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Zmiany stężenia miokin we krwi u kobiet w odpowiedzi na trening wytrzymałościowy

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Spis treści

Pragnę podziękować mojej Promotor, Pani Profesor dr hab. Ewie Ziemann
za umożliwienie mi rozwoju naukowego, motywację oraz nieocenioną pomoc.

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1. Wykaz prac stanowiących rozprawę doktorską

Przedstawiona rozprawa doktorska pod tytułem: ***„Zmiany stężenia miokin we krwi u kobiet w odpowiedzi na trening wytrzymałościowy”***, składa się z cyklu trzech prac opublikowanych w czasopismach zagranicznych o sumarycznej punktacji Impact Factor (IF) równej 12.044 oraz Ministerstwa Edukacji i Nauki (MEiN) równej 270 pkt.:

Publikacja I: ***The beneficial effects of 15 units of high-intensity circuit training in women is modified by age, baseline insulin resistance and physical capacity*** (2019) Micielska Katarzyna, Gmiąt Anna, Żychowska Małgorzata, Kozłowska Marta, Walentukiewicz Anna, Łysak- Radomska Anna, Jaworska Joanna, Rodziewicz Ewa, Duda- Biernacka Barbara, Ziemann Ewa; *Diabetes Research and Clinical Practice,* doi:10.1016/j.diabres.2019.05.009; IF= 4.234 MEiN= 100 pkt.

Publikacja II: ***Habitually inactive physically - a proposed procedure of counteracting cognitive decline in women with diminished insulin sensitivity through a high-intensity circuit training program*** (2021) Micielska Katarzyna, Kortas Antoni Jakub, Gmiąt Anna, Jaworska Joanna, Kozłowska Marta, Łysak- Radomska Anna, Rodziewicz- Flis Ewa, Żychowska Małgorzata, Ziemann Ewa; *Physiology & Behavior,* doi:10.1016/j.physbeh.2020.113235; IF= 3.244, MEiN= 70 pkt.

Publikacja III: ***Impact of 12-week moderate-intensity aerobic training on inflammasome complex activation in elderly women*** (2022) Gomarasca Marta[†], Micielska Katarzyna[†], Faraldi Martina, Kozłowska Marta, Perego Silvia, Banfi Giuseppe, Ziemann Ewa, Lombardi Giovanni; *Frontiers in Physiology,* doi: 10.3389/fphys.2022.792859; IF= 4.566, MEiN= 100 pkt.

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2. Wykaz skrótów

ACSM- Amerykańskie Towarzystwo Medycyny Sportowej (*ang. American College of Sports Medicine)*

BDNF- neurotroficzny czynnik pochodzenia mózgowego; (*ang. brain-derived neurotrophic factor*)

BMI- wskaźnik BMI (*ang. Body Mass Index*)

CATB- katepsyna B (*ang. cathepsin B*)

CRP- białko C-reaktywne (*ang. C Reactive Protein*)

DAMP- struktury molekularne związane z uszkodzeniem (*ang. damage associated molecular patterns*)

FGF21- czynnik wzrostu fibroblastów 21 (*ang. fibroblast growth factor 21*)

GLUT4- transporter glukozy-4 (*ang. glucose transporter type 4*)

GSDMD- gasdermina D (*ang. gasdermin D*)

HICT- trening oporowy z wykorzystaniem masy własnego ciała jako obciążenia (*ang. high-intensity circuit training*)

HIIT- trening interwałowy o wysokiej intensywności (*ang. high-intensity interval training)*

HMGB1- białko o dużej ruchliwości elektroforetycznej (*ang high-mobility group box 1*)

HOMA-IR- wskaźnik insulinooporności (*ang. homeostasis model assessment for insulin resistance*)

HSP27- białko szoku cieplnego 27 (*ang. heat shock protein 27*)

IGF-1- insulinopodobny czynnik wzrostu 1 (*ang. insulin like growth factor 1*)

IL-6- interleukina 6 (*ang. interleukin 6*)

IL-15- interleukina 15 (*ang. interleukin 15*)

IL-18- interleukina 18 (*ang. interleukin 18*)

IL-1β- interleukina 1β (*ang. interleukin 1β*)

LGI- niski, ogólnoustrojowy, przewlekły stan zapalny (*ang. chronic low-grade inflammation* lub *inflammaging*)

NF-κB czynnik jądrowy κB (*ang. nuclear factor κB*)

NLRP3- inflamasom NLRP3/ białko NOD-podobne 3, zawierające domenę pirynową (*ang. NOD-like receptor family, pyrin domain containing 3*)

NW- trening Nordic Walking

PAMP- wzorce molekularne związane z patogenami (*ang. pathogen associated molecular patterns*)

QUICKI- wskaźnik insulinowrażliwości (*ang. quantitative insulin sensitivity check index*)

TNFα- czynnik martwicy nowotworów α (*ang, tumour necrosis factor α*)

TLR- receptor toll- podobny (*ang. Toll-like receptor*)

T2DM- cukrzyca typu II (*ang. type 2 diabetes mellitus*)

VO_{2max} -maksymalny pobór tlenu (*ang. maximal oxygen uptake*)

3. Wstęp

Siedzący tryb życia oraz brak podejmowania aktywności fizycznej jest występującym problemem u ludzi w różnym wieku, powszechnym na całym świecie. Wiadomo, że stan ten powoduje spowolnienie procesów fizjologicznych oraz zaburzenia metabolizmu, a w konsekwencji prowadzi do rozwoju chorób przewlekłych, a nawet do przedwczesnej śmierci [1]. Takiemu stylowi życia towarzyszy nagromadzenie tkanki tłuszczowej oraz nadwaga i otyłość, które przyczyniają się do obniżenia wrażliwości tkanek na działanie insuliny, rozwoju cukrzycy typu II (T2DM) oraz zaburzeń funkcji poznawczych [2]. Istnieje silny związek między wiekiem, a występowaniem cukrzycy typu II. Obecnie wśród osób dorosłych ze zdiagnozowaną T2DM, prawie połowę stanowią osoby starsze (zdefiniowane jako osoby w wieku > 65 lat) [3]. Starzenie się jest jednym z czynników patogenezy T2DM zarówno bezpośrednio poprzez osłabienie funkcji komórek β-trzustki, która uwydatnia się w braku wydzielania insuliny, jak i pośrednio poprzez zwiększenie insulinooporności poprzez otyłość oraz inne czynniki ryzyka [4]. Jest to o tyle niebezpieczne, iż wraz ze starzeniem się organizmu, równocześnie może występować tzw. niski, ogólnoustrojowy, przewlekły stan zapalny (LGI) [5]. Dostępne dane, wskazują, że podejmowanie aktywności fizycznej jest efektywnym regulatorem przewlekłego LGI, sprzyja poprawie zaburzeń metabolicznych (m.in. stanowi prewencję T2DM [6]), ale również zapobiega występowaniu zaburzeń zdrowia psychicznego [7,8]. Niemniej jednak, może się zdarzyć, iż ludzie podejmujący zaplanowaną aktywność fizyczną nie osiągają pożądanych, prozdrowotnych efektów lub występują określone czynniki ograniczające zmiany adaptacyjne. Dlatego, prowadzenie badań w tym zakresie jest ciągle zasadne.

Amerykańskie Towarzystwo Medycyny Sportowej (ACSM), każdego roku przeprowadza badanie w celu ustalenia najbardziej skutecznych prozdrowotnych form aktywności ruchowej według specjalistów pracujących w tym obszarze. Od 2014 roku w czołówce rekomendowanych przez ACSM form znajduje się trening interwałowy o wysokiej intensywności (HIIT) [9,10]. Jest to wysiłek o małej objętości czasowej, ale wysokiej intensywności, co sprawia, iż jest to forma oszczędna czasowo dla ćwiczących dorosłych osób. Warto podkreślić, że badania dotyczące treningu HIIT, wskazują na jego skuteczność podobną do typowego treningu wytrzymałościowego, przejawiającą się m. in. w poprawie względnego maksymalnego poboru tlenu (VO_{2max}) [11,12], polepszeniu wychwytu glukozy do tkanek [13] oraz poprawie funkcji poznawczych [7]. Jedną z odmian HIIT jest trening oporowy z wykorzystaniem masy własnego ciała jako obciążenia (HICT) [14]. Dotychczas dostępne dane wykazały, że zastosowanie jednorazowego treningu HICT wpływa na zmianę funkcji

poznawczych, m. in. koncentrację i pamięć przestrzenną u kobiet, a zmiany te zależne są od wieku. Istnieją dane, które wskazują na poprawę wymienionych funkcji poznawczych w grupie młodych osób i pogorszenie w grupie kobiet w średnim wieku [15] w odpowiedzi na ten rodzaj treningu. Pytanie o kierunek zmian w wyniku regularnego powtarzania tej jednostki treningowej pozostaje otwarte. Drugą popularną formą wysiłku jest charakterze prozdrowotnym jest trening Nordic Walking (NW). Udowodniono, że trening NW ma korzystny wpływ na redukcję stanu zapalnego [16], poprawę funkcji poznawczych [17], jakość życia oraz rozwój umiejętności motorycznych [18,19].

Istotną rolę w procesie zmian adaptacyjnych pełnią białka uwalniane do krwiobiegu przez poszczególne tkanki, w wyniku podejmowanej aktywności fizycznej [20]. Należące do tej grupy białka między innymi uwalniane są przez mięśnie szkieletowe, tzw. miokiny. Dotychczas, wiadomo o istnieniu ponad 600 miokin, ale tylko nielicznym przyporządkowano określoną funkcję [21]. Ich działanie ma charakter zarówno auto, jak i parakrynny. Oznacza to, że miokiny pośredniczą w komunikacji między mięśniami, a innymi narządami, takimi jak mózg, tkanka tłuszczowa, kości, wątroba czy jelita. Dobrze udokumentowane jest przeciwzapalne działanie tych białek, jak również wpływ na regulację procesu hipertrofii mięśnia (m.in. miostatyna, dekoryna [22], insulinopodobny czynnik wzrostu 1; IGF-1, interleukina 15; IL-15), procesy uczenia się i zmiany funkcji poznawczych (m. in. neurotroficzny czynnik pochodzenia mózgowego; BDNF, katepsyna B; CATB [23]) czy metabolizm glukozy (m, in. iryzyna [24], interleukina 6; IL-6). Ponadto, IL-6 jest jedną z najlepiej poznanych cytokin, która zwiększa zarówno wychwyt glukozy, jak i translokację transporterów glukozy-4 (GLUT4) [25]. Z drugiej jednak strony, może mieć charakter cytokiny prozapalnej.

Miokiny mają znaczący wpływ na regulację czynności układu odpornościowego, szczególnie w okresie starzenia się [26]. Utrzymujący się, nieznacznie podwyższony poziom interleukin (IL-1β, IL-6), czy czynnika martwicy nowotworów α (TNFα) jest charakterystyczny dla LGI. Kluczową rolę w produkcji cytokin prozapalnych (IL-1β oraz IL-18) pełnią, znajdujące się w cytozolu, wewnątrzkomórkowe kompleksy białkowe: inflamasomy, poprzez udział w aktywacji kaspazy-1, która reguluje przejście pro-interleukiny 18 w jej aktywną formę. Kaspaza-1 należy do specyficznych endoproteaz, które zapewniają połączenia w komórkowych sieciach regulacyjnych kontrolowania stanu zapalnego oraz śmierci komórek [27].

Inflamasomy są elementem odporności nieswoistej, odpowiedzialnymi za wykrywanie molekularnych wzorców wskazujących zarówno na uszkodzenie komórki (DAMP), jak i na obecność patogenów powodujących infekcję (PAMP) oraz produkcję cytokin prozapalnych. Stan zapalny wywołany jest przez aktywację receptorów toll-podobnych (TLR), których

aktywacja następuje w odpowiedzi na PAMP lub DAMP, poprzez sygnalizację NF-κB, prowadzi do zwiększonej ekspresji białka NOD-podobnego [28] i tworzenia jednego z najlepiej poznanych inflamasomów: NLRP3. Ze względu na fakt, iż aktywacja inflamasomu jest stanem zapalnym, proces ten musi być ściśle uregulowany. Z nielicznymi wyjątkami, aktywację inflamasomu uważa się za proces dwuetapowy: najpierw trzeba go zainicjować, a potem można go aktywować. NLRP3 jest białkiem zawierającym domenę NOD, LRR i pirynę 3 i pełni rolę czujnika wewnątrzkomórkowego, który wykrywa szeroki zakres endogennych sygnałów zagrożenia, co skutkuje tworzeniem i aktywacją inflamasomu NLRP3. Formacja i aktywacja inflamasomu NLRP3 prowadzi do zależnego od kaspazy-1 uwalniania prozapalnych cytokin IL-1β i IL-18, a także do pyroptotycznej śmierci komórek, w której pośredniczy gasdermina D [27]. Ostatnie badania wskazują na szereg nowych regulatorów inflamasomu NLRP3. Aktywacja inflamasomu NLRP3, związana jest z patogenezą stanu zapalnego związanego z otyłością i insulinoopornością [29,30]. Jego aktywacja wiąże się również z gromadzeniem białka β amyloidu w chorobie Alzheimera [31]. Wpływ wysiłku fizycznego jako inhibitora inicjacji i aktywacji inflamasomów, nie został jeszcze określony. Dlatego podejmowanie badań w tym obszarze jest zasadne. Biorąc pod uwagę powyższy stan wiedzy, w swojej pracy badawczej postanowiłam zweryfikować efekt wpływu pojedynczej jednostki jak i całego cyklu treningowego HICT oraz treningu NW na zmiany adaptacyjne u kobiet z uwzględnieniem odpowiedzi anty – prozapalnej modyfikowanej przez sekrecję miokin i odpowiedź immunologiczną.

4. Pytania i hipotezy badawcze

W oparciu o dostępne dane z piśmiennictwa zadałam w swojej pracy badawczej następujące pytania badawcze:

1. Czy pojedyncza jednostka treningowa oraz regularny trening wytrzymałościowy w formie interwałowej i ciągłej zmieni istotnie stężenie wybranych miokin oraz jaki wpływ będą miały te zmiany na homeostazę glukozy oraz funkcje poznawcze?
2. Czy zmiany stężenia miokin w odpowiedzi na zastosowany trening będą uzależnione od wieku i poziomu wydolności fizycznej uczestniczek?

Dodatkowo, sformułowałam poniższe hipotezy:

1. Pojedyncza jednostka treningowa HICT jak i jej regularne powtarzanie zwiększa stężenie decorin w krwioobiegu oraz pobudza sekrecję białek zaangażowanych w proces hipertrofii mięśni szkieletowych.
2. Cykl programów HICT powoduje poprawę funkcji poznawczych w grupie ćwiczących kobiet, a kluczową rolę odgrywają wybrane białka, które w piśmiennictwie zostały wskazane jako przechodzące przez barię mózg-krew.
3. Program treningów NW wywołuje odpowiedź antyzapalną poprzez redukcję inflamasomów.

5. Materiały i metody

5.1. Charakterystyka osób badanych

Do badań zrekrutowano 139 kobiet. Badane zostały zakwalifikowane po wstępnej kontroli medycznej. Były to osoby zdrowe, prowadzące siedzący tryb życia. Z badań wykluczono 9 uczestniczek ze względu na zbyt niską frekwencję (< 85% i <90% udziału w sesjach treningowych).

W poszczególnych eksperymentach wzięło udział

1. **Publikacja I:** 36 kobiet charakteryzujących się przeciętnym poziomem wydolności fizycznej (VO_{2max}= 29 ± 6.8 mL·kg^{-1}·min^{-1}). Zostały one losowo przydzielone do grupy trenującej (HICT; n= 20, wiek: 40 ± 11 lat) oraz grupy kontrolnej (CON; n= 13, wiek: 45 ± 13 lat). W celu oceny zmian zależnych od wieku uczestniczek, grupa HICT podzielona została na osoby młode (YG; n = 11, wiek <30 lat) i w średnim wieku (MG; n= 9, wiek >30 lat). Badane przydzielone do grupy HICT wykonały 5 tygodni treningów, które dobywały się 3 razy w tygodniu. Kobiety z grupy CON wykonały 2 pojedyncze sesje HICT w odstępie 5 tygodniowym.
2. **Publikacja II:** 33 kobiety (średnia wieku= 39 ± 13 lat) o obniżonej insulinowrażliwości (zmierzonej wskaźnikiem QUICKI= 0.359 ± 0.04). Uczestniczki badań zostały losowo przydzielone do dwóch grup: trenującej (HICT; n= 21) i kontrolnej (CON; n= 12). Dodatkowo, aby ocenić zmiany zależne od wieku wśród ćwiczących kobiet, wyodrębniono dwie grupy wiekowe. W konsekwencji w grupie powyżej 30 roku życia znalazło się dziesięć uczestniczek a jedenaście w grupę poniżej 30 roku życia. Grupa HICT wykonała 15 jednostek treningowych w czasie 5 tygodni. Uczestniczki z grupy CON wykonały jedynie pierwszą i ostatnią jednostkę treningową HICT.
3. **Publikacja III:** 70 kobiet w wieku 68 ± 8 lat. Zostały one losowo przydzielone do grupy eksperymentalnej (EXP) oraz grupy kontrolnej (CTRL; n= 29). Uczestniczki z grupy EXP wykonały 12 tygodniowy program treningów Nordic Walking. Ze względu na zbyt niską frekwencję, sześć uczestniczek zostało wykluczone z analiz (EXP; n= 29). Kobiety z grupy CTRL nie podejmowały żadnej aktywności fizycznej. W celu zweryfikowania występowania zmian zależnych od BMI, uczestniczki zostały podzielone na dwie podgrupy: o normalnej masie ciała- BMI< 25 kg·m^{-2} (NW; EXP, n=

14; CTRL, n= 12) oraz z nadwagą/otyłością- BMI> 25 kg·m^{-2} (OW; EXP, n= 15; CTRL, n= 17).

5.2. Metody

W badaniach zastosowałam poniższe metody i narzędzia badawcze:

5.2.1. Metody pomiarów antropometrycznych i wydolności fizycznej

- Pomiary antropometryczne- analiza komponentów składu ciała kobiet biorących udział w badaniach dokonana została za pomocą metody spektroskopowej bio-impedancji przy użyciu analizatora InBody 720 (Biospace, Korea) [32].
- Testy oceny wydolności fizycznej u osób poniżej 54 lat wykonany został przy użyciu testu o progresywnie wzrastającej intensywności na cykloergometrze rowerowym (884E Sprint Bike Sweden, Monark) z użyciem analizatora gazów MetaMAx3B (Cortex) [33].

  Do oceny wydolności fizycznej osób starszych (powyżej 54 roku życia) zastosowany został test marszowy na 2000 m [34].

5.2.2. Metody oznaczeń biochemicznych i zmian w ekspresji genów we krwi.

- Do oznaczenia stężeń wybranych miokin we krwi użyto metodę immunoenzymatyczną ELISA przy użyciu gotowych zestawów z firm: R&D i Phoenix Pharmaceuticals Inc, Abcam, DiaMetra i Cloud-Clone Corp.
- W celu określenia poziomu ekspresji genów zastosowano metodę ilościowej łańcuchowej reakcji polimerazy (RT-qPCR). Izolacja materiału genetycznego (mRNA) została przeprowadzona na podstawie metody chemicznej Chomczyński i Sacchi [35]. Gen referencyjny stanowiła *TUBB*. W celu wyliczenia relatywnej kopii transkryptów zastowoano metodę Schmitten i Livak [36].
- Do zmierzenia aktywacji inflamasomów zastosowano metodę RT-qPCR. Dokonano pomiaru ekspresji *mRNA* dla białek *NLRP3 i TLR4* w pełnej krwi. Zastosowano geny referencyjne PPIB, PGK1 i ACTB do normalizacji danych RT-qPCR. W tym samym eksperymencie całkowitą ilość RNA wyekstrahowano z pełnej krwi za pomocą zestawu Direct-Zol (Zymo Research Co., Orange, CA, Stany Zjednoczone) zgodnie z instrukcjami producenta. Dla każdego testowanego genu obliczono x-krotność zmiany, dzieląc znormalizowaną wartość dla próbki traktowanej w stosunku do próbki nietraktowanej.

- Do pomiaru stężenia glukozy (doustnym testem tolerancji glukozy) oraz profilu lipidowego we krwi użyto analizatora Cobas INTEGRA 400 Plus firmy Roche Diagnostics. Co więcej, obliczono insulinooporność za pomocą indeksu HOMA (HOMA-IR = insulina [$\mu U \cdot mL^{-1}$]× glukoza[$mmol \cdot L^{-1}$]/22.5) [37] i insulinowrażliwość za pomocą wskaźnika QUICKI (QUICKI =1/(log insuliny [$\mu U \cdot mL^{-1}$] + log glukozy [$mg \cdot dL^{-1}$]) [38].

5.2.3. Metody treningu wytrzymałościowego

- Kobiety młodsze wykonały 5 tygodniowy program treningu wytrzymałościowego o charakterze interwałowym. Jednostkę treningową w tym cyklu stanowił pojedynczy HICT, z wykorzystaniem własnego ciała jako obciążenia[15]. Trening składał się z 15 jednostek HICT wykonanych w 5 tygodniowym okresie czasu. W ciągu każdego tygodnia uczestniczki wykonywały 3 treningi (poniedziałek, środę i piątek). Podczas pojedynczej sesji wykonane zostały 3 serie 9 ćwiczeń z 2 minutową przerwą pomiędzy seriami. Czas trwania pojedynczego ćwiczenia wynosił 30 sekund, a przerwa pomiędzy kolejnymi 10s.
- Seniorki wykonały 12 tygodniowy cykl treningowy Nordic Walking. Zastosowane zostały formy jednostek treningowych o charakterze ciągłym, uwzględniające: 10 min rozgrzewkę, 40 min części głównej i 10 min rozprężenie. Obciążenie zastosowane podczas część głównej treningu wynosiło 60-70% możliwości maksymalnych zmierzonych w trakcie testu 2 km marszu zgodnie z opublikowanymi wytycznymi [39]. Uczestniczki badań wyposażone były w standardowe kije do Nordic Walking oraz zegarki sportowe Garmin Forerunner 405 z funkcją GPS monitorujące częstość skurczów serca oraz pokonany dystans.

5.2.4. Metody oceny funkcji poznawczych

- Testy funkcji poznawczych przeprowadzone zostały z wykorzystaniem komputerowej wersji Wiedeńskiego systemu Testów firmy SCHUHFRIED (Austria), mianowicie: Test Rozpiętości Pamięci (*Block-Tapping-Test- CORSI S3*) oraz test interferencji Stroopa. Dodatkowo, przeprowadzono numeryczny test na koncentrację oraz oceniono jakość życia przy użyciu Kwestionariusza Jakości Życia (SF-36).

6. Główne wyniki

6.1. Główne wyniki badań opublikowane w pracy pt. *The beneficial effects of 15 units of high-intensity circuit training in women is modified by age, baseline insulin resistance and physical capacity.*

Zastosowany 5 tygodniowy trening obwodowy o wysokiej intensywności HICT wpłynął na poprawę metabolizmu glukozy, który przejawił się spadkiem stężenia insuliny (-20%, CI: -42% do 10%) i glukozy (-1%, Cl: -11 do 10%) we krwi, oraz obniżeniem wskaźnika HOMA-IR (-11%, CI: -27% do 9%). W grupie CON nie zaobserwowano tych zmian. Dodatkowo, w grupie HICT odnotowano poprawę wydolności fizycznej ze średniego poziomu VO_{2max}= 28.6 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$ o 20 ± 9% oraz nieznaczny spadek tkanki tłuszczowej -3% (± 4%). Co więcej, zastosowany protokół treningów HICT wpłynął istotnie na zmiany stężenia miokin w krwioobiegu. 5 tygodniowy cykl treningowy HICT obniżył istotnie stężenie miostatyny we krwi ćwiczących kobiet. Spadek ten w szczególności zaobserwowano u starszych uczestniczek (powyżej 30 roku życia), z niższą wydolnością fizyczną (VO_{2max}< 28 $mL \cdot kg^{-1} \cdot min^{-1}$) i insulinoopornością (HOMA-IR> 2.36). Dodatkowo, po zakończonym cyklu treningów, w grupie HICT odnotowano istotny wzrost stężenia IGF-1 34% (± 45%) w surowicy. Z kolei, w grupie CON wystąpiła przeciwna tendencja -29% (± -58%) a skorygowany efekt wynosił 87%, CI: 34% do 162%. Co ciekawe, zaobserwowano również znaczącą redukcję stosunku pomiędzy insuliną, a IGF-1 -21% (± 54%) wśród ćwiczących kobiet. Po upływie 5 tygodni, nie zaobserwowano żadnych istotnych zmian w obwodowym stężeniu białka szoku cieplnego 27 (HSP27), iryzyny, dekoryny oraz w ekspresji genów (*HSPB1, decorin, TNF*α) w żadnej z grup. Stężenie decorin w osoczu nie uległo zmianie w stosunku do stężenia wyjściowego, które wynosiło odpowiednio: 9.91 ± 1.9 $ng \cdot mL^{-1}$ w grupie HICT i 9.44 ± 1.7 $ng \cdot mL^{-1}$ w grupie CON.

