals with DS, the oxidative stress is postulated to be elevated due to an effect known as the gene-dosage effect, triggered by the overexpression of the superoxide dismutase, a key antioxidant enzyme located on chromosome 21 [5]. Increased oxidative stress plays a critical role in the pathophysiology of numerous diseases and can significantly impact the health and well-being of individuals with DS. Moreover, DS is also associated with various metabolic changes, particularly reflected in body mass, as well as alterations in fasting glucose and insulin levels [6–9]. Body weight is best assessed by the body mass index (BMI). When it comes to children and adolescents, interpreting BMI goes beyond the conventional metrics used for adults. It's important to consider that children's body composition varies with age and differs between sexes. Therefore, in pediatric populations, it's essential to use adjusted

Received: 18.08.2023
Accepted: 10.09.2023
Conflict of interests: none declared
This research is financed by the Wroclaw Medical University, including subsidy funds (Wroclaw Medical University, SUBK.D130.22.055) for the project "Children and young adults with DS-metabolomics".

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measurements, such as BMI Standard Deviation Scores (BMI SDS), to accurately interpret BMI. The metabolic shifts can significantly influence the health status of individuals with DS, potentially leading to a spectrum of metabolic disorders. Despite the wealth of research, understanding these metabolic shifts' full breadth and the relationship they share with oxidative stress in DS individuals is still a nascent field. While DS research has made considerable strides in understanding the disorder's physiological implications, there are still gaps. In particular, there is a dearth of comprehensive, comparative studies on the profile of oxidative stress, BMI, and metabolic changes between young people with DS and their non-DS siblings. Siblings without DS provide a unique control group due to their significant shared genetic and environmental influences with the DS individuals, offering an opportunity for a more precise understanding of trisomy 21's impact on these parameters.

Aim of the study

This study embarks on a thorough comparative analysis of oxidative stress indicators (TAS, TOS, OSI), BMI, BMI standard deviation score (BMI SDS), HOMA-IR and fasting glucose and insulin levels in children and adolescents with DS, as opposed to their non-DS siblings. The goal is to uncover a deeper understanding of the biological complexities of DS, which may guide clinical practices and spark novel therapeutic approaches.

Materials and methods

Study design and participants

The participants comprised a study group (SG) of 42 children and adolescents with DS (DS) (17 females, with a mean age of 14.17 ±6.66 years old), and a control group (CG) of 20 siblings of the individuals with DS (10 females, with a mean age of 15.92 ±8.58 years old). The study protocol adhered to the principles of the Declaration of Helsinki, and the Bioethics Committee of Wroclaw Medical University approved it (KB 674/2020). The inclusion criteria for the SG were patients diagnosed with DS (due to genetic test results) while for the CG, it was being a sibling of an individual SG. There were no specific exclusion criteria. Written informed consent was obtained from the parents or legal guardians of all the participants prior to the data collection and anthropometric measurements. Administrative approvals were also secured from each institution involved to access the necessary participants' data.

Data collection

Participants were actively recruited from various regions across Poland. Upon receiving written informed consent from the parents or guardians, these recruited children and adolescents were invited for an anthropometric examination and blood collection in a pediatric clinic located in Wroclaw, Poland.

Anthropometric measurements

Anthropometric parameters, specifically body height, and weight, were collected during these examinations. The measurements were obtained by a team of trained personnel, composed of two doctors (including the authors of this study) and the pediatric nurse. They followed standardized techniques to ensure the consistency and reliability of the measurements [10]. These standardized techniques were reviewed and agreed upon prior to the initiation of the study. Body weight and height were recorded using standardized techniques and devices. Participants were weighed using electronic digital scales (OMRON BF-515), with light clothing and barefoot, with measurements to the nearest 0.1 kg. Body height was measured to the nearest 0.1 cm using a stadiometer (SECA 264). The trained personnel ensured the correct body posture of the child during the measurement: straight back, both feet on the ground, and the back of the body pressed against the wall. The same devices were used for all measurements, and conditions were kept consistent throughout the study. BMI was subsequently calculated using the traditional formula: weight (kg) divided by the square of height (m^2).

BMI Standard Deviation Score (SDS) calculation

Body mass index was converted into SDS using the LMS-Growth Calculator [11], a Microsoft Excel add-in. The calculator applies the LMS method to fit centiles and SDS into the collected data [12]. The LMSGrowth program uses the British 1990 growth reference charts, which were used as the standard for this study [13, 14]. For the purposes of this study, the following BMI percentile (BMI PC) ranges were adopted: < 3rd PC (underweight), 3rd–90th PC (norm), 90–97th PC (overweight), and > 97th PC (obesity). For the 30% of our participants who were over the age of eighteen (the oldest had 34 years), their data were referenced to the maximum age on the percentile grids, which is 18 years. This adjustment allowed us to include these individuals in the study while maintaining consistency in the use of the growth charts.

Homeostasis Model Assessment (HOMA-IR) Index

The HOMA-IR, a method used to quantify insulin resistance and beta-cell function, was calculated from fasting glucose and insulin concentrations using the following formula:

HOMA-IR = (fasting insulin concentration (uIU/ml) × fasting glucose concentration (mmol/l)) / 22.5

The denominator "22.5" is a normalizing factor derived from the product of fasting glucose and insulin concentrations in healthy individuals. This formula provides an estimate of insulin resistance (HOMA-IR) when the beta-cell function is at 100%. HOMA-IR values above 2.5 indicate insulin resistance. To systematically present the results, the following classification was adopted: less than 2.5 is considered a physiologically normal result, while a result greater than 2.5 is generally seen as abnormal.

Laboratory methods

Subsequent to the anthropometric measurements, venous blood samples were collected from the participants by the qualified pediatric nurse. These biological samples were then processed and examined for TAS, TOS, fasting glucose, and

insulin in the laboratory of Wroclaw Medical University. To assess metabolic control, both fasting glucose and insulin levels were examined in the study participants. After an overnight fast of at least 8 hours, blood samples were collected from all participants in both groups. The TAS and TOS were expressed as equivalents of Trolox [mmol/l] [15].

Fasting glucose and insulin

The fasting blood glucose levels were results expressed in milligrams per deciliter (mg/dl). The normal reference range for children's fasting glucose is typically between 70–100 mg/dl [16]. Fasting insulin levels were determined by a radioimmunoassay method. The insulin levels were measured in micro international units per milliliter (uIU/ml), with normal fasting insulin levels in children typically falling below 15 uIU/ml [17].

Oxidative stress index (OSI) calculation

Oxidative stress index was computed as the ratio of TOS to TAS. The OSI formula used for this calculation was: OSI = (TOS / TAS) × 100. This calculation was performed for each individual participant in both groups.

Data analysis

Data analysis was performed utilizing Statistica version 13 software. The normality of the data distribution was assessed using the Shapiro-Wilk test. Subsequent analyses were guided by the results of this normality test. In instances where the data followed a normal distribution, the Student's *t*-test was employed. Alternatively, when data exhibited a non-normal distribution, the Mann-Whitney U test was utilized. To identify relationships between variables, correlation matrices were created. Pearson's correlation was employed for data with a normal distribution, whereas Spearman's correlation was used for data with a non-normal distribution. The level of statistical significance was set at $p < 0.05$.

Results

The investigation observed significant disparities in various health parameters between SG and CG. Primary data encompassing the demographic details of the study and control groups, along with the assessed parameters, are presented in Table I.

BMI SDS and BMI percentiles

Regarding BMI, the DS group showed a higher average BMI (22.20 kg/m^2) than the CG (20.20 kg/m^2). Similarly, the average BMI Standard Deviation Score (BMI SDS) for the DS group was 0.79, whereas it was –1.19 for the CG. The comparison of the BMI percentile (BMI PC) was higher for the DS group than in the

Table I. Group demographic characteristics

	Study group (n = 42)					Control group (n = 20)					p-value
	Mean	Median	Min.	Max.	SD	Mean	Median	Min.	Max.	SD	
AGE	14.17	14.5	1.58	26.92	6.58	15.92	15.25	2.33	34.17	8.36	×
BMI	22.20	21.70	10.38	43.79	5.89	20.20	20.79	4.14	30.62	5.36	0.2005
BMI SDS	0.79	1.01	–5.82	3.80	1.63	–1.19	0.23	–34.54	3.05	7.91	0.1264
BMI PC	70.56	84.34	0.00	99.99	31.42	60.97	58.78	0.00	99.89	29.67	0.1282
Fasting glucose [mg/dl]	86.62	87.50	69.29	111.26	8.80	86.42	84.49	73.28	107.16	8.66	0.9327
Fasting insulin [uIU/ml]	10.63	9.56	2.46	26.52	5.94	11.14	9.24	2.66	28.49	6.82	0.9459
TAS [mmol/l Trolox]	1.92	1.92	1.52	2.28	0.15	1.79	1.83	1.57	2.02	0.13	**0.0015**
TOS [mmol/l Trolox]	51.52	42.85	13.13	238.15	37.43	33.05	35.93	10.89	52.19	11.01	**0.0147**
OSI	2680.22	2475.02	719.82	12735.03	1952.23	1855.67	1949.75	579.09	2870.51	633.08	**0.0388**
HOMA-IR Index	2.29	2.02	0.19	6.28	1.378	2.45	1.90	0.56	6.83	1.70	0.6930

N – the amount of participants; BMI – body mass index; BMI SDS – body mass index standard deviation score; BMI PC – body mass index percentiles; TAS – total antioxidative stress; TOS – total oxidative stress; OSI – oxidative stress status

Table II. Distribution of participants in the study and control groups across different BMI categories

	N	< 3rd PC		3–90th PC		90–97th PC		> 97th PC	
		Underweight		Normal weight		Overweight		Obese	
		n	%	*n*	%	*n*	%	*n*	%
Study group	42	2.00	4.76	21.00	50.00	11.00	26.19	8.00	19.05
Control group	20	1.00	5.00	15.00	75.00	3.00	15.00	1.00	5.00

N – amount of participants; PC – percentiles

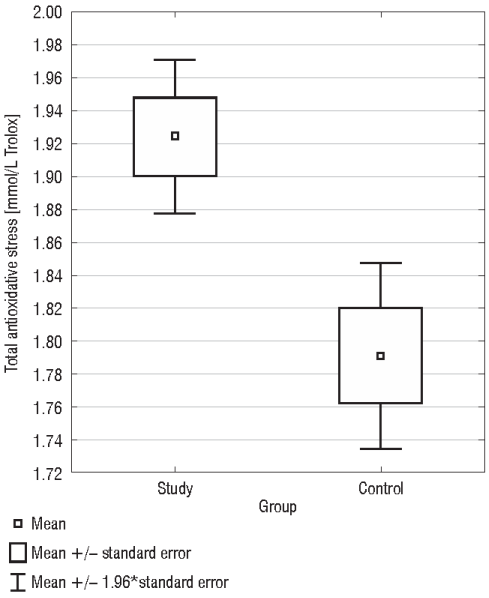

Figure 1. Comparison of TAS in the study and control group

CG (70.56 vs. 60.97). We observed no significant differences between the two groups when comparing BMI (p = 0.2005), BMI SDS (p = 0.1264), and BMI PC (p = 0.1282). Further percentile calculations based on BMI SDS and LMSGrowth (Table II) for the DS group indicate that while 2 individuals (4,76%) were underweight (below the 3rd percentile), the majority (21 individuals; 50%) fell within the normal range (3rd to 90th percentile). However, a significant proportion was overweight (11 individuals [29,19%] in the 90th to 97th percentile range) and obese (8 individuals [19,05%] exceeding the 97th percentile). When the same percentile calculations (Table II) were applied to the CG, a notably lower prevalence of overweight and obesity was found. While one individual (5%) was underweight (below the 3rd percentile), the majority (15 individuals; 75%) fell within the normal range (3rd to 90th percentile). Only three individuals (15%) were overweight (90th to 97th percentile) and one (5%) was classified as obese (above 97th percentile). The observed trends underline a generally lower prevalence of overweight and obesity in the CG compared to the SG.

