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Efekty stosowania terapii zimnem w treningu zawodników judo i piłki siatkowej

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Spis treści

1. Rozprawa doktorska

Niniejszą rozprawę doktorską oparto o cykl trzech publikacji pod wspólnym tytułem: „Efekty stosowania terapii zimnem w treningu zawodników judo i piłki siatkowej”, na który składają się trzy publikacje o sumarycznej punktacji IF 9.210 i MNiSW 205:

A. ***The Specific Judo Training Program Combined with the Whole Body Cryostimulation Induced an Increase of Serum Concentrations of Growth Factors and Changes in Amino Acid Profile in Professional Judokas*** (2021), Joanna Jaworska, Radosław Laskowski, Ewa Ziemann, Klaudia Żuczek, Giovanni Lombardi, Jędrzej Antosiewicz, Piotr Żurek; Frontiers in Physiology, DOI: 10.3389/fphys.2021.627657; IF 3.160, MNiSW 100

B. ***A 2-Week Specific Volleyball Training Supported by the Whole Body Cryostimulation Protocol Induced an Increase of Growth Factors and Counteracted Deterioration of Physical Performance*** (2018). Jaworska Joanna, Katarzyna Micielska, Marta Kozłowska, Krzysztof Wnorowski, Jerzy Skrobecki, Łukasz Radzimiński, Anna Babińska, Ewa Rodziewicz-Flis, Giovanni Lombardi, Ewa Ziemann; Frontiers in Physiology, DOI: 10.3389/fphys.2018.01711; IF 3.201, MNiSW 35

C. ***Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and Increase Isokinetic Muscle Strength*** (2020). Jaworska Joanna, Ewa Rodziewicz-Flis, Jakub Kortas, Marta Kozłowska, Katarzyna Micielska, Anna Babińska, Giovanni Lombardi, Ewa Ziemann; International Journal of Environmental Research and Public Health, DOI: 10.3390/ijerph17155496; IF 2.849, MNiSW 70

2. Streszczenie

Ogólnoustrojowa krioterapia jest dostępną oraz chętnie stosowaną metodą regeneracji wśród zawodowych sportowców. Mimo powszechnego stosowania, nadal wiedza na temat zasadności stosowania tej formy fizjoterapeutycznej w połączeniu z różnymi programami treningowymi, w tym z ćwiczeniami oporowymi, jest nie wystarczająca.

Celem moich badań było określenie wpływu różnych rodzajów specyficznego programu treningu połączonego z kriostymulacją całego ciała na stężenie białek indukowanych wysiłkiem fizycznym (ang. *exerkines*), profil aminokwasowy oraz wydolność fizyczną u osób, reprezentujących różny poziom sprawności fizycznej.

W badaniach wzięli udział zawodnicy z kadry narodowej (judocy), akademickiej (zawodnicy piłki siatkowej) oraz osoby niewytrenowane, studenci kierunku Terapii Zajęciowej. W każdej grupie badanych przeprowadzono eksperyment, w którym to uczestników podzielono na grupę eksperymentalną i kontrolną. Osoby z grup eksperymentalnych poddani zostali 10-12 sesjom kriostymulacji całego ciała, przeprowadzonych w specjalnej kriokomorze (3 min, w temperaturze -110°C). Natomiast osoby z grupy kontrolnej odpoczywały w sposób bierny. Dwie grupy sportowców zostały poddane dwutygodniowemu programowi treningowemu typowemu dla danej dyscypliny sportowej, natomiast niewytrenowani studenci zrealizowali protokół treningu oporowego. W czasie trwania każdego programu treningowego, grupa eksperymentalna korzystała z zabiegów kriostymulacji. Przed i po każdej pierwszej i ostatniej interwencji, pobierano krew w celu oceny stężenia wskaźników uszkodzenia mięśni (mioglobiny), wyselekcjonowanych białek (interleukiny 6, interleukiny 15, iryzyny, czynnika neurotroficznego pochodzenia mózgowego [BDNF]), czynników wzrostu syntezy białek miofibryli (miostatyny, insulinopodobnego czynnika wzrostu 1 [IGF-1]) oraz profilu aminokwasowego. Dodatkowo u sportowców przeprowadzono ocenę siły mięśniowej oraz testy sprawności fizycznej. Ponadto, grupa studentów wykonała dwa wysiłki ekscentryczne (uszkadzające włókna mięśniowe) na cykloergometrze rowerowym (przed i po okresie treningowym). Każdy programy treningowy był przeprowadzony pod nadzorem wykwalifikowanego trenera lub fizjoterapeuty.

Po 2-tygodniowym specyficznym programie treningowym judo połączonym z kriostymulacją całego ciała odnotowano istotny wzrost stężenia BDNF (p=0.01) i IGF-1 (p=0.00). Dodatkowo, zastosowana interwencja spowodowała znaczący spadek spoczynkowego stężenia proliny (p=0.046, $\eta_p^2 = 0.34$) i leucyny (p=0.008, $\eta_p^2 = 0.53$) w grupie poddanej kriostymulacji. Poziom sprawności fizycznej w obu grupach pozostał

niezmieniony, jednak badani dostrzegali pozytywne zmiany wywołane interwencją - nie bezpośrednio po kriostymulacji, ale w odpowiedzi na specyficzne obciążenie treningowe. Dwa tygodnie specyficznego programu treningowego piłki siatkowej połączonego z kriostymulacją całego ciała wywołało podobny wzrost stężenia BDNF i IGF-1 we krwi zawodników. Skorygowany efekt opisujący różnicę między grupami w odpowiedzi na zastosowane procedury był dla obu czynników wzrostu duży i prawdopodobny w grupie poddanej kriostymulacji w porównaniu do grupy kontrolnej. Zabiegi zimna spowodowały także spadek stężenia waliny i tryptofanu. Po interwencji treningowej, sprawność fizyczna obniżyła się u wszystkich siatkarzy, jednak w grupie poddanej kriostymulacji zmiana ta była mniejsza. U osób niewytrenowanych, zastosowanie długotrwałej kriostymulacji spowodowało zmniejszenie kosztu fizjologicznego drugiego wysiłku ekscentrycznego i obniżyło stężenie mioglobiny. W tej samej grupie zaobserwowano również obniżony poziom miostatyny ($p<0.05$) po interwencji. Trening połączony z kriostymulacją spowodował istotny wzrost maksymalnej średniej mocy w teście siły wyprostu kolana w warunkach izokinetycznych.

Uzyskane wyniki wykazały, że zastosowane protokoły kriostymulacji całego ciała w połączniu z wybranymi protokołami treningowymi, nie osłabiły procesu adaptacji treningowej. Zastosowana krioterapia spowodowała wzrost stężenia czynników wzrostu, poprawę profilu aminokwasowego oraz miała pozytywy wpływ na możliwości wysiłkowe sportowców. Zakres zmian zaobserwowany w testach sprawnościowych zależał jednak od stażu treningowego. U osób początkujących, siła mięśniowa uległa zwiększeniu w odpowiedzi na zastosowaną procedurę, natomiast u sportowców, siła mięśniowa pozostała na tym samym poziomie.

3. Wstęp

Dostępna literatura badająca wpływ kriostymulacji na możliwości wysiłkowe wykazuje zmienność jej skuteczności. Równocześnie, obecne dane zwracają uwagę na brak ogólnego protokołu stosowania kriostymulacji, który byłby dostosowany dla każdego [1]. Dostępne metody regeneracji z użyciem zimna obejmują różnego rodzaju procedury, tj. okłady z lodem, zanurzenie w zimnej wodzie o różnej temperaturze [2], zimowe pływanie [3], kriostymulację częściową, jak i kriostymulację całego ciała (ang. *whole body cryostimulation,* WBC), wykonywaną w specjalnych krio-komorach [4]. Istnieje niezliczona ilość wariantów stosowania wyżej wymienionych metod terapii zimnem, z uwzględnieniem częstotliwości zabiegów, okresu czasowego jej stosowania jak i samej temperatury zabiegu. Należy także

podkreślić, że terapia zimnem w sporcie aplikowana jest często w sposób niekontrolowany, szczególnie w okresie przygotowawczym

Wyniki badań dotyczące wpływu połączenia programów treningowych z terapią zimnem wykazały, że stosowanie kriostymulacji jako metody regeneracji po wysiłku, może przynieść różne skutki. Dostępne dane wskazują, że ekspozycja na ekstremalnie niską temperaturę (-110°C - 130°C), która jest dobrze tolerowana przez sportowców [5], wywołuje odpowiedź przeciwzapalną [6], wspiera proces regeneracji [7], oraz zmniejsza poziom zmęczenia [8]. W związku z tym, można stwierdzić, iż kriostumulacja zapobiega rozwojowi stanu przeciążenia. Uzyskane dane pokazują także, że krioterapia może być stosowana na każdym etapie procesu treningowego, w tym w fazie przygotowawczej [9], w okresie regeneracji [7] oraz po sezonie startowym [10]. Ponadto, kriostymulacja całego ciała wzmacnia proces regeneracji uszkodzonych włókien mięśniowych spowodowany wysiłkiem fizycznym (ang. *exercise-induced muscle damage*, EIMD), szczególnie wśród osób niewytrenowanych [11].

Równocześnie dostępne wyniki badań pokazują, że długotrwała terapia zimnem może mieć negatywny wpływ na zmiany adaptacyjne procesu treningowego [12, 13]. Badania Roberts i współautorów wykazały, że zanurzenie w zimnej wodzie (10 min w temperaturze 10°C), stosowane po każdej pojedynczej jednostce treningu oporowego o wysokiej intensywności, znacząco osłabiło aktywność komórek satelitarnych i stłumiło sygnalizację anaboliczną w komórkach mięśniowych po treningu, co skutkowało zmniejszoną hipertrofią we włóknach mięśniowych typu IIA oraz obniżoną poprawą siły aktywnych fizycznie mężczyzn [12]. Yamane i współpracownicy również wykazali, że po-treningowe zanurzenie w zimnej wodzie (20 min w temperaturze 10°C) zmniejszało długoterminowy przyrost masy mięśniowej, jak również zahamowało wzrost maksymalnej siły izometrycznej mięśni zginaczy nadgarstka w odpowiedzi na trening oporowy u zdrowych, młodych, niewytrenowanych mężczyzn. Ponadto, wskazali, że zanurzenie w zimnej wodzie mogło przyczynić się do zatrzymania angiogenezy, co z kolei miało negatywny wpływ na poprawę lokalnej wytrzymałości mięśniowej [13].

Wiedza na temat stosowania zamiennie różnych procedur ogólnoustrojowej krioterapii jest niespójna. Costello i współautorzy porównali wpływ pojedynczego zanurzenia w zimnej wodzie (4 min. w temperaturze 8°C) z pojedynczą sesją WBC (3.40 min. w temperaturze -110°C) w celu oceny różnic między nimi. Uzyskane wyniki wykazały, że te dwie procedury zimna wywołały podobny spadek temperatury mięśni i temperatury wewnętrznej, wyjątek w odmiennej reakcji stanowiła jedynie temperatura skóry [14]. Zastosowanie kamery

termowizyjnej wykazało, że 3 minutowa sesja w krio-komorze prowadzi do znacznego obniżenia temperatury skóry i może być zależna od wskaźnika masy ciała (ang. *body mass index*, BMI) [15].

Warto zaznaczyć, że skuteczność kriostymulacji w połączeniu z treningiem zależy od różnych czynników: m.in. rodzaju ćwiczeń, ich intensywności, czasu trwania i częstotliwości, a także charakterystyki osoby badanej, np. płci, składu ciała, poziomu sprawności fizycznej, jak i warunków środowiskowych [1]. Z jednej strony, kriostymulacja całego ciała, stosowana po treningu, skutecznie poprawiła jakość snu podczas intensywnego okresu treningowego w grupie elitarnych koszykarzy [16], jak i u synchronicznych pływaczek [8]. Z drugiej jednak strony, zanurzenie w zimnej wodzie (20 min w temp. 4°C) zastosowane przed wysiłkiem, spowodowało znaczący spadek odpowiedzi elektromiograficznej we wszystkich zanurzonych uprzednio mięśniach kończyny dolnej, co przyczyniło się do obniżenia kontroli motorycznej i czasu reakcji mięśni podczas testu skoku [17].

Proces adaptacji mięśni do treningu zależy od ekspresji i uwalniania do krwiobiegu czynników wzrostowych, interleukin oraz białek. Wciąż jednak, dane dokumentujące wpływ WBC na stężenie czynników wzrostowych oraz profil aminokwasowy u osób o różnym poziomie sprawności fizycznej są znikome [18, 19]. W związku z tym, uzasadnione jest, aby sportowcy z ostrożnością stosowali zabiegi kriostymulacji oraz wybierali sprawdzone, skuteczne na danym etapie treningu protokoły stosowania.