Analiza pojedynczych jednostek treningu HICT wykazała, że zarówno pierwsza jak i ostatnia jednostka treningowa spowodowała obniżenie stężenia iryzyny u kobiet z grupy HICT. Co ciekawe, spadek ten zależny był od wieku uczestniczek a skorygowany efekt pomiędzy młodszymi i starszymi kobietami wynosił: -27%, CI: -52% do 10%. Interesujący jest fakt, że pierwsza jednostka treningowa HICT spowodowała istotne obniżenie IL-15. Z kolei, w wyniku ostatniej sesji treningowej zaobserwowano wzrost stężenia tego białka oraz HSP27 15% (± 13%), a zmiany te utrzymywały się do 24 godzin po zakończonym treningu. W grupie CON odnotowano odwrotne tendencje: obniżenie IL-15 i HSP27, a skorygowany efekt wynosił odpowiednio 24%, CI: 9% do 40% dla IL-15 oraz 60%, CI: 29% do 99% dla HSP27. Poziom

stężenia pozostałych miokin nie uległ istotnej zmianie w odpowiedzi na pierwszą i ostatnią jednostkę treningową.

6.2. Główne wyniki badań opublikowane w pracy pt. *Habitually inactive physically - a proposed procedure of counteracting cognitive decline in women with diminished insulin sensitivity through a high-intensity circuit training program.*

Ze względu na fakt, iż w pierwszym eksperymencie nie zaobserwowano istotnych różnic w zmianie funkcji poznawczych postanowiłam przeprowadzić kolejny eksperyment z uwzględnieniem oznaczenia białek zaangażowanych w regulację czynności komórek nerwowych. Tym razem, 15 jednostek treningowych HICT spowodowało znaczącą poprawę funkcji poznawczych u kobiet z obniżoną isulinowrażliwością (QUICKI= 0.359± 0.04). Zmiany te zaobserwowano godzinę po ostatniej jednostce treningowej. W teście CORSI zwiększyła się liczba poprawnie udzielanych odpowiedzi z 9.9± 3.0 do 12.0± 2.7, a w teście interferencji Stroopa trening wpłynął istotnie na czas udzielanych odpowiedzi. Skorygowany efekt pomiędzy wynikami grup HICT i CON, wynosił 17%, CI: 7% do 43% w teście CORSI oraz -24%, -51% do 17% w teście Stroop. Dodatkowo, program treningowy HICT wpłynął korzystnie na jakość życia ćwiczących uczestniczek, w szczególności na funkcjonowanie fizyczne. Przeciwny efekt zaobserwowano w grupie CON, relacja zaobserwowana pomiędzy grupami była istotne statystycznie (p = 0.03, η_p^2 = 0.15). Dodatkowo, zmianom funkcji poznawczych towarzyszyły zmiany stężeń: czynnika wzrostu fibroblastów-21 (FGF21), BDNF, iryzyny, IL-6 i CATB, a zmiany te zależne były od indywidualnej odpowiedzi uczestniczek.

Po zastosowaniu cyklu treningów HICT odnotowano zmiany stężenia FGF21 w obu grupach (HICT: $\Delta\bar{X}$= 20.75 pg·mL^{-1} i CON: $\Delta\bar{X}$= 77.90 pg·mL^{-1}). Wśród uczestniczek z grupy HICT zaobserwowano niejednoznaczny kierunek zmian, zarówno spadek o 43,5% jak i wzrost o 157,3%, tego czynnika. Zaobserwowano również znaczącą różnicę między grupami wiekowymi (skorygowany efekt wynosił: -53%, CI: -75% do 11%). Obniżone stężenie FGF21 odnotowano u kobiet powyżej 30 roku życia, a u kobiet młodszych- wzrost. Efekt tych zmian był mały i prawdopodobny. Interesujący był fakt, iż poprawa funkcji poznawczych była bardziej wyraźna wśród kobiet, które charakteryzował spadek FGF21 w odpowiedzi na trening. Przede wszystkim poprawiła się koncentracja o 17% (± 27%) oraz wzrosła liczba udzielanych poprawnie odpowiedzi w teście CORSI o 28% (± 36%). Wyjściowa wartość FGF21 była również zależna od insulinowrażliwości uczestniczek. Spoczynkowe stężenie FGF21 przed

rozpoczęciem treningów wynosiło średnio 170.68 ± pg·mL^{-1} u kobiet ze wskaźnikiem QUICKI< 0.357 i wzrosło o 17% (±172%) po 5 tygodniach treningów. Z kolei u kobiet z QUICKI> 0.357 początkowo stężenie FGF21 wynosiło średnio 226.94 ± 110.06 pg·mL^{-1} i spadło o 19% (± 136%). Niezależnie od zmian FGF21 wywołanych 5 tygodniowym cyklem treningowym, zaobserwowano wzrost stężenia BDNF 37% (± 139%) w surowicy krwi u kobiet z grupy HICT. Spadek stężenia iryzyny o -10% (± 62%) odnotowano u młodych uczestniczek, z kolei u starszych wzrost o 21% (± 100%). Skorygowany efekt był mały i prawdopodobny. Dodatkowo, zaobserwowano wzrost o 12.55% (± 12.55%) iryzyny jedynie u kobiet z grupy HICT u których odnotowano również wzrost stężenia FGF21. 15 jednostek treningowych HICT nie wpłynęło istotnie na zmiany obwodowego stężenia IL-6 3% (± 63%) i CATB 11% (± 20%). Dodatkowo, po zastosowanym cyklu treningów HICT zaobserwowano dodatnią korelację pomiędzy stężeniem irizyny i CATB (r = 0.55, p = 0.01) oraz tendencję obniżenia ekspresji chemokiny *CCL2*.

Ostatnia sesja treningowa HICT wpłynęła istotnie na obniżenie stężenia FGF21 zarówno w grupie HICT jak i CON. Zaobserwowany spadek FGF21 w grupie HICT odnotowano w szczególności u kobiet poniżej 30 roku życia o -10% (± 43%). W tym samym czasie, zaobserwowano wzrost stężenia BDNF, a skorygowany efekt pomiędzy grupami wynosił 48%, CI: 4% do 128%. Uzyskane wyniki nie były zależne od wieku uczestniczek. Nie zaobserwowano istotnych zmian w stężeniu IL-6 i CATB w wyniku ostatniej jednostki treningu HICT.

6.3. Główne wyniki badań opublikowane w pracy pt. *Impact of 12-week moderate-intensity aerobic training on inflammasome complex activation in elderly women*

12 tygodniowy program treningów nie wpłynął na zmiany masy ciała (p= 0.46), ani BMI (p= 0.99) ćwiczących kobiet. Wpływ treningu na aktywację inflamasomów zmierzono, poprzez określenie stężenia IL-1β i IL-18 oraz TNFα i IL-6 jako markerów odpowiedzi metabolicznej. Po wykonaniu 36 jednostek treningowych zaobserwowano istotne obniżenie stężenia IL-1β u ćwiczących uczestniczek, w stosunku do wartości początkowych (p< 0.05). Podobne zmiany zaobserwowano również w wartościach średnich w podziale, ze względu na BMI (NW: przed 0.130 pg·mL^{-1} do po 0.110 pg·mL^{-1}; OW: przed 0.130 pg·mL^{-1} do po 0.080 pg·mL^{-1}). W żadnej z grup, nie zaobserwowano istotnych zmian w stężeniu spoczynkowym pozostałych cytokin po upływie 12 tygodni. Co ciekawe, poziom mRNA NLRP3 i TLR4 u uczestniczek z grupy EXP

znacząco obniżył się w odpowiedzi na zastosowany cykl treningowy i wynosiła: 0.172 i 0.090 dla NLRP3 oraz 0.184 i 0.087 dla TLR4; odpowiednio przed i po zakończeniu programu.

Pierwsza jednostka treningowa nie spowodowała zmian w ekspresji mRNA NLRP3 i TLR4. Nie zaobserwowano również zmian w stężeniu markerów odpowiedzi metabolicznej w żadnej z grup. Jednak, istotny wzrost stężenia IL-1β (przed 0.130 $pg \cdot mL^{-1}$ po 0.180 $pg \cdot mL^{-1}$) zaobserwowano u uczestniczek z grupy EXP z BMI poniżej 25 $kg \cdot m^{-2}$. Z kolei, istotny wzrost stężenia IL-1β ($p<0.0001$) i TNFα ($p<0.01$) zaobserwowano po ukończeniu ostatniej jednostki treningowej wśród uczestniczek z grupy EXP z BMI powyżej 25 $kg \cdot m^{-2}$. Średnie wartości zmierzone przed i po wykonaniu ostatniego treningu NW wynosiły kolejno: dla IL-1β: od 0.090 $pg \cdot mL^{-1}$ do 0.180 $pg \cdot mL^{-1}$ oraz dla TNFα: od 2.450 $pg \cdot mL^{-1}$ do 4.490 $pg \cdot mL^{-1}$. Dodatkowo w odpowiedzi na ostatnią sesję NW zauważono istotny wzrost ekspresji mRNA NLRP3 (przed: 0.090 po 0.237) oraz mRNA TLR4 (przed 0.087 po 0.256) u kobiet z grupy EXP.

7. Dyskusja

Badania ostatnich lat, które doprowadziły do poznania białek uwalnianych do krwiobiegu w odpowiedzi na wysiłek fizyczny umożliwiły wykorzystanie ich w profilaktyce chorób przewlekłych [40]. Miokiny mogą być znaczącymi bio-markerami w monitorowaniu rodzaju i intensywności ćwiczeń odpowiednimi do zalecenia osobom z zaburzeniami metabolizmu, takimi jak cukrzyca, choroby neurodegeneracyjne czy nowotwory [41]. Dlatego celem powyższej rozprawy doktorskiej była weryfikacja efektu zastosowania różnych programów treningów wytrzymałościowych o charakterze interwałowym (HICT) i ciągłym (NW) u nieaktywnych kobiet.

Wyniki przeprowadzonych eksperymentów wskazują, iż program treningu HICT wpływa korzystnie na homeostazę glukozy w grupie nieaktywnych kobiet. Poprawie wskaźników świadczących o insulinooporności, towarzyszył istotny spadek stężenia w surowicy, miostatyny- czynnika negatywnie regulującego przyrost tkanki mięśniowej [42]. Spadek ten był szczególnie widoczny właśnie w grupie starszych kobiet (pomiędzy 30 a 54 rokiem życia) z insulioopornością i niższym poziomem wydolności fizycznej. U kobiet w wieku 76-96 lat, wykazano podwyższony poziom miostatyny w osoczu [43], co wskazuje na zwiększenie się jej stężenia wraz z wiekiem i może świadczyć o powiązaniu z utratą masy mięśniowej. Badania dotyczące zastosowania treningu NW, wykazują jego korzystny wpływ na obniżenie stężenia miostatyny w grupie osób w wieku 61± 12 lat. Jak również, występowanie istotnej, odwrotnej korelacji pomiędzy stężeniem miostatyny a stężeniem miokiny o działaniu do niej antagonistycznym- dekoryny [44]. Dekoryna, może brać udział w regulacji procesu hipertrofii tkanki mięśniowej [22], a badania na modelach komórkowych wykazały, iż może hamować tworzenie metastaz w procesie nowotworowym [45]. Zastosowanie cyklu treningów HICT nie wpłynęło na zmiany stężenia i ekspresji dekoryny we krwi trenujących kobiet.

Dane dotyczące zastosowania pojedynczej jednostki treningu wytrzymałościowego na zmiany funkcji poznawczych, wykazują korzystny wpływ na koncentrację i proces przetwarzania informacji [46,47]. Poprawę funkcji poznawczych odnotowano w wyniku zastosowania pojedynczej jednostki treningu HICT, lecz zmiana ta zależna była od wieku kobiet i towarzyszyło jej obniżenie stężenia BDNF w surowicy krwi [15]. Efekt zastosowania programu treningu interwałowego z wykorzystaniem obciążenia masą własnego ciała na funkcje endokrynną tkanki mięśniowej i towarzyszące zmiany w funkcji mózgu, nie są do końca poznane. Przedstawione w powyższej rozprawie doktorskiej wyniki badań, dowodzą, że regularny trening HICT (minimum 3 razy w tygodniu, przez 5 tygodni) wpływa znacząco na

poprawę funkcji poznawczych m. in. pamięć krótkotrwałą oraz jakość życia. Co ciekawe, zmianom tym towarzyszyło obniżenie stężenia FGF21, na którą to redukcję miał wpływ wiek kobiet i wrażliwości tkanek na insulinę. Zaobserwowano ciekawą tendencję: w grupie kobiet w których odnotowano wzrost FGF21, funkcje poznawcze nie poprawiły się, natomiast badane, które charakteryzowały się obniżeniem FGF21, odnotowały równocześnie poprawę funkcji poznawczych. 5 tygodniowy program HICT spowodował wzrost obwodowego stężenia BDNF i CATB. Wzrost BDNF we krwi był prawdopodobnie rezultatem jego uwalniania przez komórki nerwowe mózgu lub płytki krwi. Mimo wielu lat badań nie stwierdzono, iż tkanka mięśniowa może być źródłem BDNF. Mierzone stężenie we krwi jest raczej wynikiem uwalniania BDNF przez płytki krwi lub przechodzenia tego czynnika przez barierę mózg-krew. Zaobserwowany wzrost miokiny CATB miał związek ze wzmożoną czynnością mięśni szkieletowych. Ze względu na fakt, iż CATB jest białkiem, które może przekraczać barierę krew- mózg [23], odnotowana poprawa funkcji poznawczych mogła być kompensowana również przez tę miokinę. Uzyskane wyniki wskazują, iż zmiany funkcji poznawczych i stężenia białek są zależne od indywidualnej odpowiedzi uczestniczek i dlatego w badanej grupie wyróżniłam w przypadku jakich miokin ta zindywidualizowana odpowiedź była największa.

Z kolei, jak dowodzą, wcześniejsze badania pojedyncza sesja treningu NW wpływa na zmiany w stężeniu obwodowego BDNF i obniża poziom glukozy we krwi u kobiet z długim stażem treningowym, a zastosowanie 12 tygodniowego cyklu treningów NW wpływa korzystnie na poprawę funkcji poznawczych wraz z towarzyszącym wzrostem BDNF u kobiet w wieku 68± 5 lat [17]. Ten rodzaj aktywności fizycznej zmniejszył stężenie białka regulującego proces autofagii HMGB1, a istotnym czynnikiem, który regulował tą redukcję był zarówno poziom witaminy D (>20 $ng \cdot mL^{-1}$), jak i wzrost miokiny- iryzyny [48].

Aktualne dane dotyczące zmiany statusu zapalanego i zmian stężenia miokin w krwioobiegu w wyniku zastosowania programu treningu NW, są dobrze udokumentowane [44,48,49]. Jednak, brak jest badań związanych z wpływem tego rodzaju aktywności fizycznej na aktywację inflamasomów. Dotychczas, udokumentowano, że 8 tygodniowy trening siłowy obniża ekspresję białka TLR2 i TLR4, jak również ekspresję kilku cząsteczek związanych z sygnalizacją TLR (np. MyD88, TRIF, NF-κB, MAPK) i poziom białka C-reaktywnego (CRP) [50]. Z kolei, 8 tygodniowy cykl treningu wytrzymałościowego u osób starszych, wykazują brak zmian w ekspresji TLR2, TLR4, MyD88 i TRIF w jednojądrzastych komórkach krwi [51], co może świadczyć o przeciwzapalnym efekcie tego treningu. Badania przeprowadzone w ostatnich latach, wykazały, że regularny trening wytrzymałościowy o umiarkowanej

intensywności, ćwiczenia oporowe i HIIT znacząco obniżają aktywację inflamasomu NLRP3, indukowaną przez różne czynniki patologiczne, poprawiając w ten sposób wrodzoną odporność [52]. Przedstawione w powyższej rozprawie doktorskiej wyniki, przedstawią znaczące obniżenie ekspresji mRNA dla TLR4 i NLRP3 pod wpływem 12 tygodniowego programu treningów NW. Aktywacja inflamasomu może stanowić zmianę adaptacyjną, nabywaną w trakcie wykonywania programu treningowego. Zastosowany protokół wysiłkowy obniżył stężenie prozapalnej cytokiny IL-1β, a zmiana ta widoczna była u kobiet, niezależnie od BMI. Intersujący jest fakt, że wzrost stężenia IL-1β zaobserwowano w odpowiedzi na pierwszą jednostkę treningu NW u kobiet z BMI poniżej 25 $kg \cdot m^{-2}$. Ostatnia sesja treningowa spowodowała wzrost stężenia tej cytokiny i TNFα u kobiet otyłych (BMI> 25 $kg \cdot m^{-2}$). Wyniki te mogą świadczyć o korzystnym wpływie regularnego podejmowania treningu NW na obniżenie stężenia IL-1β, jako markera aktywacji inflamasomów, u kobiet bez względu na BMI.

Podsumowując, wyniki moich badań wykazują, że zarówno program treningów HICT jak i NW mogą stanowić skuteczną metodę prewencji przed rozwojem zaburzeń metabolicznych. Efekt podejmowanych form aktywności ruchowej nie ogranicza się do poprawy sprawności układu krążeniowo-oddechowego, ale również istotną rolę odgrywają w tej regulacji miokiny i zmiany ich stężeń. Należy podkreślić, że zaproponowany, nowatorski cykl treningów HICT ze względu na bezpieczeństwo wykonania oraz przystępną metodykę ćwiczeń umożliwia podejmowanie regularnie, prozdrowotnej aktywności fizycznej w domowych warunkach.

8. Wnioski

Wyniki przeprowadzonych przeze mnie badań dowodzą, że:

1. Zmiany stężenia białek/miokin we krwi kobiet, widoczne są po zastosowaniu treningów o charakterze wytrzymałościowym.

2. 5 tygodniowy cykl treningów interwałowych HICT powoduje poprawę homeostazy glukozy oraz obniżenie stężenia miostatyny we krwi kobiet. Efekt ten widoczny był szczególnie u kobiet powyżej 30 roku życia z insulinoopornością.

3. Zastosowanie 15 jednostek treningu HICT poprawia funkcje poznawcze oraz jakość życia trenujących kobiet. Zmiany te zależne są od stężenia FGF21 oraz od indywidualnej odpowiedzi uczestniczek badań.

4. 12 tygodniowy cykl treningów NW o charakterze ciągłym zredukował ekspresję mRNA inflamasomu NLRP3, w konsekwencji czego obniżył się spoczynkowy poziom prozapalnej cytokiny IL-1β u ćwiczących kobiet.

9. Streszczenie

Wiedza na temat prozdrowotnego wpływu treningu fizycznego jest coraz bardziej obszerna. Jednak mechanizmy związane ze zmianami adaptacyjnymi indukowanymi przez różne formy wysiłku fizycznego nie są dostatecznie zweryfikowane i wyjaśnione. Szczególną rolę w tych procesach przypisuje się miokinom- białkom uwalnianym do krwioobiegu przez tkanki poddawane obciążeniu fizycznemu.

Celem przeprowadzonych przeze mnie badań była ocena wpływu różnych form treningów wytrzymałościowych w formie pojedynczej jednostki treningowej oraz cyklu treningów u kobiet w różnym wieku na poziom wybranych miokin. Oraz zweryfikowanie jak zastosowane programy treningowe mają wpływ na zmiany metabolizmu ogólnoustrojowego.

W badaniach wzięło udział 130 kobiet w wieku 21-75 lat. Uczestniczki losowo przydzielone zostały do grup trenujących i kontrolnych. Osoby z grupy trenującej mające powyżej 54 lat zostały objęte 12 tygodniowym programem treningów o charakterze ciągłym- Nordic Walking (NW). Z kolei młodsze uczestniczki wykonały 5 tygodniowy cykl treningów oporowych z wykorzystaniem masy własnego ciała jako obciążenia (HICT). Jednostki treningowe odbywały się 3 razy w tygodniu. W grupie kontrolnej znajdowały się zarówno osoby młodsze, jak i seniorki. Przed przystąpieniem do eksperymentu uczestniczki zostały poddane badaniu lekarskiemu w celu zakwalifikowania ich do dalszych badań na podstawie wywiadu i wyników morfologii krwi. Przed rozpoczęciem i po zakończeniu każdego z cykli treningowych oraz pierwszej i ostatniej jednostki treningowej, pobrano krew w celu zmierzenia stężenia wybranych cytokin.

Wyniki przeprowadzonych badań wykazały, że zaproponowane protokoły treningów wytrzymałościowych, wpływają znacząco na zmiany obwodowego stężenia miokin we krwi kobiet w różnym wieku. Zastosowanie 15 jednostek treningu HICT poprawia insulinowrażliwość, przejawiającą się spadkiem obwodowego stężenia insuliny (-20%, CI: -42% do 10%) i glukozy (-1%, Cl: -11 do 10%) we krwi, oraz obniżeniem wskaźnika HOMA-IR (-11%, CI: -27% do 9%), w porównaniu do grupy CON. A zmianom tym towarzyszy obniżenie stężenia miostatyny. Trening HICT poprawił również jakość życia u kobiet ($p = 0.03$, $\eta_p^2 = 0.15$), niezależnie od wieku. Interesujący jest fakt, że poprawa funkcji poznawczych zależna była od stężenia czynnika wzrostu fibroblastów 21 (FGF21), a poprawę funkcji poznawczych zaobserwowano u kobiet, które charakteryzował spadek FGF21. 5 tygodniowy trening HICT spowodował wzrost stężenia neurotroficznego czynnika pochodzenia mózgowego (BDNF) o 37% (± 139%) we krwi trenujących uczestniczek. Efekt ten

zaobserwowano również w wyniku pojedynczej sesji treningowej, a skorygowany efekt pomiędzy uczestniczkami z grupy HICT i CON wynosił 48%, CI: 4% do 128%. Z kolei, 12 tygodniowy cykl treningów NW wpływa na znaczną redukcję stężenia prozapalnej cytokiny IL-1β ($p < 0.05$) i ekspresji TLR4 i NLRP3 u ćwiczących kobiet. Co ciekawe zauważono istotny wzrost ekspresji TLR4 ($p < 0.05$) i NLRP3 ($p < 0.05$) w odpowiedzi na ostatnią sesje treningową NW u kobiet z grupy EXP. Zmian tych nie odnotowano u kobiet z grupy kontrolnej.

Podsumowując, powyższe wyniki dowodzą skuteczności zastosowania cyklu programów HICT i NW na poprawę metabolizmu ogólnoustrojowego w grupie kobiet. Zaproponowane protokoły programów treningowych indukują zmiany adaptacyjne, a istotną rolę w osiąganiu korzystnych, prozdrowotnych efektów odgrywają miokiny.

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Circulating myokines concentration changes in response to endurance training in women

Ph.D. thesis under the supervision of:

Professor Ewa Ziemann

Gdansk 2022

10. Abstract

Current knowledge about the health-promoting effects of physical training is becoming more extensive. However, the mechanisms related to adaptive changes induced by various forms of physical exercise are not sufficiently verified and explained, yet. A special role in these processes is assigned to myokines - proteins released into the bloodstream by tissues during performing physical activity.

The aim my study was to verify the impact of various types of endurance training (as well single training unit as whole training program) in women different age, on the selected myokines concentration. The second purpose of the study was to determine an impact on applied training programs on changes in glucose homeostasis and inflammatory response.

130 women (aged 21 to 75 years), participated in the research. Subjects were randomly assigned to training and control groups. Women from the training group aged over 54 performed 12-week continuous training program - Nordic Walking (NW). Whereas, the younger participants performed a 5-week resistance training program, using their own body weight as a workload (high-intensity circuit training; HICT). Each program, was held 3 times a week. The control group included women different age. Before beginning of the experiment, all participants underwent a medical examination and based on the interview and blood count results they were included to the study program. Blood was collected before and after each training cycle, and first and last training unit to measure the concentration of selected cytokines.

The results showed that the proposed endurance training protocols induced significant changes in the myokines concentration in women of all ages. The 15 units of HICT training improves insulin sensitivity, which was manifested by a decrease in the concentration of insulin (-20%, CI: -42% to 10%) and glucose (-1%, Cl: -11 to 10%) in the blood, and a decrease in the HOMA-IR (-11%, CI: -27% to 9%), compared to the CON group. These changes were accompanied by a decrease in the myostatin concentration. HICT training also improved the quality of life in women ($p = 0.03$, $\eta_p^2 = 0.15$), regardless of age. Interestingly, the improvement of cognitive functions was dependent on the concentration of fibroblast growth factor 21 (FGF21), and this effect was observed in women characterized by a decrease in FGF21. The 5-week HICT training increased the concentration of brain-derived neurotrophic factor (BDNF) by 37% (± 139%) in training women. This effect was observed as well after single training session, and the adjusted effect between HICT and CON participants was 48%, CI: 4% to 128%. On the other hand, 12-week NW training program significantly reduced the pro-inflammatory cytokine blood concentration IL-1β ($p < 0.05$) and the expression of mRNA of TLR4 and

NLRP3 in exercising women. Interestingly, a significant increase of TLR4 ($p < 0.05$) and NLRP3 ($p < 0.05$) expression was noticed in response to the last NW training in women from the EXP group. However, these changes were not noted in women from the control group.

Overall, abovementioned results prove the effectiveness of the application of the HICT and NW training programs in the improvement of systemic metabolism in women and myokines induced by different training protocols modified beneficial, pro-health effects.

11. Piśmiennictwo

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OŚWIADCZENIE WSPÓŁAUTORÓW PUBLIKACJI

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International Diabetes Federation

The beneficial effects of 15 units of high-intensity circuit training in women is modified by age, baseline insulin resistance and physical capacity

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ABSTRACT

Aim: To investigate the effect of a single and 15 units of high-intensity circuit training (HICT) programme on glucose metabolism, myokines' response and selected genes' expression in women.