Total antioxidant status

Total antioxidant status presented noticeable differences between the DS group and the control group. The DS group exhibited a higher TAS median as opposed to the CG (1.92 mmol/l vs. 1.79 mmol/l; 1.92 mmol/l vs 1.83 mmol/l) (p = 0.0015) (Fig. 1). Only in the CG, a positive correlation was found between BMI and TAS (r = 0.4459), but not in the SG. In Table I, basic statistics related to TAS are presented.

Total oxidant status

Total oxidant status exhibited a statistically significant difference between the SG and the CG (p = 0.0147) (Fig. 2). The DS group had a higher TOS median than the CG (51.52 mmol/l vs. 33.05 mmol/l; 42.85 mmol/l vs. 35.93 mmol/l). In terms of variability, the DS group displayed a higher standard deviation for TOS (37.43 mmol/l) compared to the CG (11.01 mmol/l), suggesting greater variation in TOS values among the DS group. The confidence intervals for the standard deviation in the DS group (30.73 mmol/l to 47.90 mmol/l) and the CG (8.37 mmol/l to 16.07 mmol/l) did not overlap, indicating the observed difference in TOS variability between the groups is statistically significant. There was a negative correlation between BMI and TOS (r = –0.3957) in the DS group. In Table I basic statistics related to TOS are presented.

Oxidative stress index

Oxidative stress index, a measure of the overall oxidative stress status, demonstrated a statistically significant difference (p = 0.0388) between the SG and CG (Fig. 3). The median OSI for the SG was also higher (2475.02) than the CG (1949.75). The confidence intervals for the standard deviation in the DS group (1602.81 to 2497) and the CG (481.45 to 924.66) did not overlap, signifying the observed difference in OSI variability

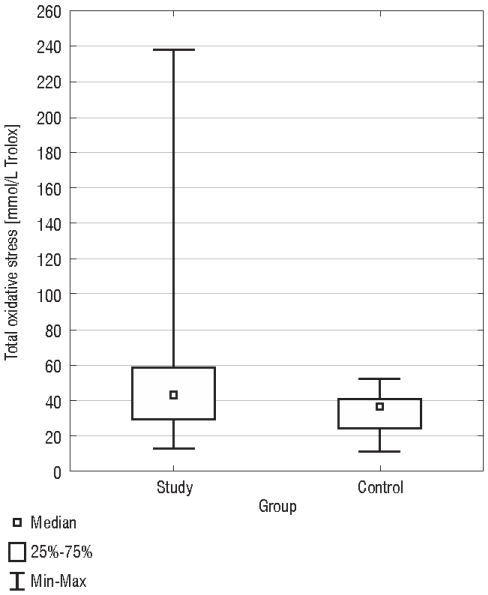

Figure 2. Comparison of TOS in the study and control group

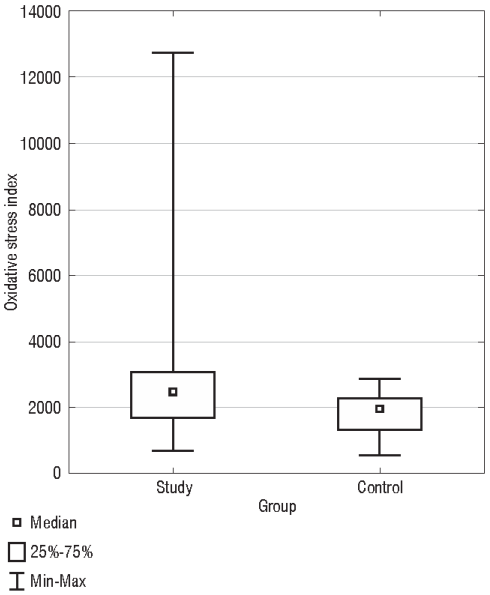

Figure 3. Comparison of OSI in the study and control group

between the groups is statistically significant. In Table I, basic statistics related to OSI are presented. We identified a negative correlation in the DS group between BMI and OSI ($r = -0.3925$) (Table III).

Fasting glucose and insulin

The analysis of fasting glucose levels revealed no statistically significant difference ($p = 0.9327$) between the SG and CG (Fig. 5). In the CG, but not in the SG, there was a negative correlation between BMI and fasting glucose levels ($r = -0.4887$).

The analysis of fasting insulin levels showed no statistically significant difference between the SG and CG ($p = 0.9459$) (Fig. 5). Additionally, in the DS group, there was a positive correlation between BMI PC and fasting insulin levels ($r = 0.3580$) (Table III). Only in the CG, we found a positive correlation between fasting insulin levels and fasting glucose levels ($r = 0.5468$). In both, the study and control groups, no statistically significant correlations were found between fasting glucose and TAS, TOS, OSI; as well as between fasting insulin and TAS, TOS, OSI. In Table I, basic statistics related to fasting glucose and insulin are presented.

HOMA-IR

In the SG the mean value of HOMA-IR was 2.29 ±1.37. No statistically significant difference was detected between the two groups ($p = 0.6930$). In Table I, basic statistics related to HOMA-IR are presented. Notably, several statistically significant relationships: positive between HOMA-IR and BMI SDS ($r = 0.4348$), HOMA-IR and BMI PC ($r = 0.4369$), and negative HOMA-IR and TOS ($r = -0.4605$), OSI ($r = -0.3692$) (Table III). In the CG, a positive statistically significant correlation was found between HOMA-IR and insulin, which is expected given that the HOMA-IR is calculated based on fasting insulin and glucose.

Discussion

Considering the link between obesity and metabolic disorders like insulin resistance, type 2 diabetes, and heart diseases, especially in those with DS, is crucial. Their higher obesity rates call for strategies to prevent excessive weight gain. This includes lifestyle changes like a balanced diet, regular exercise, and weight management. Early obesity detection can prevent severe metabolic issues. Given the DS group's unique metabolic traits, personalized obesity management is essential, as a generic approach might not meet their specific needs. In terms of BMI, despite the DS group showing higher mean BMI, BMI SDS and BMI PC values than their siblings in the CG, these differences did not reach statistical significance. This might suggest that environmental factors, shared by siblings, such as diet and activity level, play a considerable role in determining BMI, regardless of the presence of DS. However, when analysing the distribution of individuals within specific BMI categories, a distinct trend emerged. The DS group had a noticeably

Table III. Correlation matrix of BMI, fasting glucose, fasting insulin, TAS, TOS, OSI and HOMA-IR Index

	G	BMI [kg/m²]	BMI SDS	BMI PC	Glucose [mg/dl]	Insulin [ulU/ml]	TAS [mmol/l Trolox]	TOS [mmol/l Trolox]	OSI	HOMA
BMI [kg/m²]	S		**0.780769**	**0.718686**	–0.152206	0.268096	0.002883	**–0.395740**	**–0.392590**	0.225365
BMI SDS		**0.780769**		**0.904398**	–0.099885	0.243108	–0.047355	**–0.413594**	**–0.394741**	**0.434846**
BMI PC		**0.718686**	**0.904398**		–0.072195	**0.358061**	0.020542	**–0.357004**	**–0.352602**	**0.436791**
Glucose [mg/dl]		–0.152206	–0.099885	–0.072195		0.135332	–0.190641	–0.091373	–0.073209	**0.356726**
Insulin [ulU/ml]		0.268096	0.243108	**0.358061**	0.135332		–0.079789	–0.253373	–0.255693	**0.919729**
TAS [mmol/l Trolox]		0.002883	–0.047355	0.020542	–0.190641	–0.079789		0.089604	–0.009716	–0.084606
TOS [mmol/l Trolox]		**–0.395740**	**–0.413594**	–0.357004	–0.091373	–0.253373	0.089604		**0.993895**	–0.460470
OSI		**–0.392590**	**–0.394741**	–0.352602	–0.073209	–0.255693	–0.009716	**0.993895**		–0.369206
HOMA-IR		0.225365	0.434846	0.436791	0.356726	0.919729	–0.084606	–0.460470	–0.369206	
BMI [kg/m²]	C		**0.762311**	**0.704675**	**–0.488733**	–0.343101	**0.445916**	0.085573	–0.027085	–0.233170
BMI SDS		**0.762311**		**0.584655**	–0.336445	–0.298648	0.430341	–0.061357	–0.164534	0.046634
BMI PC		**0.704675**	**0.584655**		–0.020542	–0.104921	0.353643	–0.238193	–0.319371	0.046634
Glucose [mg/dl]		**–0.488733**	–0.336445	–0.020542		0.546800	–0.153649	0.109161	0.127810	0.435502
Insulin [ulU/ml]		–0.343101	–0.298648	–0.104921	**0.546800**		–0.146526	–0.000675	0.044951	0.993607
TAS [mmol/L Trolox]		**0.445916**	0.430341	0.353643	–0.153649	–0.146526		0.002389	–0.235165	–0.131678
TOS [mmol/L Trolox]		0.085573	–0.061357	–0.238193	0.109161	–0.000675	0.002389		**0.969845**	–0.063182
OSI		–0.027085	–0.164534	–0.319371	0.127810	0.044951	–0.235165	**0.969845**		–0.007522
HOMA-IR		–0.233170	0.046634	0.046634	0.435502	0.993607	–0.131678	–0.063182	–0.007522	

Bold values indicate statistically significant correlations ($p < 0.05$).
G – group; S – study group; C – control group; BMI – body mass index; TAS – total antioxidant status; TOS – total oxidant status; OSI – oxidative stress index

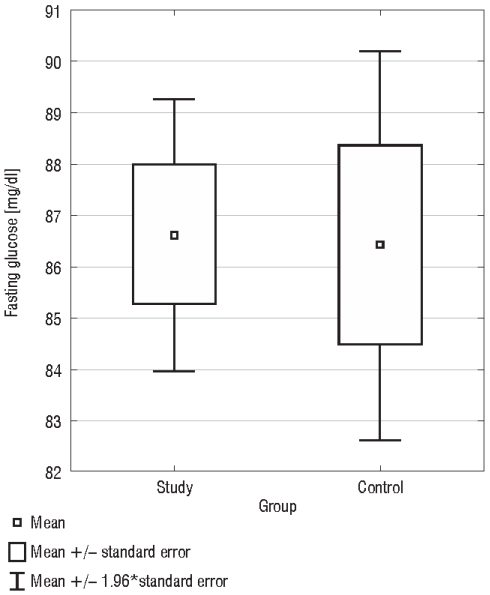

Figure 4. Comparison of fasting glucose in the study and control group

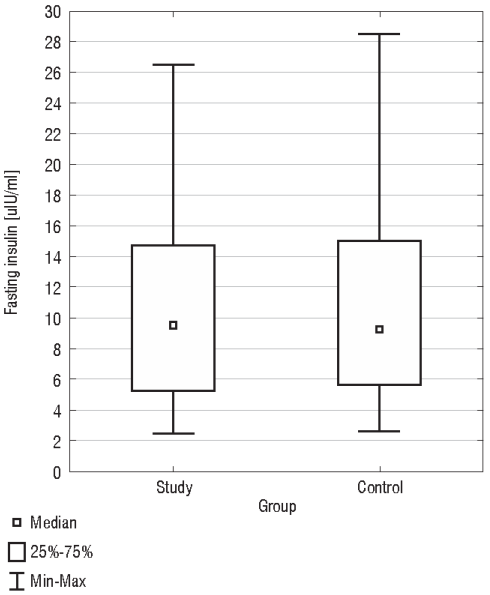

Figure 5. Comparison of fasting insulin in the study and control group

higher proportion of individuals categorized as overweight and obese, based on BMI SDS and BMI PC. Approximately half of the individuals in the DS group were within the normal range, but a significant proportion was overweight and obese. This is concerning and indicates an increased risk of weight-related health issues. Conversely, in the CG, composed of the siblings of the DS group, there was a considerably lower prevalence of overweight and obesity. This sharp contrast underlines the potential impact of DS on weight gain and distribution, beyond the shared environmental factors within families. The higher prevalence of overweight and obesity in the DS group compared to their siblings could potentially be attributed to various factors, including genetic predisposition, metabolic variations, differences in physical activity, or other DS-related health conditions. This is in line with previous studies showing that individuals with DS are at a higher risk of being overweight and obese, potentially due to factors such as hypotonia, reduced physical activity, and altered metabolism [18, 19]. The redox status, reflecting the balance between oxidants and antioxidants in the body, plays a pivotal role in maintaining physiological homeostasis. Our exploration of TAS, TOS and OSI revealed significant variations between the DS group and the CG, highlighting notable differences in their oxidative balance. The DS group consistently showed higher values across these measures, suggesting a distinct oxidative profile that might be shaped by inherent genetic condition. These findings emphasize the importance of monitoring oxidative stress markers in individuals with DS, as it could have far-reaching implications for their health outcomes. Oxidative stress has been implicated in various aspects of DS, such as intellectual disability and premature aging, suggesting the relevance of managing oxidative stress in this population [21–24]. Despite differing oxidative markers, the DS group's fasting glucose and insulin levels didn't significantly differ from their non-DS siblings. The degree of insulin resistance, gauged by HOMA-IR, also did not show a significant difference in the DS group, although a higher incidence of abnormal HOMA-IR was reported. Correlation findings suggest a complex interplay between BMI, oxidative stress, and metabolic indicators in DS individuals, with a higher BMI potentially associated with a lower oxidative stress state. These results highlight DS's complexity, where increased oxidative stress can coexist with certain preserved metabolic processes like glucose and insulin homeostasis. Alternatively, the elevated BMI observed in the DS group could potentially be a consequence of adaptive physiological responses to heightened oxidative stress. The correlations observed indeed suggest a complex interplay between BMI, oxidative stress parameters (TOS and OSI), and metabolic indicators. While we must be cautious in drawing causal relationships from correlation data, the trends offer some interesting avenues for interpretation and further exploration. The negative correlation between BMI and oxidative stress parameters in the DS group might suggest that