Jednym z najważniejszych elementów procesu treningowego, który często decyduje o sukcesie sportowym, jak również wspomaga zdrowie wśród aktywnych, nieprofesjonalnie trenujących osób jest trening oporowy [20]. Praktykowany trening siłowy nie zawsze skupia się na zwiększaniu maksymalnej siły mięśniowej czy hipertrofii mięśniowej, może również koncentrować się na rozwoju eksplozywnej siły mięśniowej u sportowców, co jest kluczowe w wielu dyscyplinach sportu, takich jak judo [21] czy też piłka siatkowa [22]. Ponadto, trening oporowy jest obecnie powszechnie stosowaną formą aktywności fizycznej wśród osób rozpoczynających regularną aktywność fizyczną. Warto zwrócić uwagę, iż u osób nieprzyzwyczajonych do dużych obciążeń, często dochodzi do uszkodzenia włókien mięśniowych wywołanych wysiłkiem fizycznym [23], którego objawy mogą utrzymywać się nawet kilka dni i negatywnie wpływać na sprawność fizyczną [24], co w konsekwencji, zniechęca początkujących do kontynuowania treningu.

Biorąc pod uwagę powyższe doniesienia, istotne jest precyzyjne ustalenie efektów stosowania zabiegów ogólnoustrojowej krioterapii w połączeniu z różnymi protokołami

treningowymi oraz ich wpływu na adaptację mięśni do treningu oporowego na każdym etapie doświadczenia treningowego.

4. Pytania i hipotezy badawcze

W świetle powyższych doniesień, w swoich badaniach postawiłam następujące pytania badawcze:

1. Czy seria zabiegów kriostymulacji całego ciała będzie miała wpływ na proces adaptacji treningowej oraz jak zmieni stężenie markerów, takich jak: czynniki wzrostu i profilu aminokwasowego w grupach sportowców – zawodników grających na poziomie akademickim oraz osób niewytrenowanych?
2. Czy 10 sesji kriostymulacji całego ciała zwiększy możliwości wysiłkowe wśród profesjonalnych judoków?
3. Czy 10 zabiegów kriostymulacji całego ciała będzie miało wpływ na zdolności motoryczne i opóźnienie pojawienia się zmęczenia ośrodkowego u siatkarzy, grających na poziomie uniwersyteckim?
4. Czy terapia zimnem, zastosowana dzień po treningu oporowym o wysokiej intensywności, będzie miała korzystny wpływ na poziom siły mięśniowej i adaptację do wysiłku fizycznego uszkadzającego włókna mięśniowe?

Biorąc pod uwagę powyższe pytania, postawiłam trzy hipotezy badawcze:

1. Seria zabiegów kriostymulacji całego ciała zmienia spoczynkowe stężenie białek indukowanych wysiłkiem (ang. *exerkines*, wg Safdar i współautorów [25]), co z kolei będzie miało wpływ na siłę mięśni i możliwości wysiłkowe badanych.
2. Ekspozycja na ekstremalnie niską temperaturę poprawi profil aminokwasowy (ze względu na potencjalnie lepszy obwodowy wychwyt aminokwasów przez tkanki).
3. Kriostymulacja całego ciała poprawi proces adaptacji mięśni do uszkodzeń wywołanych wysiłkiem fizycznym; efekt ten będzie zależał od rodzaju treningu połączonego z WBC oraz poziomu wydolności fizycznej badanych.

5. Materiały i metody

5.1.Badani

W eksperymentach wzięły udział trzy grupy uczestników reprezentujących różny poziom sprawności fizycznej:

1. Grupę profesjonalnych sportowców reprezentowało 12 wysoko wytrenowanych zawodników judo. Sportowcy (wiek 21 ± 3 lata, masa ciała 84 ± 22 kg, masa mięśni szkieletowych 34 ± 7 kg, masa tkanki tłuszczowej 22 ± 14 kg) zostali losowo przydzieleni do dwóch grup: eksperymentalnej (CRY; n= 6) i kontrolnej (CON; n= 6). Obie grupy uczestniczyły w 2-tygodniowym, jednakowym programie treningowym. Wszyscy judocy z grupy CRY uczestniczyli w 10 sesjach WBC (codziennie, w godzinach 12-13 od poniedziałku do piątku) pomiędzy dwoma sesjami treningowymi.
2. Jako grupa sportowców na akademickim poziomie wytrenowania, w badaniu wzięła udział uniwersytecka drużyna siatkarska. Zawodnicy (wiek: 22 ± 1 lata, masa ciała 72 ± 12 kg, masa mięśni szkieletowych 34 ± 7 kg, procentowa zawartość tkanki tłuszczowej 17 ± 8 %) zostali losowo przydzieleni do dwóch grup: eksperymentalnej (CRY, n=8) i kontrolnej (CON, n=12). Wszyscy siatkarze uczestniczyli w 2-tygodniowym programie treningowym, w którym sesje treningowe odbywały się w godzinach popołudniowych. Ponadto, zawodnicy z grupy eksperymentalnej wykonali 10 sesji kriostymulacji całego ciała w komorze kriogenicznej (codziennie, o godzinie 9 rano, od poniedziałku do piątku).
3. W trzecim badaniu, w którym zastosowano trening oporowy o wysokiej intensywności, wzięła udział grupa 25 niewytrenowanych, zdrowych studentów. Uczestnicy (wiek 20 ± 1, masa ciała 74 ± 2 kg, masa mięśni szkieletowych 33 ± 8 kg, masa tkanki tłuszczowej 15 ± 7 kg) zostali losowo przydzieleni do jednej z dwóch grup: eksperymentalnej poddanej kriostymulacji całego ciała (CRY; n=15) oraz kontrolnej (CON; n=10). Wszyscy badani uczestniczyli w treningach siłowych, które odbywały się 3 razy w tygodniu (w poniedziałek, środę i piątek) o godz. 16.00, dodatkowo badani z grupy CRY wykonywali 12 sesji WBC, dzień po każdej sesji treningowej w godz. 8-10.00.

5.2. Metody

W celu weryfikacji wyżej wymienionych hipotez i określenia efektu połączenia ogólnoustrojowej kriostymulacji całego ciała z różnego rodzaju programem treningowym, zastosowałam następujące metody:

- Pomiary antropometryczne zostały przeprowadzone z wykorzystaniem analizatora składu ciała z wielokrotną impedancyjną (In Body 720, Biospace, Korea).
- Stężenia białek indukowanych wysiłkiem, markerów uszkodzenia mięśni w surowicy krwi oceniano przy użyciu zestawów immunologicznych ELISA.
- Oznaczenia stężenia wybranych aminokwasów wykonano za pomocą wysokosprawnej chromatografii cieczowej z odwróconą fazą jonową połączonej z tandemową spektrometrią mas IP-RP HPLC-MS/MS.
- Poziom mleczanów (LA) oznaczano przy użyciu standardowego zestawu firmy Randox (Crumlin, Wielka Brytania) opartego na metodzie oksydazy LA (LC2389). Oznaczenia wykonywano na spektrofotometrze Cecil CE9200 (Cambridge, Wielka Brytania).
- Sprawność motoryczną zawodowych judoków oceniano za pomocą testu The Special Judo Efficiency Test (SJET) [26].
- Sprawność fizyczną siatkarek i siatkarzy oceniono za pomocą pomiaru siły eksplozywnej (skoki dosiężne w dwóch pozycjach) oraz testu dokładności zagrywki [27].
- Do oceny poziomu zdolności koncentracji zawodników piłki siatkowej zastosowano komputerowy, dwuminutowy test „*grid exercise*" [28].
- Izometryczne i izokinetyczne funkcje mięśni mierzono przy użyciu dynamometru Biodex System 4 (Biodex Medical System, Inc. Shirley, NY, USA).
- W celu oceny adaptacji mięśni do treningu oporowego oraz tolerancji na uszkodzenie włókien mięśniowych wywołanym wysiłkiem fizycznym, wykonano pojedynczą sesję wysiłku ekscentrycznego o wysokiej intensywności na ergometrze rowerowym (Cyclus2 Eccentric Trainer).
- W każdym eksperymencie sesje ogólnoustrojowej kriostymulacji całego ciała przeprowadzane były w specjalnej krio-komorze pod nadzorem medycznym w Pomorskim Centrum Reumatologicznym w Sopocie. Procedura rozpoczynała się od 30 sekundowej adaptacji ciała do niskiej temperatury w komorze przedsionka, gdzie

temperatura wynosiła -60°C, po czym badani przechodzili do głównej krio-komory, w której temperatura osiągała -110°C i pozostawał w niej przez 3 min. Podczas każdej sesji uczestnicy byli minimalnie ubrani tj. mieli na sobie szorty/strój kąpielowy, skarpetki, rękawiczki i czapkę zakrywającą małżowiny uszne.

6. Wyniki

6.1. Wyniki eksperymentu opublikowane w pracy "*The Specific Judo Training Program Combined with the Whole Body Cryostimulation Induced an Increase of Serum Concentrations of Growth Factors and Changes in Amino Acid Profile in Professional Judokas"*

Specyficzny program treningowy judo połączony z 10 sesjami kriostymulacji całego ciała spowodował znaczący wzrost poziomu neurotroficznego czynnika pochodzenia mózgowego (ang. *brain-derived neurotrophic factor*, BDNF) (z poziomu 10.23 ± 1.61ng·ml^{-1} do 15.13 ± 2.93 ng·ml^{-1}; p= 0,01) i insulinopodobnego czynnika wzrostu (ang. *insulin-like growth factor 1*, IGF-1) (z poziomu 174.29 ± 49.34 pg·ml^{-1} do 300.50 ± 43.80 pg·ml^{-1}; p= 0,00) oraz przyczynił się do spoczynkowego spadku stężenia aminokwasów w surowicy: proliny (z poziomu 157.98 ± 51.39 μmol·l^{-1} do 90.99 ± 41.26 μmol·l^{-1}; p=0.046, η_p^2=0.34) i leucyny (z poziomu 138.51 ± 37.25 μmol·l^{-1} do 93.43 ± 24.47 μmol·l^{-1}; p=0.008, η_p^2=0.53).. W grupie kontrolnej nie stwierdzono zmian w stężeniu BDNF, jak również w IGF-1 (wartości wyjściowe BDNF przed 8.74 ± 2.08 ng·ml^{-1} po 9.04 ± 2.01 ng·ml^{-1}; IGF-1 przed 175.19 ± 44.67 pg·ml^{-1} po 159.70 ± 22.33 pg·ml^{-1}). Nie stwierdzono również istotnych zmian przed i po interwencji w wyjściowych stężeniach aminokwasów w surowicy w grupie kontrolnej. Równocześnie odnotowano nieznacznie podwyższenie stężenia interleukiny 15 (ang. *interleukin 15*, IL-15) w obu grupach. Niezależnie od zastosowanej metody regeneracji (ekspozycja na zimno czy wypoczynek pasywny), spoczynkowe stężenie iryzyny nie uległo zmianie. Istotne zmiany w stężeniu IGF-1, IL-15 i iryzyny odnotowano jedynie godzinę po drugim teście SJET tylko w grupie poddanej kriostymulacji (różnice istotne odpowiednio: p=0.01, p=0.03, p=0.00). Zastosowana procedura WBC w trakcie programu treningowego nie spowodowała żadnych zmian w stężeniach białek we krwi pobranej godzinę po ostatniej sesji kriostymulacji.

Po dwóch tygodniach interwencji zaobserwowano różnice w stężeniu mleczanów we krwi pomiędzy grupami. Bezpośrednio po drugim teście SJET stężenie LA wynosiło 7.06 ± 1.23 mmol·l^{-1} w grupie poddanej kriostymulacji i 10.48 ± 1.86 mmol·l^{-1} w grupie kontrolnej. Jednocześnie ΔLA (różnica między stężeniem LA zarejestrowanym bezpośrednio po i godzinę po drugim teście SJET) wynosiła 3.74 ± 1.32 mmol·l^{-1} w grupie traktowanej zimnem i 5.72 ± 1.49 mmol·l^{-1} w grupie kontrolnej.

Średnia liczba rzutów w pierwszym teście SJET wyniosła w grupie CRY 53 ± 12, a w grupie CON 61 ± 15, przy średniej częstości skurczów serca (HR) odpowiednio:183 ± 5 bpm i 185 ± 6 bpm. Po dwutygodniowym programie treningowym średnia liczba rzutów nieznacznie wzrosła w drugim teście SJET w grupie CRY 56 ± 11, natomiast w grupie pozostała prawie niezmienna i wynosiła CON 63 ± 15, przy średnim HR 181 ± 1 bpm w grupie CRY i 179 ± 4 bpm w grupie CON.

6.2. Wyniki eksperymentu opublikowane w pracy "*A 2-Week Specific Volleyball Training Supported by the Whole Body Cryostimulation Protocol Induced an Increase of Growth Factors and Counteracted Deterioration of Physical Performance"*

Dziewięć sesji ogólnoustrojowej kriostymulacji doprowadziło do istotnego wzrostu stężenia BDNF (57 ± 112%) w grupie CRY, podczas gdy w grupie CON odnotowano odwrotną tendencję (-32 ± 74%). Skorygowany efekt był umiarkowany i prawdopodobny. Co ciekawe pierwsza pojedyncza sesja WBC spowodowała istotny spadek stężenia BDNF (-4 ± 169 % w grupie CRY, efekt skorygowany: umiarkowany i bardzo prawdopodobny). Zabiegi WBC nie wpłynęły na zmiany stężenia iryzyny. Nie odnotowano również zależności pomiędzy poziomem iryzyny, a BDNF. Zastosowany trening i kriostymulacja spowodowały wzrost stężenia IGF-1 o 14% w grupie CRY, natomiast w grupie CON odnotowano 22% spadek. Skorygowany efekt dla tych zmian był duży i bardzo prawdopodobny. Program treningowy wywołał spadek stężenia IL-15 w grupie kontrolnej, jednak połączenie treningu i WBC spowodowało wzrost stężenia tej miokiny, zwłaszcza u zawodników płci męskiej (efekt skorygowany był umiarkowany i bardzo prawdopodobny). Specyficzny trening siatkarski w połączeniu z WBC spowodował istotny spadek stężenia waliny i tryptofanu we krwi. Skorygowany efekt dla tych zmian był umiarkowanie mały (walina) i prawdopodobny (tryptofan). Poziom stężenia pozostałych aminokwasów nie uległ zmianie.