Methods: Thirty-three, non-active women (mean age: 38 ± 12) were split into a HICT (n = 20) or a control group (CON, n = 13). The training protocol included three circuits of nine exercises with own body weight as a workload performed 3 times a week for five weeks. The CON group performed HICT twice. Blood samples were taken before, 1 h and 24 h after the first and last unit to determine IGF-1, myostatin, irisin, decorin, HSP27, interleukin-15 concentrations using the ELISA immunoenzymatic method. To evaluate *HSPB1*, *TNF-α* and *DCN* mRNA, real-time PCR was used. Pre- and post-intervention, the oral glucose test and body composition assessment were completed.

Results: The following parameters tended to decrease after the 5-week HICT program: insulin and HOMA-IR Training diminished insulin/IGF-1 ratio (51% CI: −63% to −34%) and induced the drop of myostatin concentration but significantly only among middle-aged women and at baseline insulin resistance.

Conclusion: Obtained data revealed that HICT improved an insulin sensitivity and diminished myostatin concentration among older, insulin-resistant women with lower baseline physical capacity.

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1. Introduction

Physical activity deficit has become an important public health issue in recent years. In the long-term, a sedentary lifestyle is associated with an increased risk of mortality, cardiovascular disease, and type 2 diabetes mellitus (T2DM), which is commonly registered among adults as well as young patients [1]. At the same time, physical activity promotes better functioning of the human body through adaptive changes. Regular exercise supports improved insulin sensitivity, thus, helping to prevent diabetes [2].

The most common reason for not engaging in physical activity is lack of time. Since 2014, the American College of Sports Medicine (ACSM) has been consistently recommending high-intensity interval training (HIIT) as the most effective and time-efficient form of exercise [3,4]. Not only is HIIT thought to induce similar changes as moderate-intensity continuous training, but it can be practiced by people with an impaired glucose tolerance and T2DM [5]. Furthermore, a modification of HIIT, high-intensity circuit training (HICT) using one's own body weight as a workload, is considered as a safe way of practicing resistance training [6].

Data published by Gmiat et al. showed that in a group of women, a single session of HICT had a positive impact on cognitive function and immunological response, modulated by circulating myokines. Still, the effect depended on participants' age [7]. These findings carry a particular importance, as few studies so far have investigated the combined effect of resistance training and endocrine function of muscle mass (myokines released during contractions) [8].

It is worth noting that skeletal muscles are responsible for >75% of insulin-mediated glucose metabolism [9]. Moreover, reduced muscle mass contributes to skeletal muscles' insulin resistance [10]. Considering this observation and the anabolic action of some myokines (IL-15), during maintenance or development of muscle mass through resistance training [11], it appears to be crucial for counteracting adverse metabolic changes. Nonetheless, a recently published review emphasised that among 51 published papers relating to resistance training, only 12 involved female participants [12].

The potential treatment and prevention of metabolic diseases associated with regular physical activity is ascribed to the myokines released during exercise. Another protein, decorin, secreted during exercise, has been shown to have an inhibitive impact on breast cancer progression [13] and a blinding effect on myostatin that consequently conduces to muscle hypertrophy [14].

Myostatin as a member of the TGF-β superfamily is a negative regulator of muscle development [15], and is also mentioned to be involved in energy expenditure and glucose homeostasis [16]. Higher expression of myostatin mRNA in skeletal muscles has been observed in obese, insulin-resistant subjects [17]. Moreover, a study on mice has indicated that the animals lacking the myostatin gene (*Mstn* $^{-/-}$) have exhibited an improved glucose uptake and insulin sensitivity [18]. Data published by Dong et al. revealed that inhibition of myostatin improves insulin sensitivity via irisin-mediated crosstalk between muscles and adipose tissue [9]. Involvement of irisin in glucose homeostasis [19] may be modified by signalling between insulin and insulin-like growth factor 1 (IGF-1) [20].

Interestingly, insulin resistance (IR) may be affected by heat shock protein beta 1 (gene expression as *HSPB1*), also known as heat shock protein 27 (HSP27). Yuan et al. have demonstrated that AMPK-mediated *HSPB1* expression enhanced insulin sensitivity in skeletal muscles [21]. Hence, the increase of HSP27 24 h after a single bout of HICT recorded by Gmiat et al. should encourage further investigations to verify this form of exercise as an effective, health-promoting strategy [7].

We have decided therefore to investigate the effect of a single session as well as a 5-week HICT programme applied in adult women on glucose homeostasis and myokines' secretion, which can modulate and enhance the response to training. Given that blood cells are known to be the best way of describing the whole-body response to exercise [22], together with proteins: HSP27, IGF-1, IL-15, irisin, myostatin and decorin, a genetic assessment of *HSPB1*, *DCN* and *TNF-α* mRNA was also evaluated.

2. Materials and methods

2.1. Subjects

Thirty-six healthy, inactive women (within last year) without diabetes characterised by a fair level of relative maximal oxygen uptake (average VO_{2max} 29 ± 6.8 mL·kg^{-1}·min^{-1}) [23] participated in the experiment. At baseline women were examined by a doctor in order to eliminate those with medical contraindications. Written, informed consent was obtained from all participants. Based on the values of relative maximal oxygen uptake, women were divided into two groups trained HICT (n = 20, age: 40 ± 11 years) or control CON (n = 13, age: 45 ± 13 years). Women were additionally divided according to the young (YG n = 11, age ≤30 years) and middle-aged (MG n = 9, age >30 years) group, in order to assess age-depended changes [7]. Only women whose training attendance exceeded 85% were included in to statistical analysis and three women were therefore excluded in final analysis.

One week prior to the start of the experiment and directly (within four days) after 5 weeks of completed training the following tests were performed: body composition, aerobic capacity assessment, blood collection and functional movement screen (FMS).

Women were asked to not change their daily habits. The study was approved by the Bioethical Committee of the Regional Medical Society in Gdansk KB-14/17 in accordance with Declaration of Helsinki.

2.2. Preliminary testing

2.2.1. Body composition assessment

Skeletal muscle mass (SMM), body fat mass (BFM), and percent body fat (PBF) were evaluated by using a multi-frequency impedance Analyser In Body 720 (Biospace, Korea). Impedance of segments of the body parts (trunk, arms and legs) was measured at diverse 6 frequencies (1, 5, 50, 250,

500, and 1000 kHz) using an eight-polar tactile-electrode. Percent of body fat mass repeated measurement precision was expressed as the coefficient of variation, which was on average, 0.6% [24]. Additionally, the amount of the visceral fat area (VFA) expressed in cm^2 was determined [25,26].

2.2.2. *Cardiorespiratory fitness measurement*

To assign a VO_{2max} value participants performed a graded cycle test on an electromagnetically-braked, cycle ergometer (884E Sprint Bike Monark, Sweden). The test began with 5-minute warm up with the intensity 1 $W \cdot kg^{-1}$ and pedalling cadence 60 rpm. Directly after the warm up, participants began VO_{2max} testing by cycling with the same pedalling cadence but with progressively increased workload by 25 $W \cdot min^{-1}$ until the subject reached the point of volitional exhaustion. During testing breath by breath pulmonary gas exchange was measured (MetaMax 3B, Cortex, Germany) [27]. The highest values of relative oxygen uptake were considered to calculate maximal aerobic power. Participants were identified according to cardiorespiratory fitness level. The cut-off point was set at the level of 28 $mL \cdot kg^{-1} \cdot min^{-1}$, women above were described as high fitness level (VO_{2max}) and those below as low fitness level (VO_{2max}).

2.2.3. *Functional Movement Screen (FMS)*

FMS was used to assess participants' movement patterns, the mobility and stability of certain joints and the coordination of certain kinetic chains. It includes seven tasks: deep squat, hurdle step, inline lunge, shoulder mobility, active straight leg raises, trunk stability push-up and rotary stability. Each task is evaluated by the quantity and the quality of the movement by the scale from 0 to 3. The sum score of all tasks is calculated [28].

2.3. *Training procedure*

HICT training protocol was the same as in our previous study [7] based on ACSM recommendations and the original protocol [6]. Before the main experiment we recorded a movie with instructions (whole units of training) with music and time visualisation, that was easy to play on mobile devices. One week before beginning the experiment, participants had been familiarised with the protocol in order to perform each exercise correctly at an appropriate intensity (80%–90% of maximal heart rate HR_{max}). The first and last unit of HICT were performed under instructor's supervision, in case of the need to correct and control the quality of the training. Directly after the first and the last HICT sessions participants were asked to assess the rating of perceived exertion (RPE), based on the Borg scale [29]. During the training programme participants were allowed to train individually at home, and they were also asked to train always at the same time of a day. Each week the instructor called (or directly asked) to confirm the attendance of HICT sessions and assess the RPE values. After 2 weeks of training the additional series were added. Subjects from training group completed 15 HICT sessions 3 times per week (on Monday, Wednesday and Friday) within 5 weeks. One HICT session consisted of 3 circuits with 2-minute break between. Each HICT training consisted 9 exercises with one's body as a workload performed as follow: jumping jacks, push-ups, sit-ups, side plank, squats, plank, running in place, lunges and push-ups with rotation. Subjects from the CON group completed HICT twice at the baseline and after 5 weeks. Supplementary Material 1 shows the schedule of the study.

2.4. *Blood collection*

Blood samples were taken at two time points during the experiment: before and after the whole intervention and also before and after single bout of HICT. A professional nurse collected the blood from the antecubital vein into the vacutainer tubes: with $EDTAK_3$ for plasma analysis, Vacutainer SST™ II Advance for serum analysis and into the vacutainer tubes with sodium fluoride to estimate glucose concentration. One week before and two days after 5 weeks of training blood samples were collected to perform oral glucose tolerance test (OGTT). Before, 1 h and 24 h after the first and last HICT sessions blood samples were collected. Samples were centrifuged at 2000 g for 10 min at 4 °C then stored at −80 °C by the time of immunoenzymatic analysis. Serum concentrations of irisin, IL-15, myostatin, IGF-1, HSP27 and plasma level of decorin were measured. Additionally, gene expression was performed.

Serum IL-15, myostatin, and IGF-1 were evaluated using ELISA kits (R&D Systems, USA, catalogue no. D1500, DGDF80, and DG100 respectively) in accordance with manufacturer's instructions. The maximal intra-assay coefficient of variability (CV) for IL-15 was 5.3%, for myostatin was 5% and for IGF-1 4.3%. The inter-assay coefficient and detection sensitivity were as follows: 9.1% and 2 $pg \cdot mL^{-1}$ for IL-15; 6% and 5.32 $pg \cdot mL^{-1}$ for myostatin and 8.3% and 0.056 $ng \cdot mL^{-1}$ for IGF-1. The serum concentration of irisin was evaluated using competitive enzyme immunoassay sandwiches from Phoenix Pharmaceuticals Inc (catalog no EK 067-16). Intra-assay CV was 4%–6% and inter-assay CV was 8%–10%. The procedure was the same as described previously [7].

Quantification of plasma decorin was determined via Human Decorin DuoSet ELISA (R&D Systems, USA, catalogue no. DY143) and DuoSet ELISA Ancillary Reagent Kit (catalogue no. DY008) according to the manufacturer´s protocol.

Serum HSP27 was evaluated using a Cloud-Clone Corp. ELISA kit (USA). The minimum detection limits were 0.31 $ng \cdot mL^{-1}$ and the intra-assay and inter-assay CV were <10% and <12% respectively.

Glucose level was assessed using the Cobas 6000 analyser. To evaluate insulin values the immunoassay kit from DiaMetra (catalogue no DKO076) was used. The within intra-assay CV was ≤5% and the inter-assay CV was ≤10%.

Homoeostasis model assessment HOMA-IR (fasting serum insulin $\mu U \cdot mL^{-1}$ × fasting plasma glucose $mmol \cdot L^{-1}/22.5$), was calculated [30].

2.5. *Genetic research*

Genetic methodology was conducted according to a protocol described previously by Zychowska et al. [31].

2.5.1. RNA isolation

During analysis 2 mL of blood was collected into the vacutainer tube with $EDTAK_3$ and used for mRNA extraction. Erythrocytes were lysed and discarded using RBCL buffer (A&A Biotechnology, Poland) and the obtained leukocytes were lysed using Fenozol (A&A Biotechnology, Poland). Further isolation of RNA was performed according to the methodology described by Chomczynski and Sacchi [32].

2.5.2. Reverse transcription and quantitative real-time polymerase chain reaction

Quality and quantity obtained RNA was analised using spectrophotometery (photometer, Eppendorf, BioPhotometer Plus, Germany), and 1000 ng of total RNA were used to reverse transcription (Transcriptor First Strand cDNA Synthesis Kit, Roche, Poland).

To quantitative real-time PCR (qRT-PCR) 10-fold diluted cDNA was applied. To amplify tested genes, a reaction mix containing the following was used: 5 μL polymerase (LightCycler polymerase; Roche, Poland), 0.4 μL reverse and forward primers and 2.2 μL H_2O for each reaction. The thermal profile was used according to the manufacturer's instructions. qRT-PCR was applied using AriaMx real-time PCR System (Agilent, Department Poland) and amplification of tested genes was performed in three replications for each sample. *DCN* (decorin) and *TNF-α* (tumor necrosis factor α) expression were checked using two different primer pairs.

For amplification of tested genes following the primers were used:

For *TUBB*: R:TCTGTCGGCTCCGCTCTGAGAT and F:ACTCCC GTTGTCCCAAGGCTCT

For *DCN*: F: AAGTTCCTGATGACCGCGACTT and

R: TTGCAGGTCTAGCAGAGTTGTG (F: GATGAGGCTTCTGGG ATA

and R: CAATGCGTGAAGGTTCTT)

For *TNF-α*: F:GCCATTGGCCAGGAGGGC and

R:CGCCACCACGCTCTTCTG (F:TTCTCCTTCCTGATCGTGCA R:TACAGGCTTGTCACTCGG)

For *HSPB1*: F: AAGGATGGCGTGGTGGAGATCA and

R: GAGGAAACTTGGGTGGGGTCCA

Target genes expression were calculated to the expression of the reference gene *TUBB* according to the delta Ct method [33].

2.6. Statistical calculation

All measures were compiled in a spreadsheet for the analysis of parallel-group trials and the effects were interpreted using magnitude-based inferences [34]. All data were log- transformed to reduce bias arising from non-uniformity of the error. To improve precision of estimates, mean changes were adjusted to the overall mean of baseline in the CON and HICT groups. Baseline values were expressed in measurement units. Means of the observed and adjusted changes, standard deviations of the observed changes, and adjusted effects (differences in changes of the means and their confidence intervals) were back-transformed to percentages. Magnitudes of the effects were evaluated with the log-transformed data by standardizing with the standard deviation of the overall baseline values. Threshold values for assessing magnitudes of standardised effects were 0.20, 0.60, 1.2 and 2.0 for small, moderate, large and very large respectively Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial or trivial as follows: * – possible, ** – likely, *** – very likely and **** – most likely [35].

3. Results

Overall, data obtained from thirty-three participants were evaluated. Table 1 presents the anthropometrical and physiological data. Although any significant changes were recorded in body composition, it is worth noting that the applied HICT programme reduced body fat content expressed in kg as well as in percentage of body mass. Women who completed 15 training sessions gained better results in FMS and were characterised by the elevated relative maximal oxygen consumption (VO_{2max}) compared to the baseline values (Table 1). Ratings of perceived exertion differed between groups. Among the HICT group values of RPE decreased from 18 ± 3 after the first to 16 ± 2 after the last training session, whereas in the CON group values remained unchanged (16 ± 2 and 16 ± 8, respectively).

The training programme tended to improve glucose homeostasis in the HICT group. The 15th units of HICT caused some decrease in resting glucose, insulin concentrations and diminished the HOMA-IR (insulin −20% CI: −42% to 10%; glucose −1% Cl: −11 to 10% and HOMA-IR −11% CI: −27% to 9%, respectively) as depicted in Fig. 1 which in fact only shows a slight difference among the two groups. Still, in the CON group those indicators remained unchanged or presented an opposite tendency.

The applied training programme induced a significant 34% ±45% rise of IGF-1, whereas in the CON group the decrease (−29% ±58%) of this protein was noted and the adjusted effect for this change was 87%, CI: 34% to 162%. This effect was firstly noted after single HICT, especially prominent 24 h post training. Furthermore, the ratio insulin to IGF-1 declined significantly (−21% ±54%) only in the HICT group (Fig. 1d). These alterations were independent of participants age, VO_{2max} and IR. The exercise-induced changes of myostatin in response to a single session of HICT were small, nonsignificant and registered only 24 h after effort. Still, the whole HICT program caused a decrease in resting serum myostatin (Fig. 2a). Interestingly, the drop in resting values of myostatin, recorded after 5 training weeks, was much more pronounced and significant among the MG than YG group (Fig. 2b). Based on diversification of HOMA-IR in the HICT group, the higher decline of myostatin was observed in women, who were classified at baseline as IR and the range of changes was higher compared to non-insulin-resistant group (nonIR) (Fig. 2c). Moreover, when considering individual values of relative oxygen uptake of women from the HICT group, a higher and significant decrease of myostatin was registered in participants with VO_{2max} below 28 $mL \cdot kg^{-1} \cdot min^{-1}$ (Fig. 2d).

The intervention did not modify the resting concentration of decorin in either group (9.91 ± 1.9 $ng \cdot mL^{-1}$ and 9.44 ± 1.7 $ng \cdot mL^{-1}$ in HICT and CON group, respectively). Values recorded at rest as well after first and last training session

Table 1 – Characteristic of participants divided into groups.

	Group	Baseline	Adjusted change	Adjusted effect		
				Mean	CI	Inference
Anthropometric parameters						
BFM (kg)	CON	25.9 ± 10.5	4 ± 3%	−8%	−10 to −5%	Trivial
	HICT	20.5 ± 9.6	−4 ± 5%			
SMM (kg)	CON	26.4 ± 3.3	−1 ± 3%	2%	0 to 3%	Trivial
	HICT	26.6 ± 3.5	1 ± 3%			
PBF (%)	CON	33.9 ± 8.4	4 ± 3%	−7%	−9 to −5%	Small*
	HICT	28.6 ± 8.5	−3 ± 4%			
VFA (cm^3)	CON	110.9 ± 48.9	−1 ± 4%	0%	−3 to 3%	Trivial
	HICT	80.8 ± 35.4	−2 ± 7%			
Physiological performance						
VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$)	CON	30.2 ± 8.7	5 ± 5%	15%	10 to 20%	Moderate****
	HICT	28.6 ± 5.1	20 ± 9%			
AP ($W \cdot kg^{-1}$)	CON	2.03 ± 0.6	−1 ± 10%	9%	2 to 16%	Small**
	HICT	2.25 ± 0.5	8 ± 12%			
FMS	CON	13.5 ± 2.1	6 ± 9%	6%	1 to 11%	Small**
	HICT	14.9 ± 1.5	12 ± 5%			

HICT – high intensity circuit training group, CON – control group, BFM – body fat mass, SMM – skeletal muscle mass, PBF – percentage body fat, VFA – visceral fat area, VO_{2max} – maximal oxygen uptake, AP – aerobic power, FMS – functional movement screen. Asterisks indicate effects clear at the 5% level (90% CL) and likelihood that the true effect is substantial or trivial. as follows: * possible, ** likely, **** most likely. Magnitude thresholds (for difference in means divided by SD of HICT group): <0.20, trivial; 0.20–0.59, small; 0.60–1.19, moderate; >1.20, large.

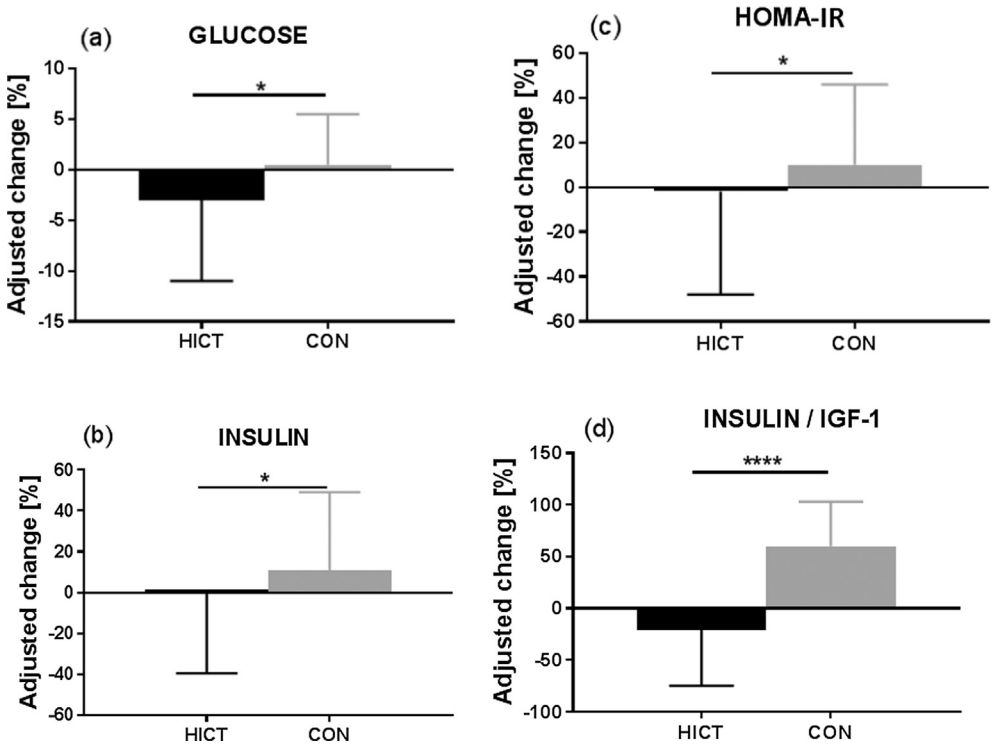

Fig. 1 – Adjusted changes in indicators of glucose homeostasis in response to 5 week high- intensity circuit training in high-intensity interval in group (HICT) and control group (CON): (a) Glucose level. (b) Insulin level. (c) the Homeostasis Model Assessment (HOMA-IR). (d) Insulin/IGF-1 ratio. Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial: * – possible, ** – likely, * – very likely, **** – most likely.**

remained unchanged. On the other hand, the single HICT unit significantly altered IL-15 concentration, and this change was different among groups. The first HICT session involved a decrease of IL-15 in the HICT as well as in the CON group (Fig. 3a), whereas after the last HICT training session, concentration of IL-15 increased only in the HICT group with the tendency being opposite in the CON (24%, CI: 9% to 40%) (Fig. 3b). Notably, obtained changes were sustained 24 h post exercise.

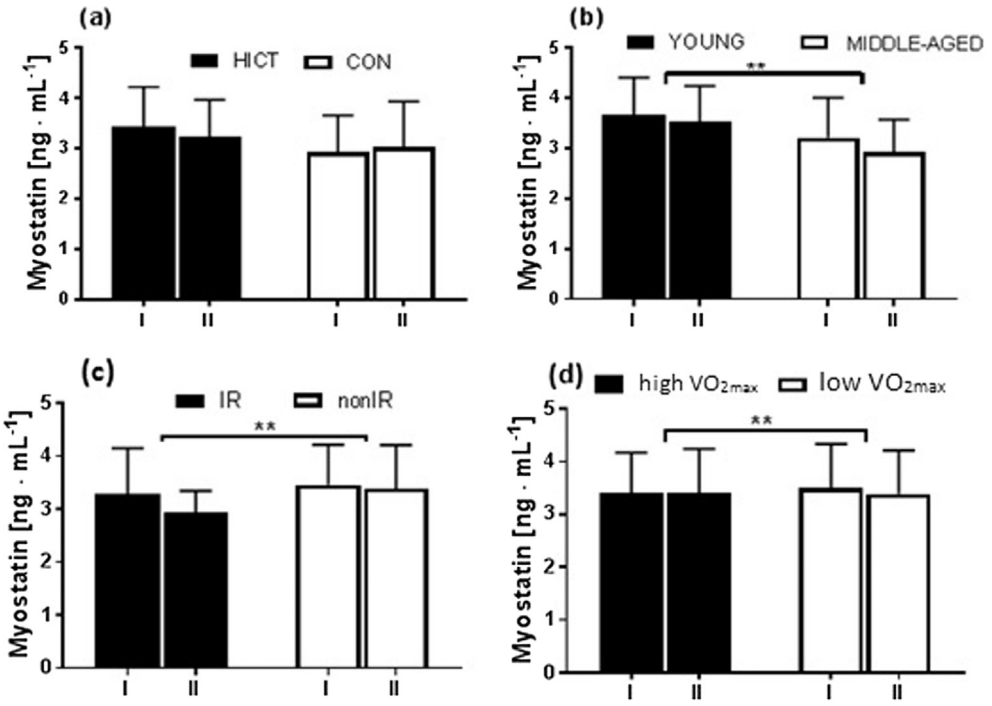

Fig. 2 – Changes in resting myostatin concentration recorded before (I) and after 5 weeks (II): (a) among trained (HICT) and control (CON) groups. (b) among young and middle-aged group. (c) among women with insulin resistance (IR) and without insulin resistance (nonIR). (d) among women with high and low relative oxygen uptake (VO_{2max}).

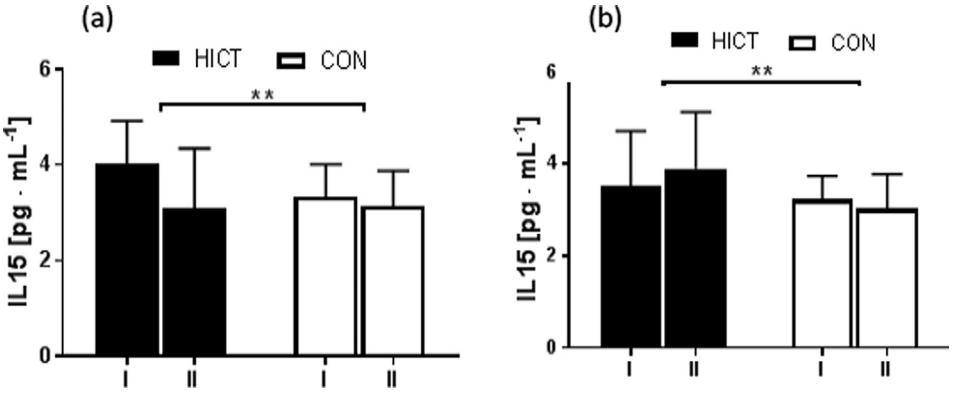

Fig. 3 – Comparison of shifts in circulating IL-15 between high-intensity interval training (HICT) and control group (CON). Values registered at rest (I) and 1 h after (II) single training: (a) First HICT session. (b) Last HICT session.