oxidative stress parameters decrease as BMI increases. This could potentially be due to a protective mechanism, wherein individuals with higher BMI produce more antioxidants to mitigate the damaging effects of oxidative stress. Adipose tissue has been shown to express antioxidant enzymes and may play a role in modulating oxidative stress [25, 26]. However, it is also possible that the increased oxidative stress observed in individuals with DS could stimulate physiological changes that result in increased BMI. Chronic oxidative stress has been linked with inflammation and insulin resistance, which can drive changes in metabolism and energy storage, potentially leading to increased body weight [27–29]. The positive correlation between BMI percentile and fasting insulin in the DS group may imply increasing insulin resistance as BMI rises. However, in the CG, a negative correlation between BMI and fasting glucose was observed, suggesting fasting glucose levels increase as BMI decreases – a seemingly counterintuitive finding given that higher BMI often correlates with increased glucose levels due to insulin resistance. This unexpected result calls for further investigationThe positive correlation between BMI and TAS in the CG could potentially suggest that as BMI increases, so does the antioxidant status. The multifaceted and intricate interplay between BMI, oxidative stress parameters, and metabolic indicators in individuals with DS uncovered in the study reinforces the necessity for continued research in this area to elucidate the mechanisms underlying these correlations. Future studies could explore these relationships in greater depth, potentially incorporating longitudinal designs, larger sample sizes, and more diverse participant groups. The observed positive link between BMI percentile and fasting insulin in the DS group suggests that higher BMI could increase insulin resistance risk. In contrast, the CG showed an inverse correlation between BMI and fasting glucose, and a positive correlation between fasting insulin and glucose, indicating a normal insulin response. A positive correlation between BMI and TAS may mean that as BMI increases, antioxidant activity rises potentially to offset metabolic stress. These interactions underscore the complex relationship between genetic, environmental, and metabolic factors in DS and potential risk factors for future health complications. In the conducted study, BMI had an impact on many laboratory parameters. It's important to remember that entire families should be involved in the problem of controlling excessive body weight [30, 31].

Limitations and strengths of the study

This study, unique in comparing DS children to their non-DS siblings, provides broad insights into their health and potential genetic and environmental influences. It identifies meaningful correlations that contribute to DS research and future healthcare management, setting the stage for more studies. Limitations include a small sample size potentially affecting results' power and applicability, possible bias from using non-DS siblings as controls, uncontrolled variables like diet and physical activity, and lack of established causality from correlations. Single-point measurements may not reflect long-term health. However, acknowledging these constraints strengthens the study's credibility and guides future research.

Conclusions

Despite similar family environments, DS children and adolescents were more often overweight, suggesting DS's role in weight gain. They showed higher oxidative stress values, indicating a distinct redox state, potentially impacting their health. Even with comparable fasting glucose and insulin levels to the CG, the DS group showed higher incidences of abnormal HOMA-IR values, suggesting potential insulin resistance. There were complex correlations between HOMA-IR, BMI, and oxidative stress, suggesting protective mechanisms or potential risks in DS. The link between higher BMI and fasting insulin in DS may indicate a risk of insulin resistance. These findings emphasize the need for further research and tailored health strategies for DS individuals.

Acknowledgments

Our greatest thanks go to the children and their families who participated in this study, the parents' interest groups and organizations that assisted in reaching those families.

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7. ARTYKUŁ CZWARTY

Pediatric Endocrinology Diabetes and Metabolism
Endokrynologia, Diabetologia i Choroby Przemiany Materii Wieku Rozwojowego

Original paper | Praca oryginalna
Pediatr Endocrinol Diabetes Metab 2023; 29
DOI: https://doi.org/10.5114/pedm.2023.131162

Predisposition to atherosclerosis in children and adults with trisomy 21: biochemical and metabolomic studies

Predyspozycje do miażdżycy u dzieci i dorosłych z trisomią 21: badania biochemiczne i metabolomiczne

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Abstract

Introduction: Atherosclerosis, a precursor to cardiovascular disease (CVD), is deeply intertwined with lipid metabolism. The metabolic process in the Down syndrome (DS) population remain less explored.

Aim of the study: This study examines the lipid profiles of DS in comparison to their siblings (CG), aiming to uncover potential atherosclerotic and CVD risks.

Material and methods: The study included 42 people with DS (mean age 14.17 years) and the CG – 20 individuals (mean age 15.92 years). Anthropometric measurements: BMI, BMI SDS, and TMI were calculated. Lipid profile (LP) and metabolomics were determined.

Results: LP: DS display significantly reduced HDL (DS vs CG: 47± 10 vs 59 ± 12 mg/dl; $p = 0{,}0001$) and elevated LDL (104 ±25 vs. 90 ±22 mg/dl; $p = 0{,}0331$). Triglycerides, APO A1, and APO B/APO A1 ratio corroborate with the elevated risk of CVD in DS. Despite no marked differences in: TCH and APO B, the DS group demonstrated a concerning BMI trend. Of 31 identified metabolites, 12 showed statistical significance (acetate, choline, creatinine, formate, glutamine, histidine, lysine, proline, pyroglutamate, threonine, tyrosine, and xanthine). However, only 8 metabolites passed the FDR validation (acetate, creatinine, formate, glutamine, lysine, proline, pyroglutamate, xanthine).

Conclusions: Down syndrome individuals show distinct cardiovascular risks, with decreased HDL and increased LDL levels. Combined with metabolomic disparities and higher BMI and TMI, this suggests an increased atherosclerosis risk compared to controls.

Key words:

Introduction

Atherosclerosis is a chronic inflammatory disease, marked by the accumulation of lipids and fibrous materials within arterial walls, leading to cardiovascular disease (CVD) – one of the major health concerns worldwide. The development of this disease is influenced by a combination of genetic factors, environmental exposures, and various metabolic processes. Notably, lipid metabolism plays a significant role. Excessive levels of low-density lipoprotein cholesterol (LDL), known as "bad cholesterol", accumulate in arterial walls, forming the basis of atherosclerotic plaque [1]. Conversely, high-density lipoprotein (HDL), termed "good cholesterol", helps in removing cholesterol from arterial plaques, directing it towards elimination from the body [2]. The relationship between apolipoprotein A1 (APO A1) and apolipoprotein B (APO B) is crucial in understanding atherosclerotic risk [2]. Besides, emerging studies have highlighted the roles of homocysteine (HCY) and lipoprotein(a) (LP(a)) in vascular health, given their correlation with arterial damage and atherosclerosis [3, 4].

Metabolomics, which focuses on identifying and quantifying small molecules in biological samples, has become instrumental in cardiovascular research [5–12]. Through metabolomics, researchers can gain a clearer picture of the numerous metabolic processes taking place at a cellular level. In the context of atherosclerosis, this approach has identified new path-

Received: 18.08.2023
Accepted: 10.09.2023
Conflict of interests: none declared

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ways and molecules of interest [13–16]. Specific metabolites, including acetate, choline, and xanthine, might offer insights into the relationship between Down syndrome (DS) metabolism and atherosclerosis [17, 19].

There's a notable gap in our understanding when considering unique populations like those with trisomy 21 (DS). Historically, individuals with DS display a reduced incidence of atherosclerosis [20, 21], but the metabolic reasons behind this remain elusive.

This study seeks to fill the knowledge gap by providing a comprehensive evaluation of the lipid profiles and related metabolites in individuals with DS. We aimed to discern whether the DS population (study group; DS) is at a heightened risk for atherosclerosis and, by extension, CVD, by comparing their lipid and metabolomic profiles to a control group (CG) – their siblings.

Material and methods

Study design and participants

The participants comprised a study group (DS) of 42 children and adolescents with DS (17 females; mean age of 14.17 ±7 years old) and the control group (CG) of 20 siblings of the individuals with DS (10 females; mean age of 15.92 ±9 years old). The study protocol adhered to the principles of the Declaration of Helsinki, and the Bioethics Committee of Wroclaw Medical University approved it (KB 674/2020). The inclusion criteria for the DS were patients diagnosed with DS (due to genetic test results) while for the CG, it was being a sibling of an individual DS. There were no specific exclusion criteria. Written informed consent was obtained from the parents or legal guardians of all the participants before the data collection and anthropometric measurements. Administrative approvals were also secured from each institution involved to access the necessary participants' data.

Data collection

Participants were actively recruited from various regions across Poland. Upon receiving written informed consent from the parents or guardians, these recruited children and adolescents were invited for an anthropometric examination and blood collection in a pediatric clinic located in Wroclaw, Poland.

Anthropometric measurements

The methodology and procedures for anthropometric measurements have been detailed in our prior research [22].

BMI Standard Deviation Score (SDS) and Tri-Ponderal Mass Index (TMI) Calculation

BMI was converted into SDS in line with our previously outlined methodologies [20]. The TMI was calculated according to the methodology described by Peterson *et al.* [23]. For this study, the threshold to determine elevated TMI was set at the 95th percentile – specifically, values surpassing 18.8 kg/m^3 were considered elevated for boys, while for girls, the 95th percentile value was set at 19.7 kg/m^3 [23]. Before proceeding with the TMI analysis, participants aged below 7 years were excluded from the dataset. This led to the exclusion of nine individuals: 6 from the DS and 3 from the CG. Upon extending the analysis of the TMI to a larger cohort, we incorporated participants older than 18 years by categorizing them within the 17-year-old bracket. This methodological choice was made to expand our sample size and assess broader patterns without the age restrictions strictly imposed by the traditional TMI guidelines [23].

Biochemical tests

All laboratory parameters were determined under fasting conditions. For lipid profile routine laboratory methods were used. APO A1 and APO B were measured by immunotubidimetric method with goat anti-human apolipoprotein A1 antibody and anti-human apolipoprotein B antibody, respectively, whereas for Lp(a) determination particle enhanced immunoturbidimetric test was applied. Homocystein was measured with Diazyme Laboratories, Poway, USA reagent kit on the Konelab 20i analyser (ThermoScientific, Vantta, Finland). The research results were analyzed according to the established reference ranges presented in Table I.

Metabolomic tests

Samples

Metabolomic studies were conducted at the Department of Biochemistry, Molecular Biology, and Biotechnology, Faculty of Chemistry, Wroclaw University of Science and Technology. Serum samples were received from the Medical University of Wroclaw and were stored at –80°C before analysis.