Zmianom stężenia miokin i aminokwasów towarzyszyły zmiany w sprawności fizycznej i psychicznej sportowców. W obu grupach program treningowy spowodował spadek mocy osiąganej w testach sprawnościowych, jednak spadek ten był bardziej wyraźny w grupie kontrolnej. Skorygowany efekt był duży i najbardziej prawdopodobny u zawodniczek oraz mały i prawdopodobny u zawodników. Zawodniczki z grupy CRY uzyskały nieco lepsze wyniki w teście koncentracji w porównaniu z zawodniczkami z grupy CON (obserwowane zmiany wynosiły odpowiednio 46 ± 51% i 5 ± 28%).

6.3. Wyniki eksperymentu opublikowane w pracy *"Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and Increase Isokinetic Muscle Strength"*

Czterotygodniowy program treningu siłowego połączony z serią 12 zabiegów kriostymulacji spowodował istotny spadek stężenia miostatyny w krwi w spoczynku (p= 0.03, η_p^2=0.20). Podobną tendencję odnotowano w przypadku iryzyny w grupie CRY, jednak efekt ten nie był istotny. W obu grupach odnotowano spadek IGF-1, przy czym był wyraźniejszy w grupie CON (p=0.57, η_p^2=0.01). Poziomy IL-6 i BDNF nie uległy zmianie (odpowiednio: p=0.10, η_p^2=0.11; p=0.61, η_p^2=0.01). Jednocześnie uzyskanym zmianom w stężeniach białek towarzyszył przyrost siły mięśniowej w grupie eksperymentalnej. Trening połączony z kriostymulacją spowodował istotny wzrost maksymalnej średniej mocy w teście siły wyprostu kolana w warunkach izokinetycznych (standaryzowane średnie różnice [SMD]: 0.42 - lewa noga, 0.32 - prawa noga), podczas gdy w grupie kontrolnej poziom siły pozostał niezmieniony (SMD: 0.11 - lewa noga, 0.03 - prawa noga). Podobne zmiany zaobserwowano w szczytowych wartościach maksymalnego momentu siły w teście siły izokinetycznej prostowników stawu kolanowego.

Odnotowany wzrost siły mięśniowej w grupie poddanej kriostymulacji, mógł przyczynić się do zaobserwowanego zmniejszenia kosztu fizjologicznego drugiej sesji EIMD. Średnie HR zarejestrowane na początku drugiego EIMD było niższe (135 ± 36 bpm) w porównaniu do pierwszego wysiłku ekscentrycznego (148 ± 37 bpm), zmiany te były jednak zależne od grupy. Różnice między grupami w fizjologicznym koszcie wysiłku były szczególnie widoczne w ósmej minucie drugiego EIMD, kiedy to grupa poddana kriostymulacji utrzymywała częstość akcji serca na poziomie 157 ± 12 uderzeń na minutę, a grupa kontrolna

na poziomie 171 ± 9 uderzeń na minutę. Równocześnie, po miesiącu treningu w grupie kontrolnej odnotowano istotny wzrost mioglobiny (zmiany te były istotne w stosunku do wartości wyjściowych, odpowiednio 2 godz. po EIMD p=0.02 i 24 godz. po EIMD, p=0.01). Grupa eksperymentalna charakteryzowała się niskim poziomem stężenia mioglobiny, który pozostał niezmieniony. Interesujący jest również fakt, że wykonanie po raz kolejny wysiłku ekscentrycznego zróżnicowało odpowiedź w stężeniu miokin i czynników wzrostu w zależności od zastosowanej strategii regeneracji. Wysiłek ekscentryczny spowodował istotny wzrost stężenia miostatyny w grupie CON, podczas gdy w grupie CRY poziom miostatyny pozostał niezmieniony przez cały okres obserwacji powysiłkowej (różnica między grupami była istotna, p=0.27, η_p^2=0.05). W grupie CRY stężenie IL-15 wzrosło 2 godziny po ostatnim EIMD, natomiast w grupie CON stężenie tej miokiny uległo obniżeniu i utrzymywało się na niskim poziomie do 24 godzin po zakończonym wysiłku ekscentrycznym (p=0.21, η_p^2=0.07).

7. Dyskusja

W przedstawionym zestawieniu badań, zastosowanie kriostymulacji całego ciała miało pozytywny wpływ na możliwości wysiłkowe oraz zmiany stężeń czynników wzrostu i profilu aminokwasowego u badanych, którzy reprezentowali różny poziom sprawności fizycznej.

Ogólnoustrojowa kriostymulacja spowodowała wzrost stężenia BDNF i IGF-1 w surowicy krwi sportowców wyczynowych i zawodników trenujących amatorsko (liga akademicka). Zaobserwowane zmiany w stężeniach czynników wzrostowych mogły przyczynić się do uzyskania lepszych wyników w testach sprawnościowych (u zawodników piłki siatkowej), jak również utrzymania poziomu siły mięśniowej i niewielkiej poprawy wyników w teście rzutów (w grupie judoków). Długotrwała kriostymulacja podczas treningu oporowego, wśród niewytrenowanych uczestników, miała pozytywny wpływ na proces adaptacji mięśniowej poprzez obniżenie stężenia miostatyny, która znana jest jako negatywny regulator hipertrofii mięśniowej [29]. Równocześnie, obniżonemu stężeniu miostatyny w grupie poddanej kriostymulacji towarzyszył spadek IGF-1 [19].

Miostatyna i IGF-1 regulują wielkość mięśni szkieletowych i ekspresję typów miofibryli poprzez różne mechanizmy, przy czym miostatyna hamuje, a IGF1 stymuluje wzrost syntezy białek miofibryli [30]. Hennebry i współautorzy udowodnili, że miostatyna reguluje

hiperplazję, a z kolei IGF-1 doprowadza do hipertrofii miofibryli, przy czym te dwa procesy mogą zachodzić czasowo niezależnie od siebie [30]. Dlatego też w badaniu "*Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and Increase Isokinetic Muscle Strength*" terapia zimnem, przeprowadzona dzień po treningu oporowym, mogła poprawić wyniki siłowe i wzmocnić adaptację do EIMD, niezależnie od poziomu IGF-1. Ponadto, w tym przypadku zastosowany protokół zimna nie wpłynął na zmianę poziomu BDNF w krwi.

Kolejnym czynnikiem, który reguluje stężenie BDNF, jest miokina iryzyna. Dostępne dane wskazują, iż miokina ta ma może mieć wpływ na adaptację wywołaną wysiłkiem fizycznym [31] poprzez regulację wychwytu glukozy i poprawę metabolizmu lipidów w mięśniach [32]. Badanie na modelu zwierzęcym wykazało możliwe połączenie pomiędzy mózgiem, a mięśniami poprzez ekspresję iryzyny i jej wpływ na stymulację BDNF [33]. Z kolei badanie przeprowadzone w warunkach laboratoryjnych na ludziach wskazało, że stężenie iryzyny może być modyfikowane w odpowiedzi na ekspozycję na niską temperaturę [34]. We wspomnianym eksperymencie zastosowanie zimna (za pomocą termo-kocy; gdzie temperatura z 27°C obniżona została do 18°C i dalej obniżała się o 2°C co 3 min, aż do osiągnięcia temperatury 12°C) spowodowało stymulację wydzielania iryzyny. Dulian i współpracownicy wykazali również, że po serii 10 sesji WBC (raz dziennie o 9:30, przez 3 min, w temperaturze -110°C) zwiększył się poziom iryzyny u osób otyłych [35]. Opierając się na powyższych wynikach, założyłam, że niska ilość tkanki tłuszczowej wśród uczestników moich badań, wywoła większy efekt termogeniczny, a drżenie mięśni doprowadzi do wzrostu poziomu iryzyny, co w konsekwencji zwiększy poziom BDNF. Niemniej jednak, w moich badaniach nie odnotowałam żadnych statystycznych zmian stężenia tego białka w odpowiedzi na zastosowane procedury WBC, a także nie zauważyłam żadnej korelacji pomiędzy iryzyną, a BDNF.

Dodatkowym celem moich badań było sprawdzenie wpływu kriostymulacji całego ciała na profil aminokwasowy osób trenujących. Co ciekawe, kriostymulacja całego ciała spowodowała spadek stężenia wszystkich wybranych aminokwasów we krwi w warunkach spoczynku. Istotne statystycznie różnice odnotowano w poziomie leucyny i proliny u judoków oraz waliny i tryptofanu u siatkarzy.

Leucyna i walina, jako składowe aminokwasów rozgałęzionych (BCAA) mogą być potencjalnymi stymulatorami syntezy białek mięśniowych i sygnalizacji kompleksu 1 (mTORC1) w mięśniach szkieletowych człowieka [36]. Uzyskane przeze mnie wyniki sugerują, że ogólnoustrojowa kriostymulacja może zwiększać wychwyt aminokwasów przez tkanki, wspierając tym utrzymanie zdolności motorycznych u judoków i siatkarzy w krótkich

dwutygodniowych mikro cyklach treningowych. Kolejnym aminokwasem, który zmienił swoje stężenie w odpowiedzi na ekspozycję na zimno była prolina, główny składnik kolagenu. Ma to szczególne znaczenie w dyscyplinie takiej jak judo, w której walka o uchwyt jest stałym i kluczowym elementem walk, a urazy palców są najczęstszą chronicznie występującą kontuzją wśród zawodników. Zaobserwowałam obniżenie poziomu proliny w grupie judoków poddanych krio-stymulacji. Zmiana ta może sugerować, że terapia zimnem zwiększyła aktywność enzymu katalizującego reakcję prolinazy, co w konsekwencji wzmocniło strukturę kolagenu [37].

Co ciekawe, specjalistyczny program treningowy piłki siatkowej połączony z krio-sesjami doprowadził do obniżenia poziomu tryptofanu, szczególnie wśród trenujących kobiet. Następstwem tego mogły być ich lepsze wyniki w testach celności serwisu i koncentracji w porównaniu do trenujących mężczyzn.

W świetle uzyskanych wyników, kriostymulacja całego ciała nie tylko poprawiła możliwości wysiłkowe uczestników badań, ale również zwiększyła poziom czynników wzrostu we krwi. Ponadto, uzyskane zmiany stężenia aminokwasów we krwi sugerują, że kriostymulacja może, poprzez stymulację mikrokrążenia, wpływać na obwodowy tkankowy wychwyt aminokwasów. Zastosowane protokoły kriostymulacji całego ciała były prawidłowo dobrane do potrzeb badanych i nie osłabiały procesu adaptacji do treningu.

8. Wnioski

Podsumowując, uzyskane wyniki z trzech różnych eksperymentów przeprowadzonych na trzech różnych grupach uczestników pozwoliły mi na wyciągnięcie następujących wniosków:

1. Wykonanie 9/10 sesji kriostymulacji dzień po dniu, podczas specyficznego programu treningowego judo, spowodowało wzrost stężenia BDNF i IGF-1 w surowicy krwi.
2. Codzienna stymulacja niską temperaturą zmieniła stężenie wybranych aminokwasów w krwi obwodowej.
3. 10 zabiegów kriostymulacji całego ciała nie pogorszyło zdolności motorycznych profesjonalnych judoków.
4. Siatkarze, którzy poddani zostali 10 sesjom WBC uzyskali lepsze wyniki w testach sprawnościowych w porównaniu z zawodnikami z grupy kontrolnej.

5. Seria 12 zabiegów WBC, wykonywanych dzień po treningu oporowym, spowodowała istotny wzrost poziomu średniej mocy mięśni prostujących w stawie kolanowym w warunkach izokinetycznych.
6. Trening oporowy połączony z kriostymulacją spowodował obniżenie stężenia miostatyny u młodych, niewytrenowanych osób.
7. Trening oporowy połączony z kriostymulacją całego ciała spowodował lepszą adaptację do wysiłku uszkadzającego włókna mięśniowe, poprzez obniżenie wartości częstości skurczów serca podczas wykonywania EIMD oraz obniżenie, powysiłkowego stężeniem mioglobiny we krwi.
8. Zastosowane zabiegi zimna nie wpłynęły istotnie na zmianę stężenia iryzyny u wszystkich badanych.