The first and last session of HICT caused a decline of irisin, especially visible 24 h after effort in the trained group. This range was also depended on age, IR and the level of relative oxygen uptake of women. The adjusted effect between YG and MG was −27%, CI: −52% to 10%. Interestingly, IR women from the HICT group had significantly lower baseline values of irisin ($3.2 \pm 2.58\ ng{\cdot}mL^{-1}$) compared to those without IR ($9.01 \pm 8.44\ ng{\cdot}mL^{-1}$). Moreover, shifts of irisin were dependent on oxygen uptake and the adjusted effect was 54%, CI: −7% to 54%. In women with higher fitness level a drop of irisin was noted (-13% ±40%), whereas in women with a VO_{2max} below $28\ mL{\cdot}kg^{-1}{\cdot}min^{-1}$ the adverse shift was noted (34% ±100%). This change was significant. After 5 weeks of HICT independently from the age and insulin resistance, resting irisin concentration significantly correlated with HOMA-IR ($r = 0.48$, $p = 0.03$). At the same time point measurements of resting decorin in exercising women correlated with glucose ($r = 0.46$, $p = 0.04$). Similar to irisin, at the beginning of the intervention, this correlation was inverse. In the CON group the above described relationships were not presented.

Resting serum HSP27 values remained unchanged after 5 weeks, in both groups. Interestingly, the last HICT session contributed a significant increase in HSP27 in the HICT group (15% ±13%), whereas in the CON group the tendency was opposite (−28% ±42%). The adjusted effect was moderate and most likely (60%, CI: 29% to 99%). Still, those changes were most pronounced 24 h post training. To assess the whole-body response to the exercise, genes analyses in blood

cells were performed. Any significant differences were observed in *HSPB1* expression in response to intervention (Fig. 4a). Still, in HICT group the *HSPB1* mRNA was higher than in the CON group (2^0.59 ± 0.50 and 2^0.24 ± 0.19, respectively). This effect was also visible at the transcriptional level of *HSPB1* mRNA, but these changes in leukocytes were insignificant. Low expression of pro-inflammatory cytokine *TNF-α* was detected in response to the first and last HICT session (Fig. 4c, 4d). Despite no significant changes and a low number of copies of *TNF-α* mRNA after the last HICT session, a slight average reduction of *TNF-α* mRNA (from 2^0.06 to 2^0.02 in HICT and from 2^0.05 to 2^0.02 in CON) was observed. However, large individual differences in *TNF-α* expression between individuals were noted. Additionally, *DCN* mRNA was assessed. Only below cut-off *DCN* mRNA was detected in leukocytes in all participants at every stage of the experiment (mean value for groups was 0.001 and in many participants no Qt was received).

4. Discussion

Our study shows that 15 sessions of HICT significantly improved glucose homeostasis, with the effect being accompanied by shifts in myokines. Although muscle mass did not change significantly, all indicators of characterised muscle sensitivity on insulin were ameliorated. The drop of glucose, insulin, and HOMA-IR was accompanied by a decrease of serum myostatin concentration. Notably, this alteration depended on age and IR at the baseline. Sharma et al. have pointed out that myostatin is associated with obesity and T2DM [36]. Data from an animal study indicated that inhibition of myostatin improves insulin sensitivity [9]. Moreover, in humans (advanced-aged women) there was reported elevation of serum myostatin, compared with younger subjects [37]. Thus, this observation may explain the potential role of increased myostatin concentration, occurring along with ageing, in muscle-wasting processes. Neither Peng et al. has not proven myostatin age-related dependence in elderly women [38]. According to this myokine secretion during exercise, Hittel et al. have claimed that in insulin-resistant men, 6 months of low intensity endurance training contributed to a decrease in the expression of and plasma myostatin concentration, with the effect correlating with an improvement of insulin sensitivity [39]. In our experiment, 15 units of the applied training programme, using subjects' body weight as a workload, contributed to a decrease of myostatin. This effect was mostly visible among the women characterised by higher age, IR and lower VO2max. This response may suggest the anti-inflammatory effect of the applied procedure. Moreover, the lower expression of *TNF-α* can support this reasoning.

The action of myostatin can be modified by IGF-1 and irisin concentration [9,40]. Recent studies have shown that IGF-1 stimulated beneficial glucose metabolism and its level increased post-exercise [41–43]. Gregory et al. have shown that 8 weeks of resistance training (3 RM to 12 RM, 90–180 s rest between sets) caused the level of IGF-1 to rise in young women, whereas no similar effect occurred in participants who attended endurance training instead [41]. In our study, based on using body weight as a workload, 15 units of HICT caused a significant elevation of IGF-1. This rise was accompanied by an improvement of glucose and HOMA-IR, with such a beneficial effect absent in the CON group. Until now, the assessment of IGF-1 in glucose homeostasis has been focused on patients with type 1 diabetes [42,43]. A reduction of physical activity induced IR [44] and contributed to T2DM development [10]. Thus, the pursuit of effective preventative methods is justified. Furthermore, we noted a statistically significant decline of the insulin/IGF-1 ratio after completion of the training programme. To our knowledge, the decrease of this ratio reported in this study is to the first observed in

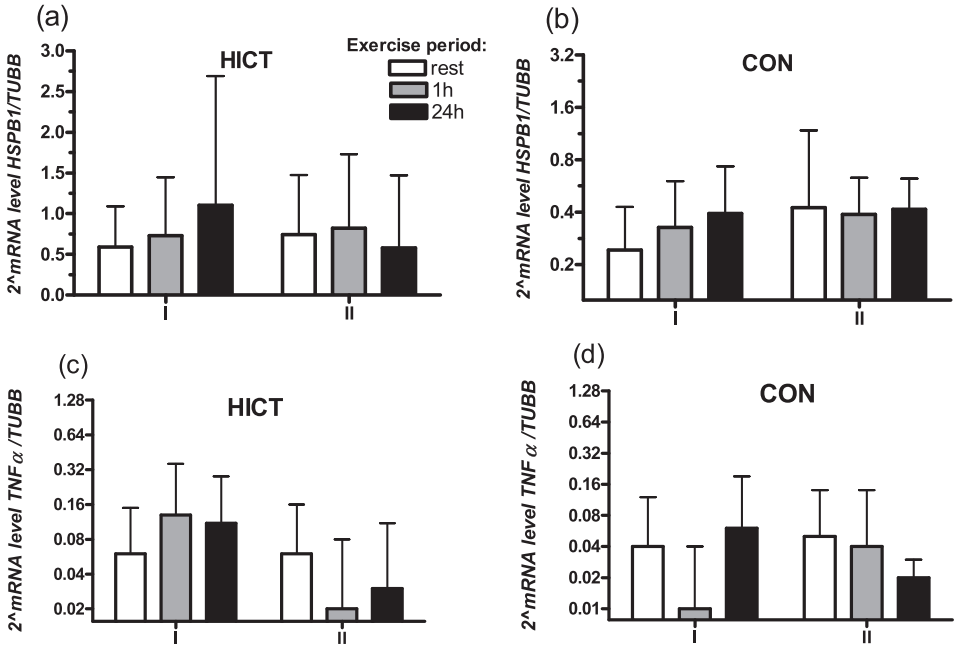

Fig. 4 – Relative expression of genes *HSPB1* and *TNF-α* in response to first (I) and last (II) of HICT session: (a) *HSPB1* expression in HICT group. (b) *HSPB1* expression in CON group. (c) *TNF-α* expression in HICT group. (d) *TNF-α* expression in CON group.

human subjects. Most available data about insulin/IGF-1 signalling are based on animal studies [45]. Thus, further investigations are needed to verify our results.

Together with IGF-1, another myokine, irisin, is the link between myostatin and glucose metabolism [9]. Recent studies have demonstrated that irisin was not only involved in maintaining glucose homeostasis [19], but it could also regulate exercise-induced adaptation [46]. Results obtained in our experiment appear ambiguous. Still, both the first and the last training session caused a drop of irisin in both groups and changes recorded after the last session of training were significant only in the HICT group. The small decrease of irisin was noted among training women with a higher level expressed in $VO_{2max,}$ which suggests applied training is insufficient for inducing adaptive changes in women who had presumably better cardiovascular fitness and higher muscle capacity. It might suggest that active women have lower circulating irisin concentration due to muscle adaptation process. On the other hand, obtained results revealed that decreased irisin was characteristic at the baseline for subjects with IR and the HICT program did not affect its circulating concentration. Nonetheless, irisin values correlated significantly and positively with HOMA-IR results. Previously published data indicated that differences in HOMA-IR are age-and sex-dependent in subjects, who do not suffer from diabetes. However, the HOMA-IR threshold is not clearly established, and the consideration is needed in relationship to the population. Following Gayoso-Diz et al. averaged smooth HOMA-IR on 75 percentile for Caucasian women aged 30–39 years is 2.36 [47] and this value was considered as a cut-off level in our study. To validate the accuracy of our results, we have also checked individual shifts in analysed blood proteins. It transpired that in women with IR, who attended 15 HICT units, there was a significant correlation between insulin and irisin level.

Modulations of glucose metabolism are known to be connected with circulating irisin [19]. It is worth establishing that irisin modulations induced by training might be crucial in the prevention of many metabolic disease due to the fact that irisin is a well-known metabolic biomarker and even a detector of breast cancer [48]. According to the latest data, patients with T2DM exhibited lower levels of irisin [49]. This condition may be modified by lifestyle habits such as physical activity [19]. Thus, a drop of HOMA-IR and irisin in women might have had an influence on an improvement of tissue' insulin sensitivity via better muscle endocrine function promoting glucose uptake after the applied training. So far, the tissue mechanism of irisin uptake remains unknown. Loffer et al have recorded an increase of irisin, detectable only immediately after the exercise. It dissipated considerably within 30 min time following the exercise, suggesting a growing uptake of this myokine. According to the results presented by Loffer et al., we cannot rule out a possibility that the training period was too short to achieve significant resting rise of irisin [50].

Previous studies have demonstrated that irisin, myostatin and IL-15 are all exercise-modulated myokines that participate in the regulation of glucose metabolism [51,52]. While Nadeau and Aguer have reported that IL-15 treatment protected against the development of IR in rodents, the link between circulating IL-15 levels and IR in humans remains unclear [51]. Some data showed that circulating IL-15 levels increased after resistance exercise [53,54]. In our study, the first session of HICT induced a drop, while the last session induced a rise of IL-15 among women in the HICT group. This inconsistent response to exercise of IL-15 may have resulted from not completely understood secretion and regulation of IL-15, which can be released by several kinds of tissues and regulated by its receptor isoforms in diverse responsible [11]. Still, elevated circulating IL-15 is considered to be a response to muscle contraction [51].

Moreover, our study verifies if HICT sessions may have induced changes of HSP concentration and expression. Exercise stimulates an increase of HSP expression and this response may contribute to beneficial metabolic effects in insulin-resistant tissues [21]. In the current experiment, increased HSP27 concentration was recorded in the HICT group after the whole HICT programme along with the higher expression in leukocytes. In contrast, serum HSP27 was observed to be significantly lower in subjects with T2DM compared to normal glucose tolerance [55]. It has also been postulated that changes in the expression of *HSPB1* in leukocytes can be associated with the intensity and duration of exercise [56]. However, high stability of *HSPB1* mRNA was noted after moderate exercise [57].

To the best of our knowledge our study is the first, to investigate the effect of high intensity circuit training protocol on decorin secretion and blood cells *DCN* expression in women. Available data showed that a single resistance training session caused an elevation of circulating decorin and an increase of the muscle expression of *DCN* in response to 12 weeks of combined resistance and endurance training in T2DM men [14]. On the other hand, previously published data revealed that decorin might be a good marker of pathophysiology of breast cancer [13] or obesity and accompanying its IR [58]. The higher expression of *DCN* was documented only in chronic lymphocytic leukaemia patients [59]. This discrepancy might be caused by different time points of blood collection and diverse physical workload of exercise. In our study blood samples were collected 1 h and 24 h after training and women performed exercise without extra weights, whereas among T2DM men, who performed strength training with an 8 RM load blood samples were taken during 3 set of exercise, directly after training and every half hour to 2 h after exercise. Thus, further investigations are needed, to assess the appropriative mode, frequency and duration of exercise to find out the best stimuli to elevate/or diminish decorin concentration among women. Moreover, the optimal time point of blood collection is required to determine to assess the decorin changes because data are inconsistent.

A few limitations of this study must to be mentioned. Firstly, women from the HICT group were allowed to train at home, with the indirectly supervision. Secondly, the number of participants might have impacted on the statistical power and disturbed detection of significant differences between the groups, hence further investigations are needed.

To summarize, 15 units of HICT led to an improved glucose homeostasis via a reduction of resting glucose concentration, insulin level and HOMA-IR. Induced changes were modified by myostatin, IGF-1 and irisin shifts. Myostatin dropped mainly in older women and especially among those with IR

and low cardiorespiratory fitness at the baseline. Moreover, the insulin/IGF-1 ratio decreased after the applied training program, suggesting that it may be a good predictor for future investigations of any improvements of insulin sensitivity mechanism after training. Consequently, HICT programme with own body weight as a workload can be considered a time-efficient and health-promoting form of physical activity.

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Declaration of Competing Interest

No conflict of interests regarding the publication of this manuscript is declared by the authors.

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Habitually inactive physically – a proposed procedure of counteracting cognitive decline in women with diminished insulin sensitivity through a high-intensity circuit training program

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ABSTRACT

This study verified the impact of five weeks of high-intensity circuit training (HICT) on changes in concentration of exerkines in relation to cognitive functions. Sedentary women (n = 33; age=39±13 years) were randomly assigned into the HICT (n = 21) group or the control group (n = 12). The HICT group performed 15 training sessions; meanwhile, the control group performed the HICT twice, only at baseline and at the end of the experiment. Blood samples were collected before, 1 h and 24 h after the first and last HICT, to evaluate the concentration of exerkines: brain-derived neurotrophic factor (BDNF), irisin, fibroblast growth factor-21 (FGF-21), interleukin-6 (IL-6) and cathepsin B (CATB) using enzyme immunoassay method. Cognitive functions and quality of life were assessed using the Vienna Test System and the Short Form Health Survey. HICT induced improvement of cognitive function and quality of life, and these changes were accompanied by an increase of BDNF and shifts in CATB concentration. HICT program caused a decrease in FGF-21 concentration, which was modified by age and insulin sensitivity. The improvement of cognitive functions was more pronounced in females, who experienced a drop in FGF-21. In summary, HICT program, that can be performed during pandemic, enhanced cognitive functions and this response was related to changes in exerkines.

1. Introduction

Diminished insulin sensitivity is growing and becoming a global health burden among children, teenagers as well as adults. It is a result of fat tissue accumulation, obesity and particularly physical vasculature, and consequentially results in a decline in cognition [1]. Thus, skeletal muscle metabolic response induced by exercising can be responsible for counteracting of the progression of these dysfunctions [2,3]. Available data shows that exercise-induced outcomes might be considered according to the inter-individual response to training [4]. Recently, it was reported that responsiveness and glucose homeostasis modulation are dependent on the type of physical performance [5]. Therefore, the effectiveness of varied training programs with different durations and intensities should be still verified. Similar to typical endurance training, high-intensity interval training (HIIT), is recognized as a powerful factor to ameliorate a relative maximal oxygen uptake (VO_{2max}) [6,7] as well as a beneficial metabolic stimulus for better glucose uptake [8,9] and cognitive functions improvement [10,11]. One of HIIT variations is the high-intensity circuit training (HICT), using a person's own body weight as a workload [12]. Our previous data revealed that a single session of HICT improved concentration and special memory among young women. Meanwhile, among older females, these functions were

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deteriorated. At the same time, HICT caused a significant drop of brain-derived neurotrophic factor (BDNF) [11]. BDNF belongs to the neurotrophins that regulate neurogenesis and synaptic plasticity, particularly in the hippocampus [13,14]. It is found to be an important factor, both in central and peripheral metabolism regulations [15]. Recently it was shown that both myokines, FNDC5/irisin and cathepsin B (CATB), contribute to the modification of BDNF concentration [16] and are involved in brain function improvement [17]. A significant correlation between BDNF and irisin was recorded in animal models, whereas such a relationship is inconsistent among human participants [18]. Previous studies indicated that irisin may improve cardiovascular/endothelial function and is involved in glucose homeostasis [19,20,21]. It was also suggested that in sedentary subjects, irisin is positively associated with insulin resistance [20]. Prediabetic stage and an impairment in glucose tolerance are also associated with elevated blood fibroblast growth factor-21 (FGF-21), which regulates glucose and lipid metabolism in type 2 diabetes mellitus [22]. A recent study in rodent's brain tissue demonstrated that FGF-21 is expressed in the brain, specifically the midbrain regions, which involves dopaminergic neurons located in the substantia nigra, striatum, hippocampus, and cortex [23] and it can easily cross the blood-brain barrier [24]. Previous published study showed that high levels of targeting *chemokine (C—C motif) ligand 2* (*CCL2*) could be also associated with a faster rate of cognitive decline in early stages of Alzheimer's disease [25]. It is also involved in neuroinflammatory processes characterized by neuro-degradation of the central nervous system [26].

Available data propose physical activity as a strategy to improve metabolic as well mental health [10,27]. New insight into factors modifying connection between physical effort and cognitive functions was obtained by identifying proteins called exerkines and myokines [28]. While there is no doubt that endurance training has a health-promoting effect, data regarding the effectiveness of resistance training programs are limited, especially including individual response. Thus, we hypothesized that in women with diminished insulin sensitivity, regular performed HICT with resistance of own body, will have beneficial effect on cognitive functions, but the effect will be regulated by shifts in myokines and exerkines concentration. Therefore, this study aimed to assess, which proteins (BDNF, IL-6, irisin, FGF-21 or CATB) will have the highest impact on obtained results.

2. Materials and methods

2.1. Subjects

Thirty-tree healthy, sedentary, adult females (age =39±13 years) with diminished insulin sensitivity, calculated by a quantitative insulin sensitivity check index (QUICKI = 0.359 ± 0.04) [29,30], were enrolled into the study. The recruitment was carried among office-working women and the participants by local journal announcement and telephone contact. Before the experiment started, each participant was medically examined. Subjects with any cardiovascular disease, diabetes or other acute or chronic disease, and those who train regularly were excluded from the study. The female participants were non-smokers and did not take any medications. Throughout the experiment, it was recommended that participants not to change their daily diet and habits. All participants were characterized by their age (30 years was the cut-off point for the young/old-age group) and according to Heyward, by fair level of relative maximal oxygen uptake (VO_{2max} 28–30 $mL \bullet kg^{-1} \bullet min^{-1}$, respectively for age) [31]. The participants were divided randomly into two groups: the HICT group ($n = 21$) and the control (CON) group ($n = 12$). Anthropometric data of participants are shown in Table 1. In order to verify age-dependent response to the training program, women from HICT group were divided into young ($n = 11$) and old ($n = 10$) group.

2.2. Ethics statement

This study was approved by the Bioethical Committee of the Regional Medical Society in Gdansk KB-28/17 according to Declaration of Helsinki. Before the study initiation, the participants received the description of training program (verbal and short movie). Prior to commencing the experiment, the subjects were informed about the procedures, risks and benefits of the study. Eligible subjects were asked to give their written inform consent to participate and underwent a medical examination. The experiment was carried out as a part of clinical trials registered ClinicalTrials.gov (identifier: NCT04375969).

2.3. Experimental design

Body composition, blood analysis, quality of life and daily activities assessments were determined one week before the experiment started and after five weeks of training. Additionally, blood samples were collected a total of six time in the experiment: three times at the first training session (before, 1 hour after, and 24 h after the first session) and three times at the final training session (before, 1 h after, and 24 h after the final training session). Additionally, cognitive functions were assessed before and 1 hour after the first and last training sessions. On experimental days all participants followed the same light-breakfast and routine.

2.4. Training program

The HICT training program was based on a previous study [9] and contained nine exercises chosen from the original HICT protocol [12]. Women from training group completed 15 HICT sessions during the five-week period (three training sessions per week). Women from CON group completed two HICT sessions (at the beginning and the end of the experiment). One week before the experiment started, all participants

Table 1
Characteristic of participants.

	HICT $n = 21$				CON $n = 12$			
	Before		After 5 weeks		Before		After 5 weeks	
	$\overline{X}$±*SD*	*CV*	$\overline{X}$±*SD*	*CV*	$\overline{X}$±*SD*	*CV*	$\overline{X}$±*SD*	*CV*
BM (kg)	69.5 ± 13.6	20%	69.3 ± 13.5	19%	71.3 ± 13.5	19%	71.7 ± 13.8	19%
SMM (kg)	26.7 ± 3.5	13%	26.9 ± 3.7	14%	26.0 ± 3.3	13%	25.6 ± 3.3	13%
BFM (kg)	21.2 ± 9.9	47%	20.5 ± 9.7	47%	24.1 ± 10.6	44%	24.9 ± 10.1	41%
BMI ($kg \bullet m^{-2}$)	24.9 ± 3.9	16%	24.7 ± 3.9	16%	26.5 ± 4.9	18%	26.6 ± 5.1	19%
PBF (%)	29.2 ± 8.9	30%	28.4 ± 8.8	31%	32.7 ± 8.5	26%	33.7 ± 8.1	24%
Glucose homeostasis indicators								
Fasting glucose ($mg \bullet dL^{-1}$)	94.4 ± 11.7	12%	91.0 ± 7.1	8%	93.3 ± 12.6	14%	93.5 ± 11.5	12%
Fasting insulin ($\mu U \bullet mL^{-1}$)	7.7 ± 4.2	55%	7.7 ± 3.5	45%	8.0 ± 4.0	50%	8.0 ± 3.5	44%
QUICKI	0.362 ± 0.05	14%	0.360 ± 0.03	8%	0.356 ± 0.03	8%	0.356 ± 0.02	6%

Values are expressed as mean± standard deviation (SD), CV-coefficient of variation, HICT-high-intensity circuit training group, CON- control group, BM- body mass, SMM-skeletal muscle mass, BFM- body fat mass, BMI- Body Mass Index, PBF- percentage body fat, QUICKI- quantitative insulin sensitivity check index.

were familiarized with the experiment design. Participants also received a link to the online film with exercise instructions (whole single bout of training procedure) with music and time feedback. Before beginning the exercise protocol, research team and all participants had watched the movie and discussed correct performance, pointing mistakes that can be made. During each exercise session, participants were encouraged to make as many repetitions as possible to achieve the highest intensity. Each HICT training session consisted of three circuits with a two-minute break in between. Following two weeks of training, additional series were added. The data of participants with training attendance less than 80% were not included in the statistical analyzes.

2.5. Anthropometric and fitness level measurement

Body mass and body composition (fat expressed in kg and percentage, and skeletal muscle mass) were measured by a multi-frequency impedance analyzer (In Body 720, Biospace, Korea) [32]. The body mass index (BMI) was also calculated. The measurements were performed twice, before and after the entire intervention, except the cardiorespiratory fitness measurement. Level of relative maximal oxygen uptake (VO_{2max}) was evaluated only at the beginning of the experiment, using a graded cycle ergometry test. Maximal oxygen uptake was measured (breath by breath) by an ergo spirometer (Cortex Metamax3B R2, Leipzig, Germany) and analyzed using Metasoft Studio v. 5.1.0 Software [33].

2.6. Assessment of cognitive functions and quality of life

The battery of cognitive tests was the same as in previous published study [11] and involved:

1) response inhibition based on the color-word interference tendency (Stroop Interference Test – Stroop S8);
2) storage capacity of spatial short-term memory, learning in spatial middle-aged memory (Corsi Block-Tapping-Test – CORSI S3), and cognitive control, including selective attention;
3) the ability to concentrate was tested using the Grid exercise by Harris (2 min Arithmetic Distraction Test).

The CORSI and Stroop tests were taken from the computerized battery Vienna Test System offered by SCHUHFRIED Company.

The quality of life was assessed using the Short Form Health Survey (the SF-36) questionnaire. This questionnaire detects the amount of change in participants' general health and comprised of both physical and mental aspects of health. The questionnaire comprises eight scored subscales such as vitality, bodily pain, general health, physical functioning, social functioning, physical role functioning, emotional role functioning and mental health [34].

2.7. Blood samples

The blood samples were taken by a professional nurse into vacutainer tubes with EDTAK3 (BD Vacutainer catalog no 368,860) for plasma analysis, Vacutainer SST™ II Advance (catalog no 367,955) for serum analysis and into the vacutainer tubes with sodium fluoride to estimate glucose concentration (BD Vacutainer catalog no 368,920). One of the samples was immediately transferred to centrifuge tubes with aprotinin for irisin measurement. The blood was centrifuged (2000 x g at 4 °C) for 10 min to get plasma and serum. The received samples were frozen at −80 °C till immunoenzymatically analysis.

To evaluate the concentration of serum irisin, the competitive enzyme immunoassay kits from Phoenix Pharmaceuticals Inc. (catalog no. EK 067–29) was used. Intra-assay coefficients of variability (CVs) and inter-assay CVs reported by the manufacturer were <10% and <15%, respectively.