Samples preparation

The volume of 300 μl serum was transferred into the new Eppendorf tube. To each sample, 600 μl of methanol was added. After 1 min mixing, the samples were stored at –20°C for 20 min and centrifugated (15 min, 11000 rpm, 4°C). Next, 750 μl of supernatant was transferred into the new Eppendorf tube, and samples were evaporated (1100 rpm, 4.5 h, 40°C). After that, 600 μl of PBS buffer (0.5 M, 50% D_2O, pH = 7.4, TSP = 0.03 mM) were added to each sample and mixed for 3 min. After centrifugation (5 min, 11000 rpm, 4°C), 550 μl of solution were transferred into 5-mm NMR tubes (Norell®) for measurements. Before analysis, the samples were stored at 4°C.

^{1}H NMR spectroscopy analysis of the bacterial metabolites

Standard ^{1}H NMR experiments were performed on a Bruker AVANCE II 600.58 MHz spectrometer equipped with a 5 mm NMR probe at 298 K. All one-dimensional ^{1}H NMR spectra were carried out using the noesy1dpr (in Bruker notation) pulse sequence by suppression of water resonance by presaturation. Acquisition parameters were as follows: spectral width, 19.82 ppm; the number of scans, 128; acquisition time, 2.75 s per scan; relaxation delay, 3.5 s; and time-domain points, 65.5 K. The spectra were referenced to the TSP resonance at 0.0 ppm and manually corrected for phase and baseline (MestReNova v. 14.0.2).

Table I. Reference ranges used for result interpretation

Parameter	Age/Sex	Below norm	Norm	Above norm
TCH [mg/dl]	Children	×	< 170	≥ 170
	Adults	×	< 200	≥ 200
HDL [mg/dl]	Children	≤ 35	> 35	×
	Adults (women)	< 50	≥ 50	×
	Adults (men)	< 40	≥ 40	×
LDL [mg/dl]	Children	×	< 110	≥ 110
	Adults		<100	≥ 100
TG [mg/dl]	Children	×	<100	≥ 100
	Adults	×	<150	≥ 150
HCY [umol/L]	x	×	<15	≥ 15
APO A1 [mg/dl]	Women	< 108	108-125	> 125
	Men	< 104	104-202	> 202
APO B [mg/dl]	x	< 50	50-150	> 150
Lp(a)	x		< 30	≥ 30
APO B/APO A1	Women	×	< 0.8	≥ 0.8
	Men	×	< 0.9	≥ 0.9

TCH – total cholesterol; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglycerides; APO A1 – apolipoprotein A1 ; APO B – apolipoprotein B; HCY – homocysteine; Lp(a) – lipoprotein(a); APO B/APO A1 – ratio.

Data processing and multivariate statistical data analysis

Data analysis was performed utilizing Statistica version 13 software. The normality of the data distribution was assessed using the Shapiro-Wilk test. Subsequent analyses were guided by the results of this normality test. In instances where the data followed a normal distribution, the Student's t-test was employed. Alternatively, when data exhibited a non-normal distribution, the Mann-Whitney U test was utilized. To identify relationships between variables, correlation matrices were created. Pearson's correlation was employed for data with a normal distribution, whereas Spearman's correlation was used for data with a non-normal distribution. The level of statistical significance was set at $p < 0.05$. All spectra were exported to Matlab (Matlab v. 8.3.0.532) for preprocessing. Regions affected by solvent suppression were excluded (4.7–5.1 ppm) and alignment procedures involving the correlation of optimized warping (COW) and interval correlation shifting (icoshift) algorithms were applied [24, 25]. The spectra consisted of 8910 data points and were normalized using the probabilistic quotient method to overcome the issue of dilution [26]. The multivariate and statistical data analysis were performed on a set of the 30 assigned metabolites (methanol wasn't included in the analysis, because it was used for the extraction). The concentration of metabolite measured by NMR was obtained as the sum of the intensities of the no-overlapping resonances (or a part of partly overlapping resonances). The input for SIMCA-P software was a transformed data matrix (v 17.0.2, Umetrics, Umeå, Sweden). The data sets were unit variance scaled before the chemometric analysis. For T21 samples, principal component analysis (PCA), and partial least square analysis (OPLS) were carried out. The OPLS-DA model reliability was tested with CV-ANOVA at the level of significance of < 0.05. Univariate analysis was performed by the use of MATLAB software (v R2014a, Mathworks Inc.) by use of Student's *t*-test (equal/unequal variance) for data originated from a normal distribution, and Mann-Whitney-Wilcoxon test was performed for data that does not meet these requirements. The normality of distribution was assessed by the Shapiro-Wilk test. The Student's *t*-test was performed for metabolites recurring in both samples. The correction for multiple comparisons was preceded by the Benjamini-Hochberg procedure (FDR). All univariate statistics were carried out at the level of significance of $\alpha < 0.05$.

Results

Anthropometric data

BMI: The DS demonstrated a higher mean BMI and BMI SDS than the CG. Despite the absence of significant statistical disparities, it's imperative to highlight the contrast in overweight and obesity prevalence: about half of the DS group compared to just 20% in the CG. This accentuates the prominent weight concerns in the DS group. For a comprehensive view, refer to Tables II and IV.

TMI: The mean TMI for the DS was significantly higher than the CG (16.22 ±4.04 kg/m^3 vs 13.67 ±1.59 kg/m^3; $p = 0.02$) emphasizing a potential concern regarding body fat distribution within the DS compared to the CG.

Biochemical tests

Basic descriptive statistics are presented in Table II. Percentage data regarding specific parameters in both groups are presented in Table III.

HDL: The DS displayed a significantly lower concentration compared to the CG (46.88 ±10.37 mg/dl vs. 59.19 ±12.28 mg/dl; p = 0.0001). **Triglycerides (TG):** The DS had a noticeably elevated median TG level than the CG (87.02 mg/dl vs 63.34 mg/dl; p = 0.0588). **LDL:** A heightened concentration was observed in the DS when juxtaposed with the CG (104.30 ±25.41 mg/dl vs 89.78 ±22.43 mg/dl; p = 0.0331). **APO A1:** The DS exhibited a markedly lower concentration than the CG (119.64 ±20.17 mg/dl vs 158.21 ±40.61 mg/dl; p = 0.000005).

Other parameters: Statistical examination revealed no significant contrasts between DS and CG for **TCH** (p = 0.3747); **APO B** (p = 0.1612); **HCY** (p = 0.5320); **Lp(a)** (p = 0.3466), **APO B/APO A1 Ratio** (p = 0.3466).

Differential correlations between DS and CG

The correlation patterns among the various parameters can be found in Table V.

Homocysteine: Present in DS with correlations to body metrics, absent in CG; **HDL:** In DS, mainly correlated with APO

Table II. Comparative parameters analyzed for the DS and CG

Parameter	DS (n = 42)			CG (n = 20)			p-value
	Mean	Median	SD	Mean	Median	SD	
Age [years]	14.17	14.50	6.7	15.92	15.25	8.58	0.3804
Body weight [kg]	44.57	48.75	19.3	49.52	51.35	23.80	0.3852
Body height [m]	1.37	1.42	0.2	1.50	1.59	0.26	**0.0377**
BMI [kg2/m]	22.20	21.70	5.9	20.20	20.79	5.36	0.2006
BMI SDS	0.79	1.01	1.6	-1.19	0.23	7.91	0.1264
BMI PC	70.56	84.34	31.4	60.97	58.78	29.67	0.1283
TCH [mg/dl]	182.08	180.47	34.5	173.64	174.27	35.15	0.3747
HDL [mg/dl]	46.99	47.08	10.4	59.19	61.62	12.28	**0.0001**
LDL [mg/dl]	104.30	101.41	25.4	89.78	90.05	22.43	**0.0331**
TG [mg/dl]	97.57	87.02	53.4	68.42	63.34	27.58	**0.0588**
APOA1 [mg/dl]	119.64	119.14	20.2	158.21	149.69	40.61	**0.0000**
APOB [mg/dl]	67.40	64.18	16.8	61.08	63.60	15.52	0.1612
HCY [umol/l]	9.60	9.40	2.6	10.17	9.64	3.07	0.5320
Lp(a) [mg/dl]	22.63	7.66	33.8	21.24	4.27	38.39	0.3466
APOB/APOA1	0.58	0.54	0.2	0.39	0.40	0.10	0.3466
TMI [kg/m3]	16.22	15.22	4.0	13.67	13.30	1.59	**0.0200**

DS – study group; CG – control group; N – amount of participants; SD – standard deviation; BMI – body mass index; SDS – standard deviation scores; PC – percentiles; TCH – total cholesterol; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglycerides; PO A1 – apolipoprotein A1 ; APO B – apolipoprotein B; HCY – homocysteine; Lp(a) – lipoprotein(a); APO B/APO A1 – ratio; TMI – tri-ponderal mass index

Table III. Group demographic characteristics

Group	TCH (%)	HDL (%)	LDL (%)	TG (%)	APOA1 (%)	APOB (%)	HCY (%)	Lp(a) (%)	APOB/APOA1 (%)
DS									
Below norm	×	12.5	×	10	21.95	12.20	×	×	×
Norm	51.22	87.5	46.34	90	78.05	87.80	97.56	80.49	90.244
Above norm	48.78	×	53.66	×	×	×	2.44	19.51	9.756
CG									
Below norm	×	10	×	×	5	10	×	×	×
Norm	80	90	90	95	90	90	95	80	100
Above norm	20	×	10	5	5	×	5	20	×

DS – study group; CG – control group; PC – percentile; TCH – total cholesterol; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglycerides; APO A1 – apolipoprotein A1; APO B – apolipoprotein B; HCY – homocysteine; Lp(a) – lipoprotein(a); APO B/APO A1 – ratio.

Table IV. Percentage distribution of participants in the study and control groups across different BMI categories

Group	*N*	< 3rd PC		3–90th PC		90–97th PC		>97th PC	
		Underweight		Normal weight		Overweight		Obese	
		n	%	*n*	%	*n*	%	*n*	%
Study	42	2	4,76	21	50	11	26,19	8	19,05
Control	20	1	5	15	75	3	15	1	5

PC – percentile

A1; in CG, correlated with age and BMI; **TCH:** DS had multiple correlations (e.g., LDL, APO B, Lp(a)); CG's were fewer and included age; **APO B:** In DS, primarily linked to lipids; in CG, showed broader links including age and body metrics; **APO A1:** DS correlations centered around lipids; in CG, they were more diverse, involving HDL, TCH, TG, LDL, APO B; **LDL:** DS showed correlations including APO B and Lp(a); CG had fewer, like age; **APO B/APO A1 Ratio:** In DS, both positive and negative correlations were seen, including TCH and BMI metrics. In CG, it was mainly linked to Lp(a).

Metabolomics

In total, 31 metabolites were identified (3-hydroxybutyrate, acetate, acetone, alanine, choline, creatinine, ethanol, formate, glucose, glutamine, glycine, histidine, isobutyrate, isoleucine, lactate, leucine, lysine, methionine, methylamine, oxypurinol, phenylalanine, proline, pyroglutamate, pyruvate, succinate, threonine, tryptophan, tyrosine, valine, xanthine, methanol). All assignments were verified using the following databases (HMDB). Information about a chemical shift for each metabolite is available in Table VI. The representative ^{1}H NMR spectrum is presented below (Fig. 1).