Akademia Wychowania Fizycznego i Sportu im. Jędrzeja Śniadeckiego w Gdańsku

M.Sc. Joanna Jaworska

" Effects of using cold therapy in judo and volleyball training"

Ph.D. thesis under the supervision of:

Prof. dr hab. Ewa Ziemann

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1. Dissertation summary

This doctoral dissertation is based on the series of three publications under a common title: "Effects of using cold therapy in judo and volleyball training", consisting of IF 9.210 and MNiSW 205 points:

A. ***The Specific Judo Training Program Combined with the Whole Body Cryostimulation Induced an Increase of Serum Concentrations of Growth Factors and Changes in Amino Acid Profile in Professional Judokas*** (2021), Joanna Jaworska, Radosław Laskowski, Ewa Ziemann, Klaudia Żuczek, Giovanni Lombardi, Jędrzej Antosiewicz, Piotr Żurek; Frontiers in Physiology, DOI: 10.3389/fphys.2021.627657; IF 3.160, MNiSW 100

B. ***A 2-Week Specific Volleyball Training Supported by the Whole Body Cryostimulation Protocol Induced an Increase of Growth Factors and Counteracted Deterioration of Physical Performance*** (2018) Jaworska Joanna, Katarzyna Micielska, Marta Kozłowska, Krzysztof Wnorowski, Jerzy Skrobecki, Łukasz Radzimiński, Anna Babińska, Ewa Rodziewicz-Flis, Giovanni Lombardi, Ewa Ziemann; Frontiers in Physiology, DOI: 10.3389/fphys.2018.01711; IF 3.201, MNiSW 35

C. ***Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and Increase Isokinetic Muscle Strength*** (2020). Jaworska Joanna, Ewa Rodziewicz-Flis, Jakub Kortas, Marta Kozłowska, Katarzyna Micielska, Anna Babińska, Giovanni Lombardi, Ewa Ziemann; International Journal of Environmental Research and Public Health, DOI: 10.3390/ijerph17155496; IF 2.849, MNiSW 70

2. Abstract

Whole body cryostimulation has recently become more available and widely used recovery method among professional athletes. The validity of cryostimulation application combined with different types of training, including resistance exercise, remains largely inconclusive.
The aim of my study was to determine the effect of different types of specific sports training programs in combination with the whole body cryostimulation on proteins induced by exercise (exerkines) and amino acid profile as well as physical performance among subjects who represented different fitness level.

In my studies, participants were recruited from national (judo), academic (volleyball) team and amateur-beginners student occupation therapy. All groups performed the experiment, where each one was divided into experimental and control one. Participants from experimental groups executed 10-12 sessions of whole body cryostimulation, performed in special cryo-chamber (3-min, at -110°C), whereas the subjects from the control groups rested passively.

Two groups of athletes had a two-week specific training programs and the untrained students conducted a resistance training protocol. At the same time, the experimental group underwent the cryosession. Prior and post this intervention, blood collection was done in order to assess serum concentrations of muscle damage marker (myoglobin), exerkines (interleukin 6, interleukin 15, irisin, brain-derived neurotrophic factor [BDNF]), growth factors (myostatin, insulin-like growth factor 1 [IGF-1]) and amino acid profile. Additionally, the athletes performed strength and physical capacity assessment. The student group performed two eccentric cycling bouts (both before and after the training). The training programs were applied under the supervision of a qualified physiotherapist or a coach.

After the two-week specific judo training program combined with the whole body cryostimulation a significant increase of BDNF p=0.01) and IGF-1; p=0.00) was noted. Additionally, the applied intervention resulted in a significant decrease of resting concentrations of proline (p=0.046, $\eta_p^2 = 0.34$) and leucine (p=0.008, $\eta_p^2 = 0.53$) in the cryo-stimulated group. Athletes' performance remained unchanged in both groups. However, subjects perceived positive changes induced by the intervention – not directly after cryostimulation, but, rather, in response to the specific training workload. Two weeks of specific volleyball training program including whole body cryostimulation sessions also caused an elevation of BDNF and IGF-1 among university players. The adjusted effect describing the difference between groups in response to applied procedures was for both growth factors large and very likely for cryo-stimulated group than the control group. The cold treatment caused a

drop of valine and tryptophan concentration. After the training intervention physical performance dropped in all volleyball players, although, in the cryo-stimulated group, the magnitude of change was smaller. In untrained participants, the application of long-term cryostimulation reduced the physiological cost of the second eccentric cycling bout and diminished myoglobin concentration. The same group also exhibited significant decreased levels of myostatin ($p<0.05$). Resistance training combined with cryostimulation induced a positive and likely significant improvement of isokinetic muscle strength among students.

The obtained results demonstrated that the whole body cryostimulation applied together with training protocol did not attenuate the adaptation of the training process. The applied cryotherapy caused an increase of growth factors, improved amino acid profile as well as had a positive impact on athletes' physical performance. The range of changes in physical performance depended on training experience. The muscle strength among beginners was enhanced in response to applied procedure, whereas among athletes muscle strength remained at the same level.

3. Introduction

The available literature demonstrates a variability of the effectiveness of the cold therapy for physical performance benefits. At the same time some reports are focused on a lack of general cold protocol, which could be adapted for everyone [1]. The cold intervention involves diverse procedures such as ice bags, cold water immersion [2], winter swimming [3] partial- and whole body cryostimulation (WBC), performed in special cryo-chambers [4]. Cold treatments can be applied with different amount of sessions, and frequency with diverse temperature. Additionally, it should be highlighted that cold therapy in sports is often used indiscriminately and uncontrollably.

The studies exploring the impact of training programs combined with the cold therapy have shown that the post exercise cold application, mainly used as recovery support, might induced various effects. Extremely cold exposure (-110°C – 130°C), well-tolerated by athletes [5], causes an anti-inflammatory response [6], supports the recovery process [7] and reduces the perceived level of fatigue [8]. Consequently, published evidence shows that cold therapy can counteract the onset of the overreaching state. Additionally, obtained data demonstrate that the cryotherapy can be applied on every stage of training process including the preparation phase [9], the regenerative period [7] and as well as after the competition season [10].

Moreover, the whole body cryostimulation enhanced the regeneration process after exercise-induced muscle damage (EIMD), especially among untrained subjects [11].

Available reports revealed that the long-term cold therapy might have a negative impact on adaptive changes to training process [12, 13]. Study by Roberts et al. showed that cold water immersion with 10 min duration at the temperature of 10°C applied after each high-intensity resistance training unit, substantially attenuated satellite cells' activity and suppressed the acute anabolic signalling in muscle cells after training which resulted in lower range of hypertrophy in muscle fibers IIA and improvement in strength among physically active men [12]. Also, Yamane and co-workers demonstrated that post-training cold water immersion (20 min at 10°C) diminished a long-term gain in muscle mass as well as inhibited an increase in maximal isometric strength of the wrist flexors in response to resistance training in healthy, young, untrained male subjects [13]. Moreover, they pointed that cold-water immersion may have inhibited vascular remodeling with subsequent negative effects on an improvement of local muscle endurance, after resistance training [13].

The knowledge how to use various cold procedures interchangeably is inconsistent. Costello and co-authors compared the impact of a single cold-water immersion (4 min at 8°C) and a single session of WBC (3.40 min at -110°C) in order to assess differences between them. Obtained results demonstrated that these two cold procedures induced a similar decrease of muscle and core temperature with one exception in the skin temperature [14]. The thermal vision camera imaging emphasized that the 3 min session in the cryo-chamber lead to a substantial reduction of skin temperature and can be reliant on body mass index [15].

It is worth to note that the effectiveness of the cryostimulation is depended on various factors: including type of exercise, its intensity, duration and frequency as well as subject's characteristic for example: gender, body composition, fitness level and environmental conditions [1]. On the one hand, whole body cryostimulation, applied after training session, can successfully improve sleep quality during a heavy-load training period in elite basketball players [16] as well as synchronized swimmers [8]. On the other hand, the cold-water immersion (20 min at 4°C), performed before exercise, caused a significant decrease in the electromyographic response in all immersed muscles that consequently decreased motor control and muscle reaction during unipodal vertical jump [17].

The process of muscle adaptation to training depends upon the expression and release of circulating anabolic factors, interleukins and proteins. Still, there limited data describing the influence of WBC on growth factors concentration and amino acid profile in subjects with

different level of physical fitness [18, 19]. Thus, the assumption that athletes should have apply this treatment very carefully and have to choose verified, is justified.

One of the most important part of the training process, which often determines success in sport as well supports the health among active, unprofessional trained individuals is resistance training [20]. The applied strength training does not always focus on increasing maximum muscle strength or muscle hypertrophy, it can also concentrate on a development of explosive muscle power in athletes, what is crucial in many discipline of sport including, judo [21] or volleyball [22]. Moreover, the resistance training is a widely used form of physical activity among exercise novices presently. At the same time unaccustomed to heavy loads individuals may experience a muscle damage [23], which symptoms can persist even several days and negatively affect physical performance [24]. It consequently, might discourage beginners to continue physical activity.

Considering the above, it is important to establish the effects of cold treatments application applied with different training protocols, its impact on muscle adaptation to resistance training on every stage of training experience.

4. Questions and hypotheses

In the light of the presented reasoning, I have asked the following questions in my research:

1. If the series of whole body cryostimulation sessions can affect the training adaptation process and its markers, such as: growth factors and amino acid profile to different type of training among professional athletes, academic players and untrained subjects?
2. Do the 10 sessions of whole body cryostimulation enhance the physical performance among high level judokas?
3. Do the 10 sessions whole body cryostimulation change the motor abilities and delay mental fatigue in college volleyball players?
4. Is the cold treatment applied one day after the high-intensity resistance training a beneficial application for muscle strength and adaptation to exercise-induced muscle damage?

Considering aforementioned questions, I have defined three hypotheses:

1. The series of whole body cryostimulation sessions alter the resting concentration of proteins induced by exercise (ang. *exerkines*, according to Safdar et al. [25]) what in turn will have an impact on the muscle strength and physical performance.
2. The extremely cold exposure changes the circulating amino acids profile owing to its increased possible peripheral uptake.
3. Whole body cryostimulation improves the muscle adaptation to exercise-induced muscle damage, still the effect is depended on type of training combined with the procedure and physical performance level of subjects.

5. Materials and methods

5.1 Subjects

Three groups of participants representing different fitness level took part in my experiments:

1. As a group of professional athletes, 12 highly trained judo fighters participated in my study. Athletes (age 21 ± 3 years, body weight 84 ± 22 kg, skeletal muscle mass 34 ± 7 kg, body fat 22 ± 14 kg) were randomly assigned into two groups: the experimental group (cryo-stimulated group, CRY; n= 6) and the control group (CON; n= 6) and attended in the 2-week training program. All participants from the CRY group participated in 10 sessions of WBC (daily, between 12 and 1 pm from Monday to Friday) between two training sessions.
2. As a group of athletes at the academic level of training, the university volleyball team took part in the investigation. Participants (age: 22 ± 1 years, body weight 72 ± 12 kg, skeletal muscle mass 34 ± 7 kg, percentage body fat 17 ± 8%) were randomly assigned in to two groups: the experimental group (CRY, n=8) and the control group (CON, n=12). All players attended in the 2-week training program, where training sessions were executed in the afternoon. Moreover, participants from the experimental group performed 10 sessions of whole body cryostimulation in a cryogenic chamber (daily, at 9 am, from Monday to Friday).

3. In the third investigation, where high-intensity resistance training was applied, a group of 25 untrained, healthy students took part. Participants (age 20.3 ± 1.1, body weight 74 ± 12 kg, skeletal muscle mass 33 ± 8 kg, body fat 15 ± 7 kg), were randomly assigned to one of the two groups: the experimental group treated with whole body cryostimulation (CRY; n=15) and the control group (CON; n=10). Training took place 3 times a week (on Monday, Wednesday and Friday) and started at 4 pm, additionally, subjects from the CRY group executed 12 sessions of WBC, day after each training session between 8 to 10 am.

5.1. Methods

In order to verify these hypotheses and determine the effect of different type of training program combined with whole body cryostimulation, I have used the following methods:

- Anthropometric measurements were established by impedance body composition analyzer (In Body 720, Biospace, Korea).
- Serum concentrations of exerkines, muscle damage markers were assessed using immunoassay ELISA kits.
- Quantification of chosen amino acids was based on using ion-pair reversed phase high performance liquid chromatography combined with tandem mass spectrometry IP-RP HPLC-MS/MS.
- The blood lactate level (LA) was determined using a standard Randox (Crumlin, United Kingdom) kit based on the LA oxidase method (LC2389). Assays were performed on the Cecil CE9200 spectrophotometer (Cambridge, United Kingdom).
- The motor skills of professional judokas performed The Special Judo Efficiency Test (SJET) [26].
- The physical performance among volleyball players was settled by explosive power measurement (countermovement jumps in two positions) and serve accuracy test [27].
- A computerized two-minute test - grid exercise was used to assess the level of concentration ability of volleyball players [28].
- Isometric and isokinetic muscle functions were measured using the Biodex System 4 dynamometer (Biodex Medical System, Inc. Shirley, NY, USA).

- In order to assess muscle adaptation to resistance training and the tolerance to exercise-induced muscle damage (EIMD), a single bout of high-intensity eccentric exercise on the cycle ergometer (Cyclus2 Eccentric Trainer) was performed.
- On each conducted experiment the whole body cryostimulation sessions were performed in special cryo-chamber always under medical supervisor at the Pomeranian Rheumatologic Center in Sopot, Poland. The procedure started from body adaptation in a vestibule chamber at -60°C for 30 s then the subject passed to the main cryo-chamber where the temperature reached above -110°C and stayed there for 3 min. During this cold session's participants were minimally dressed, wore shorts/swimsuit, socks, gloves and hat which covered their auricles.