The concentration of serum FGF-21, BDNF and IL-6 were assessed using sandwich ELISA, according to manufacturers' instructions (R&D Systems, USA, catalog no. DF2100, DBD00 and HS600B, respectively). The precision of intra-assay and inter-assay CV were as follows: 3.9% and 10.9% for FGF-21; 6.2% and 11.3% for BDNF and 7.8% and 9.6% for IL-6. The detection sensitivity was as follows: 8.69 pg•mL^{-1} for FGF-21; 20 pg mL^{-1} for BDNF and 0.11 pg mL^{-1} for IL-6. The plasma CATB concentration was measured using Cathepsin B Human ELISA Kit (Abcam, catalog no. ab119584). The sensitivity was <5 pg mL^{-1}.

In order to assess the glucose concentration, analyzer Cobas INTEGRA 400 Plus Roche was used. To assess insulin, the insulin- immunoassay kit from DiaMetra (catalog no. DKO076) with the intra-assay CV ≤5% and the inter-assay CV ≤10% was used.

The quantitative insulin sensitivity check index QUICKI (QUICKI = 1/(log serum insulin μU mL^{-1} + log fasting plasma glucose mg•dL^{-1}) was estimated as insulin resistance assessment methods [29].

2.8. Genetic research

A total of 2 mL of venous whole blood was collected to special tubes with EDTA as a coagulant. To eliminate erythrocytes, red blood cell buffer (RBCL, AA Biotechnology, Gdynia, Poland) was used immediately after the collection. Obtained leukocytes were lysed using fenozol (AA Biotechnology, Gdynia, Poland). RNA isolation was carried out according to the Chomczynski and Sacchi method [35]. The purity and quality of RNA was marked using a spectrophotometer (Eppendorph, Germany). Altogether 1000 ng of pure RNA (A260/A230 ≥ 1.7) was used for reverse transcription (Transcriptor First Strand cDNA Synthesis Kit, Roche, Department Poland) with oligo dT primers, according to manufacturer's instruction. 10-fold diluted cDNA was used to qRT-PCR. In order to assess the *CCL2* and housekeeping gene expression, the following primers were used:

For *TUBB*: F: CTA GAA CCT GGG ACC ATG GA
R: TGC AGG CAG TCA CAG CTC T
For *CCL2*: F: CAG CCA GAT GCA ATC AAT GCC
R: CTT GGC CAC AAT GGT CTT GAA

The reaction mixture contained: 2 μL of diluted cDNA; 0.4 μL of each primer; 5 μL polymerase (Fast Start Polymerase, Roche, Department Poland); and 2.2 μL of water. Temperature-time profile was compatible to the manufacturer's instruction. The method was based on previously published papers [36,37].

2.9. Statistical analysis

The efficacy of the applied training program and the direction of causality between obtained results were interpreted using magnitude-based decision (MBD) [38,39–40]. All data were log-transformed for analysis to reduce the bias arising from the non-uniformity of errors and after analysis were back-transformed to percent units. Threshold values for assessing the magnitude of standardized effects were 0.20, 0.60, 1.2 and 2.0 for small, moderate, large and very large, respectively. The uncertainty of each effect was expressed as 90% confidence limits and as probabilities that the true value of the effect was beneficial, trivial or harmful [41]. Effects were deemed clinically clear and were expressed as the chance of the true effect being trivial, beneficial or harmful using the following scale: 25–75%, possibly; 75–95%, likely; 95–99.5%, very likely; >99.5%, most likely.

Statistical analysis of psychometrics from questionnaires was performed using the Statistica 13.1 software (StatSoft, Tulsa, OK, USA). Separate 2 (group: HICT, CON) × 3 (time: before, 1 h and 24 h after the first and the last HICT session) repeated analyzes of variances (ANOVA Friedman) with the Dunn-Bonferroni post-hoc method were applied. Effect sizes (partial eta-squared,η_p^2) were additionally calculated with $\eta_p^2 \geq 0.01$ indicating small, ≥ 0.059 indicating medium and ≥0.138 indicating large effects [42].

In order to calculate the gene expression, the Schmitten`s and Livak`s

method [43] was applied (Microsoft Excel 2016). Next, all data was transformed to linear values (GraphPad Prism 6.0). The normality of all data was analyzed with the Shapiro-Wilk normality test. The paired *t*-test and ANOVA 2-way were calculated.

2.10. Classification of responders and non-responders

To evaluate participants in terms of their inter-individual variability and responsiveness to the exercise the typical error measurement was used. Women in the HICT and CON group were classified by their inter-individual variability in the response and recognized as responders and non-responders. A non-responder was defined as one who did not reveal of any rise nor drop higher than two times the typical errors away from zero. A change beyond two times the typical error means there is a high probability that this response is a true physiological adaptation beyond what might be expected to result from a technical and/or biological variability [4,5].

3. Results

3.1. Anthropometric measurement and glucose homeostasis indicators

Anthropometric characteristics of both groups (HICT and CON) did not reveal any significant differences between the exercising females before and after the intervention (Table 1). Insulin resistance indicator (QUICKI) did not express any significant changes between both groups (24%, CI: −15% to 80%). The five-week HICT program caused a decreasing tendency in the resting glucose concentration. This change was noted only among exercising females −3% (± 7%).

3.2. Cognitive function measurement

The applied training program consisting 15 HICT units caused a significant improvement in cognitive functions (17%, CI: 7 to 43% and −24%, CI: −51 to 17% in CORSI and Strop tests, respectively). However, the whole intervention did not improve concentration (Table 2). After five weeks of training, better resting results in an immediate block span and the number of correct answers in the CORSI test were noted (Table 2). Diversification by age indicated that the applied HICT program induced better results in reading and naming interference in the Stroop test among older females (−42%, CI: −67% to 1% and −17%, CI: −53% to 49%, respectively). This effect was moderate and likely, and it was recorded 1 h after the last HICT. The first single HICT improved results significantly in the CORSI task among all women. The number of better answers recorded 1 h after the exercise increased in both groups in the CON group 7% (± 23%) and in the HICT group 22% (± 23%). The last training session induced a significant amelioration in the HICT group in their ability to concentrate (13%, CI: −4% to 39%), meanwhile in the CON group these results were unchanged.

3.3. Quality of life measurement

The SF-36 questionnaire score calculations showed significant changes in physical functioning of participants after five weeks. The opposite significant result between the groups was noted in physical functioning ($p = 0.03$, η_p^2=0.15); elevation in HICT group, whereas in the CON group the decrease of this aspect was noted. No more significant changes were recorded after 5 weeks of training in quality of life measurements (Table 3).

3.4. Serum and plasma metabolic markers

3.4.1. FGF-21

Data obtained after five weeks of HICT indicated that the change of baseline FGF-21 concentration was recorded in both groups (HICT: $\Delta\overline{X}$ = 20.75 pg·mL^{-1}and CON: $\Delta\overline{X}$ = 77.90 pg·mL-^{1}). Interestingly, among participants in the HICT group, the 43.5% drop, as well the 157.3% increase, were noted in FGF-21 concentration (Fig. 1A). What is more, the amelioration of cognitive functions was more pronounced among those participants who experienced a drop of FGF-21. This was observed especially in adjusted changes of improvement of concentration 17% (±27%) and number of better answers in the CORSI task 28% (± 36%), respectively. Moreover, a significant change was observed in vitality between women with elevated and decreased FGF-21 in response to the HICT program (28%, CI: −7 to 75%), and this effect was small and likely. Additionally, in the HICT group, a discrepancy between young and old participants was observed (−53%, CI: −75% to 11%). It was registered that in women over 30 years old (older participants) the FGF-21 decreased, but increased in younger participants. Interestingly, according to participants baseline insulin resistance, the FGF-21 concentration was lower in participants with QUICKI ⟨0.357 than those with QUICKI ⟩0.357 (170.68 ± 77.36 pg mL^{-1}and 226.94 ± 110.06 pg·mL^{-1}, respectively). After five weeks of HICT, an 17% elevation (±172%) of FGF-21 was observed only in women with impaired insulin sensitivity. In participants with QUICKI > 0.357, a −19% decrease (± 136%) was noted.

The first HICT unit did not change the FGF-21 concentration in both groups. Interestingly, 24 h after the first session, the significant decrease in HICT as well in CON group (26%, CI: −7% to 72%) was recorded.

The last HICT unit deteriorated FGF-21 concentration 1 h after training and the change between the groups was significant (Fig. 2A). Decrease of this factor noted 1 h post last training was more pronounced among the younger than older HICT participants (Fig. 2B). The drop of FGF-21 recorded 24 h after last HICT was noted only among older women −13 (± 96%).

Table 2
Changes in cognitive functions in response to five weeks of the high-intensity circuit training among exercising and control group.

		Before		After 5 weeks		Adjusted effect mean, 90% CI
		$\overline{X}$±*SD*	95% CI	$\overline{X}$±*SD*	95% CI	
Concentration	CON	15.50 ± 5.23	12.18 to 18.82	17.80 ± 3.89	15.28 to 20.22	−4, −14 to 7%
	HICT	16.90 ± 5.50	14.40 to 19.41	18.00 ± 5.05	15.59 to 20.31	
CORSI 1 (immediate block span)	CON	5.00 ± 0.90	4.51 to 5.66	5.60 ± 1.21	4.73 to 6.36	8, −7 to 25%
	HICT	5.70 ± 1.00	5.26 to 6.17	6.10 ± 1.20	5.60 to 6.69	
CORSI 2 (correct answers)	CON	9.10 ± 3.10	7.10 to 11.06	9.80 ± 3.34	7.57 to 12.06	**17, 7 to 43%****
	HICT	9.90 ± 3.00	8.58 to 11.32	12.00 ± 2.70	10.77 to 13.23	
STROOP 1 (reading interference) (s)	CON	0.15 ± 0.24	0.00 to 0.31	0.09 ± 0.13	0 to 0.18	26, 28 to 46%
	HICT	0.07 ± 0.04	0.05 to 0.08	0.07 ± 0.04	0.06 to 0.09	
STROOP 2 (naming interference) (s)	CON	0.07 ± 0.04	0.04 to 0.10	0.10 ± 0.08	0.05 to 0.15	**−24, −51 to 17%***
	HICT	0.05 ± 0.03	0.03 to 0.06	0.06 ± 0.04	0.04 to 0.08	

HICT-high-intensity circuit training group, CON- control group.
90% CI, 90% confidence interval. Asterisks indicate effects clear at the 5% level (90% CL) and likelihood that the true effect is substantial, as follows:
* possible,
** likely.Effects in bold are clear at the 0.5% level.

Table 3
The quality of life changes reported after 5 weeks in women from high-intensity circuit training (HICT) and control (CON) groups.`

		Before		After 5 weeks		ANOVA p (η_p^2)
		$\overline{X} \pm SD$	95% CI	$\overline{X} \pm SD$	95% CI	Group x Time Interaction
Vitality	CON	51.0 ± 18.4	39.3 to 62.8	56.6 ± 17.9	44.2 to 68.3	0.48 (0.02)
	HICT	56.5 ± 19.4	47.7 to 65.4	66.3 ± 17.0	58.3 to 74.2	
Bodily pain	CON	56.9 ± 18.7	45.2 to 68.8	63.9 ± 18.9	51.2 to 76.6	0.16 (0.07)
	HICT	70.0 ± 20.1	60.6 to 79.4	69.9 ± 19.0	61.0 to 78.7	
General health	CON	59.9 ± 12.1	52.2 to 67.6	68.2 ± 20.2	54.6 to 81.7	0.19 (0.01)
	HICT	74.6 ± 12.9	68.7 to 80.5	80.4 ±11.9	74.8 to 86.0	
Physical functioning	CON	94.6 ± 4.0	92.1 to 97.1	93.2 ± 6.4	88.9 to 97.5	**0.03 (0.15)**
	HICT	94.8 ± 6.0	92.0 to 97.5	97.4 ± 4.4*	95.3 to 99.4	
Social functioning	CON	59.4 ± 23.9	44.2 to 74.6	70.0 ± 21.9	55.3 to 84.7	0.11 (0.09)
	HICT	88.7 ± 13.6	82.5 to 94.9	88.1 ± 19.2	79.1 to 97.1	
Physical role functioning	CON	75.3 ± 9.0	69.6 to 80.9	77.0 ± 11.9	69.0 to 85.1	0.19 (0.02)
	HICT	83.6 ± 9.1	79.5 to 87.7	86.2 ± 8.8	82.1 to 90.3	
Emotional role functioning	CON	57 ± 17.2	46.1 to 67.9	64.9 ± 15.4	54.6 to 75.3	0.26 (0.01)
	HICT	72.7 ± 10.9	67.9 to 77.7	76.4 ± 14.4	69.7 to 83.2	
Mental health	CON	53.7 ± 21.0	40.5 to 67.0	65.9 ± 14.1	56.4 to 75.4	0.16 (0.08)
	HICT	70.9 ± 12.0	65.5 to 76.4	74.0 ± 15.0	67.0 to 81.0	

Values are presented as mean ± SD; 95% CI – confidential interval, CON – control group, HICT – high-intensity circuit training;.
* significant differences from before measurement, $p < 0.05$. Eta squared effects (η_p^2)for interaction are presented in brackets.

3.4.2. BDNF

The resting BDNF concentration did not change in response to the complete training program. In participants in the HICT group, the elevation of this factor was observed regardless of FGF-21 changes (Fig. 1B). The first unit of HICT did not induce any significant changes in BDNF concentration, whereas the last HICT unit increased BDNF concentration 1 h after session (Table 4) and its elevated concentration was maintained also 24 h after the exercise. Similar shifts were registered in both groups 24 h after last HICT: 12% (± 135%) in the HICT group and 21% (± 70%) in the CON group and were significant. Still, the range of changes was age-independent.

3.4.3. Irisin

There were no significant differences in the baseline values of irisin before and after five weeks (Table 4). The 15 units of HICT training caused changes only relevant to the age of the participants. In young participants in the HICT group, adjusted change indicated on reduction −10% (±62%), meanwhile in the old participant the same effect revealed an increase 21% (±100%). This effect was small and possible. This change was not registered in participants in the CON group. At the same time, in women, who finished the applied training program with an increment of FGF-21, the elevation 12.55% (±12.55%) of irisin was observed. The opposite tendency, occurred in participants with decreased FGF-21 concentrations (Fig. 1C). In the HICT group, a slight drop of irisin was noted 1 h after the last training session (Table 4) and it maintained until 24 h −26 (±71%). Although the drop was not significant, an opposite correlation between irisin and CORSI task results was recorded ($r = -0.45$, $p = 0.03$).

3.4.4. IL-6

According to our expectation, the first session of HICT induced a significant rise of IL-6 in CON 47% (±62%) and in HICT group 20% (±42%), respectively. The effect of change was small and possible. Still, this change was not significant after 24 h. The whole training program did not alter the resting IL-6 concentrations (Table 4). Additionally, in the same table, the effect of last session of training is presented. In regularly trained group, the 20% elevation of IL-6 was noted, whereas among female CON group, the 17% drop was registered.

3.4.5. CATB

The average baseline plasma mean value of CATB in all participants was 31.08 ± 12.40 ng·mL^{-1}. After applied training program CATB concentration was slightly elevated 11% (±20%) in HICT group, and 4% (±22%) in CON, still this change was not significant. The age-diversification of training participants showed that the increase was observed both in young 5% (±24%) and in older 10% (±18%) women. The significant correlation between baseline values CATB and irisin was noted after 15 training units ($r = 0.55$, $p = 0.01$) in exercising women. Among participants from HICT group, who experienced the increase of FGF-21, the CATB elevated 20.9%, whereas among them with the drop of FGF-21, CATB declined −3.2% (Fig. 1D).

3.5. Responders and non-responders

Fig. 3 shows the inter-individual variability in cytokines and cognitive function, and the percentage of responsiveness to apply the five-week HICT training program of exercising women. Cognitive function had a better positive response than circulating proteins.

3.6. CCL2 gene expression

Before the intervention the expression of *CCL2* mRNA was comparable in both groups ($p = 0.5$) (Fig. 4A). After applied training program the tendency to decrease in *CCL2* mRNA was observed only in HICT group (resting values at baseline vs post $p = 0.24$; 1 h post first vs 1 h post last session of HICT $p = 0.24$; and 24 h post first and last HICT unit $p = 0.35$) (Fig. 4B). Still data was not significant.

4. Discussion

The obtained results revealed that the applied five-week resistance training program significantly improved the cognitive functions and quality of life in the exercising women. Moreover, this study showed that the effect size of information processing and attention depended on the percentage of responsiveness to the applied HICT program. This is the first study to assess an individual approach and the rate of individual response of cognitive functions in response to the specific training program using the load of own body. Different types of regular interval

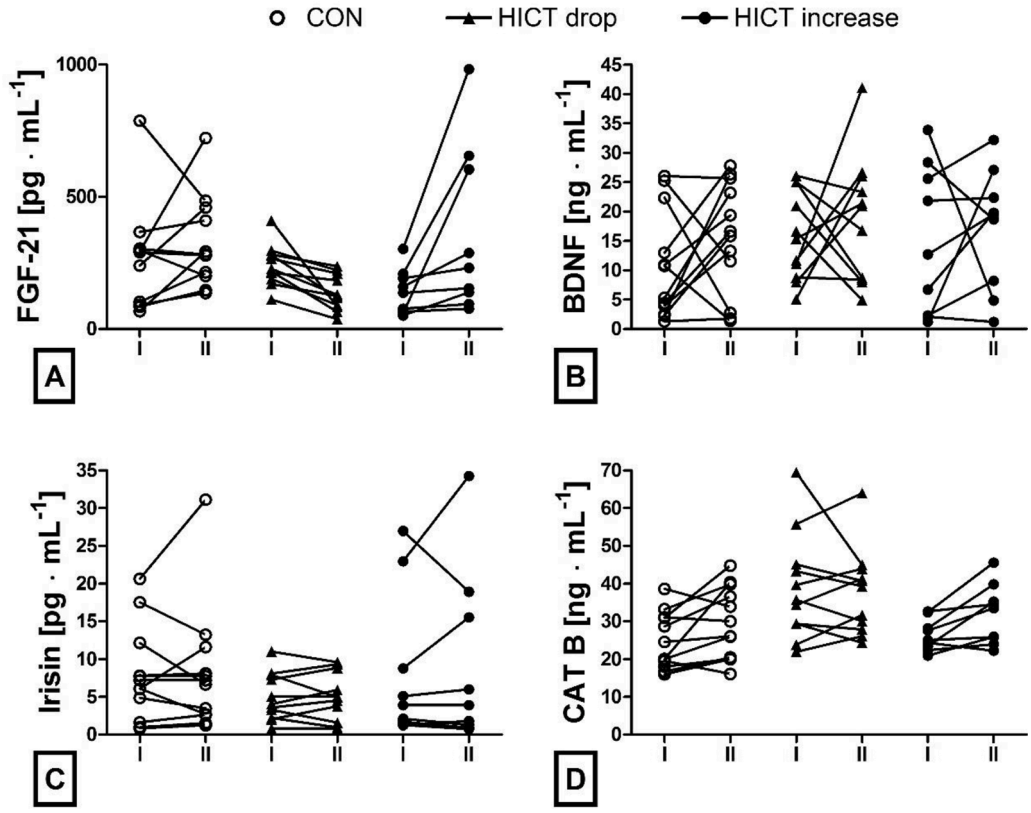

Fig. 1. The individual values registered before and after five weeks of HICT among women from control (CON) and high-intensity circuit training (HICT) group. HICT participants were divided into those who experienced the increase and drop of FGF-21. (A) Fibroblast-growth factor 21 (FGF-21) (B) Brain-derived neurotrophic factor (BDNF) (C) irisin (D) Cathepsin B (CATB).

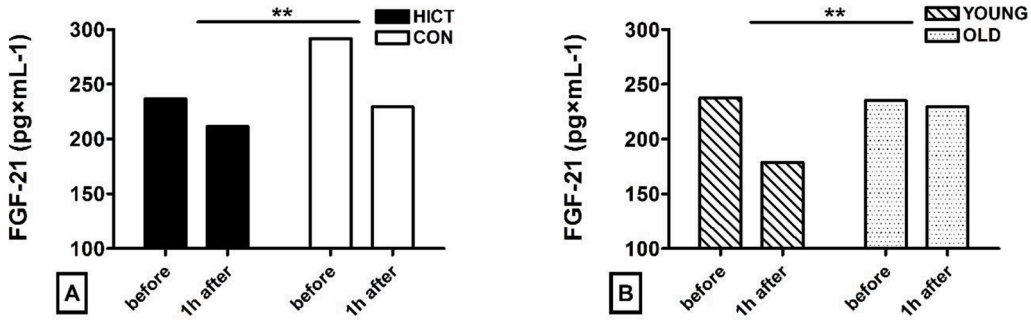

Fig. 2. Changes of the fibroblast growth factor-21 concentration in response to High- Intensity Circuit Training. (A) the effect of single training in women from HICT and control group (CON) (B) the effect of single training in women different age from HICT group: ** *Asterisks indicate effects clear at the 5% level (90% CL) and likelihood that the true effect is substantial or trivial ** likely*.

training are known as non-drug strategies to inhibit disease progression connected to glucose metabolism impairment [8] or brain dysfunction [44,45]. However, the impact of resistance training and muscle activity on brain function remains unclear. There are still doubts whether this type of training could affect cognitive functions. In order to define if regular HICT might affect executive functions, such as information processing speed, learning, short-term memory processes and concentration ability among healthy participants, we used particular tests [11] . Previous studies have shown that acute exercise had a positive impact on cognition and information processing [46,47]. However, significant changes in the Stroop test were only noted in adolescents in response to high intensity intermittent sprint exercise (ten sessions of 10 s sprints with 50 s break between each sprint) [48]. Among our exercising adult women, the correct answers have increased together with the reduced time needed to achieve them [49,50], what is expressed in drop of STROOP 2 results. At the same time, the amelioration of cognition was accompanied by FGF-21 shifts. Interestingly, the improvement of cognitive functions and vitality, were more pronounced among exercised participants who have experienced a drop of FGF-21. FGF-21 is primarily expressed and produced by the liver, however, its expression is also induced in skeletal muscle during exercise [51]. As we mentioned before, it is known that FGF-21 can easily cross the blood-brain barrier [24] and correlates with reduced dopamine neurotransmission within the nucleus accumbens [16]. He and co-workers showed that in men, resistance training induced more significant changes in FGF-21 than a high-intensity interval training [4]. Cuevas-Ramos et al. reported that in healthy, young women, an increase in FGF-21 was noted after two weeks of five times a week supervised daily physical activity. Additionally, the same author contended that serum FGF-21 concentration positively correlated with age [52]. In our study, FGF-21 baseline values recorded

Table 4
The concentration of proteins induced by 5 weeks HICT program and single session.

	Baseline values before and after 15 units of HICT								The effect of last unit of HICT recorded 1 h after							
	Group	Baseline			Adjusted change			Adjusted effect Mean	CI	Group	Before HICT			Adjusted change			Adjusted effect Mean	CI
BDNF ($ng \cdot mL^{-1}$)	CON	10.7	±	9.2	15	±	212%	19%	40 to 136%	CON	15.5	±	9.6	28	±	91%	**48%****	4 to 128%
	HICT	15.2	±	9.7	37	±	139%			HICT	17.4	±	10.5	6	±	114%		
Irisin ($pg \cdot mL^{-1}$)	CON	12.1	±	16.3	8	±	61%	−4%	−30 to 32%	CON	13.8	±	21.5	4	±	47%	−26%	−42 to −4%
	HICT	6.8	±	7.3	3	±	74%			HICT	10.9	±	20.6	−23	±	49%		
IL-6 ($pg \cdot mL^{-1}$)	CON	1.3	±	0.6	6	±	40%	−3%	−26 to 27%	CON	1.5	±	0.5	−17	±	248%	45%	−29 to 193%
	HICT	2.1	±	1.4	3	±	63%			HICT	2.1	±	1.9	20	±	43%		

HICT-high- intensity circuit training, group, CON- control group, BDNF-brain-derived neurotrophic factor, IL-6- interleukin-6. Asterisks indicate effects clear at the 5% level (90% CL) and likelihood that the true effect is substantial or trivial as follow as
** likely. Effects in bold are clear at the 0.5% level (99% CL).