Metabolites relative concentration and statistical analysis

For each metabolite, relative concentration and standard deviation were obtained. Statistical analysis was performed to obtain statistically important metabolites. Two groups were compared – Study Group (DS; 39 samples) and the Control Group (CG; 20 samples) (Table VI). The results showed that 12 metabolites were statistically important according to *p*-value (acetate, choline, creatinine, formate, glutamine, histidine, lysine, proline, pyroglutamate, threonine, tyrosine, and xanthine), while only 8 metabolites were statistically important according to FDR results, where choline, tyrosine, threonine, and histidine didn't pass the Benjamini-Hochberg procedure

Table V. Correlation matrices in DS and CG

		Age [years]	Body weight [kg]	Body height [m]	BMI [kg/m²]	BMI SDS	BMI PC	HDL [mg/dl]	TCH [mg/dl]	TG [mg/dl]	LDL [mg/dl]	APO A1 [mg/dl]	APO B [mg/dl]	HCY [umol/L]	Lp(a) [mg/dL]	APO
Age [years]	SG		**0.8136**	**0.7702**	**0.6346**	0.3048	0.3042	0.0164	0.0797	−0.2599	0.1028	−0.0533	0.0776	B/APO	0.0789	0.1083
Body weight [kg]		**0.8136**		**0.8649**	**0.8246**	**0.5697**	**0.6195**	−0.0973	0.0726	−0.0211	0.0805	−0.1658	0.0437	**0.3422**	0.0599	0.1470
Body height [m]		**0.7702**	**0.8649**		**0.4637**	0.2493	**0.3850**	−0.0899	0.1431	0.0064	0.1477	−0.0871	0.0381	0.2624	0.0252	0.0843
BMI [kg/m²]		**0.6346**	**0.8246**	**0.4637**		**0.7808**	**0.7187**	−0.0816	−0.0769	−0.1017	−0.0570	−0.1777	−0.0369	**0.3103**	0.0221	0.0941
BMI SDS		0.3048	**0.5697**	0.2493	**0.7808**		**0.9044**	−0.1521	−0.1938	0.0694	−0.1401	**−0.3901**	−0.1233	**0.3929**	0.0186	0.1269
BMI PC		0.3042	**0.6195**	**0.3850**	**0.7187**	**0.9044**		−0.2572	−0.0361	0.0662	0.0172	**−0.3552**	−0.0078	**0.3916**	0.0127	0.2014
HDL [mg/dl]		0.0164	−0.0973	−0.0899	−0.0816	−0.1521	−0.2572		0.0694	−0.2596	−0.1167	**0.7767**	−0.1838	−0.0406	−0.1681	**−0.6457**
TCH [mg/dl]		0.0797	0.0726	0.1431	−0.0769	−0.1938	−0.0361	0.0694		**0.4511**	**0.9695**	**0.3575**	**0.8622**	0.0605	**0.4036**	**0.5318**
TG [mg/dl]		−0.2599	−0.0211	0.0064	−0.1017	0.0694	0.0662	−0.2596	**0.4511**		**0.4158**	−0.0446	**0.4307**	0.0798	0.2477	**0.4676**
LDL [mg/dl]		0.1028	0.0805	0.1477	−0.0570	−0.1401	0.0172	−0.1167	**0.9695**	0.4158		0.1857	**0.9139**	0.1007	**0.4480**	**0.6756**
APO A1 [mg/dl]		−0.0533	−0.1658	−0.0871	−0.1777	**−0.3901**	**−0.3552**	**0.7767**	**0.3575**	−0.0446	0.1857		0.1252	0.0126	−0.0066	**−0.5088**
APO B [mg/dl]		0.0776	0.0437	0.0381	−0.0369	−0.1233	−0.0078	−0.1838	**0.8622**	**0.4307**	**0.9139**	0.1252		0.1354	**0.4840**	**0.7737**
HCY [umol/l]		0.1516	**0.3422**	0.2624	**0.3103**	**0.3929**	**0.3916**	−0.0406	0.0605	0.0798	0.1007	0.0126	0.1354		0.2352	0.1252
Lp(a) [mg/l]		0.0789	0.0599	0.0252	0.0221	0.0186	0.0127	−0.1681	**0.4036**	0.2477	**0.4480**	−0.0066	**0.4840**	0.2352		0.1672
APO B/APO A1		0.1083	0.1470	0.0843	0.0941	0.1269	0.2014	**−0.6457**	**0.5318**	**0.4676**	**0.6756**	**−0.5088**	**0.7737**	0.1252	0.1672	
Age [years]	CG		**0.6812**	**0.7840**	**0.5701**	−0.0526	−0.0526	**0.4963**	**0.5197**	−0.0658	**0.4845**	0.3489	**0.6922**	0.2117	0.0677	0.3698
Body weight [kg]		**0.6812**		**0.9440**	**0.9025**	0.4415	0.4415	0.3679	0.3467	0.2745	0.2376	0.2546	**0.5246**	0.3556	−0.0903	0.3225
Body height [m]		**0.7840**	**0.9440**		**0.8206**	0.2385	0.2385	0.4189	0.3961	0.2377	0.3059	0.2568	**0.5701**	0.4066	−0.0143	0.3849
BMI [kg/m²]		**0.5701**	**0.9025**	**0.8206**		**0.6767**	**0.6767**	**0.4776**	0.2446	0.0961	0.0697	0.2140	0.3563	0.2843	−0.0797	0.1798
BMI SDS		−0.0526	0.4415	0.2385	**0.6767**		1.0000	0.1759	0.2060	−0.0902	−0.0331	−0.0316	0.1624	0.0015	−0.2586	−0.0638
BMI PC		−0.0526	0.4415	0.2385	**0.6767**	1.0000		0.1759	0.2060	−0.0902	−0.0331	−0.0316	0.1624	0.0015	−0.2586	0.0822
HDL [mg/dl]		**0.4963**	0.3679	0.4189	**0.4776**	0.1759	0.1759		**0.6143**	0.1160	0.3681	**0.7091**	0.4012	−0.0980	0.0677	−0.2483
TCH [mg/dl]		**0.5197**	0.3467	0.3961	0.2446	0.2060	0.2060	**0.6143**		**0.4445**	**0.9469**	**0.7603**	**0.8361**	−0.1313	0.2120	0.2289
TG [mg/dl]		−0.0658	0.2745	0.2377	0.0961	−0.0902	−0.0902	0.1160	**0.4445**		0.3561	**0.4978**	0.2937	−0.0576	0.0346	−0.1000
LDL [mg/dl]		**0.4845**	0.2376	0.3059	0.0697	−0.0331	−0.0331	0.3681	**0.9469**	0.3561		**0.5977**	**0.8589**	−0.1178	0.3534	0.4216
APO A1 [mg/dl]		0.3489	0.2546	0.2568	0.2140	−0.0316	−0.0316	**0.7091**	**0.7603**	**0.4978**	**0.5977**		**0.5508**	−0.3302	0.2947	−0.3450
APO B [mg/dl]		**0.6922**	**0.5246**	**0.5701**	0.3563	0.1624	0.1624	0.4012	**0.8361**	0.2937	**0.8589**	**0.5508**		−0.0628	0.4376	**0.5651**
HCY [umol/l]		0.2117	0.3556	0.4066	0.2843	0.0015	0.0015	−0.0960	−0.1313	−0.0576	−0.1178	−0.3302	−0.0628		−0.2121	0.2965
Lp(a) [mg/l]		0.0677	−0.0903	−0.0143	−0.0797	−0.2586	−0.2586	0.0677	0.2120	0.0346	0.3534	0.2947	0.4376	−0.2121		**0.5837**
APO B/APO A1		0.3698	0.3225	0.3849	0.1798	−0.0638	0.0822	−0.2483	0.2289	−0.1000	0.4216	−0.3450	**0.5651**	0.2965	**0.5837**	

DS – study group; CG – control group; BMI – body mass index; SDS – standard deviation scores; PC – percentiles; TCH – total cholesterol; HDL – high-density lipoprotein; LDL – low-density lipoprotein; TG – triglycerides; APO A1 – apolipoprotein A1 ; APO B – apolipoprotein B; HCY – homocysteine; Lp(a) – lipoprotein(a); APO B/APO A1 – ratio

Table VI. Statistical analysis of metabolite levels: distinguishing between Study Group (DS) and Control Group (CG) using *p*-value and FDR criteria

Metabolite	*p*-value	FDR	Mean/ Median DS	Mean/ Median DS	RSD DS [%]	SD DS	RSD CG [%]	SD CG
Creatinine	1.67E-07	5.01E-06	0.111	0.203	24.69	0.027	26.30	0.053
Pyroglutamate	6.10E-07	9.15E-06	0.231	**0.677**	14.19	0.033	30.58	0.207
Glutamine	1.75E-06	1.75E-05	1.075	**0.120**	12.39	0.133	28.54	0.034
Formate	8.21E-06	5.31E-05	**2.246**	0.153	15.38	0.345	14.18	0.022
Proline	8.85E-06	5.31E-05	**1.630**	**2.033**	22.65	0.369	45.64	0.928
Acetate	1.28E-05	6.40E-05	**0.115**	**0.208**	25.57	0.029	38.83	0.081
Xanthine	3.30E-05	1.41E-04	0.117	0.143	15.61	0.018	17.57	0.025
Lysine	1.97E-04	7.38E-04	**0.125**	0.276	19.68	0.025	18.80	0.052
Choline	1.82E-02	6.06E-02	**5.280**	1.997	26.47	1.398	22.63	0.452
Tyrosine	2.07E-02	6.20E-02	0.224	**0.789**	15.28	0.034	15.66	0.124
Threonine	2.55E-02	6.95E-02	**1.041**	0.308	22.51	0.234	21.59	0.067
Histidine	4.21E-02	1.05E-01	0.152	0.134	19.66	0.030	24.15	0.032
Acetone	7.95E-02	1.84E-01	**0.692**	**0.687**	60.33	0.418	155.21	1.066
Succinate	1.30E-01	2.79E-01	0.249	**0.292**	43.86	0.109	34.91	0.102
Ethanol	1.48E-01	2.96E-01	0.528	0.503	10.83	0.057	14.48	0.073
Valine	2.27E-01	4.25E-01	1.140	**0.904**	15.30	0.174	20.03	0.181
Pyruvate	2.80E-01	4.94E-01	**0.326**	**0.271**	31.84	0.104	22.55	0.061
Isoleucine	3.11E-01	5.18E-01	0.328	0.308	22.31	0.073	20.37	0.063
3-hydroxybutyrate	3.46E-01	5.19E-01	0.148	0.142	16.56	0.025	17.25	0.024
Methionine	3.33E-01	5.19E-01	0.132	**0.100**	16.18	0.021	24.70	0.025
Glucose	3.66E-01	5.22E-01	**0.267**	5.208	15.88	0.042	14.30	0.745
Oxypurinol	3.83E-01	5.22E-01	**0.101**	**0.149**	33.28	0.033	28.64	0.043
Phenylalanine	4.04E-01	5.26E-01	0.264	0.274	14.78	0.039	18.52	0.051
Lactate	5.59E-01	6.99E-01	**0.129**	16.235	36.54	0.047	25.42	4.127
Isobutyrate	7.79E-01	8.71E-01	0.708	**0.186**	18.04	0.128	22.79	0.042
Methylamine	7.84E-01	8.71E-01	0.053	0.054	23.50	0.012	29.45	0.016
Tryptophan	7.78E-01	8.71E-01	0.150	0.152	18.82	0.028	17.31	0.026
Alanine	9.46E-01	9.46E-01	2.282	2.289	17.22	0.393	19.48	0.446
Glycine	9.04E-01	9.46E-01	**1.148**	**1.083**	22.13	0.254	21.98	0.238
Leucine	9.30E-01	9.46E-01	0.937	**0.138**	17.90	0.168	17.82	0.025

DS – study group; CG – control group

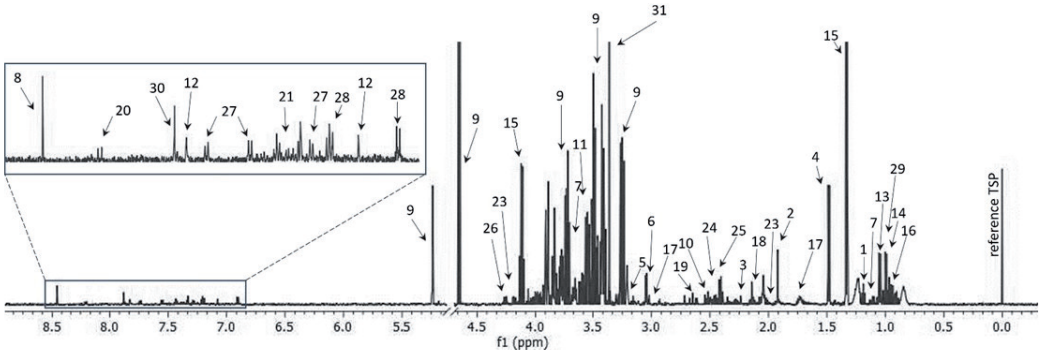

Figure 1. The representative 1D 1H NMR spectra of T21 sample (1 – 3-hydroxybutyrate, 2 – acetate, 3 – acetone, 4 – alanine, 5 – choline, 6 – creatinine, 7 – ethanol, 8 – formate, 9 – glucose, 10 – glutamine, 11 – glycine, 12 – histidine, 13 – isobutyrate, 14 – isoleucine, 15 – lactate, 16 – leucine, 17 – lysine, 18 – methionine, 19 – methylamine, 20 – oxypurinol, 21 – phenylalanine, 22 – proline, 23 – pyroglutamate, 24 – pyruvate, 25 – succinate, 26 – threonine, 27 – tryptophan, 28 – tyrosine, 29 – valine, 30 – xanthine, 31 – methanol)

Discussion

The findings of this study provide an elucidative insight into the biochemical and metabolic parameters that might predispose children and young adults with DS to atherosclerosis and subsequent CVD.