6. Results

6.1. Results of the study "The Specific Judo Training Program Combined with the Whole Body Cryostimulation Induced an Increase of Serum Concentrations of Growth Factors and Changes in Amino Acid Profile in Professional Judokas".

The specific judo training program supported by 10 sessions of whole body cryostimulation induced a significant increase in the circulating, resting levels of brain-derived neurotrophic factor (BDNF) (from 10.23 ± 1.61ng·ml^{-1} to 15.13 ± 2.93 ng·ml^{-1}; p= 0.01) and insulin-like growth factor 1 (IGF-1) (from 174.29 ± 49.34 pg·ml^{-1} to 300.50 ± 43.80 pg·ml^{-1}; p= 0.00), together with a decrement of the circulating amino acids: proline (from 157.98 ± 51.39 µmol·l^{-1} to 90.99 ± 41.26 µmol·l^{-1}; p=0.046, $\eta_p^2 = 0.34$) and leucine (from 138.51 ± 37.25 µmol·l^{-1} to 93.43 ± 24.47 µmol·l^{-1}; p=0.008, $\eta_p^2 = 0.53$) in blood serum. In the control group any changes in BDNF as well as in IGF-1 were noted (baseline values maintained from 8.74 ± 2.08 ng·ml^{-1} to 9.04 ± 2.01 ng·ml^{-1}; from 175.19 ± 44.67 pg·ml^{-1} to 159.70 ± 22.33 pg·ml^{-1}, respectively). Additionally, there were no significant changes in baseline amino acid serum concentrations before and after intervention in the CON group. At the same time, only the slightly elevated tendency of interleukin 15 (IL-15) concentration in both groups was recorded. Independently of the applied recovery method, the resting irisin concentration remained unchanged. Significant alternations were recorded in IGF-1, IL-15, and irisin concentration after 1 h post second SJET only in the CRY group (p=0.01, p=0.03, p=0.00, respectively). The applied WBC

procedure and training program did not cause any changes in measured proteins in blood taken 1h after the last cryo-session. The differences in blood lactate concentration after two weeks of intervention between groups were observed. Immediately after the first SJET, LA concentrations reached 7.06 ± 1.23 mmol·l-1 in CRY group and 10.48 ± 1.86 mmol·l-1 in CON respectively. Also, ΔLA (difference between the LA concentration recorded immediately after the SJET and 1 h rest), were 3.74 ± 1.32 mmol·l-1 in the CRY and 5.72 ± 1.49 mmol·l-1 in CON group, respectively. The average number of throws in the first SJET reached in the CRY group 53 ± 12 and in the CON group 61 ± 15 with the average heart rate (HR) during the test 183 ± 5 bpm and 185 ± 6 bpm, respectively. After the two-week training program, the average number of throws increased slightly in the second SJET in the CRY group 56 ± 11, whereas in the CON group remained almost the same 63 ± 15 with the average HR 181 ± 1 bpm in the CRY and 179 ± 4 bpm in the CON group.

6.2. Results of the study "A 2-Week Specific Volleyball Training Supported by the Whole Body Cryostimulation Protocol Induced an Increase of Growth Factors and Counteracted Deterioration of Physical Performance"

After nine sessions of cryostimulation the BDNF concentration significant increased (57 ± 112 %) in CRY group, while in the CON group the opposite tendency was noted (-32 ± 74 %). The adjusted effect was moderate and likely. Interestingly, the first single session of WBC caused a significant drop of BDNF levels (CRY group: -4 ± 169 %, adjusted effect: moderate and very likely). Still, cold procedure did not alter significantly the concentration of irisin. Additionally, no relationship was reported between the level of irisin and BDNF. The applied training and cold treatment produced induced a 14% rise of IGF-1 concentration in CRY group, whereas in the CON group, a 22% drop was recorded. The adjusted effect for those changes was large and very likely. The training program provoked a drop of IL-15 concentration in the control group, but the combination of training and WBC caused an increase of this myokine, especially in the male athletes (the adjusted effect was moderate and very likely). The specific volleyball training with the WBC application resulted in a significant drop of valine and tryptophan concentrations. The adjusted effect for these changes was moderate small and likely, respectively. The remaining amino acids assessed were not changed. The myokines and amino acid shifts were accompanying by changes in physical and mental athletes' performance. In both groups, the training program resulted in a drop of power achieved in

countermovement jumps, but this drop was much more pronounced in the control group. The adjusted effect was huge and most likely in female athletes and small and likely in male players. Also, female players in the CRY group achieved slightly better results in the concentration test compare to females in CON group (observed change were 46 ± 51 % and 5 ± 28 %, respectively).

6.3. Results of the study "Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and Increase Isokinetic Muscle Strength"

The applied training combined with 12 session of whole body cryostimulation caused a significant drop of myostatin concentration at rest (p= 0.03, $\eta_p^2 = 0.20$). A similar tendency was registered in irisin in the CRY group, yet the effect was not significant. In both groups, IGF-1 dropped, still the magnitude of changes was more pronounced in the CON group (p=0.57, $\eta_p^2 = 0.01$). Levels of IL-6 and BDNF did not change across the observation (p=0.10, $\eta_p^2 = 0.11$; p=0.61, $\eta_p^2 = 0.01$, respectively). At the same time obtained protein changes were accompanied by an increment of muscle strength. Training combined with CRY induced significant increase of maximal average power in the knee isokinetic extension strength test (standardized mean differences [SMD]: 0.42 - left leg, 0.32 - right leg), while in control group the level of strength has remained stable (SMD: 0.11 - left leg, 0.03 - right leg). Similar, but not significant changes have been observed in maximal peak torque in the knee isokinetic extension strength test.

In this study, the increase of muscle strength would have also contributed to a diminished physiological cost of the second bout of EIMD in the cryostimulation group. The average HR recorded at the beginning of second EIMD was lower (135 ± 36 bpm) in compare to the first eccentric bout (148 ± 37 bpm); still, changes were depended on the group. The differences between the groups' physiological cost of exercise were especially visible in the eight minutes of the second EIMD, when the cryostimulation group maintained their heart rate at 157 ± 12 bpm, and the control group at 171 ± 9 bpm. At the same time, after a month of training in the control group, a significant increase of myoglobin was noted (baseline vs 2 h post: p=0.02; baseline vs 24 h post: p=0.01), whereas, experimental group was characterized by low myoglobin, which remained unchanged. Interestingly, the second bout of exercise-induced

muscle damage caused different responses in myokines and growth factors concentrations depending on the recovery strategy applied. This eccentric effort induced a significant increase of myostatin concentration in the CON group, while in the CRY group, level of myostatin remained unchanged over the entire post-exercise observation (the difference between groups was significant, p=0.27, $\eta_p^2 = 0.05$). In the CRY group, the concentration of IL-15 increased 2 h after the last EIMD, whereas in the CON group it was decreased and remained at this low level until 24 h after the eccentric bout (p=0.21, $\eta_p^2 = 0.07$).

7. Discussion

In the presented compilation of studies, the application of the whole body cryostimulation had a positive effect on the physical performance and changes in growth factors and amino acid profile concentration among subjects, who represented different fitness level.

The whole body cryostimulation caused an increase of BDNF and IGF-1 serum level among professionally athletes and college players. Those observed changes in growth factors concentration could resulted in better volleyball performance as well as maintain the strength level and slightly improved the specific judo test results. Also, long-term cryostimulation during a resistance training, among untrained participants, supported the muscle adaptation process by a decreased myostatin concentration, which is known as a negative regulator of muscle hypertrophy [29]. In this investigation, a decrease in myostatin levels in the cryostimulation group was accompanied by a decline in IGF-1 [19].

Myostatin and IGF-1 regulate skeletal muscle size and myofiber type expression through different mechanisms, with myostatin inhibiting, IGF1 stimulating growth [30]. Precisely, Hennebry and co-authors proofed that myostatin regulates hyperplasia and IGF1 regulates hypertrophy of myofibres, with the two processes being temporarily separated [30]. Thus, in study "*Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and Increase Isokinetic Muscle Strength*" the cold treatment, performed day after resistance training unit might improve the strength outcomes and enhanced the adaptation to EIMD, regardless of IGF-1 secretion [19]. Also, in this case, applied cold protocol did not change the BDNF concentration.

Another factor, which may have impact on the BDNF shifts, can be irisin. This exerkine has impact on exercise-induced adaptations [31] via regulating muscle's glucose uptake and lipid metabolism [32]. Additionally, the study on animal model demonstrated a possible

connection between the brain and the muscles through irisin expression and its impact on BDNF stimulation [33]. What is more, a study performed in laboratory settings on humans indicated that the irisin concentration may be modified in response to cold exposure [34]. In this experiment, cold application (water-infused thermo-blankets; from 27°C cooled to 18°C and cooled further by 2°C every 3 min until 12°C temperature was reached) resulted in stimulated irisin secretion. Moreover, Dulian and co-workers demonstrated that after a series of 10 sessions of WBC (once a day at 9:30 am, for 3 min, at temperature -110 °C) enhanced the level of irisin among obese subjects [35]. Based on aforementioned findings, I assumed that low amount of fat tissue among my participants would induce a bigger thermogenic effect, muscle shivering and finally, lead to an increase of irisin, what consequently increase the BDNF level and enhanced participants' performance. Nevertheless, in my investigations no statistical alternations of this exerkine concentrations were recorded in response to the applied WBC procedures, additionally, any correlation between irisin and BDNF was noted.

The additional aim of my study was to investigate the effect of the applied cold procedure on the amino acid profile in trained subjects. Interestingly, the whole body cryostimulation caused a drop of all chosen amino acids concentrations at rest condition. Most pronounced differences were noted in the level of leucine and proline among all judokas, valine and tryptophan concentration in volleyball players.

Leucine and valine, as a part of branched-chain amino acids (BCAA) proteins may be a potential stimulators of muscle protein synthesis and mammalian target of rapamycin complex 1 (mTORC1) signalling human skeletal muscle [36]. Thus, my findings suggest that cryostimulation may enhance the amino acid uptake, supporting the maintenance of motor abilities in judokas as well as volleyball players after short two-week training programs. Another amino acid that changed its concentration in response to cold exposure was proline, the main component of collagen [38]. This is particularly important in a discipline such as judo, where permanent grip fight is a crucial part of competitive fights and finger injuries are the most common chronic problem among judokas. After the whole intervention, the level of proline was significant decreased in the cryo-stimulated group. This change may suggest that cold treatment enhanced the prolinase activity, and consequently, enhance collagen structure [37].

Interestingly, the program combined with cryo-sessions led to decrease of tryptophan in the volleyball cryo-stimulated group, especially among women. This can provide an explanation for their better results in the serve accuracy and concentration tests compared to men.

In the lights of collected results, whole body cryostimulation did not only enhance participants' physical performers but also increased the level of growth factors in circulating blood. Additionally, the obtained changes of amino acids concentration suggest that cryostimulation may, by stimulating microcirculation, have the impact of the peripheral amino acid tissue uptake. The applied whole body cryostimulation protocols were correctly fitted to the subjects needs and did not attenuated the adaptation training process.

8. Conclusions

In summary, the obtained results from three different groups of participants have enabled me to draw the following conclusions:

1. Daily performed 9/10 sessions of whole body applied specific judo training cryostimulation induced an increase in serum BDNF and IGF-1 concentrations.
2. Daily performed cold stimulation altered the amino acid concentration in circuit blood.
3. The 10 sessions of cold application did not impair the professional judokas' motor abilities.
4. Volleyball players who executed 10 sessions of WBC achieved better results from physical performance tests compared to the players from control group.
5. The series of 12 WBC sessions, performed day after the resistance training caused significant increase of maximal average power in the knee isokinetic extension strength test.
6. The resistance training combined with WBC caused a decrease of myostatin concentration among young, untrained subjects
7. The resistance training and cold application resulted in better adaptation to exercise-induced muscle damage, which characterize lover heart rate values during EIMD performing as well as lower, post-exercise concentration of myoglobin
8. The applied cold procedures did not significant change irisin concentration among all participants.

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10. Załączniki

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The Specific Judo Training Program Combined With the Whole Body Cryostimulation Induced an Increase of Serum Concentrations of Growth Factors and Changes in Amino Acid Profile in Professional Judokas

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This study aimed to evaluate the effect of a specific training program, supported by 10 sessions of whole body cryostimulation, on growth factors concentrations, amino acids profile and motor abilities in professional judokas. Ultimately, twelve athletes took part in the study. They were randomly assigned to the cryostimulation group (CRY, $n = 6$) or the control group (CON, $n = 6$). During 2 weeks of the judo training program, the CRY group performed 10 cryo-sessions (3-min, at a temperature of −110°C) and the CON group rested passively. Anthropometric measurements, a strength test, the Special Judo Efficiency Test (SJET) were assessed 2 days before and after the judo training program. Blood samples were collected at rest, 1 h after the first and the second SJET and 1 h after the first and the last cryo-session to establish growth factors and amino acid concentrations. Lactate level was measured before, immediately after and 1 h after the first and the second SJET. The applied intervention resulted in a significant increase of resting concentrations of brain-derived neurotrophic factor (from 10.23 ± 1.61 to 15.13 ± 2.93 ng·ml^{-1}; $p = 0.01$) and insulin-like growth factor 1 (IGF-1; from 174.29 ± 49.34 to 300.50 ± 43.80 pg·ml^{-1}; $p = 0.00$) in the CRY group. A different response was registered 1 h directly post SJET in the CRY group (a significant increase of IGF-1, interleukin 15 and irisin: $p = 0.01$; $p = 0.00$; $p = 0.03$). Additionally, the significant drop of proline and leucine concentrations in the CRY group was obtained. Athletes' performance remained unchanged in both groups. However, subjects perceived positive changes induced by the intervention – not directly after cryostimulation but in response to the specific training workload. The increase of growth factors concentrations and the improvement of amino acid profile (proline and leucine) contributed to maintaining a high level of muscle function.