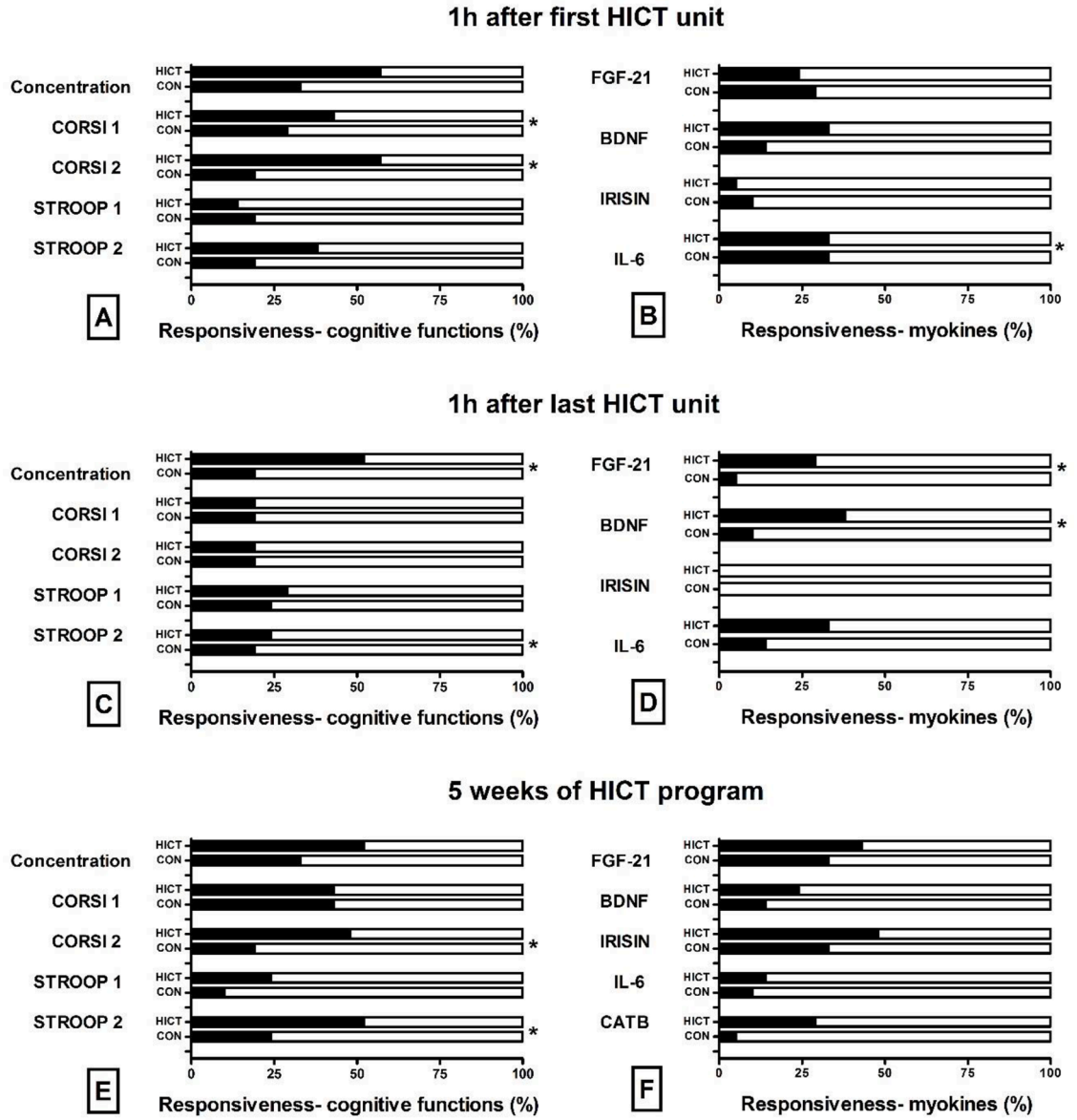

Fig. 3. Percentage of responsiveness to HICT in participants from high-intensity circuit training (HICT) and control (CON) group: *positive change greater than the smallest worthwhile change was calculated as responsiveness (for each indicator, based on measurement of twice the typical error -expressed as a percentage). Asterisks indicate the significant change observed in response to training in HICT group. Corsi 1- immediate block span; Corsi 2- correct answers; Stroop 1- reading interference; Stroop 2- naming interference; FGF-21- fibroblast growth factor 21; BDNF-brain-derived neurotrophic factor; IL-6- interleukin-6; CATB- cathepsin B.*

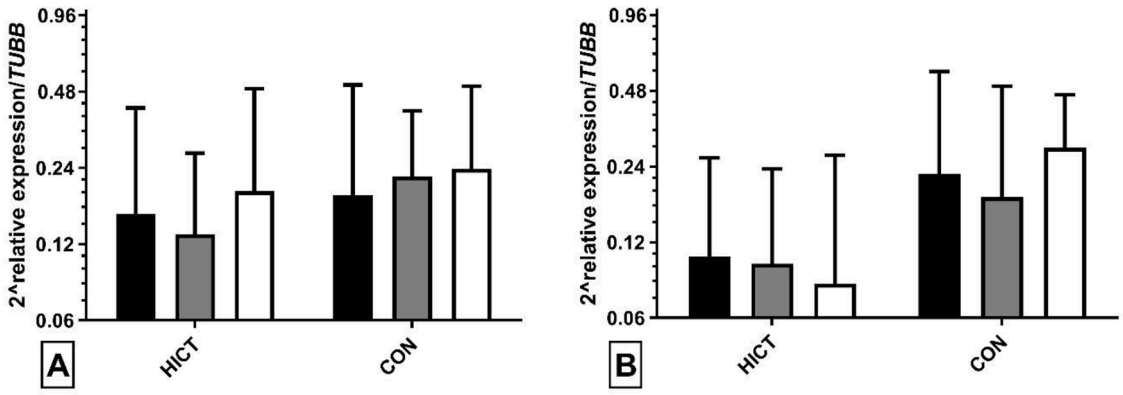

Fig. 4. Changes in CCL2 mRNA before (A) and after (B) 15 units of High Intensity Circuit Training: dark bars – before training unit, gray bars – 1 h after training, white bars – 24 h after training, HICT- high- intensity circuit training group, CON- control group.

after five weeks of HICT were different between older and younger participants. Interestingly, in response to an applied training program, the FGF-21 concentration of participants above 30 years old reduced significantly, opposite to that of younger women. The lack of changes in FGF-21 concentration recorded 24 h post exercise could be related to its short half-life in blood [53]. We wanted to find out which factor contributed the most differentiation in response to the training expressed in FGF-21 changes. The analysis showed that, particularly in women with an elevated visceral fat area, elevated glucose level and low physical performance, a drop in FGF21 was noted. Still, none of these factors exhibited statistically significant correlation. Our participants with insulin resistance had exhibited a lower concentration of FGF-21 at baseline, and the later-recorded increase of this growth factor was smaller in comparison to women without this syndrome.

In order to find a link between changes in mental health, cognitive functions, and muscle activity, the concentrations of BDNF, CATB and irisin were measured. It is known that CATB can cross the blood-brain barrier [54] and modulate BDNF synthesis [16,55], but there is a limited data regarding irisin. In this study, the increment of CATB was observed after five weeks of HICT– a shorter time than the four months of treadmill running applied previously in healthy young adults [54]. Moreover, to the best of our knowledge, this is the first study to assess CATB shifts and verify its impact on cognitive functions in response to resistance training. Interestingly, in the HICT group, among women who was characterized by decrement of FGF-21 after five weeks, a drop in CATB was noted in contrast to those who experienced an increase of FGF-21. This drop was accompanied by the improvement of cognitive functions. It is worth adding that a significant positive correlation between CATB and irisin among women after 15 HICT units was observed. A previous study considered FNDC5, as a precursor of irisin, in the formation of dendrites [56] that suppresses neurodegenerative diseases by increasing the BDNF mRNA level in the hippocampus. It also promotes the expression of the BDNF gene in the cerebral cortex [14]. This study results indicate that a single HICT session led to increased circulating concentrations of BDNF in all women. In contrast, a previous study showed that in men immediately after a single bout of high-intensity training, a decrease of BDNF was noted [57]. As recently reviewed by Dinoff et al., BDNF response to a single training can be different between sexes [58], however, the authors assumed that the increment in BDNF was observed mostly in male participants. This study showed that an elevation of BDNF was also significant in women.

Regular aerobic exercise is also known to correlate with reduced anxiety and increased levels of irisin in the brain of rats [55]. In this study, we noted a drop of serum irisin in parallel with a significant correlation between the irisin and results of the Corsi Block Tapping Test. We could not rule out that uptake of irisin had taken place triggering an improvement of participants' attention. Recently, Li et al. revealed that irisin protects against neuronal injury via activation of the Akt and ERK1/2 signaling pathways, which contributes to the neuroprotection of physical exercise [59]. Moreover, an integrin αV/β5 was identified as a receptor for irisin however, its presence was only noted in the osteocytes [60]. Owing the fact that positively associated to the irisin in obese people [61], its concentration in response to applied procedures was assessed.

Inseparably, exercise has an impact on inflammation and can modulate neuroinflammation throughout pro-inflammatory cytokines and its genes expression, such as IL-6 or *CCL2* mRNA [62]. In this study, an increase of IL-6 in response to a single HICT unit was noted in agreement with the previous published data. Recently, it was reported that in middle-aged people, IL-6 is significantly associated with depression and anxiety symptoms, still the role of IL-6 pathogenesis in such dysfunctions is not clear [63]. There is no correlation between IL-6 and the mental health aspects assessed observed in this study.

In order to evaluate how the applied procedure affects induction of macrophages, changes in *CCL2* gene expression was assessed. It was reported that protein and RNA levels correlate with neuronal, especially cognitive function. An animal-based study demonstrated that the astrocyte expression of *CCL2* can influence the synaptic function within the hippocampus, which plays a very important role in cognitive functions [64]. Xu et al. suggested that *CCL2* released by astrocytes has an impact on cognitive dysfunctions and brain inflammation [65]. This study's results demonstrated a clear tendency of *CCL2* expression to decrease in the leukocytes after 15 units of HICT. It is possible that a lower mRNA level of this cytokine could be associated with improved cognitive functions. Still, those changes were statistically insignificant. Thus, more research is needed to investigate if obtained results of *CCL2* expression are consist or temporary.

Still, some limitations of the study should be mentioned. One of them is the small sample size of subjects especially when individual response was evaluated. Thus, in the future investigations it is need to expand the number of responders. Moreover, further limitation is unbalanced participants distribution among groups as well as the wide range of age in both study groups. It is necessary for future investigations to increase the number of participants to support obtained results. At the same time, it is worth mentioning that although we had not performed any depression questionnaire, the subjective mood assessment among the exercising women indicted a significant improvement to their well-being. Additionally, the SF-36 questionnaire revealed a significant improvement in physical functioning only among exercising participants. Thus, this observation proved a beneficial impact of the HICT program on both metabolic and mental aspects of health.

5. Conclusion

Overall, the obtained results revealed that a simple procedure of resistance training, which can be performed anywhere especially

considering home conditions, had induced an improvement in cognition and quality of life among females exhibiting impaired insulin sensitivity. This improvement was supported by changes in FGF-21 concentration, especially by its drop. Given the growing number of insulin resistance and depression cases in modern society, it can be considered as a simple treatment. The proposed training program could be universally practiced, requiring no access to a gym or specialized equipment. Due to the COVID-19 pandemic it is particularly important that proposed home-based exercise can be a simple strategy not only for metabolic dysfunction but also for brain health.

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Declaration of Competing 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.

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Impact of 12-Week Moderate-Intensity Aerobic Training on Inflammasome Complex Activation in Elderly Women

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Aging often associates with a chronic low-grade inflammatory status that can be consequent to the activation of Toll-like receptors (TLRs) and the downstream NLR family pyrin domain containing 3 (NLRP3) inflammasome and causes a chronic secretion of pro-inflammatory cytokines. Since exercise has known anti-inflammatory effects, we investigated the effect of Nordic walking training on inflammasome activation and downstream effectors in elderly women. A population of elderly women was divided into EXP (n = 29) that completed 12 weeks of the moderate-intensity aerobic training program and CTRL (n = 29), performing no activity. Blood samples were taken before and after the first (T1-pre and T1-post, respectively) and last (T2-pre and T2-post, respectively) exercise unit. Inflammasome activation status was assessed by whole blood NLRP3 and TLR4 expression by RT-qPCR. Serum levels of IL-1β, IL-6, TNFα, and IL-18 cytokines were assayed by multiplex fluorescent beads-based immunoassays or ELISA. NLRP3 and TLR4 levels were reduced 2 folds between T1-pre and T2-pre and induced at T2-post, compared to T2-pre, by 2.6- and 2.9-fold, respectively. A single exercise bout elicited a 1. 38-, 1. 5-, and 1.36-fold rise of IL-1β, TNFα, and IL-6 concentration, respectively, although not significant, at the beginning of the training (T1-pre vs. T1-post), a 1.4-fold decrease for IL-1β and TNFα at the end of the training (T1-pre vs. T2-pre), and a 2-, 1.8- and 1.26-fold increase after the last exercise session (T2-pre vs. T2-post) for the three cytokines. When stratifying the population based on BMI in normal weight (NW) and overweight (OW), NLRP3 and TLR4 expression was affected only in NW. As for inflammatory cytokines, IL-1β was modulated in NW at the beginning of the training, whereas in OW at the end of the training; for TNFα, this time-dependent modulation was significant only in OW. Applied aerobic training affected the resting expression of inflammasome constituents (NLRP3 and TLR4) and levels of

downstream effectors (IL-1β, TNFα, and IL-6). However, at the end of the program, participants acquire an acute inflammatory response to exercise that was absent at baseline. Future studies would have to define the molecular mechanisms associated with, and how to potentiate, the exercise-associated inflammatory response.

Keywords: aging, NLRP3 inflammasome, pro-inflammatory cytokines, physical activity, aerobic exercise

INTRODUCTION

Aging is associated with several biological changes that profoundly affect cell and tissue functions and hesitates in the increased risk of developing diseases, frailty, injuries, disability, hospitalization, and, consequently, mortality. Among the plethora of altered functions, aging associates with a persistent state of chronic low-grade inflammation (LGI), also referred to as inflammaging (Franceschi et al., 2000). LGI is, in turn, associated with onset and development of most diseases including metabolic dysfunctions (e.g., obesity, impaired glucose tolerance, metabolic syndrome, and type 2 diabetes), muscle-skeletal failure (e.g., osteopenia, osteoporosis, and sarcopenia), cancers, neurodegenerative diseases, and aging itself (Franceschi et al., 2007).

Inflammation is a complex homeostatic response to harmful stimuli that protects the organism and promotes tissue repair and regeneration after injury, by orchestrating the innate immune response (Franceschi et al., 2007). The innate immune response is primed by the activation of pattern-recognition receptors (PRRs), as the Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and NOD-like receptors (NLRs), by pathogen-associated molecular patterns (PAMPs). Injured cells can also activate the innate inflammatory response through the release of endogenous damage-associated molecular patterns (DAMPs), in the absence of pathogens (Gomarasca et al., 2020). During aging, alterations in T cell function, immune-senescence, extracellular matrix alterations, unfavorable changes in body composition (increased fat mass), and foci of chronic infections feed the presence of DAMPs and PAMPs into the circulation and keep the inflammatory response chronically activated (Franceschi et al., 2007; Mejias et al., 2018). This condition, known exactly as the LGI, is characterized by slightly and chronically increased plasma levels of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)α, and C-reactive protein (CRP). This pro-inflammatory state is caused by the activation of TLRs, upon PAMPs and DAMPs recognition, and the downstream signaling that culminates in the activation of NF-κB and MAPK pathways that promotes the expression of pro-inflammatory cytokines (Kawasaki and Kawai, 2014).

A key modulator of age-related systemic LGI is the NLR family pyrin domain containing 3 (NLRP3) inflammasome. Inflammasomes are multimeric protein complexes that assemble in the cytosol after sensing both PAMPs and DAMPs. Inflammasomes are involved in the activation of caspase-1 that in turn processes the pro-inflammatory cytokines IL-1β and IL-18 into their bioactive mature forms. The NLRP3 inflammasome needs to be primed by the activation of the TLR4 that, throughout NF-κB signaling, leads to the increased expression of the NLRP3 protein (Guo et al., 2015). Among others, aging is associated with elevation of intracellular and extracellular levels of uric acid, reactive oxygen species (ROS), free fatty acids (fFAs), ceramides, free cholesterol, oxidized low-density lipoproteins (LDL), advanced glycation end products, as well as with the alteration of the microbial community and the consequent increase in the level of microbial-derived constituents in blood. All these age-associated danger signals, contribute to the activation of the NLRP3 inflammasome and the subsequent triggering of the so-called age-related inflammation (or inflammaging) (Gritsenko et al., 2020). Thus, the NLRP3 inflammasome is a major sensor of age-related accumulation of DAMPs, in absence of apparent infection (Youm et al., 2013).

Powerful and effective regulators of chronic LGI are physical exercise and training. Both aerobic and resistance chronic exercises exert beneficial effects on the modulation of the inflammatory response (Gerosa-Neto et al., 2020; Padilha et al., 2021), reduction of chronic LGI in the elderly population (Dalle et al., 2017; Duggal et al., 2019), as well as, on the improvement of the metabolic profile (Lira et al., 2017; Da Silva et al., 2020). Exercise, which is defined as a planned, structured, and repeated physical activity (PA), can reduce not only the baseline inflammatory status of chronic LGI but also the acute inflammatory response against harmful stimuli (Beyer et al., 2012). However, only a few studies investigated the effects of PA on inflammasome activation and none has focused on Nordic walking. A resistance training (RT) program in healthy elderly reduced the protein expression of TLR4 and its downstream signaling effectors, leading to an overall improvement of the inflammatory status (Rodriguez-Miguelez et al., 2014). Similarly, another study related to RT intervention in elderly demonstrated the downregulation of NLRP3 protein and a decreased caspase-1-to-pro-caspase-1 ratio in peripheral blood mononuclear cells (PBMCs), highlighting the possible beneficial effect of RT in limiting the inflammatory reactivity (Mejias-Pena et al., 2017). A recent study has demonstrated that also moderate-intensity chronic aerobic exercise may reduce TLR4 and NLRP3 mRNA expression in PBMCs and circulating levels of IL-1β and IL-18 in young males (Khakroo Abkenar et al., 2019). Moreover, it was revealed that 12 weeks of moderate-intensity aerobic training combined with RT brought cardiometabolic benefits in adults with metabolic syndrome (Da Silva et al., 2020). Previously published papers indicated that Nordic walking training induces a reduction in circulating levels of the autophagy protein high mobility group box 1 (HMGB1) in elderly women and an increase the myokine

irisin (Gmiat et al., 2017). Thereby, moderate-intensity aerobic training, as Nordic walking, seems to positively affect the inflammatory status and it happens mainly throughout the modulation of the innate immunity function (Padilha et al., 2021), whose activation mainly relay on TLR4 signaling and the consequent inflammasome activation, especially in the elderly subjects that experience an age-associated deregulation of the TLR4-associated inflammatory pathways (Kawasaki and Kawai, 2014; Mejias et al., 2018). Nordic walking, also known as "Scandinavian walking with poles," is a popular outdoor activity based on specially designed poles for the purpose of activating the upper body during walking. It combines and stimulates skiing, sport walking, and trekking skills: by activating the upper body muscles, the use of poles may increase the length of each step, finally resulting in a faster gait and improved metabolism. Elderly are the most enthusiastic performers maybe because of its open-air, nature friendly, and social behaviors but also because perceived exertion and joint overload are limited thanks to the use of poles, despite the higher heart rate and oxygen consumption compared to a standard walk (Skorkowska-Telichowska et al., 2016). Interestingly, although the moderate intensity, Nordic walking is emerging as a powerful and effective strategy to counteract frailty and, particularly, metabolic- (Muollo et al., 2019) and mobility-associated aspects of frailty, as increased risk of fracture and skeletal muscle wasting (Ossowski et al., 2016; Xu et al., 2016). Within this context, this study aims to unravel the effect of a 12-week Nordic walking moderate aerobic training program on the expression of the main components of inflammasome complex (TLR4 and NLRP3) in whole blood and the downstream cytokine effectors (IL-1β, IL-18, TNFα, and IL-6) in elderly women.

MATERIALS AND METHODS

Study Design

In this intervention case-control study, 70 elderly women (age = 68 ± 8 years old), with a sedentary behavior [according to the American College of Sports Medicine guidelines (ACSM, 2010)] were recruited among church communities, senior citizens' clubs, and universities of the third age. Participants were randomly assigned to the experimental (EXP, n = 35) group, engaged in a 12-week Nordic walking training program, and control (CTRL, n = 35) group, not involved in any activity. Recruitment and testing of EXP and CTRL subjects took place at the same period. At enrolment, all subjects underwent a medical examination and were asked to provide information regarding prescribed medications. Exclusion criteria were: uncontrolled hypertension (diastolic blood pressure > 100 mmHg), history of cardiac arrhythmia, cardio-respiratory disorders, and orthopedic problems. Body composition and 2,000 m walking test were determined 1 week prior to the start of the experiment and after 12 weeks of training. Participants belonging to the EXP group were familiarized with the right technique for walking with Nordic walking poles. All subjects were characterized for weight, BMI, and hematological and biochemical markers (as reported in the subsection "Blood Collection and Sample Preparation").

From the original cohorts, six subjects who either did not attend one blood sampling or did not meet the compliance criteria to training (i.e., <90% participation), were excluded. By the end, 29 subjects were included in the EXP group and 29 in the CTRL group. In order to verify the existence of a BMI-dependent response to the training program, participants were sub-grouped in normal weight (NW; EXP, n = 14; CTRL, n = 12), with BMI < 25 kg/m^{-2}, and overweight/obese (OW; EXP, n = 15; CTRL, n = 17), with BMI ≥ 25 kg/m^{-2}.

The population was not stratified according to Vitamin D intake, since many participants used to take this supplement as a general recommendation from the Ministry of Health for seniors in Poland. The levels of Vitamin D were measured to be in the range of 32–55 ng/mL for the whole population.

The study received the official approval of the Bioethical Committee of the Regional Medical Society in Gdansk (KB-34/18) and was registered as clinical trial with the ID: NCT03417700, in accordance with the Declaration of Helsinki. All participants were given detailed information about experiment, procedures, risks, and benefits of the study and gave their written consent to participation.

Training Protocol

Participants from EXP group met three times a week (Monday, Wednesday, and Friday), 1 h after eating a light breakfast. In order to avoid the impact of different diets on training response, all the participants were given the same breakfast on the day the tests were collected and were asked not to change their eating habits during the training period. CTRL subjects were also asked to maintain unaltered their lifestyle habits and to keep their PA level below 150 min/week (ACSM, 2010). Both groups were instructed not to perform any additional physical activity during the study period. Each training session lasted 1 h, and consisted of 10-min warm-up, 40-min specific Nordic walking training, and 10-min cool-down. Subjects were equipped with standard Nordic walking poles. The same group of research assistants and coaches checked attendance of participants, supervised all training sessions, and performed the tests. Nordic walking training was performed with 60–70% intensity of the maximal heart rate (HR) obtained during the supervised 2000 m walking test. This test was performed on a flat floor, according to the previously described procedure (Mieszkowski et al., 2018). This test, as well as each training unit, was monitored using Garmin Forerunner 405 with a built-in GPS in order to record distance. The model of Garmin Forerunner 405 was equipped with additional HR sensor. The participants were encouraged to maintain the highest possible pace during the 2,000 m walking test to achieve the highest intensity, but they were not allowed to run. Time was measured using photoelectric cells (Racetime 2 SF, Microgate, Bolzano, Italy) with an accuracy of 0.001 s. The start of the movement was signaled by the instructor. The information about 60–70% HR max intensity achieved during every training session was monitored for each participant individually by coach. To evaluate the maximal oxygen capacity a mathematic formula

was applied: VO_2 max = 116.2-2.98 Time-0.11HR-0.14Age-0.39BMI (Laukkanen et al., 2000; Kortas et al., 2015). The EXP group completed 12 weeks of Nordic walking training, which included 36 training units. During the entire training program, participants in the EXP group covered a total distance of almost 120 km. Only subjects, who attended at least 90% of the total amount of training units, were considered as completing the protocol.

Blood Collection and Sample Preparation

Blood samples were taken from the antecubital vein by two professional nurses. For the EXP group, blood samples were collected at baseline, before and after the first session of Nordic walking (T1-pre and T1-post, respectively), immediately before the last exercise session, after 12 weeks of training (T2-pre), and immediately after the last training session (T2-post). Post-exercise blood drawings were performed within 15 min from the end of the exercise session. For the CTRL group, blood was sampled only at T1 and T2, corresponding to the T1-pre and T2-pre, respectively, of the EXP cohort. The blood was collected at rest, under fasting condition, between 7:00 and 8:00 a.m. Ethylendiaminotetraacetate dipotassium salt (K2EDTA)-anticoagulated blood (K2EDTA Vacutainer®, Becton Dickinson, and Co., Franklin Lakes, NJ, United States) was used for hematological characterizations and RNA extraction. The hematological assessment was performed only at T1-pre and T2-pre for the EXP group and at both T1 and T2 for the CTRL group, and included: hemoglobin [Hb], hematocrit (Ht%), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin content (MCHC), red cells distribution width – coefficient of variation (RDW-CV), platelets count (Plt), mean platelet volume (MPV), total white blood cell count (WBC), absolute and relative counts of neutrophils (Neu), lymphocytes (Ly), monocytes (Mo), eosinophils (Eo), and basophils (Ba). Serum was obtained from blood collected into SST II Advance™ tubes with clot activator (Becton Dickinson, and Co.) and was used for the characterization of the metabolic [total cholesterol (TChol), high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), iron, and ferritin] and inflammatory (as described below) profiles, measured only at T1-pre and T2-pre for the EXP group and at both T1 and T2 for the CTRL group. Samples were centrifuged at 2,000 × *g*, for 10 min, at 4°C and stored at −80°C until later analysis. Guidelines for the correct management of the pre-analytical phase were strikingly followed (Banfi et al., 2010; Dugue et al., 2018; Faraldi et al., 2020). Since [Hb], Ht%, RBC, MCV, MCH, MCHC, RDW-CV, Plt, and MPV remained stable during the observation and did not differ among any of the groups, and are, however, mostly irrelevant to the aim of the current study, they were not further discussed.

Further, 25-hydroxy vitamin D [25-(OH)D], the most reliable marker of vitamin D status (Ferrari et al., 2017), was measured by high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) on a Shimadzu LCMS 8050 HPLC system Nexera X2 column with Agilent Eclipse Plus C18 1.8 μm 2.1 × 100 mm columns, according to Gmiat et al. (2017). The measurement was performed at baseline in order to verify that all participants were in a state of sufficiency. Importantly, according to Polish guidelines for seniors, all participants used to be supplemented with vitamin D.