BMI implications on health profiles

The DS group displayed a higher tendency towards overweight and obesity compared to the CG group, a trend linked to considerable health risks including cardiovascular and metabolic issues [27, 28]. The heightened prevalence of increased weight in the DS population points to their potential vulnerability to related health complications [29, 30]. In contrast, the CG group showed a more balanced BMI, possibly due to a mix of metabolic, genetic, and lifestyle factors [31]. It is critical to recognize that being underweight, although less common in both groups, can also lead to health issues [32]. To analyze adolescent body fat distribution more accurately, the study introduced the TMI metric [23]. This method suggests the DS group might be prone to dangerous fat distribution patterns, indicative of greater health risks including visceral fat accumulation. The findings emphasize the need for targeted interventions and monitoring strategies to counter potential health threats.

HDL-cholesterol and its cardiovascular implications

The DS group exhibited notably lower HDL cholesterol levels compared to their CG siblings, potentially increasing their risk of cardiac issues due to the associated higher likelihood of developing atherosclerosis, a precursor to CVD [33]. However, the HDL distribution was similar between the two groups, suggesting that both groups might still benefit from the cardiovascular protective features generally associated with optimal HDL properties. It's important to consider not just the HDL concentration but also its functionality, which includes aspects like particle size and antioxidant enzymes, in assessing its cardioprotective potential [34, 35].

LDL-cholesterol: atherogenic risk

The DS group showed a significant increase in LDL levels compared to the CG group, which is worrisome given that LDL-cholesterol is a well-established indicator for cardiovascular risk and even slight elevations can substantially increase this risk over time [36, 37]. These elevated levels could mean a higher susceptibility to arterial plaque build-up and consequently, a greater risk of CVD [38]. The observed discrepancy prompts further exploration into the metabolic or genetic factors in individuals with DS that might be causing these higher LDL levels, indicating a potential predisposition to atherosclerotic developments [39].

Triglycerides: a borderline concern

While our study reveals an approaching significance in TG levels between the DS and CG. Elevated TG levels have been universally recognized as a marker for cardiovascular risk [40]. In the DS population, understanding the particular significance of these levels is crucial due to their unique metabolic and genetic profiles. A seminal paper by Capone et al. [39] highlighted the increased frequency of congenital heart disease among individuals with DS. With such a pre-existing predisposition, even borderline variations in TG levels can compound potential risks.

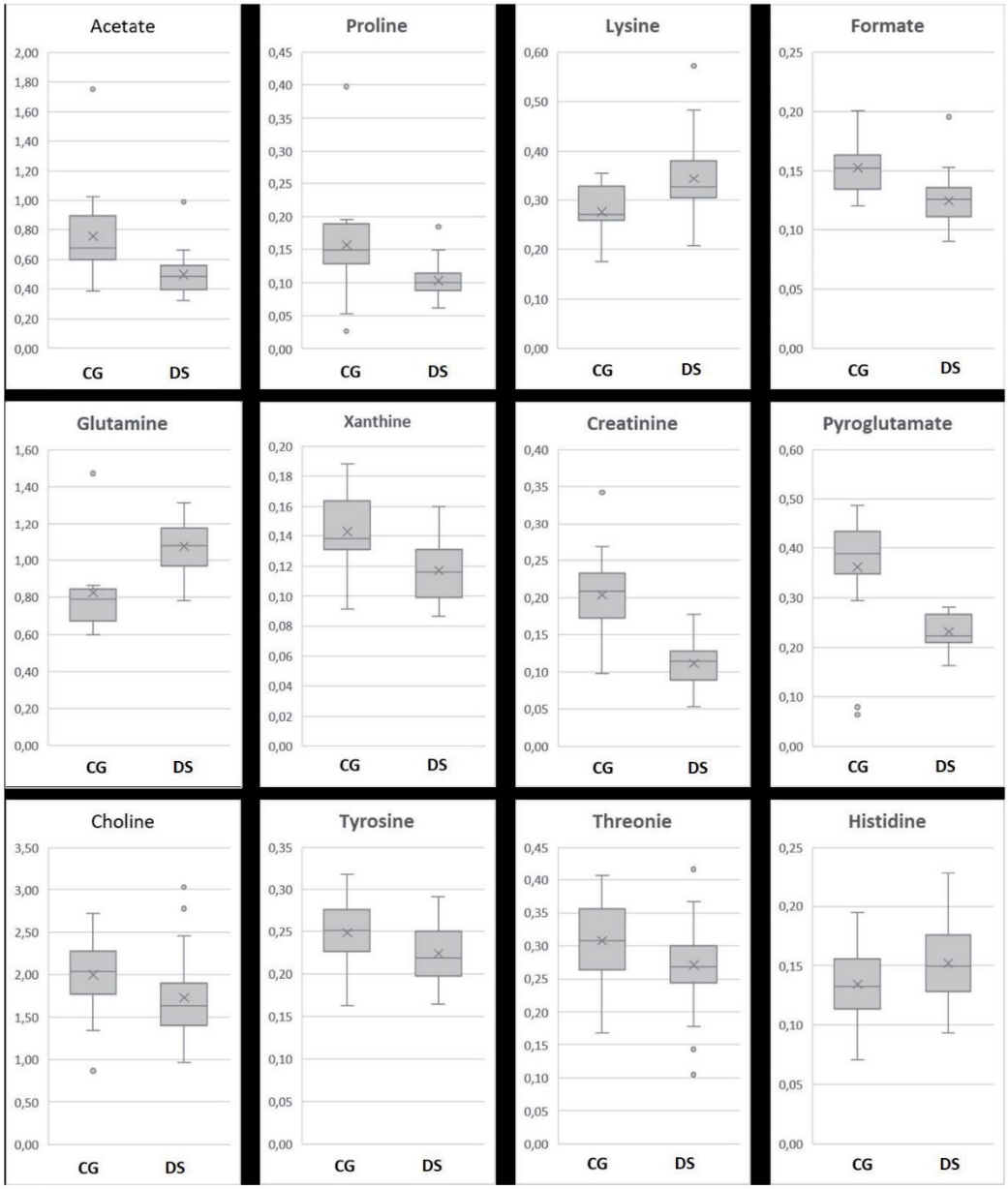

Figure 2. The differences in relative concentration for statistically important metabolites
DS – study group, CG – control group

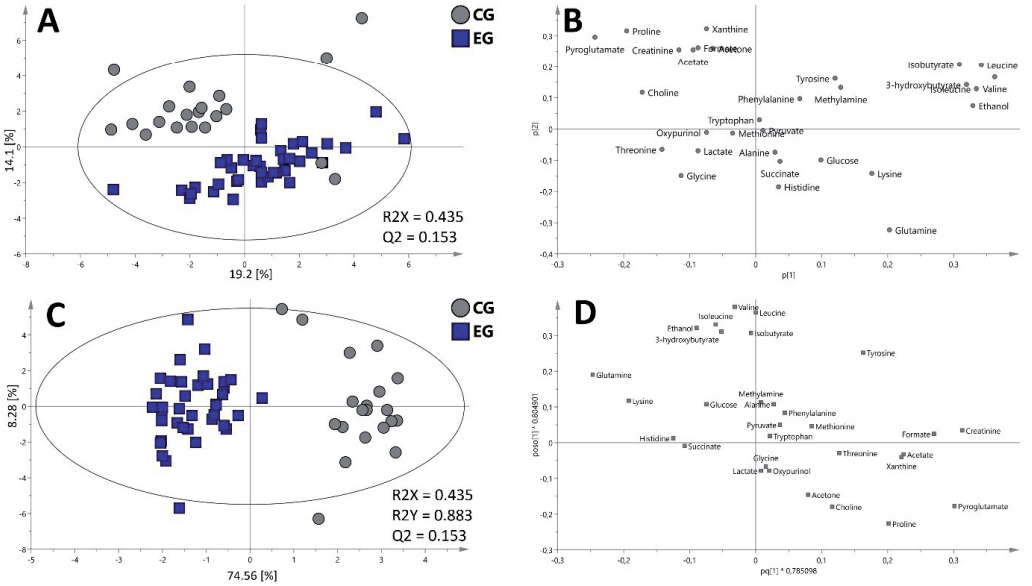

Figure 3. PCA model plot (A) with corresponding loading plot (B) and OPLS-DA model (C) of 1H NMR data of T21 samples

Balancing atherogenic and anti-atherogenic particles

The DS group exhibited significantly lower APO A1 levels compared to the CG group, which corroborates with the reduced HDL cholesterol findings and indicates a diminished defensive mechanism against atherosclerosis [41, 42]. Further, the DS group had a higher APO B/APO A1 ratio, revealing an increased predisposition to cardiovascular risks as this ratio serves as a recognized predictor of such risks [43]. Despite a distinct difference in the mean ratios between the groups, it was observed that a larger percentage of the DS group had ratios above the norm, highlighting a noticeable shift towards higher values and emphasizing the augmented risk of CVD in the DS group.

Other parameters

While TCH, APO B, HCY, and Lp(a) did not display pronounced differences between the two groups, a deeper analysis into their distributions can reveal nuanced disparities [44]. Even if average values appear similar, the proportion of individuals within or outside the recommended ranges can vary significantly between groups [45]. This underscores the importance of evaluating both overarching trends and individual data distributions to comprehensively understand cardiovascular health in the DS [46]. Such patterns hint at the potential for hidden cardiovascular risks, even when overall averages seem reassuring, emphasizing the need for a holistic assessment approach [47].

Interconnected metabolic relationships: insights from correlation patterns in study and control groups

In the DS group, homocysteine's connection to body metrics hints at a relationship between body composition and metabolic implications for cardiovascular health. There is a notable complex relationship between the APO B/APO A1 ratio and various health markers, emphasizing the necessity for a comprehensive view of metabolic health. In the CG, age appears to significantly influence lipid metabolism, impacting various lipid and apolipoprotein markers and consequently cardiovascular risk assessment. The observed relationships between different lipid markers, such as the interplay between APO A1 and other markers, suggest a balanced lipid regulatory environment and reiterate LDL's central role in cholesterol transport. Moreover, associations between HDL and factors like age and BMI necessitate further investigation into the effects of lifestyle or dietary habits on beneficial cholesterol. The connection between the APO B/APO A1 ratio and Lp(a) also indicates underlying lipid metabolic pathways deserving of further study.

Discussion on metabolomic analysis in the context of atherosclerosis and cardiovascular disease risk

Twelve out of 31 metabolites showed significant differences between the DS and CG groups, but applying the Benjamini-Hochberg procedure for FDR correction highlighted eight metabolites with physiological relevance, offering a deeper understanding of potential health implications. Acetate variations point to altered lipid metabolism, which is associated with the formation of atherosclerotic plaques and increased CVD risks [48, 49]. Creatinine fluctuations indicate that apart from kidney function, muscle metabolism and its impact on cardiac performance should be considered [50]. Format is linked with amino acid metabolism and hints at changes in methylation pathways that, when disrupted, can lead to vascular inflammation and early atherosclerosis. Glutamine, crucial for protein synthesis and cellular energy, suggests broader metabolic shifts potentially affecting vascular health and stress responses [51, 52]. Lysine is vital for collagen synthesis essential for arterial wall structure, and its deficiency could result in weaker arterial walls prone to plaque accumulation [53, 54]. Proline, also involved in collagen production, can affect vessel flexibility, with inconsistent levels potentially leading to hypertension and early atherosclerosis stages [55]. Pyroglutamate plays a role in glutamate metabolism, possibly influencing vascular tone and responsiveness. Xanthine oscillations reflect purine metabolism state, where increased uric acid levels are associated with higher risks of hypertension and vascular inflammation, raising CVD risks [4]. These insights suggest a complex interplay of various metabolic pathways influencing cardiovascular health in the DS group.