Keywords: physical therapy, martial arts, irisin, interleukin 15, recovery

INTRODUCTION

Competitive judo is a demanding Olympic discipline, which requires a high level of technical and tactical skills (Franchini et al., 2008) as well as physical fitness (Franchini et al., 2011). Judokas follow various complex training programs, geared toward technical and tactical practice, traditional strength and endurance training as well as fight simulation such as *Randori* (Franchini and Takito, 2014), which evoke muscle damage even among experienced judo fighters (Laskowski et al., 2011). In the last few years, the frequency of judo competitions has increased significantly (Sikorski, 2011). Athletes thus face the challenge of maintaining a steady, high level of physical performance and decision-making skills for an extended period (Garatachea et al., 2012).

The specificity of the judo training process can pose a challenge for coaches and physiotherapists as they need to determine an optimal training program for their athletes, involving appropriate recovery methods. Meanwhile, our knowledge of the recovery methods adequate for judokas is lacking, particularly in the aspects of fostering adaptation to a judo training program or tailoring recovery to athletes' special needs such as eliminating chronic injuries, aiding progressive weight loss or maintaining muscle mass size. At the same time, deficient recovery practices within a professional training program, may lead to functional overreaching and, in consequence, trigger an overtraining syndrome (Meeusen et al., 2013).

Cold therapy is one of the most popular recovery methods used by athletes (Murray and Cardinale, 2015; Lombardi et al., 2017b). However, literature data have not unequivocally verified its impact on regeneration, physical performance and muscle adaptation. On the one hand, cold treatment was demonstrated to enhance the muscle recovery process and attenuate muscle damage including delayed-onset muscle soreness (DOMS) (Siqueira et al., 2018). On the other hand, its application does not always have a positive effect on muscle recovery or a visible impact on the recovery index (Malta et al., 2018). This differentiated effect of cold therapy depends on the applied procedure, the type of training and tasks executed simultaneously. Attenuated anabolic signaling and muscle hypertrophy in response to cold water immersion (CWI) was mainly recorded after a one-repetition exercise test and less so after regular training or endurance exercise (Malta et al., 2021). Interchangeably with CWI, whole body cryostimulation or partial exposure to extremely low temperature is also applied. Although the increasing accessibility of cryo-chambers is making the implementation of whole body cryostimulation in training more feasible and convenient, reports on the application of this treatment as part of a professional training program are limited (Rose et al., 2017). Therapies based on exposure to extremely low temperatures (−110 to −130°C) are known to be well-tolerated by professional athletes (Bouzigon et al., 2018), to limit exercise-associated inflammation and to enhance athletes' performance during demanding competition and training periods (Ziemann et al., 2012; Schaal et al., 2015; Bouzigon et al., 2020). Positive impact on levels of growth factors such as brain-derived neurotrophic factor (BDNF) or insulin-like growth factor (IGF-1), and blood concentrations of amino acids (Jaworska et al., 2018), linked with this temperature range, have been recorded mainly in academic, non-professional athletes. Therefore, the question of the effectiveness and validity of applying this kind of recovery strategy in professional training remains open.

Therefore, this study aimed to investigate the effect of 10 sessions of whole body cryostimulation on athletes' performance, levels of growth factors and amino acid concentrations in professional judokas during a 2-week high-intensity specific judo training program.

MATERIALS AND METHODS

Study Design

The study design and timeline are presented in **Figure 1**. Professional judokas participated in a 2-week judo training program including various kinds of high intensity practices. In addition, the experimental group underwent 10 sessions of whole body cryostimulation, while the control group did not use any dedicated methods of recovery. 2 days prior to beginning the intervention and 2 days after its completion, blood samples were collected, anthropometric measurements were taken, muscle performance was assessed and the Special Judo Efficiency Test (SJET) was performed (Laskowski, 2007).

Subjects

Thirteen highly trained judokas (all members of the Polish National Judo Team, age: 21 ± 3 years, height: 176 ± 8 cm, body weight 84 ± 22 kg, skeletal muscle mass 34 ± 7 kg, body fat 22 ± 14 kg, VO_2max 47 ± 11 ml·kg^{-1}·min^{-1}) took part in the study. The average training experience of each judoka amounted to 10 ± 2 years. Athletes were randomly split into two groups: the experimental group (CRY; n = 7) and the control group (CON; n = 6). Subjects stayed in a university dorm and followed a predetermined, balanced diet, taking their meals in the same place and at the same time of day (daily average intake of 3,600 ± 600 calories included protein at 1.5 g·kg^{-1} body mass). This approach was based on the previously published protocol (Laskowski and Antosiewicz, 2003). None of the participants had any previous experience with whole body cryostimulation, and did not take any supplementation or prescribed drugs during the intervention. Subjects were informed about the risks associated with the study and provided a written consent to their participation. The Bioethical Committee of the Regional Medical Society in Gdańsk approved the investigation before its onset (KB-28/17). The study was conducted under the Declaration of Helsinki. Athletes knew well the venue where all tests, the training protocol and recovery sessions took place.

Anthropometric Measurements

Body mass and body composition were assessed using a multi–frequency impedance body composition analyser (In Body

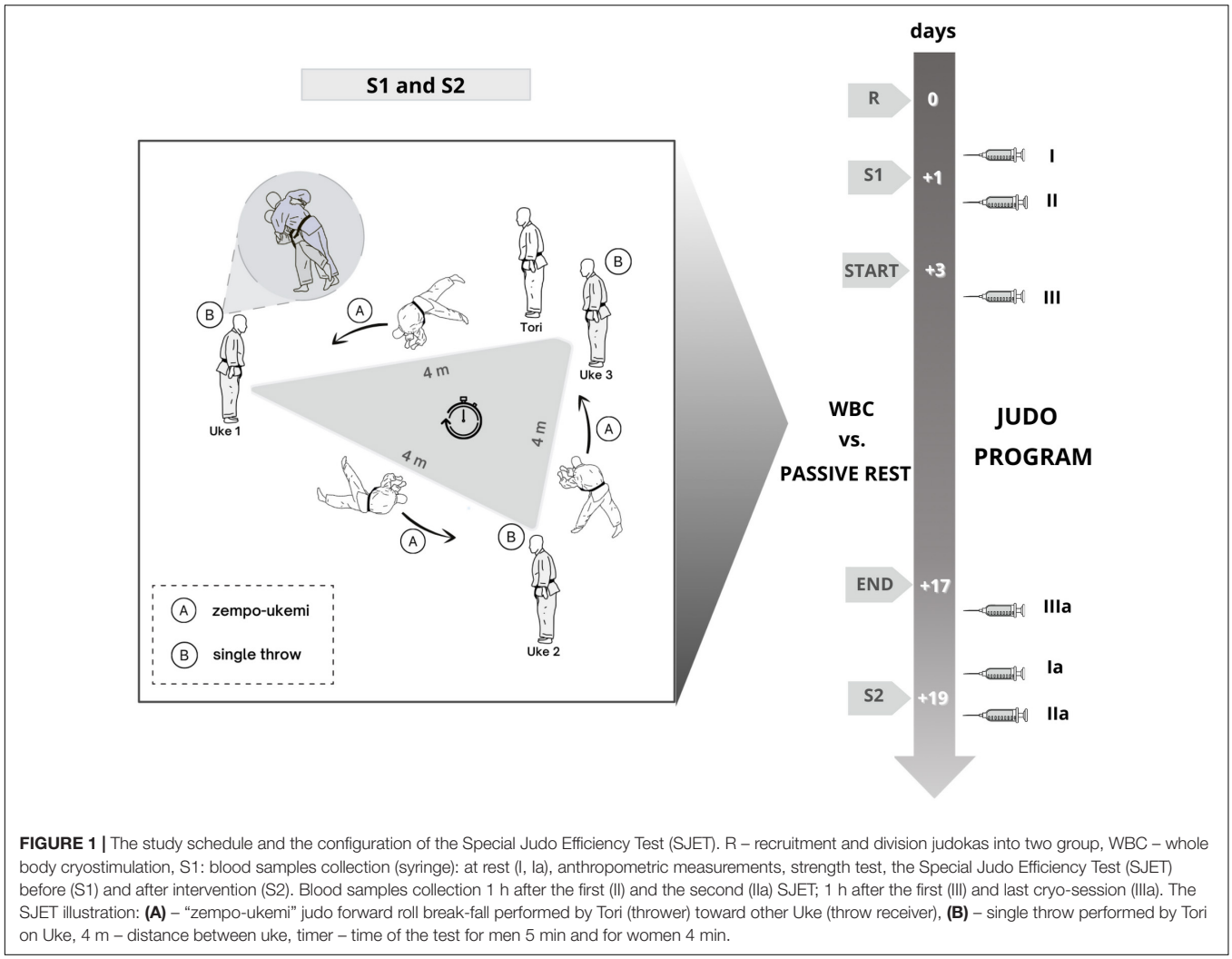

FIGURE 1 | The study schedule and the configuration of the Special Judo Efficiency Test (SJET). R – recruitment and division judokas into two group, WBC – whole body cryostimulation, S1: blood samples collection (syringe): at rest (I, Ia), anthropometric measurements, strength test, the Special Judo Efficiency Test (SJET) before (S1) and after intervention (S2). Blood samples collection 1 h after the first (II) and the second (IIa) SJET; 1 h after the first (III) and last cryo-session (IIIa). The SJET illustration: **(A)** – "zempo-ukemi" judo forward roll break-fall performed by Tori (thrower) toward other Uke (throw receiver), **(B)** – single throw performed by Tori on Uke, 4 m – distance between uke, timer – time of the test for men 5 min and for women 4 min.

720, Biospace, Korea). Impedance of segments of the body parts (trunk, arms, and legs) was measured at diverse six frequencies (1, 5, 50, 250,500, and 1000 kHz), using an eight-polar tactile electrode (Volgyi et al., 2008). Measurements were taken according to the manufacturer's protocol (McLester et al., 2020), following an overnight fasting (12 h after the last meal and drink). Athletes were also instructed to drink 400 ml of water, additional to their daily intake, and avoid any physical exercise 1 day before to the measurements.

The Assessment of Muscle Performance

Muscle function of the lower and upper limbs was estimated using a Biodex System four dynamometer (Biodex Medical System, Inc. Medical System, NY, United States). After 10 min of standardized warm-up, athletes were positioned in the equipment, according to the manufacturer's manual. Each test was performed in a sitting position and belts were used for body stabilization. Athletes received standardized verbal instructions before each test, and verbal encouragements throughout it. Subjects were asked to contract "as hard as possible" (to their maximum) to obtain their maximal peak torque. First, we measured muscle strength in isometric conditions, and recorded the peak contraction during flexion and extension in the knee and shoulder joints (of dominant limbs) in the conditions of 5-s isometric contractions. Then, we immediately measured the peak torque for the flexion and extension of the knee and shoulder joints (of dominant limbs) in the isokinetic conditions (velocity $90°{\cdot}s^{-1}$) in a three-time repeated movement. This assessment was repeated twice before and after the whole training program intervention.

The Assessment of Specific Physical Performance

The SJET was used to establish participants' specific judo performance abilities (Laskowski, 2007). The configuration of the SJET is shown in **Figure 1**. During the test, judokas performed an effort similar to a competitive match, characterized by the high-intensity, the approximate number of actions and changing body positions (standing "nage-komi" – repetitive throwing practice with a partner executing the technique and groundwork position; "zempo-ukemi" – judo forward roll break-fall). The

SJET was based on performing the highest number of throws in 5 min for men and 4 min for women. Before the test, athletes performed a standardized 15-min warm-up. During the SJET, subjects received verbal encouragements. SJET involved four judokas – one performing the throws and three athletes partnering (partners had a similar height and body mass as the athlete performing the test). Before every single throw, "zempo-ukemi" toward other judokas was done (**Figure 1**). Judokas' heart rate during the SJET was measured using a Polar Team^2; Pro device (Polar, Kempele, Finland).

Training Program

The training protocol was conducted at the beginning of the training preparation phase. Every judoka attended two training sessions a day with various loads applied, including special judo technical training (standing position – *tachi waza* and ground position – *ne waza*) as well as endurance and resistance training components. Each training session begun with 15–20 min of warm-up and ended with 15 min of stretching exercises. The endurance training units consisted of a continued 50-min run with the intensity of 60% of maximum heart rate (MHR). Sprint training units performed at the track included sprints at 100, 200, 400, and 800 m (90% MHR). The whole-body resistance training focused on strength improvement and included four main exercises: clean and jerk, squat, bench press and barbell row. Each training consisted of five sets of five repetitions maximum (RM) of each exercise. During the current intervention athletes were under the strict control of the team's coach (seven DAN judo, master class trainer) and a physiologist. The detailed schedule of the training program is shown in **Table 1**.