RNA Extraction

Total RNA was extracted from whole blood using the Direct-Zol miniprep Kits (Zymo Research Co., Orange, CA, United States), following manufacturer instructions. Briefly, three volumes of TRI Reagent® were added to 250 μl of whole blood. After mixing thoroughly and centrifuging at 12,000 × *g* for 30 s at RT, the supernatant was transferred into RNase-free tubes for the subsequent RNA purification. After adding an equal volume of ethanol 95% and mixing, the sample was transferred into the Zymo-Spin™ IC Column and centrifuged at 12,000 × *g*, for 30 s, at RT. Thereafter, the sample was digested with DNase I (6 U/μl) for 15 min at RT. The columns were further washed and the RNA was eluted in 15 μl of DNase/RNase-Free Water by centrifugation at 12,000 × *g* for 30 sat RT. RNA concentration was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States). RNA purity and integrity were assessed by considering the 260/280 nm and 260/230 nm absorbance ratios, visualized at NanoDrop spectrophotometer and through 1% agarose gel electrophoresis.

Gene Expression Analysis in Whole-Blood

Total RNA was reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, United States). RT-qPCR was carried out on a StepOne Plus instrument (Applied Biosystems, Foster City, CA, United States), using TaqMan™ Gene Expression Master Mix and premade 6-Carboxyfluorescein (FAM)-labeled TaqMan assay for *TLR4* (Hs00152939_m1), *NLRP3* (Hs00918082_m1), *PPIB* (Hs00168719_m1), *PGK1* (Hs99999906_m1), *ACTB* (Hs99999903_m1) (Thermo Fisher Scientific). The thermal protocol was as follows: 50°C for 2 min, 95°C for 10 min, followed by 40 amplification cycles at 95°C for 15 s and 60°C for 60 s. Results, reported as quantification cycle (Cq) values, were analyzed by the GenEx software ver. 6 (Exiqon A/S, Vedbaek, Denmark). The relative expression of each gene was calculated by the $2^{-\Delta\Delta Cq}$ method, using PPIB and PGK1 as reference genes. Analysis of target genes was performed on the overall included subjects and on subjects stratified for BMI. Results on gene expression are reported as median (minimum value to maximum value).

Selection of Reference Genes

PPIB, PGK1, and ACTB were assayed as reference genes. Expression level analysis was performed comparing normalized expression levels calculated as follows:

$$\Delta Cq = Cq\ rg - geomean\ rg \quad (1)$$

Cq rg: quantification cycle of a reference gene in a sample.

geomean rg: geometrical mean of the Cq of PPIB, PGK1, and ACTB of all samples.

The heatmap analysis was performed using the tool provided by GenEx software (Exiqon). An in-depth analysis of the normalization strategies was performed as previously described (Faraldi et al., 2019). Expression stability of PPIB, PGK1, and ACTB, was analyzed using the NormFinder (Andersen et al., 2004) and GeNorm (Vandesompele et al., 2002) algorithms provided by the GenEx software.

Cytokines Analysis

The pro-inflammatory cytokines IL-1β, IL-6, and TNFα were quantified in serum with a multiplex customized Human High Sensitivity Cytokine B Premixed Mag Luminex Performance Assay (R&D Systems, Minneapolis, MN, United States). Samples were analyzed in duplicates and read on a MAGPIX® Multiplex System (Luminex® Co., Austin, TX, United States). The assay sensitivities were 0.146 pg/mL for IL-1β, 0.135 pg/mL for IL-6, and 0.250 pg/mL for TNFα. Values under the last point of the standard curve, but above the blank and the minimum detectable dose (0.03 pg/mL for IL-1β, 0.08 pg/mL for IL-6, and 0.13 pg/mL for TNFα) were derived by the Bio-Plex Manager Software. The intra-assay (CV_i) and inter-assay (CV_b) coefficients of variation of each analyte were 1.7 and 11.1% for IL-1β, 2.0 and 11.4% for IL-6, and 1.7 and 11.6% for TNFα, respectively.

IL-18 concentrations were measured in serum by Human Total IL-18 ELISA (R&D Systems), following manufacturer instructions. The assay-specific sensitivity was 5.15 pg/mL. Maximum intra-assay (CV_i) and inter-assay (CV_b) coefficients of variation were 3.1% and 8.7%, respectively. Readings were performed at λ = 450 nm subtracted of the corresponding readings at λ = 570 nm on a Victor X3 (PerkinElmer, Waltham, MA, United States). Body mass, body composition, and body mass index (BMI) were determined using a multi-frequency impedance analyzer (In $Body_{720}$, Biospace, South Korea). The measurements were performed twice, 1 week before and after the entire intervention, according to McLester et al. (2020).

Statistical Analysis

The minimum sample size was determined with G*Power (v3.1.9.7) based on IL-6 serum concentrations as the primary endpoint index, being this cytokine largely described as the prototypic mediator of innate immune response and exercise-dependent metabolic regulation (Chowdhury et al., 2020). For sample size calculation was assumed a two-tailed type I α error of 0.05, a power (1-β error probability) > 0.95, a pre-to-post-intervention difference of 18%, and a standard deviation of ±18.54 pg/mL, according to Gmiat et al. (2018). The total estimated sample size was 22.

Statistical analysis was performed with Prism® v6.01 (GraphPad Software Inc., La Jolla, CA, United States). The D'Agostino-Pearson's normality test (omnibus K2 test) defined non-parametric distributions for most of the parameters analyzed. Thereby, in the descriptive analysis, data are reported as the median and range (minimum to maximum), while the statistical analysis was conducted with non-parametric tests.

In EXP and CTRL groups, age, height, and Vitamin D were analyzed by non-parametric Mann–Whitney test.

In the EXP group, time-dependent changes were analyzed by non-parametric repeated measures Friedman's test with Dunn's multiple comparisons (T1-pre vs. T1-post vs. T2-pre vs. T2-post). Comparison of time-dependent changes between EXP and CTRL group and comparison between NW and OW within and between EXP and CTRL groups were performed by two-way ANOVA with Sidak's multiple comparison test. Differences were considered statistically significant if p-values < 0.05, and only significant data were discussed in the text.

The effect size has been calculated by Kendal W for Friedman's tests, Cohen's eta-squared for two-way ANOVA, and Cohen's d for the *post hoc* tests.

RESULTS

Characterization of the Study Cohort

The characterization of EXP subjects before the beginning (T1-pre) and before the last exercise session of the 12-week Nordic walking training (T2-pre), and CTRL subjects over the same period (T1 and T2, respectively) is detailed in **Tables 1**, **2** and **Supplementary Table 1**. The characterization of the cohorts at these two time-points allows avoiding the alteration of some hematological markers due to the effect of acute exercise, meaning after a session of training, rather than highlighting the differences due to the chronic exercise.

The two cohorts, compared before and after the intervention (T2-pre for EXP), resulted homogenous for age and most of the measured parameters. Within each cohort, NW and OW subjects differed for weight and BMI at T1 and T2 in both EXP and CTRL. Basophils were increased in OW subjects at both T1 and T2 of the CTRL cohort, while they were reduced between T1 and T2 in the EXP-NW cohort. No time-dependent change was observed in CTRL, although lymphocyte absolute count increased from T1 to T2 in the entire group and in the OW cohort. The results of the ANOVA tests and the related effect size are reported in **Supplementary Table 2**.

Considering the metabolic markers, HDL was lower in EXP-OW compared to EXP-NW at both time-points, and decreased in NW subject at the end of the training (**Supplementary Table 1**). In the EXP group, a time-dependent, although clinically irrelevant, reduction of serum iron was recorded in OW subjects, and at T2 compared to CTRL considering the whole population or the OW subgroup. Importantly, baseline 25-(OH)D concentrations did not differ among any of the cohorts.

Expression and Stability of PPIB, PGK1, and ACTB Genes

According to previously published studies (Dheda et al., 2004; Falkenberg et al., 2011), we selected PPIB, PGK1, and ACTB as possible candidates reference genes to normalize RT-qPCR data in whole blood samples. Following a cluster analysis to exclude co-regulation, ΔCq analysis revealed a more scattered expression of ACTB compared to PPIB and PGK1 (**Figures 1A–C**) and a more constant of PPIB and PGK1 in all samples at all

TABLE 1 | Anthropometrical characteristic of the study cohort.

	CTRL (n = 29)					EXP (n = 29)						
	T1		T2		p-value T1 vs. T2	T1-pre		T2-pre		p-value T1-pre vs. T2-pre	p-value T1 CTRL vs. T1-pre EXP	p-value T2 CTRL vs. T2-pre EXP
	Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW		Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW			
Age (years)	68.0 (60.00–78.0)		/		/	69.0 (60.0–78.0)		/		/	0.568	/
NW	67.5 (62.0–76.0)	0.763	/	/	/	70.0 (60.0–78.0)	0.502	/	/	/	0.446	/
OW	71.0 (60.0–78.0)		/		/	67.0 (60.0–76.0)		/		/	0.836	/
Height (m)	1.63 (1.50–1.74)		/			1.63 (1.53–1.78)		/		/	0.243	/
NW	1.64 (1.53–1.74)	0.360	/	/	/	1.65 (1.53–1.78)	0.883	/	/	/	0.962	/
OW	1.61 (1.50–1.70)		/		/	1.63 (1.53–1.75)		/		/	0.398	/
Weight (kg)	67.80 (54.40–93.00)		67.70 (54.10–93.80)		0.928	67.35 (54.40–94.20)		65.80 (54.80–95.60)		0.460	0.731	0.664
NW	63.90 (54.40–73.30)	**0.004**	63.25 (54.10–73.80)	**0.004**	1.000	58.70 (54.40–73.80)	**<0.001**	57.60 (54.80–73.80)	**<0.001**	0.996	0.966	0.971
OW	71.80 (58.50–93.00)		72.20 (57.70–93.80)		0.986	68.50 (65.80–94.20)		68.70 (65.10–95.60)		0.258	1.000	1.000
BMI (kg/m^2)	26.00 (20.70–34.60)		25.90 (20.60–34.90)		0.909	25.30 (19.80–33.00)		24.80 (19.60–33.10)		0.995	0.261	0.280
NW	23.75 (20.70–24.90)	**<0.001**	23.43 (20.60–25.30)	**<0.001**	1.000	22.60 (19.80–24.70)	**<0.001**	22.20 (19.60–24.70)	**<0.001**	0.941	0.681	0.816
OW	27.90 (25.30–34.60)		27.10 (24.90–34.90)		0.992	26.90 (25.20–33.00		26.50 (24.50–33.10)		0.915	0.813	0.771

Description of weight, BMI, and hematologic markers in the entire study cohort and for the sub-cohorts stratified based on BMI (BMI < 25 kg/m^2: NW; BMI > 25 kg/m^2: OW, as determined at recruitment). Data are expressed as median (range) since the non-parametric distribution, as assayed by D'Agostino-Pearson's test. Comparison of age and height between EXP and CTRL were assessed using Mann–Whitney test. Within-group (EXP and CTRL) time-dependent changes in NW and OW subjects were performed by the means of two-way ANOVA with Sidak's multiple comparison post hoc test. Statistically significant (p-values < 0.05) differences are indicated in bold. NW, normal weight subjects; OW, overweight subjects; BMI, body mass index.

TABLE 2 | White blood cell counts.

	CTRL (n = 29)					EXP (n = 29)						
	T1		T2		p-value T1 vs. T2	T1-pre		T2-pre		p-value T1-pre vs. T2-pre	p-value T1 CTRL vs. T1-pre EXP	p-value T2 CTRL vs. T2-pre EXP
	Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW		Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW			
WBC ($\times 10^9$/L)	5.58 (3.93–7.90)		5.74 (4.02–9.21)		0.475	5.73 (3.53–8.89)		5.52 (3.71–10.18)		1.000	0.997	0.821
NW	5.83 (5.06–7.90)	0.819	5.69 (4.47–8.80)	1.000	0.999	5.30 (3.95–7.56)	0.641	4.79 (3.79–6.86)	0.232	0.968	0.716	0.577
OW	5.53 (3.93–7.41)		5.74 (4.02–9.21)		0.375	6.00 (3.53–8.89)		6.25 (3.71–10.18)		0.977	0.751	0.985
Neu (%)	50.80 (38.40–70.10)		50.80 (31.90–66.00)		0.635	52.60 (31.70–77.40)		50.70 (38.10–69.30)		0.323	0.905	0.999
NW	49.30 (38.40–65.40)	0.995	50.60 (31.90–66.00)	0.974	0.932	51.40 (31.70–77.40)	0.998	48.50 (41.00–67.20)	0.986	0.747	1.000	1.000
OW	53.60 (39.90–70.10)		51.40 (42.20–65.90)		0.977	53.50 (35.90–67.60)		52.00 (38.10–69.30)		0.874	1.000	1.000
Ly (%)	35.40 (19.10–48.20)		37.10 (21.80–54.90)		0.296	34.85 (13.20–54.90)		37.95 (20.50–49.20)		0.280	0.989	0.995
NW	38.70 (23.10–47.20)	0.964	37.05 (23.80–54.90)	0.957	0.834	37.20 (13.20–54.90)	0.999	39.00 (20.50–49.20)	0.976	0.662	1.000	1.000
OW	33.80 (19.10–48.20)		37.10 (21.80–46.30)		0.758	34.10 (25.80–49.10)		36.70 (21.50–47.60)		0.874	1.000	1.000
Mo (%)	8.20 (6.10–11.00)		8.20 (5.60–11.00)		0.431	8.60 (5.50–13.30)		8.90 (5.60–13.40)		0.889	0.462	0.277
NW	8.05 (6.90–10.70)	1.000	7.70 (5.60–10.90)	0.999	0.711	8.50 (6.20–12.00)	1.000	8.70 (5.70–13.40)	1.000	1.000	0.972	0.709
OW	8.50 (6.10–11.00)		8.30 (5.90–11.00)		0.966	8.80 (5.50–13.30)		9.30 (5.60–11.90)		0.938	0.966	0.978
Eo (%)	2.90 (0.90–15.90)		2.70 (0.80–6.30)		0.256	2.70 (0.60–5.10)		2.45 (0.70–5.00)		0.987	0.154	0.583
NW	3.00 (1.20–8.00)	0.998	2.95 (1.40–5.70)	0.988	1.000	2.60 (1.10–5.10)	1.000	2.30 (0.80–5.00)	1.000	1.000	0.865	0.854
OW	2.50 (0.90–15.90)		2.70 (0.80–6.30)		0.196	2.70 (0.60–5.00)		2.50 (0.70–4.50)		1.000	0.712	1.000
Ba (%)	0.70 (0.30–1.60)		0.70 (0.20–1.50)		0.346	0.70 (0.30–1.30)		0.60 (0.20–1.10)		0.113	0.621	0.381
NW	0.55 (0.30–1.30)	**0.050**	0.45 (0.20–1.10)	**0.028**	0.756	0.70 (0.40–1.30)	0.986	0.60 (0.20–0.80)	0.812	**0.021**	0.793	1.000
OW	0.80 (0.40–1.60)		0.80 (0.40–1.50)		0.853	0.70 (0.30–1.10)		0.60 (0.30–1.10)		1.000	0.166	0.454

(Continued)

TABLE 2 | (Continued)

	CTRL (n = 29)					EXP (n = 29)						
	T1		T2		p-value T1 vs. T2	T1-pre		T2-pre		p-value T1-pre vs. T2-pre	p-value T1 CTRL vs. T1-pre EXP	p-value T2 CTRL vs. T2-pre EXP
	Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW		Median (min-to-max)	p-value NW vs. OW	Median (min-to-max)	p-value NW vs. OW			
Neu (×10^9/L)	2.92 (1.58–4.55)		3.04 (1.74–5.29)		0.975	2.84 (1.80–5.01)		2.86 (1.69–7.06)		0.839	0.936	0.981
NW	3.02 (2.24–4.16)	0.998	3.14 (1.74–4.64)	1.000	0.988	2.37 (1.89–5.01)	0.820	2.45 (1.75–4.13)	0.341	0.857	0.989	0.931
OW	2.92 (1.58–4.55)		2.88 (2.12–5.29)		0.953	3.39 (1.80–4.89)		3.07 (1.69–7.06)		1.000	0.889	0.984
Ly (×10^9/L)	1.93 (0.98–3.05)		2.22 (1.24–3.28)		**0.025**	1.82 (0.83–3.32)		2.06 (1.11–3.17)		0.367	0.986	0.654
NW	2.34 (1.17–3.05)	0.412	2.17 (1.24–3.28)	0.968	0.933	1.78 (0.83–3.32)	0.937	2.07 (1.11–2.91)	0.878	0.921	0.572	0.576
OW	1.90 (0.98–2.92)		2.24 (1.25–3.13)		**0.030**	2.05 (1.16–2.95)		2.19 (1.27–3.17)		0.745	0.852	1.000
Mo (×10^9/L)	0.48 (0.34–0.67)		0.48 (0.27–0.76)		0.903	0.48 (0.32–0.92)		0.45 (0.30–0.84)		0.910	0.733	0.937
NW	0.51 (0.40–0.61)	0.929	0.47 (0.30–0.76)	1.000	0.897	0.47 (0.33–0.69)	0.835	0.44 (0.31–0.84)	0.770	0.993	0.990	0.999
OW	0.44 (0.34–0.67		0.50 (0.27–0.76)		0.665	0.51 (0.32–0.92)		0.49 (0.30–0.78)		1.000	0.588	0.952
Eo (×10^9/L)	0.15 (0.07–1.06)		0.16 (0.06–0.33)		0.341	0.13 (0.04–0.30)		0.13 (0.03–0.34)		0.994	0.114	0.503
NW	0.19 (0.07–0.48)	1.000	0.21 (0.08–0.27)	0.989	0.998	0.13 (0.06–0.27)	0.986	0.11 (0.03–0.23)	0.971	1.000	0.582	0.642
OW	0.14 (0.07–1.06)		0.15 (0.06–0.33		0.448	0.15 (0.04–0.30)		0.15 (0.05–0.34)		1.000	0.828	1.000
Ba (×10^9/L)	0.04 (0.02–0.10)		0.04 (0.01–0.09)		0.740	0.04 (0.02–0.07)		0.04 (0.01–0.07)		0.374	0.507	0.306

White blood cell counts in the entire study cohort and for the sub-cohorts stratified based on BMI (BMI < 25 kg/m^2: NW; BMI > 25 kg/m^2: OW, as determined at recruitment). Data are expressed as median (range) since the non-parametric distribution, as assayed by D'Agostino-Pearson's test. Within-group (EXP and CTRL) time-dependent changes in NW and OW subjects were performed by the means of two-way ANOVA with Sidak's multiple comparison post hoc test. Statistically significant (p-values < 0.05) differences are indicated in bold. NW, normal weight subjects; OW, overweight subjects; WBC, white blood cell count; Neu, neutrophils count; Mo, monocytes count; Ly, lymphocytes count; Eo, eosinophils count; Ba, basophils count.

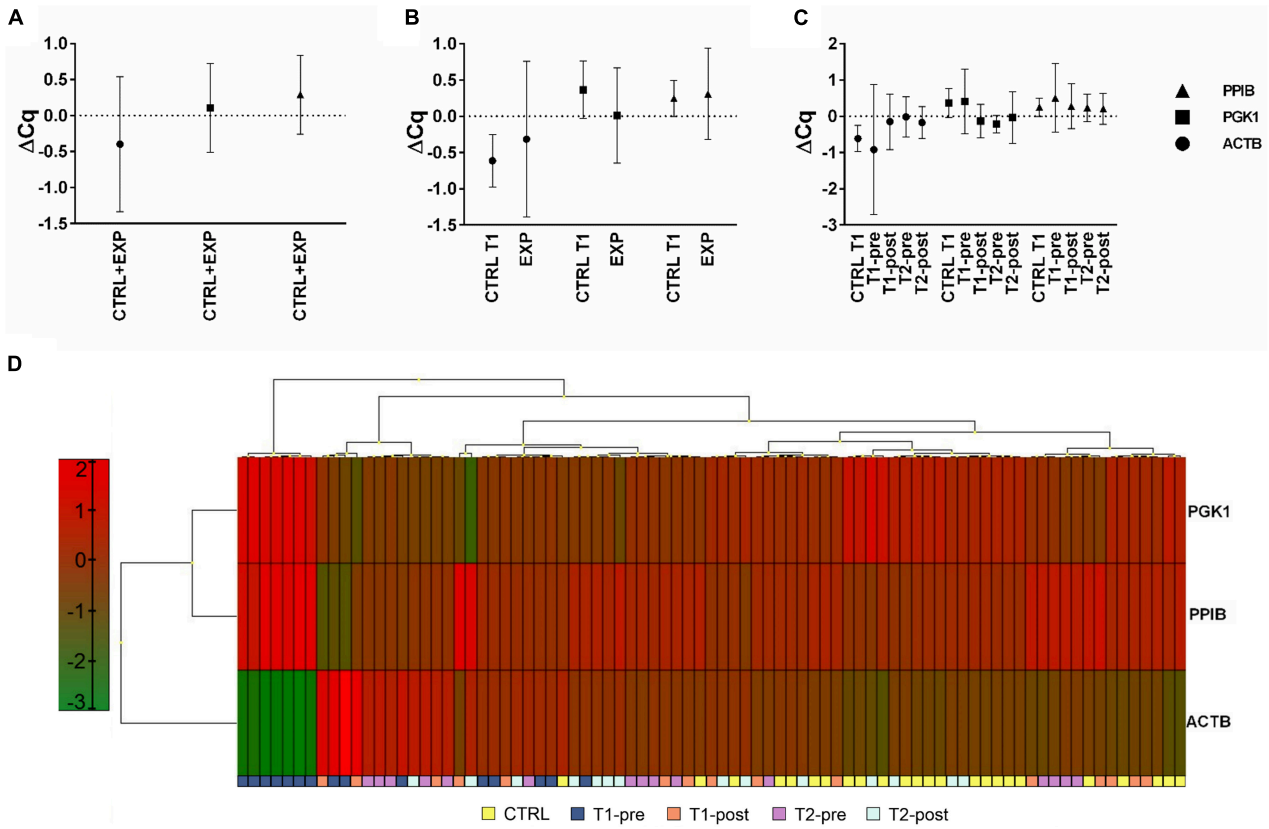

FIGURE 1 | Expression profile of potential housekeeping genes, ACTB, PGK1, and PPIB, in whole blood. Expression profile of the three selected potential reference genes (ACTB, PGK1, and PPIB). Expression level of each potential reference gene was analyzed considering **(A)** samples from all groups (CTRL+EXP), **(B)** samples from the EXP (T1-pre, T1-post, T2-pre, and T2-post), and CTRL groups separated, and **(C)** samples from each time-point separated. **(D)** Heat-map of the expression profiles of ACTB, PGK1, and PPIB, from each group (CTRL, T1-pre, T1-post, T2-pre, and T2-post). Different colors represent differential gene expression.

time-points (**Figure 1D**). Descriptive characteristics of the potential reference genes and the relative expression stability analysis are summarized in **Supplementary Tables 3**, **4**.

The expression stability of candidate genes was evaluated by NormFinder and GeNorm algorithms: PPIB and PGK1 were the best ranked genes, by NormFinder, while the combination PPIB-PGK1 was identified by GeNorm. Therefore, PPIB and PGK1 were used as reference genes.

Effect of Training on NLRP3 and TLR4 Expression in Whole Blood

NLRP3 and TLR4 mRNA expression levels were determined in whole blood. Both NLRP3 and TLR4 showed similar expression profiles: while a single bout of aerobic exercise at the beginning of the training protocol (T1-pre vs. T1-post) did not induce any significant changes in the expression of both genes, they were induced at T2-post, compared to T2-pre [NLRP3 T2-pre: 0.090 (0.049–0.459) vs. NLRP3 T2-post: 0.237 (0.064–0.594); TLR4 T2-pre: 0.087 (0.046–0.765) vs. TLR4 T2-post: 0.256 (0.047–0.660)]. Additionally, both NLRP3 and TLR4 expression levels were significantly reduced between T1-pre and T2-pre [NLRP3: 0.172 (0.072–1.680) vs. 0.090 (0.049–0.459); TLR4: 0.184 (0.068–1.296) vs. 0.087 (0.046–0.765)] (**Figure 2A**).

However, when comparing EXP and CTRL, the two groups differed at the beginning of the training (T1-pre vs. T1) for both genes. While in the CTRL group, no time effect was recorded, the EXP group showed a significant reduction of both genes at T2-pre compared to T1-pre (**Figure 2B**).

When analyzing the trained cohort sub-grouped in NW and OW, no differences were found at any time-point between the two sub-groups (**Figure 2C**).

The NW sub-population was affected by the acute intervention since it showed a significant increase in the NLRP3 gene expression [T1-pre vs. T1-post) (0.180 (0.072–1.679) vs. 0.218 (0.069–0.528), respectively] and this acute effect was seen also at the end of the training, when comparing T2-pre vs. T2-post, even though not significantly (**Figure 2C**). The chronic exercise, on the other hand, induced a significant decrease in both NLRP3 and TLR4 expression at the rest time-points (T1-pre vs. T2-pre) [NLRP3: 0.180 (0.072–1.679) vs. 0.088 (0.062–0.237); TLR4 0.216 (0.068–1.300) vs. 0.081 (0.046–0.292)] in the NW cohort (**Figures 2C,D**).