Oxidative stress and its implications in DS

Oxidative stress is a known contributor to the pathogenesis of atherosclerosis and CVD. Relevant to this discussion is our previous work where we demonstrated that individuals with DS exhibit higher levels of oxidative stress compared to their siblings without DS [22]. This increased oxidative stress in the DS population might be a significant factor contributing to their distinctive cardiovascular risk profile.

Conclusions

Individuals with DS demonstrate distinct cardiovascular risk profiles, marked by reduced HDL cholesterol and heightened LDL cholesterol levels, pointing to an increased susceptibility to atherosclerosis and CVD. Their metabolomic analysis further emphasizes potential metabolic imbalances that could predispose them to vascular complications. Moreover, a pronounced tendency towards elevated BMI and TMI in DS group signals additional health challenges. It can be concluded that individuals with DS might be at a greater risk of developing atherosclerosis compared to the CG.

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8. STRESZCZENIE W JĘZYKU POLSKIM

Wstęp: Zespół Downa (ZD, Trisomia 21) to najczęstsze zaburzenie chromosomalne o globalnej częstości występowania 1:1000-1100 urodzonych noworodków. Dodatkowy chromosom 21, lub przynajmniej jego część, powoduje szereg zaburzeń klinicznych. Osoby z ZD (PWSD) mogą różnić się od siebie zarówno pod względem cech zewnętrznych i występowania przewlekłych chorób. Ponadto z powodów genetycznych są narażone na zwiększony stres oksydacyjny, który nasila procesy degeneracyjne tkanek. Dodatkowo zaburzenia hormonalne występujące w tej populacji są ściśle powiązane z regulacją procesów antyoksydacyjnych i metabolicznych.

Cel badań: Celem badań przedstawionych w rozprawie doktorskiej jest ocena procesów metabolicznych i metabolomicznych u osób z ZD i ich wpływu na rozwój miażdżycy w populacji pediatrycznej oraz ocena rozwoju dzieci i młodzieży z ZD z wykorzystaniem różnych siatek centylowych.

Metodologia i Wyniki: Badaniami objęto 411 pacjentów z ZD (94% w wieku <18lat; 6% dorosłych). Oceniano rozwój wykorzystując siatki standardowe populacyjne i wyspecjalizowane dla ZD. Dodatkowo ramach badania przeprowadzono analizę rozwoju i stanu zdrowia dzieci z ZD oraz ich rodziców. Wykazano, że znaczący odsetek ojców (69%) i matek (42%) prezentuje co najmniej nadwagę lub otyłość. Nie wykazano istotnej korelacji między masą ciała rodziców a masą ich dzieci. U pacjentów z ZD przeanalizowano masę, wysokość ciała oraz Wskaźnik Masy Ciała (BMI) w trzech kategoriach. Stwierdzono istotne różnice statystyczne: prawidłowa masa ciała (70% wg. siatki centylowej standardowej vs 89% wg. siatki wystandaryzowanej dla ZD; $p<0,02$); niedobór masy ciała (27% vs 5%; $p<0,0001$); nadmierna wysokość ciała: (35% vs 23%; $p<0,005$); otyłość (7% vs 2; $p<0,005$). Pozostałe parametry nie wykazały istotnych różnic statystycznych. Na podstawie przeprowadzonych analiz, stwierdzono różnice w klasyfikacji masy, wysokości oraz BMI w zależności od zastosowanego rodzaju siatki centylowej. Dodatkowo wśród 42 osób z ZD (grupa badana, SG; średnia wieku 14,17 lat) i CG – 20 osób (średnia wieku 15,92 lat) analizowano profil metaboliczny i metabolomiczny. Obliczono BMI, Standaryzowany Wskaźnik Masy Ciała (BMI SDS), Wskaźnik Masy Ciała Tri-Ponderal (TMI), Współczynnik Stresu Oksydacyjnego (OSI) i Wskaźnik Insulinooporności (HOMA-IR). Porównując grupę badaną (SG) do grupy kontrolnej (CG) stwierdzono, że: BMI - nadwaga (29,19% vs 15%), otyłość (19,05% vs 5%); Całkowity Potencjał Antyoksydacyjny (TAS; 1,92 mmol/l vs 1,79 mmol/l; $p=0,0015$); Całkowity Potencjał Oksydacyjny (TOS; 51,52 mmol/l vs 33,05 mmol/l; $p=0,014$); OSI (2475,02 vs 1949,75; $p=0,038$), natomiast brak istotnych różnic w stężeniu glukozy na czczo, insuliny i HOMA-IR. Osoby z ZD wykazują znacząco obniżony HDL (SG vs CG: 47± 10 vs 59 ± 12 mg/dl; $p= 0,0001$) i podwyższony LDL (104 ± 25 vs 90 ± 22 mg/dl; $p= 0, 0331$). Uzyskane wyniki analizy trójglicerydów, apolipoproteiny A1 (APO A1) i wskaźnika APO B/APO A1 potwierdzają zwiększone ryzyko chorób sercowo-naczyniowych (CVD) w ZD. Dodatkowo w grupie ZD stwierdzono wysokie wartości BMI oraz TMI. W analizie metabolomicznej, spośród 31 zidentyfikowanych metabolitów 12 wykazało istotność

statystyczną (octany, cholina, kreatynina, mrówczan, glutamina, histydyna, lizyna, prolina, piroglutaminian, treonina, tyrozyna i ksantyna). Jednak tylko 8 metabolitów przeszło walidację FDR (octany, kreatynina, mrówczan, glutamina, lizyna, prolina, piroglutaminian, ksantyna).

Wnioski: Wyniki przeprowadzonych badań wskazują różnicowanie procesów metabolicznych między grupą badaną a grupą kontrolną, przy zbliżonych warunkach środowiskowych. Przypuszcza się, że nadmiarowa kopia chromosomu 21 może być fundamentalnym czynnikiem sprzyjającym zaburzeniom metabolicznym, powodującym zwiększone ryzyko chorób sercowo-naczyniowych osób z ZD. Wykazane różnice metaboliczne, współistniejące z wyższymi wskaźnikami BMI i TMI, wskazują, że osoby z ZD mogą być bardziej narażone na rozwój miażdżycy niż ich rodzeństwo. Podkreśla to konieczność wnikliwego monitorowania i oceny zaburzeń rozwojowych u osób z ZD. Jednocześnie zwraca uwagę brak jednego, kompleksowego narzędzia diagnostycznego dedykowanego tej grupie pacjentów. Stwierdzone różnice w wynikach uzyskiwanych za pomocą standardowych siatek centylowych w porównaniu z wynikami z siatek dedykowanych dla osób z ZD, podkreślają tę lukę w dostępnych metodach diagnostycznych. Osoby z ZD wykazują zwiększone ryzyko nadwagi, otyłości oraz CVD, co wskazuje na potrzebę stworzenia indywidualnych programów dietetycznych. Wyraźnie zaznaczone zaburzenia lipidowe oraz podwyższone wartości HOMA- IR u osób z ZD sugerują konieczność regularnych kontroli profilu lipidowego oraz gospodarki węglowodanowej. Analiza metabolomiczna podkreśla złożoność interakcji metabolicznych w ZD. Metabolomika może być kluczowym narzędziem do identyfikacji markerów ryzyka i zrozumienia ścieżek metabolicznych związanych z patogenezą miażdżycy, otwierając drogę do rozwoju spersonalizowanej strategii profilaktyki i leczenia.

9. STRESZCZENIE W JĘZYKU ANGIELSKIM

Introduction: Down syndrome (DS, Trisomy 21) is the most common chromosomal disorder with a global incidence of 1:1000-1100 newborns. The extra chromosome 21, or at least part of it, causes a number of clinical disorders. People with DS (PWSD) may differ from each other both in terms of external characteristics and the presence of chronic diseases. Moreover, for genetic reasons, they are exposed to increased oxidative stress, which intensifies tissue degenerative processes. Additionally, hormonal disorders occurring in this population are closely related to the regulation of antioxidant and metabolic processes.

Aim of the study: The aim of the research presented in the doctoral dissertation is to assess metabolic and metabolomic processes in people with DS and their impact on the development of atherosclerosis in the pediatric population and to assess the development of children and adolescents with DS using various growth charts.

Methodology and Results: The study consisted of 411 patients with DS (94% aged <18 years; 6% adults). Growth was assessed using standard population and specialized DS charts. Additionally, the study analyzed the development and health status of children with DS and their parents. It has been shown that a significant percentage of fathers (69%) and mothers (42%) are at least overweight or obese. There was no significant correlation between the body weight of parents and the weight of their children. Weight, height and Body Mass Index (BMI) were analyzed in three categories in patients with DS. Significant statistical differences were found in: normal body weight (70% according to the standard growth chart vs. 89% according to the standardized growth chart for DS; $p<0.02$); body weight deficiency (27% vs 5%; $p<0.0001$); excessive body height: (35% vs 23%; $p<0.005$); obesity (7% vs 2; $p<0.005$). The remaining parameters did not show any significant statistical differences. Based on the analyses, differences were found in the classification of weight, height and BMI depending on the type of growth chart used. Additionally, the metabolic and metabolomic profiles were analyzed among 42 people with DS (study group, SG; mean age 14.17 years) and CG - 20 people (mean age 15.92 years). BMI, Standardized Body Mass Index (BMI SDS), Tri-Ponderal Body Mass Index (TMI), Oxidative Stress Index (OSI) and Insulin Resistance Index (HOMA-IR) were calculated. Comparing the study group (SG) to the control group (CG), it was found that: BMI - overweight (29.19% vs 15%), obesity (19.05% vs 5%); Total Antioxidant Potential (TAS; 1.92 mmol/l vs 1.79 mmol/l; $p=0.0015$); Total Oxidative Potential (TOS; 51.52 mmol/l vs 33.05 mmol/l; $p=0.014$); OSI (2475.02 vs 1949.75; $p=0.038$), but there were no significant differences in fasting glucose, insulin and HOMA-IR levels. People with DS show significantly reduced HDL (SG vs CG: 47 ± 10 vs 59 ± 12 mg/dl; $p= 0.0001$) and increased LDL (104 ± 25 vs 90 ± 22 mg/dl; $p= 0.0331$) . The obtained results of the analysis of triglycerides, apolipoprotein A1 (APO A1) and the APO B/APO A1 ratio confirm the increased risk of cardiovascular diseases (CVD) in DS. Additionally, high BMI and TMI values were found in the DS group. In the metabolomics analysis, of the 31 identified metabolites, 12 showed statistical significance (acetate, choline, creatinine, formate, glutamine, histidine, lysine, proline, pyroglutamate, threonine, tyrosine and xanthine). However, only 8 metabolites passed FDR validation (acetate, creatinine, formate, glutamine, lysine, proline, pyroglutamate, xanthine).

Conclusions: The results of the conducted research indicate differences in metabolic processes between the study group and the control group, under similar environmental conditions. It is believed that an excess copy of chromosome 21 may be a fundamental factor contributing to metabolic disorders, causing an increased risk of cardiovascular diseases in people with DS. The demonstrated metabolic differences, coexisting with higher BMI and TMI, indicate that people with DS may be at greater risk of developing atherosclerosis than their siblings. This emphasizes the need for careful monitoring and assessment of developmental disorders in people with DS. At the same time, the lack of a single, comprehensive diagnostic tool dedicated to this group of patients is noteworthy. The differences found in the results obtained using standard percentile charts compared to the results from charts dedicated to people with DS highlight this gap in the available diagnostic methods. People with DS have an increased risk of overweight, obesity and CVD, which indicates the need to create individual dietary programs. Clearly marked lipid disorders and elevated HOMA-IR values in people with DS suggest the need for regular monitoring of the lipid profile and carbohydrate metabolism. Metabolomics analysis highlights the complexity of metabolic interactions in DS. Metabolomics may be a key tool to identify risk markers and understand metabolic pathways involved in the pathogenesis of atherosclerosis, opening the way to the development of personalized strategies.