TABLE 1 | The judo specific training protocol.

Day	Training at 10 am	Intensity	Recovery 12–1 pm	Training at 4 pm	Intensity
Monday	**A**	60–70% MHR	CRY/PR	**B**	60–70% MHR
Tuesday	**C**	90% MHR	CRY/PR	**D**	5 × 5 RM
Wednesday	**E**	60% MHR	CRY/PR	**F**	80–90% MHR
Thursday	**A**	60–70% MHR	CRY/PR	**B**	60–70% MHR
Friday	**C**	90% MHR	CRY/PR	**D**	5 × 5 RM
Saturday	**E**	60% MHR		Rest	
Sunday	**Rest**				
Mondaycy i	**A**	60–70% MHR	CRY/PR	**B**	60–70% MHR
Tuesday	**C**	90% MHR	CRY/PR	**D**	5 × 5 RM
Wednesday	**D**	5 × 5 RM	CRY/PR	**F**	80–90% MHR
Thursday	**A**	60–70% MHR	CRY/PR	**B**	60–70% MHR
Friday	**C**	90% MHR	CRY/PR	**D**	5 × 5 RM
Saturday	**E**	60% MHR		Rest	

*CRY, single session of cryostimulation; PR, passive rest; MHR, maximal hart rate; RM, repetitions maximum. Training **A:** special judo training ne-waza base on repetitive technical exercise; 90 min. Training **B:** special judo training tachi-waza based on repetitive throwing exercise; 90 min. Training **C:** high-intensity sprint training at the running track, 100, 200, 400, and 800 m; 60 min. Training **D:** whole-body resistance training at the gym; four exercise, five sets of 5 RM, 2 min break between sets; 60 min. Training **E:** endurance training based on continuous moderate-intensity run; 50 min. Training **F:** fight practice – Randori; four sparring in ne-waza and 8 sparring in tachi-waza, every lasted 5 min, 2 min break between fight; 120 min.*

Whole Body Cryostimulation

All athletes in the CRY group participated in 10 sessions of whole body cryostimulation in a cryogenic chamber (Zimmer, Medizine-systeme, German) at the Pomeranian Rheumatologic Centre in Sopot, Poland. Sessions took place between 12 and 1 pm from Monday to Friday under medical supervision. Every session lasted 3 min. Before entering the main cryo-chamber, participants followed the adaptation procedure in the vestibule at a temperature of −60°C (approx. 30 s). The temperature in the main cryogenic chamber was −110°C. During the treatment, participants wore only shorts, socks, gloves and hats to cover their auricles. The sessions were conducted following the standardized protocol of whole body cryostimulation (Lombardi et al., 2017b).

Blood Collection and Analysis

In order to assess growth factors concentrations, blood samples were collected 2 days before and after the specific training program, at baseline and 1 h after the first and second SJET test (**Figure 1**). Additionally, blood samples were taken 1 h after the first and last cryo-session. Samples were taken from the antecubital vein into vacutainer tubes with K2EDTA (Becton Dickinson and Co., Franklin Lakes, NJ, United States) by professional medical staff. Immediately following blood collection, samples were centrifuged at 2,000*g* for 10 min at 4°C and the serum was stored at −80°C until later analysis, according to the currently available pre-analytical warnings (Lombardi et al., 2017a).

For blood lactate concentration (LA) analysis, samples were collected from capillary blood taken from the right index fingertip before the warm-up, immediately after and 1 h after the first and the second SJET. Directly after the collection, samples were deproteinized adding ice-cold 0.4 M perchloric acid. After being thoroughly mixed, samples were centrifuged at 12,000*g* for 10 min. Blood LA was determined using a standard Randox (Crumlin, United Kingdom) kit based on the LA oxidase method (LC2389). Assays were performed on the Cecil CE9200 spectrophotometer (Cambridge, United Kingdom).

Serum concentrations of BDNF, IGF-1 and interleukin-15 (IL-15) were assessed using sandwich ELISA kits according to manufacturer's instructions (R&D System, United States; catalog no. DBD00, DG100, and D1500, respectively). Irisin concentration too was determined with an ELISA kit (Phoenix Pharmaceutical Inc. United States; catalog no. EK 067-16).

Quantification of serum amino acids was done through the ion-pair reversed phase high performance liquid chromatography combined with the tandem mass spectrometry IP-RP HPLC-MS/MS triple state quadrupole (TSQ Vantage Thermo Scientific) and was performed following the same procedure as in the previous study (Gmiat et al., 2018).

Statistical Analysis

Statistical analysis was performed using Statistica 13.1 software. All values are expressed as a mean ± standard deviation

(SD). Shapiro–Wilk test was used to assess the homogeneity of dispersion from the normal distribution. Brown–Forsythe test was used to evaluate the homogeneity of variance. For homogenous results, the analysis of variance (ANOVA) for repeated measures and the *post-hoc* Tukey's test for unequal sample sizes were performed to identify significantly different results. For heterogenous results, ANOVA Friedman's test and Dunn-Bonferroni *post-hoc* test were used. The effect size (partial eta squared, η_p^2) was also calculated, with $\eta_p^2 \geq 0.01$ indicating small effect; ≥0.059 indicating medium effect; and ≥0.138 indicating large effect (Cohen, 1988). The significance level was set at $p < 0.05$. Additionally, due to the small size of the study group, all measurements were compiled in a spreadsheet for the analysis of parallel-group trials and the effects were interpreted using the magnitude-based inferences decision. All data was log-converted to reduce bias arising from the error non-uniformity. Probabilistic conclusions about the true (large-sample) value of effects were provided in the spreadsheet as clinical magnitude-based inferences (Hopkins et al., 2009). We expressed uncertainty in each effect as 90% confidence limits and as probabilities that the true effect was beneficial (e.g., a substantial increase in irisin level) and harmful (e.g., a substantial decrease in irisin level). Clinically clear beneficial effects were those for which the benefit was at least possible (>25% chance) and the risk of harm was acceptably low (<0.5%). Effects where the chance of benefit outweighed the risk of harm (odds ratio of benefit to harm >66) were also deemed clear. Other effects were either clearly non-beneficial (chance of benefit <25%) or unclear (chance of benefit >25% and risk of harm >0.5%). Clear effects were reported as the magnitude of the observed value, with the qualitative probability that the true effect was beneficial, trivial or harmful for the change (e.g., in irisin level). The scale for interpreting the probabilities was as follows: * – possible, ** – likely, *** – very likely, **** – most likely (Hopkins et al., 2009).

RESULTS

Subjects

Twelve athletes completed the intervention. One judoka from the CRY group was excluded from the study due to his injury (CRY, $n = 6$; CON, $n = 6$). After the intervention, no changes in body composition or body mass were recorded (data not shown).

Changes in Physical Performance

Muscle performance test data indicated that judo training did not affect athletes' muscle function, regardless of the applied recovery strategy. Among all analyzed parameters characterizing muscle function no significant changes were noted. Parameters remained unchanged, regardless of the rest procedure (**Table 2**). Results of the SJET also did not differ significantly. The average number of throws in the first SJET reached 57 ± 14 (CRY 53 ± 12; CON 61 ± 15) with the average HR during the test 183 ± 7 bpm (CRY 183 ± 5; CON 185 ± 6). After the 2-week training program, the average number of throws increased slightly in the second SJET and totaled at 60 ± 14 (CRY 56 ± 11; CON 63 ± 15) with the average HR 180 ± 3 bpm (CRY 181 ± 1; CON 179 ± 4).

Changes in Blood Lactate Concentration

The fatigue level was expressed by blood LA. Recorded values indicated that the intensity of SJET was high and above the anaerobic threshold for both groups. The average blood LA concentration immediately reached 7.06 ± 1.23 mmol·l^{-1} in the CRY group and 10.48 ± 1.86 mmol·l^{-1} in the CON group. ΔLA (the difference between the LA concentration recorded immediately after the SJET and 1 h rest) after the first SJET was 4.47 ± 1.02 mmol·l^{-1} in the CRY group and 4.37 ± 0.86 mmol·l^{-1} in the CON group. After the 2-week intervention, ΔLA were 3.74 ± 1.32 mmol·l^{-1} in the CRY group and 5.72 ± 1.49 mmol·l^{-1} in the CON group.

Changes in Growth Factors Levels

Changes in resting serum growth factors concentrations before and after the intervention are presented in **Figure 2**. The applied whole body cryostimulation, resulted in a significant increase of resting BDNF concentration from 10.23 ± 1.61 to 15.13 ± 2.93 ng·ml^{-1} ($p = 0.01$; **Figure 2B**). This change was accompanied by a significant elevation of IGF-1 concentration in the CRY group (pre: 174.29 ± 49.34; post: 300.50 ± 43.80 pg·ml^{-1}; p = 0.00; **Figure 2D**). The effect size p $\left(\eta_p^2\right)$ of delta values recorded pre vs post of resting concentration of BDNF was 0.47 and, respectively, 0.81 of IGF-1. In the CON group, no changes in BDNF (pre: 8.74 ± 2.08 ng·ml^{-1} vs post: 9.04 ± 2.01 ng·ml^{-1}) and IGF-1 (pre: 175.19 ± 44.67 pg·ml^{-1}, post: 159.70 ± 22.33 pg·ml^{-1}) were recorded. At the same time, only a slightly elevated tendency of IL-15 concentration was noted in both groups (**Figure 2E**). Independently of the applied recovery method, the training program did not alter resting irisin concentration (**Figure 2F**). Interestingly, a correlation between the resting level of irisin and ΔLA after the second SJET was noted ($r = 0.90$, $p < 0.05$).

The applied intervention did not cause changes in proteins in samples taken 1 h after cryo-session (data not shown). Still, significant differences were observed in serum taken 1 h after the second SJET (**Figure 3**). These were recorded for IGF-1, IL-15, and irisin concentrations.

Changes in Amino Acids Concentration

Changes of amino acid serum concentrations are shown in **Table 3**. Following the specific judo training program and whole body cryostimulation, a significant decrease of proline concentration was observed (**Table 3**). The magnitude-based inference indicated that the difference between groups was significant and the effect was very likely. Also, a statistically significant decrease of leucine concentration in the CRY group was noted and the effect was likely. The intervention too affected concentrations of isoleucine, valine and tryptophan, but the changes were not significant. There were no significant changes in baseline amino acid serum concentrations before and after the intervention in the CON group.

TABLE 2 | The muscle functions results before and after 2-week of specific judo training program combined with 10 sessions of cryostimulation or passive rest in both groups.

		PRE		POST		SMD	MBI	ANOVA p (η_p^2)
		$\bar{X} \pm SD$	95% CI	$\bar{X} \pm SD$	95% CI	d – Cohena	Practically worthwhile effect	Group × Time Interaction
PT max isometric [Nm] extension								
Dominant leg	CON	348.47 ± 74.42	270.37– 426.56	393.62 ± 125.85	261.55–525.69	0.44	Unclear	0.12 (0.23)
	CRY	306.63 ± 89.71	212.48 – 400.77	307.16 ± 99.38	202.87–411.46	0.01		
Dominant shoulder	CON	135.22 ± 43.69	89.37–181.07	137.38 ± 41.06	79.51–193.32	0.05	Unclear	0.99 (0.00)
	CRY	131.63 ± 47.32	81.97– 181.29	133.93 ± 49.98	81.48–186.38	0.05		
PT max isometric [Nm] flexion								
Dominant leg	CON	153.43 ± 29.83	122.13–184.74	157.59 ± 37.15	118.60–196.58	0.12	Unclear	0.43 (0.06)
	CRY	155.20 ± 32.95	120.61– 189.78	149.46 ± 24.68	123.56– 175.36	0.20		
Dominant shoulder	CON	105.01 ± 38.58	64.52–145.49	98.50 ± 23.49	73.85–123.15	0.20	Unclear	0.98 (0.06)
	CRY	94.27 ± 28.72	64.14– 124.41	95.54 ± 35.51	58.27– 132.81	0.04		
PT max isokinetic 90°● s^{-1} [Nm] extension								
Dominant leg	CON	245.36 ± 43.94	199.24–291.47	229.16 ± 35.11	192.31–266.00	0.41	Unclear	0.59 (0.03)
	CRY	235.12 ± 64.49	167.44– 302.80	224.64 ± 60.09	161.58– 287.70	0.17		
Dominant shoulder	CON	107.83 ± 31.55	74.72–140.94	111.63 ± 23.67	86.79–136.47	0.14	Unclear	0.33 (0.01)
	CRY	98.32 ± 33.59	63.07–133.57	98.75 ± 29.78	67.50– 130.00	0.01		
PT max isokinetic 90°● s^{-1} [Nm] flexion								
Dominant leg	CON	137.91 ± 21.92	114.91–160.91	129.17 ± 17.50	110.80–147.53	0.44	Unclear	0.44 (0.06)
	CRY	136.35 ± 35.58	99.02– 173.69	134.33 ± 38.00	94.45– 174.22	0.05		
Dominant shoulder	CON	96.40 ± 23.48	71.76–121.04	96.60 ± 13.46	82.48–110.73	0.01	Unclear	0.61 (0.02)
	CRY	84.92 ± 28.01	55.53– 114.31	82.07 ± 25.70	55.10– 109.04	0.11		
AP max isokinetic 90°● s^{-1} [W] extension								
Dominant leg	CON	147.07 ± 57.69	86.52– 207.61	149.87 ± 10.61	138.73–161.00	0.07	Unclear	0.54 (0.04)
	CRY	167.95 ± 48.49	117.06–218.84	157.43 ± 40.27	115.17– 199.69	0.24		
Dominant shoulder	CON	85.25 ± 27.59	56.30– 114.20	80.27 ± 23.65	55.44–105.09	0.19	Unclear	0.12 (0.02)
	CRY	69.32 ± 29.15	38.72–99.91	69.70 ± 29.31	38.94–100.46	0.01		
AP max isokinetic 90°● s^{-1} [W] flexion								
Dominant leg	CON	101.42 ± 32.64	67.16– 135.67	106.25 ± 16.48	88.96– 123.54	0.19	Unclear	0.70 (0.02)
	CRY	108.98 ± 30.13	77.37– 140.60	108.43 ± 32.39	74.44– 142.42	0.02		
Dominant shoulder	CON	87.77 ± 36.88	49.07– 126.47	71.55 ± 17.75	52.92–90.18	0.56	Unclear	0.09 (0.07)
	CRY	64.25 ± 24.40	38.64–89.86	62.60 ± 23.88	37.54–87.66	0.07		

PRE, before 2-week of special judo training program; POST, after 2-week of special judo training program; PT max, peak torque maximal, AP max, average power maximal; CRY, cryostimulation group; CON, control group; 95%CI – confidence interval; η_p^2, effect size, partial eta-squared; SMD, standardized mean difference; MBI, Magnitude-based inference.