When comparing the NW and OW cohorts between the EXP and CTRL groups, besides the lack of differences in NLRP3, the

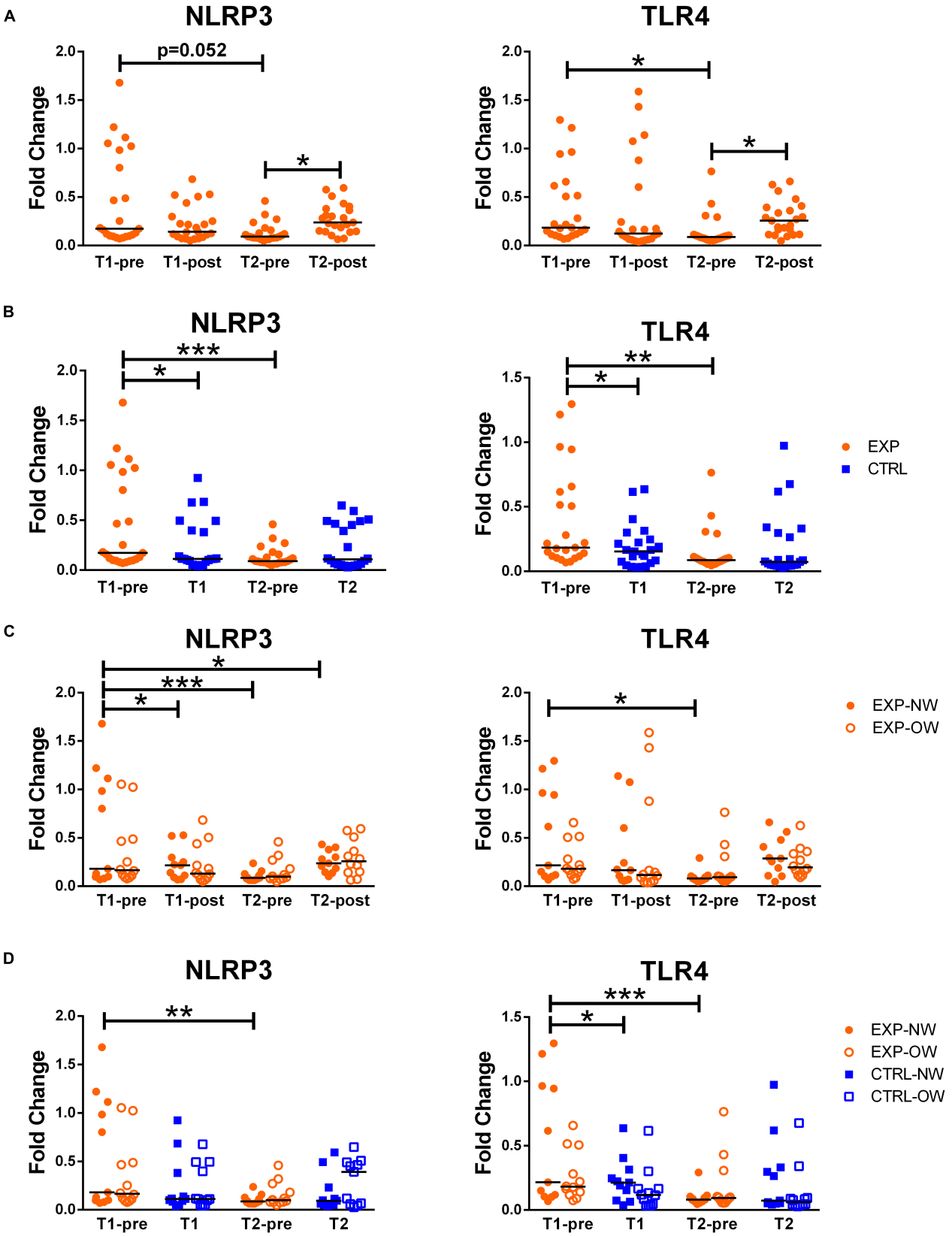

FIGURE 2 | Continued

FIGURE 2 | Expression of inflammasome-related genes in whole blood samples. Fold change of NLRP3 and TLR4 gene expression, normalized on PPIB and PGK1, in whole blood from elderly women underwent a 12-week aerobic Nordic walking training program (EXP, $n = 29$) and untrained controls (CTRL, $n = 35$). **(A)** Expression of NLRP3 and TLR4 in the whole EXP group before and after the first (T1-pre and T1-post) and the last (T2-pre and T2-post) sessions of Nordic walking. **(B)** Expression of NLRP3 and TLR4 in the whole EXP group (orange dots), before the first and the last (T1-pre and T2-pre) Nordic walking session, and in the CTRL (blue squares) over the same observation period (T1, T2). **(C)** Expression of NLRP3 and TLR4 in the whole EXP cohort, grouped based on BMI in normal weight (NW, full dots) and overweight (OW, empty dots) subjects. **(D)** Expression of NLRP3 and TLR4 in the EXP (orange dots) and CTRL (blue squares) cohorts, grouped based on BMI in normal weight (NW, full symbols) and overweight (OW, empty symbols) subjects. Asterisks indicated significant differences according to the different statistic tests applied: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

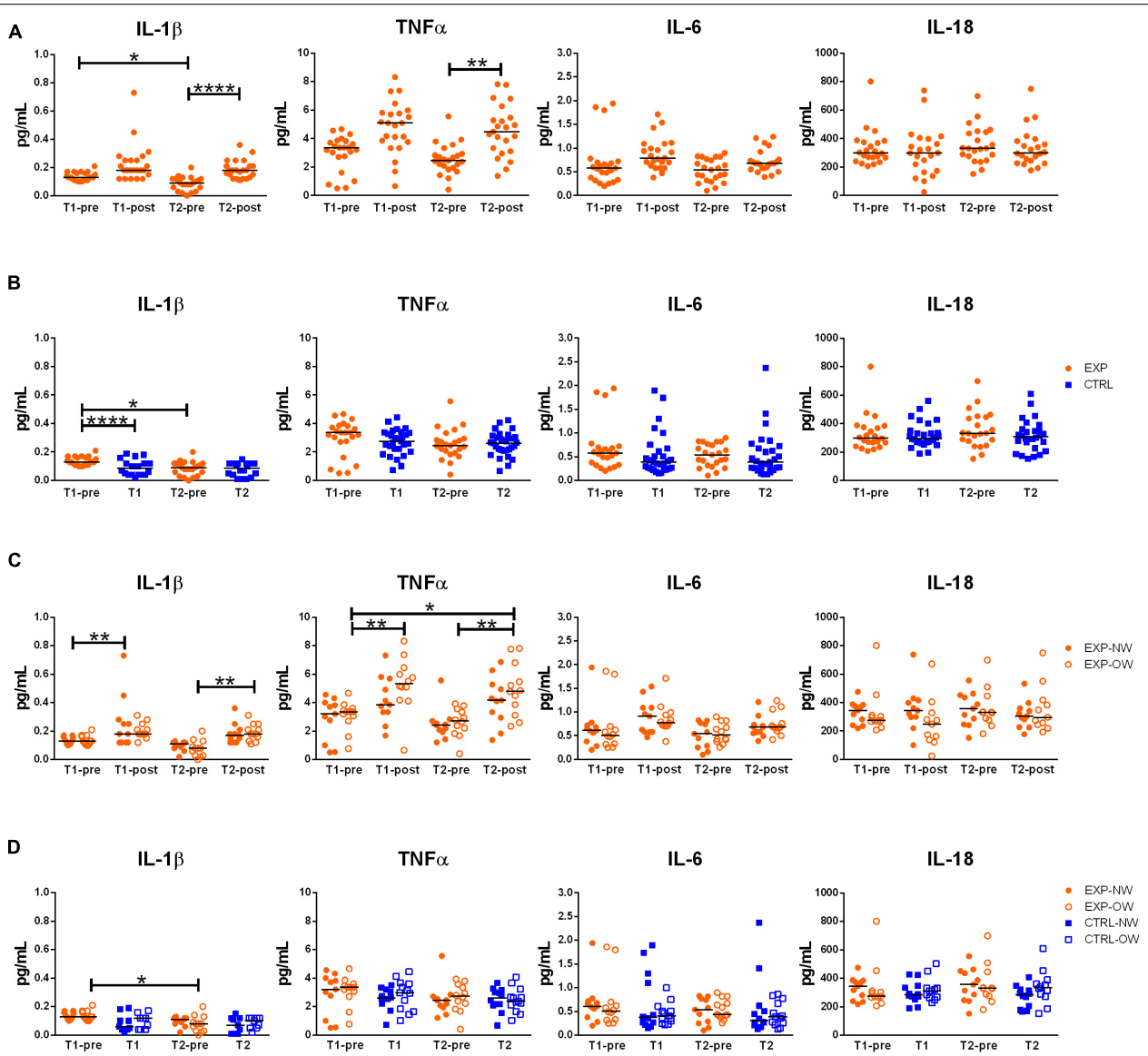

FIGURE 3 | Circulating levels of relevant cytokines associated to inflammasome pathway activation. Concentrations of IL-1β, TNFα, IL-6 and IL-18 in sera from elderly women underwent to a 12-week aerobic Nordic walking training program (EXP, $n = 29$) and untrained controls (CTRL, $n = 35$). **(A)** Circulating levels of the assayed cytokines in the whole EXP group before and after the first (T1-pre and T1-post) and the last (T2-pre and T2-post) sessions of Nordic walking. **(B)** Circulating levels of the assayed cytokines in the whole EXP group (orange dots), before the first and the last (T1-pre and T2-pre) Nordic walking session, and in the CTRL (blue squares) over the same observation period (T1, T2). **(C)** Circulating levels of the assayed cytokines in the EXP cohort, grouped based on BMI in normal weight (NW, full dots) and overweight (OW, empty dots) subjects. **(D)** Circulating levels of the assayed cytokines in the EXP (orange dots) and CTRL (blue squares) cohorts, grouped based on BMI in normal weight (NW, full symbols) and overweight (OW, empty symbols) subjects. Asterisks indicated significant differences according to the different statistic tests applied: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

expression level of TLR4 resulted slightly higher, but significant, in EXP-NW than CTRL-NW at the beginning of the training (**Figures 2C,D**).

The results of the ANOVA tests and the related effect size are reported in **Supplementary Table 5**.

Effect of Training on the Release of Inflammasome- and Metabolic Inflammation-Related Cytokines

In order to determine the effect of the aerobic training program on inflammasome activation and inflammatory status, the circulating concentrations of related cytokines (IL-1β and IL-18, as markers of inflammasome activation, and TNFα and IL-6, as markers of metabolic inflammation) were determined. IL-18 levels neither were affected by the intervention in the EXP group, nor were different between EXP and CTRL groups, or between NW and OW cohorts (**Figure 3**). A single exercise bout elicited a rise in blood concentrations of the other cytokines, although not significant at the beginning of the training, but highly significant for IL-1β and TNFα at the end of the training (T2-pre vs. T2-post) [IL-1β: 0.090 pg/mL (0.000–0.200) pg/mL vs. 0.180 pg/mL (0.110–0.360) pg/mL; TNFα: 2.450 pg/mL (0.410–5.560) pg/mL vs. 4.490 pg/mL (1.380–7.820) pg/mL], while for IL-6, this increase is not significant (**Figure 3A**). No changes were detected between the CTRL and the EXP groups at the two time-points except for IL-1β that decreased at the beginning of the training (T1-pre vs. T1, **Figure 3B**).

When considering the EXP group divided between NW and OW sub-cohorts (**Figure 3C**), a single bout of exercise induced a general increase of IL-1β in both sub-cohorts, however, significant at the beginning of the training (T1-pre vs. T1-post) for NW [0.130 pg/mL (0.100–0.170) pg/mL vs. 0.180 pg/mL (0.120–0.730) pg/mL] and at the end of the training (T2-pre vs. T2-post) for OW [0.080 pg/mL (0.000–0.200) pg/mL vs. 0.180 pg/mL (0.110–0.310) pg/mL]. On the other hand, as effect of the chronic intervention (T1-pre vs. T2-pre), the cytokine decreased, though not significantly, in both sub-cohorts [NW: 0.130 pg/mL (0.100–0.170) pg/mL vs. 0.110 pg/mL (0.020–0.130) pg/mL; OW: 0.130 pg/mL (0.100–0.210) pg/mL vs. (0.080 pg/mL (0.000–0.200) pg/mL]. For TNFα, only the OW subjects were affected by a modulation of the cytokine, characterized by a strong increase after the first bout compared to baseline (T1-pre vs. T1-post) [3.360 pg/mL (0.770–4.670) pg/mL vs. 5.340 pg/mL (0.660–8.330) pg/mL], a decrease after the 12-week training (T1-pre vs. T2-pre) [2.740 pg/mL (0.410–3.930) pg/mL], and an increase after the last bout of exercise (T2-pre vs. T2-post) [4.830 pg/mL (2.350–7.820) pg/mL]. Additionally, an overall increase of TNFα was observed between the beginning (T1-pre) and the end of the training (T2-post). Even though IL-6 followed the same pattern of the two other cytokines, no differences were significant. Curiously, no differences were observed between the NW and OW cohorts (**Figure 3C**).

Finally, no differences were observed between EXP and CTRL groups within the stratification in NW and OW (**Figure 3D**).

The results of the ANOVA tests and the related effect size are reported in **Supplementary Table 5**.

DISCUSSION

Our study aimed at analyzing the behavior of inflammasome activation in post-menopausal women performing 12-week moderate-intensity aerobic Nordic walking training. Obtained findings reveal that Nordic walking training program is associated with a reduced expression of inflammasome components at rest and, importantly, the acquisition of a post-exercise pro-inflammatory response at the end of the training period, as indicated by the modulation of NLRP3 and TLR4 mRNA, and IL-1β and TNFα levels. Interestingly, when participants are grouped based on BMI it emerges that NLRP3 response is more pronounced in NW subjects than in OW. The downstream markers of inflammasome activation status, IL-1β, and of the inflammatory status, TNFα, showed a similar course: their level increases after the first bout of exercise, decreases after the 12-week training, and newly increases in response to the last exercise session. Noteworthy, in case of IL-1β, this modulation is significant for NW at the beginning of the training, whereas for OW at the end of the training; in case of TNFα, this time-dependent modulation was significant only in OW. This suggests a beneficial effect of Nordic walking in both NW and OW due to the reduction of inflammasome marker IL-1β. Importantly, vitamin D status, which is known to potentially affect the innate immunity response (Arababadi et al., 2018), was comparable among the EXP and CTRL groups and the relative sub-cohorts.

The importance of studying inflammasome resides in the fact that its activation may be the cause of (or may contribute to) the onset and development of several diseases (Guo et al., 2015) and particularly, since the involvement of NLRP3 inflammasome in age-associated chronic LGI, linked to the onset of several age-related diseases (Mejias-Pena et al., 2017). Aging may represent a key determinant of the responsivity to aerobic exercise. In a recent study, an 8-week Nordic walking training reduced the TLR4 and NLRP3 mRNA expression and circulating levels of IL-1β and IL-18 in young males (Khakroo Abkenar et al., 2019) while, in the present study, the most relevant result, is represented by the (re)acquisition of a post-acute exercise inflammatory response. In order to investigate whether the activation status of the inflammasome machinery, in response to aerobic activity in elderly women, was reflected into the circulation, mRNA expression level of NLRP3 and TLR4 was analyzed in whole blood. The choice of whole blood, as the assay matrix, was driven by the fact that inflammasome pathways can be activated in virtually all blood cells, but red blood cells (Tran et al., 2019), other than in other tissues. Therefore, whole blood expression of inflammasome markers may picture the integrated innate response potential against danger signals which may drive, in turn, LGI (Gomarasca et al., 2020). Gene expression analysis is completed by a normalization study to determine the best suitable reference genes in RT-qPCR analysis, in order to obtain the most reliable results (Mahoney et al., 2004; Faraldi et al., 2018).

Exercise training-dependent alteration of immune function is associated with the activation of several local and systemic responses. For instance, exercise activates purinergic signaling (ATP, ADP, adenosine, related receptors, and enzymes) (Moritz

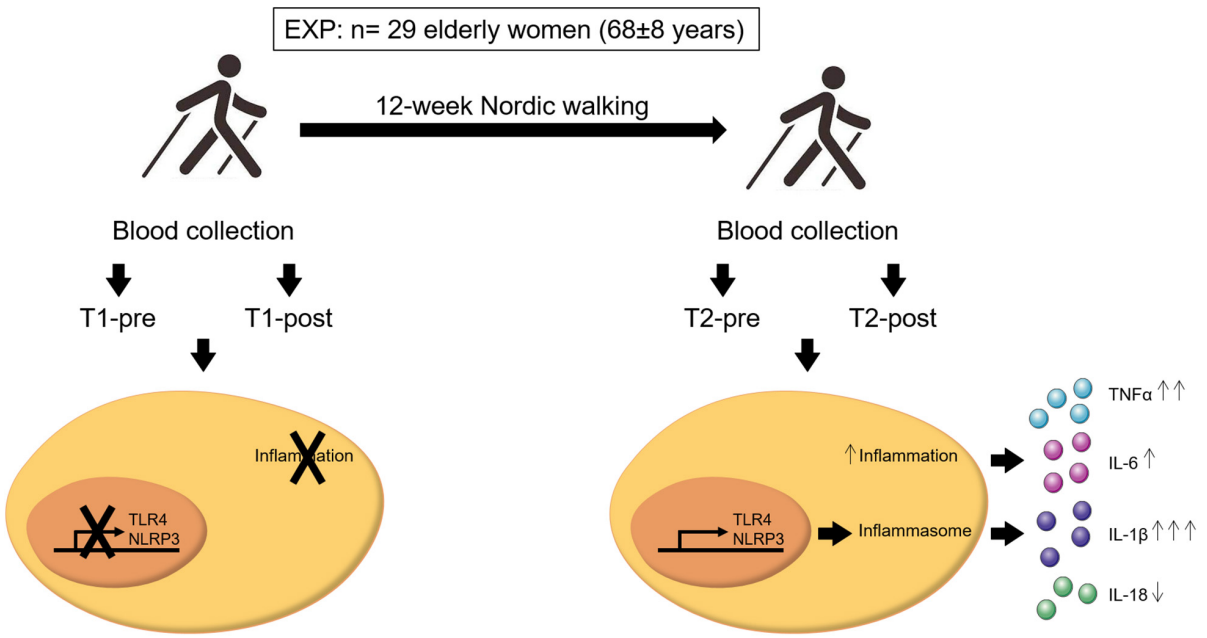

FIGURE 4 | Schematic representation of the important findings of this study. A population of 29 elderly women (age 68 ± 8 years old) completed 12 weeks of Nordic walking moderate-intensity aerobic training program (EXP). Blood samples were taken before and after the first (T1-pre and T1-post, respectively) and last (T2-pre and T2-post, respectively) exercise unit, and inflammasome- and inflammation-related markers were assessed. Aerobic training mildly affected the inflammasome-related inflammatory response. Participants acquired an acute inflammatory response to exercise, that was absent at baseline, that resulted in the activation of NLRP3 and TLR4 gene expression and the consequent release in the blood stream of the inflammasome- and inflammation-related cytokines IL-1β, IL-6, TNFα, and IL-18. In particular, a single bout of exercise at the end of the training induced a significant upregulation of IL-1β (↑↑↑) and TNFα (↑↑), a non-significant increase of IL-6 (↑), and a non-significant down-modulation of IL-18 (↓). The arrows next to the cytokines refer to the entity of the effect of the upregulation or downregulation of these cytokines.

et al., 2021). The purinergic system has relevance in inflammatory response and, particularly, in the shift from the pro-inflammatory response to acute, intense exercise to the anti-inflammatory response associated with chronic exercise (Cardoso et al., 2021). Exercise improves immune functions via the stimulation of the neuroendocrine secretion of catecholamines and the activation of their signaling (Simpson et al., 2021). Similarly, PA, together with diet, affects the composition of gut microbiota with profound effects on immune function, but also on muscle strength and dynamics (Strasser et al., 2021).

Many pieces of evidence have demonstrated that PA has inhibitory effects on inflammasome activation. This control may take place in different ways, indirectly by targeting pro-inflammatory compounds (fFA, ceramides) that are increased in LGI and aging (Ringseis et al., 2015) or, as it happens consequently to neuronal stimulation of myofibers, via the perturbation of plasma membrane integrity and potassium gradient across the membrane (Gaidt and Hornung, 2018), and directly by triggering TLRs expression and their downstream signaling. For instance, IL-18, the designated marker of NLRP3 inflammasome activation status was decreased by 43% in men and women with metabolic syndrome in response to a 12-week aerobic interval training program (three times a week) (Stensvold et al., 2012). Similarly, IL-18 was decreased in patients with metabolic syndrome undergone 12-week combined (endurance and strength) training program (three times a week) (Troseid et al., 2009), in T2DM subjects following a 6-month aerobic moderate-intensity exercise training program (four times/week) (Kadoglou et al., 2007a,b), and 8-week high-intensity exercise training on a rowing ergometer (three times/week), in obese subjects (Leick et al., 2007). However, similarly to our results, other authors failed in evidencing any putative improvement of the inflammatory status marked by IL-18. For instance, Christiansen et al. (2010) did not observe any decrease in circulating IL-18 concentrations in obese men and women following a 12-week aerobic exercise training program performed three times a week, possibly because of the relatively moderate intensity of the exercise. Also, RT has been shown to target innate immunity and inflammasome activation: in healthy elderly 8-week RT decreased the protein expression of TLR2 and TLR4 as well as the expression of several TLRs signaling-associated molecules (e.g., MyD88, TRIF, NF-κB, and MAPK) and plasma levels of the CRP (Rodriguez-Miguelez et al., 2014). On the contrary, Mejias-Pena et al. (2016) did not record any change in the expression of TLR2, TLR4, MyD88, and TRIF, in peripheral blood mononuclear cells (PBMCs) from older subjects after 8 weeks of aerobic exercise training, suggesting the possibility that the type of exercise might be a determinant of the TLRs-mediated anti-inflammatory effect of exercise. The lack of control for confounding variables, in the available studies, prevents a definitive elaboration on potential benefits of this interventional practice, according to a recently

published systematic review (Sanchez-Lastra et al., 2020). However, Nordic walking is a potentially beneficial exercise strategy for overweight and obese people. Based on the twelve good-to-fair quality selected studies, the authors of this study evidenced that subjects performing Nordic walking experienced significant improvement in parameters such as fasting plasma glucose, abdominal adiposity, and body fat compared with the values recorded at baseline, but no significant improvements were found when compared with control groups (Sanchez-Lastra et al., 2020). Notably, adiposity in the elderly may be associated with a deregulated glucose metabolism, and hence to an increased risk of T2DM, via a deregulation of immune cells and, specifically of CD8+ cytotoxic subsets (Bosslau et al., 2021).

In regards to the inflammasome activation status, our results revealed that the last bout of exercise determined an increase of both NLRP3 and TLR4 mRNA, indicating that, possibly, the ability to activate inflammasome is acquired during the training program, since such a response was absent after only one bout of exercise at the beginning of the training. This effect may be linked to the restoration of whole blood NLRP3/TLR4 mRNA expression at the end of the program, compared to the first sampling. As a consequence, these results demonstrated that although the circulating inflammatory profile may be fairly affected by the moderate-intensity aerobic training, the system alertness to endogenous and exogenous danger signals (TLR4 and NLRP3 mRNA) may be restored by the activity. Still, few limitations must be mentioned. The CTRL group, in our study, was not involved in any training program and, hence, the comparison was made between Nordic walking and sedentary lifestyle. Therefore, future investigations should aim at comparing the effect of different kinds of physical activities. This study lacks an objective and standardized method of assessment of the participants' effort, as well as of their baseline physical activity level [e.g., via the international physical activity questionnaire (IPAQ)] and thereby, it is not possible to define if the effects on inflammasome activation and inflammatory response may be related to the effort spent. Only females have been considered and for future research gender-dependent differences should also be considered. Other limitations are related to the eventual lack of comparison between subjects with different inflammatory statuses at the beginning of the training. This additional comparison would highlight the effect of Nordic Walking training on inflammation. Further, gene expression analysis of cytokines would have given additional information related to the inflammatory status of PBMCs while their circulating levels represent the net result of their systemic expression. Finally, body composition analysis would have a greater significance than anthropometrical measures in describing the cohorts and, eventually, their intervention-related changes. An important strength of this study is represented by the robust study of normalization, based on validated algorithms, applied to gene expression analysis: indeed, different normalization strategies may lead to different results and, for each experimental set, it is recommended to select the most appropriate method.

CONCLUSION

Despite a fair effect on the resting whole blood expression of inflammasome constituents (NLRP3 and TLR4) and circulating levels of the downstream inflammasome related cytokine IL-1β, a 12-week moderate-intensity aerobic training program (Nordic walking) allows the partial acquisition of the acute exercise-induced inflammatory response at the end of the training compared to the total absence of response observed at the beginning of the program (**Figure 4**). Specifically, a post-training acute exercise-induced response was recorded for NLRP3 and TLR4 expression, while IL-1β and TNFα changes were driven by the overweight participants.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://zenodo.org/, doi: 10.5281/zenodo.5789001.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethical Committee of the Regional Medical Society in Gdansk. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EZ and GL: conception. MG, KM, EZ, and GL: study design. KM and MFl: data acquisition. MG, KM, MFa, MFl, and SP: data analysis. MG, KM, MFa, EZ, and GL: data interpretation. MG, KM, MFa, and GL: manuscript drafting. MFl, SP, GB, and EZ: manuscript revision. MG, KM, MFa, MFl, SP, GB, EZ, and GL: final approval. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.792859/full#supplementary-material

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Adnotacja

Gdańsk, 05.02.2022

Zapoznałam się z niniejszą rozprawą doktorską przygotowaną pod moją opieką przez doktorantkę mgr Katarzynę Micielską. Praca została przeze mnie zaakceptowana.

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