10. CURRICULUM VITAE

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- Hematologia Dziecięca
- Zaburzenia Krzepnięcia Krwi

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01.10.2019- 30.09.2023 – Szkoła Doktorska, Wrocławski Uniwersytet Medyczny im. Piastów Śląskich

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Szkolenia i Kursy:

2022- Late Effects in Childhood and Adolescent Cancer Survivors – A Course by PanCare and SIOP Europe

2021- MJHS Institute for Innovation in Palliative Care LMS

2021- Pacjent Pediatryczny w NPL

2021- Pediatra na dyżurze

2021- Statystyka w medycynie (metody podstawowe)

2021- Psychoonkologia praktyczna

2021- Clinical Terminology for International and U.S. Students

2021- Kurs Efektywnej Nauki BeThink

Stypendia naukowe:

01.10.2022-30.09.2023 – Stypendium Rektora dla Najlepszych Doktorantów w Szkole Doktorskiej

01.03.2022-31.12.2022 - Uniwersytet Medyczny we Wrocławiu, SUBK.D130.22.055; Dzieci i młodzież z trisomią 21- badania metabolomiczne; 47 500PLN

01.10.2020-31.12.2021 - Dolnośląscy liderzy medycyny" – Europejskie stypendium wspierające doktorantów medycyny, 25000 PLN

Publikacje naukowe:

Opublikowanych 16 artykułów zarówno w Polskich, jak i międzynarodowych czasopismach (8 jako pierwszy autor). Sumaryczny IF = 14,446, punktacja MEiN = 646.

Towarzystwa naukowe:

2023 – obecnie – Członek the European Association for Haemophilia and Allied Disorders (EAHAD)

2022 – obecnie – Członek the Pan-European Network for Care of Survivors after Childhood and Adolescent Cancer

2022 – obecnie – członek the European Society for Paediatric Oncology (SIOPE)

2020- obecnie- Członek Polskiego Towarzystwa Onkologów i Hematologów Dziecięcych

11. DOROBEK NAUKOWY

(z wyłączeniem prac stanowiących cykl publikacji do Rozprawy Doktorskiej)

11.1. LISTA PUBLIKACJI

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2. **Hetman Marta**, Jura Maksym, Krakowska Kornelia, Barg Ewa: X-linked adrenoleukodystrophy diagnosed in three brothers, Pediatric Endocrinology, Diabetes and Metabolism, 2019, vol. 25, nr 2, s. 95-98, DOI:10.5114/pedm.2019.85821, 70 punktów
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6. **Hetman Marta**, Fułek Michał, Zajączkowska Katarzyna, Żarczyńska Anna, Łagosz Piotr, Barg Ewa: The central diabetes insipidus associated with septo-optic dysplasia (de Morsier syndrome), Pediatric Endocrinology, Diabetes and Metabolism, 2018, vol. 24, nr 4, s. 197-203, DOI:10.5114/pedm.2018.83367, 8 punktów
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1. Fułek Michał, Jura Maksym, Krakowska Kornelia, Zajączkowska Katarzyna, **Hetman Marta**: Powikłania endokrynologiczne wśród pacjentów poddanych leczeniu ziarnicy złośliwej, W: Czwarta Wrocławska Konferencja Ogólnopediatryczna. Wrocław, 22-23 marca 2019. Książka streszczeń, Wrocław 2019, s. 20
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4. **Hetman Marta**, Galik Katarzyna, Jura Maksym, Fułek Michał, Krakowska Kornelia: Can we be more precise in evaluation the growth of children and adolescents with Down syndrome? Comparison of the Down syndrome growth charts with the populational growth charts, W: The 22d International Medical Congress of Students and Young Scientists. Ternopil, April 23-25, 2018. [Book of abstracts], (red.) M.M. Korda, Ternopil 2018, Ukrmedknyha, s. 142
5. Jura Maksym, Krakowska Kornelia, **Hetman Marta**, Galik Kasia, Zajączkowska Katarzyna: Cri du chat syndrome in 10 years observation, W: The 22d International Medical Congress of Students and Young Scientists. Ternopil, April 23-25, 2018. [Book of abstracts], (red.) M.M. Korda, Ternopil 2018, Ukrmedknyha, s. 33

12.3. DONIESIENIA ZJAZDOWE

1. **Hetman Marta**, **Latos-Grażyńska Elżbieta**: Does the hemophilia gene protect against the symptoms development of congenital thrombocytopenic purpura? A 2-year-old boy, homozygous in the ADAMST-13 gene, suffering from severe hemophilia B complicated by an inhibitor and anaphylactic shock.12th Midsummer Meeting on Pediatric Hematology, Oncology and Stem Cell Transplantation, June, 16-18 2023, Wroclaw, Poland. Pretty Kettle of Fish Session
2. **Hetman Marta**: Polskiej Grupie Guzów Litych (Konferencja ogólnopolska; 6- 7.10.2022) na sesji: Infantile choriocarcinoma – trudności terapeutyczne – opis przypadku. Sesja: Siatkówczak, nerwiak zarodkowy, guzy nerek, guzy wątroby, guzy germinalne wieku dziecięcego – aktualne wyniki leczenia, propozycje współpracy wieloośrodkowej.

13. OŚWIADCZENIA WSPÓŁAUTORÓW

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Hetman Marta, Moreira Helena, Barg Ewa: The best tool for the assessment of developmental disorders in children with down syndrome: comparison of standard and specialized growth charts - cross sectional study, Frontiers in Endocrinology, 2022, vol. 13, art.928151 [11 s.], DOI:10.3389/fendo.2022.928151, 100 punktów, IF(5,2)

Hetman Marta, Barg Ewa: Pediatric population with Down syndrome: obesity and the risk of cardiovascular disease and their assessment using omics techniques - review, Biomedicines, 2022, vol. 10, nr 12, art.3219 [14 s.], DOI:10.3390/biomedicines10123219, 100 punktów, IF(4,7)

Hetman Marta, Placzkowska Sylwia, Barg Ewa: Comparative Analysis of Obesity Prevalence, Antioxidant and Oxidant Status in Children with Down Syndrome - A Sibling-Controlled Study, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100.

Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100.

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Hetman Marta, Moreira Helena, Barg Ewa: The best tool for the assessment of developmental disorders in children with down syndrome: comparison of standard and specialized growth charts - cross sectional study, Frontiers in Endocrinology, 2022, vol. 13, art.928151 [11 s.], DOI:10.3389/fendo.2022.928151, 100 punktów, IF(5,2);

Hetman Marta, Barg Ewa: Pediatric population with Down syndrome: obesity and the risk of cardiovascular disease and their assessment using omics techniques - review, Biomedicines, 2022, vol. 10, nr 12, art.3219 [14 s.], DOI:10.3390/biomedicines10123219, 100 punktów, IF(4,7);

Hetman Marta, Placzkowska Sylwia, Barg Ewa: Comparative Analysis of Obesity Prevalence, Antioxidant and Oxidant Status in Children with Down Syndrome - A Sibling-Controlled Study, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100;

Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100;

mój udział polegał na:

- Koncepcja i projekt badania
- Analiza i interpretacja danych
- Krytyczne zrecenzowanie artykułu
- Zatwierdzenie ostatecznej wersji artykułu

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Ja, Helena Moreira, wyrażam zgodę na włączenie poniższych prac naukowych, których jestem współautorem, do serii publikacji, na podstawie których będzie oparta praca doktorska głównego autora, Marty Hetman.

Hetman Marta, Moreira Helena, Barg Ewa: The best tool for the assessment of developmental disorders in children with down syndrome: comparison of standard and specialized growth charts - cross sectional study, Frontiers in Endocrinology, 2022, vol. 13, art.928151 [11 s.], DOI:10.3389/fendo.2022.928151, 100 punktów, IF(5,2)

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OŚWIADCZENIA WSPÓŁAUTORA

Dr Helena Moreira

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Wrocław, 12.09.2023

OŚWIADCZENIE

Oświadczam, że w pracy:

Hetman M, Moreira H, Barg E: The best tool for the assessment of developmental disorders in children with Down syndrome: comparison of standard and specialized growth charts - cross sectional study, Frontiers in Endocrinology, 2022, vol. 13, art.928151 [11 s.], MNiSW 100, IF 5,2.

mój udział polegał na:

- Analiza statystyczna i interpretacja danych

H. Moreira

Podpis

UNIWERSYTET MEDYCZNY
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Wydział Lekarski Kształcenia Podyplomowego
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Wrocław, 2023-09-12

DEKLARACJA WSPÓŁAUTORA

Ja, Aleksandra Bodetko, wyrażam zgodę na włączenie poniższych prac naukowych, których jestem współautorem, do serii publikacji, na podstawie których będzie oparta praca doktorska głównego autora, Marty Hetman.

Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol 29, MNSiW 100.

12.09.2023 r. Aleksandra Bodetko

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mój udział polegał na:

- Gromadzenie danych,
- Kontakt z uczestnikami badania.

Podpis

Aleksandra Bodetko

UNIWERSYTET MEDYCZNY
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Hetman Marta, Placzkowska Sylwia, Barg Ewa: Comparative Analysis of Obesity Prevalence, Antioxidant and Oxidant Status in Children with Down Syndrome - A Sibling-Controlled Study, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100.

Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100.

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Hetman Marta, Placzkowska Sylwia, Barg Ewa: Comparative Analysis of Obesity Prevalence, Antioxidant and Oxidant Status in Children with Down Syndrome - A Sibling-Controlled Study, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol 29, MNSiW 100.

mój udział polegał na:

- wykonanie badań laboratoryjnych (badania biochemiczne) oraz wstępne opracowanie uzyskanych danych wraz z opisem metodyki.

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mój udział polegał na:

- wykonanie badań laboratoryjnych (badania biochemiczne) oraz wstępne opracowanie uzyskanych danych wraz z opisem metodyki.

Podpis

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Wrocław, 12.09.2023

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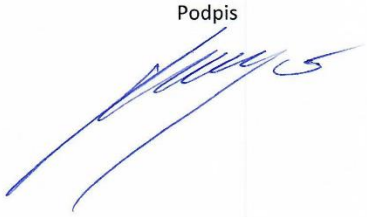
Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100.

mój udział polegał na:

konsultowaniu otrzymanych wyników

Podpis



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Wrocław, 2023-09-12

DEKLARACJA WSPÓŁAUTORA

Ja, Piotr Młynarz, wyrażam zgodę na włączenie poniższych prac naukowych, których jestem współautorem, do serii publikacji, na podstawie których będzie oparta praca doktorska głównego autora, Marty Hetman.

Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100.

20.09.23

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Wrocław, 12.09.2023

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Hetman Marta, Mielko Karolina, Placzkowska Sylwia, Bodetko Aleksandra, Młynarz Piotr, Barg Ewa: *Predisposition to atherosclerosis in children and adults with Trisomy 21: Biochemical and metabolomic studies*, Pediatric Endocrinology, Diabetes and Metabolism, 2023, vol. 29, MNSiW 100

mój udział polegał na:

- przeprowadzeniu procedury ekstrakcji próbek surowicy;
- przygotowaniu próbek do pomiarów techniką NMR;
- wstępnej obróbce uzyskanych danych;
- identyfikacji metabolitów;
- przeprowadzeniu analiz statystycznych;
- analizie chemometrycznej uzyskanych wyników;
- opracowaniu części manuskryptu (metodologia i wyniki analiz metabolomicznych);
- przygotowaniu Tabeli 6, a także rysunków nr 1, 2 oraz 3.

Karolina Mielko

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12.09.2023
Karolina Mielko

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