DISCUSSION

The aim of this study was to determine the effect of the specific judo training, supported by 10 sessions of whole body cryostimulation, on muscle adaptation as reflected in growth factors concentrations, amino acids levels and motor abilities of professional judokas. The applied cryo-intervention caused a significant increase in circulating levels of the two growth factors (BDNF and IGF-1), together with a decrease of the circulating amino acids (proline and leucine) in blood after overnight fasting. Those ameliorations were not observed in judokas in the CON group, who rested passively in-between training units.

Recently published studies, involving martial arts athletes, analyzed only a single application of cold therapy (CWI, 15 min at 10°C) after a single sparring training /combat stimulation unit (Lindsay et al., 2017; Tabben et al., 2018). No studies, however, examined the effect of chronic cold therapy (repeated daily for a certain time) on adaptation to training in professional judo athletes. Malta and co-workers (Malta et al., 2021) based on results obtained from eight papers, reported that effects of CWI (temperature <15°C) are modified by the specificity of strength training (duration, frequency, number of sets). Findings of Roberts et al. (2015) showed that CWI (10 min at 10°C) applied after each unit of high-intensity strength training (12 weeks, 2 units per week, 3 – 5 sets of 8 – 12 RM) substantially attenuated satellite cells' activity as well as suppressed the acute anabolic signaling in muscle cells after training. This resulted in a small improvement of strength and hypertrophy, mainly in type II muscle fiber. On the other hand, Fyfe and co-workers (Fyfe et al., 2019) observed a negative impact of cold application (15 min at 10°C) performed after resistance training (7 weeks, 3 units per week, 3 – 5 sets of 12 RM) on muscle fibers hypertrophy but had no effect on maximal strength performance. Compared with the above-mentioned studies, in the this study cryostimulation was

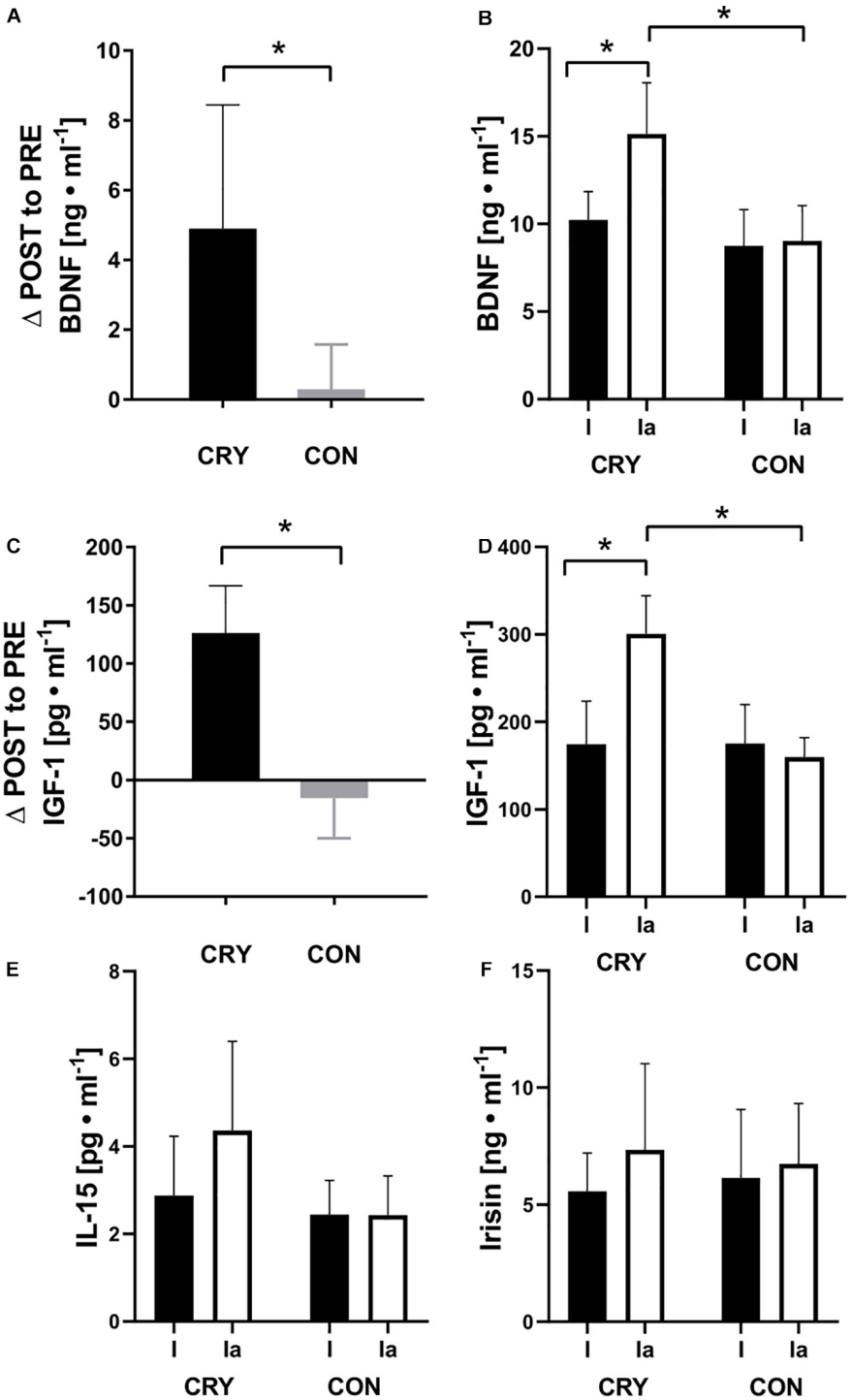

FIGURE 2 | The immunological response induced by training and whole body cryostimulation. **(A)** delta changes (Δ POST to PRE) of brain-derived neurotrophic factor BDNF (effect size $p\ \eta^2_p$ 0.47 and **(C)** insulin-like growth factor 1 – IGF-1 (effect size $p\ \eta^2_p$ 0.81); comparison of serum growth factors concentration at rest: **(B)** BDNF, **(D)** IGF-1, **(E)** IL-15, **(F)** irisin; *significant changes ($p < 0.05$); CRY, cryostimulation group; CON, control group; POST, post intervention values, PRE, pre-intervention values; I, baseline, Ia, after 2-week the training program.

applied 2 h post rather than immediately after training and the administered temperature was much lower. Based on the fact that CWI (8°C, 4 min) caused a similar decrease of muscle and trunk temperature as a single session of whole body cryostimulation in a special cryo-chamber (Costello et al., 2012), it was possible that the applied cold treatment protocol could decrease judokas'

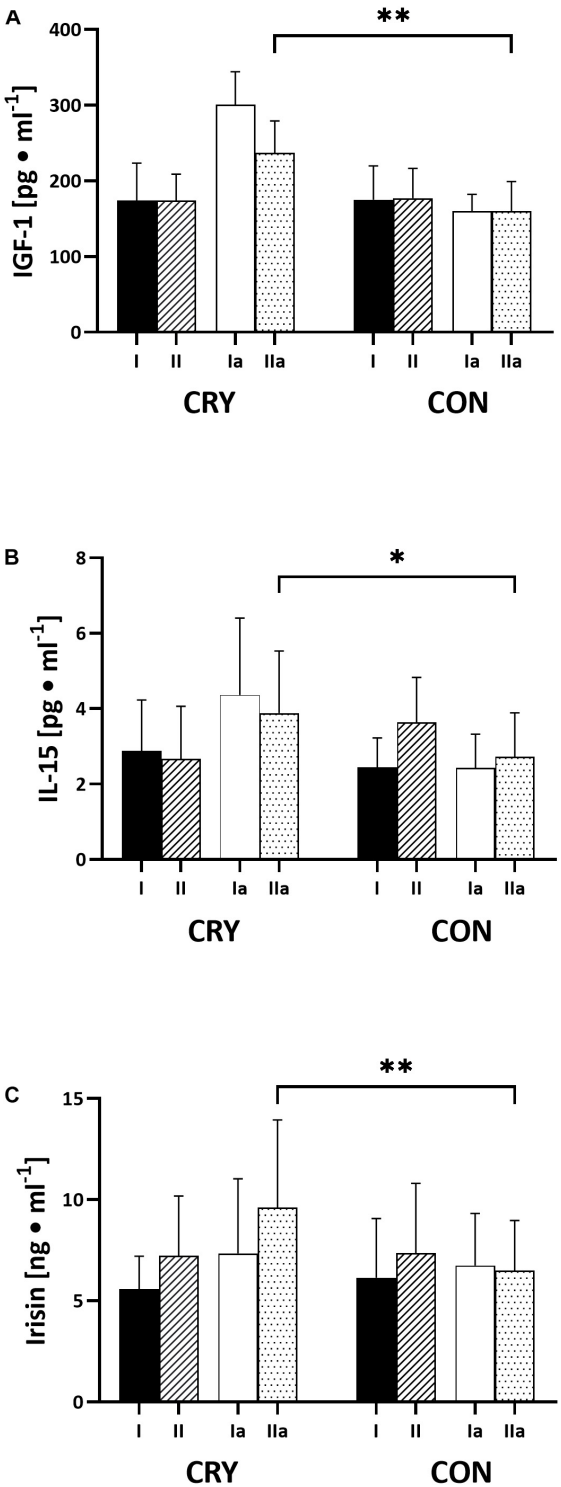

FIGURE 3 | The effect of Special Judo Efficiency Test (SJET) performed before and post 2 weeks of intervention. Comparison serum concentration of resting (I, Ia) and 1 h post SJET values (II, IIa): **(A)** insulin-like growth factor 1 – IGF-1 (*CRY IIa vs CON IIa: p* = 0.01, *effect size* 0.52); **(B)** IL-15, interleukin 15 (*CRY IIa vs CON IIa: p* = 0.03*, effect size* 0.38); **(C)** irisin (*CRY IIa vs CON IIa: p* = 0.00, *effect size* 0.63); CRY – cryostimulation group; CON – control group with passive rest.

motor abilities. Meanwhile, the obtained data showed that the applied intervention did not alter muscle performance, sustained muscle function and even slightly improved the SJET results. This is particularly important as our participants represented a very high level of physical capacity.

Alongside high physical capacity demands, mental strength and psychological skills are an integral training component in professional sport. BDNF is one of the growth factors involved in cognitive functions, which has an impact on brain plasticity (Cotman and Berchtold, 2002). Physical activity has been reported to enhance its level (Dinoff et al., 2017), which manifests in boosted brain functions, improved mood and inhibited depression (Teixeira et al., 2010; Phillips, 2017). A recent paper by Schor et al. (2019) reported that combat exercise, like *Randori*, can have a far more significant impact on BDNF release into the bloodstream than other kinds of exercise in elite judo athletes. A single fight induced a much pronounced increase of BDNF in both female and male judokas in comparison to changes after a maximal incremental effort. The authors have explained that judo fights can engage the nervous central system far more than other types of exercise owing to the tactical preparation and quick reactions to sudden changes of action provoked by the opponent (Schor et al., 2019). In our study, a significant difference between groups in the BDNF serum after the intervention was recorded. The 10 sessions of whole body cryostimulation caused an increase in the resting level in BDNF in judokas, yet without changes in body composition or muscle strength. In our previous paper, the specific resistance volleyball training supported by whole body cryostimulation, induced a significant increase of resting BDNF concentration among volleyball players, together with a less pronounced drop of maximal power in the countermovement jump in both female and male athletes (Jaworska et al., 2018). Both studies prove that whole body cryostimulation (9–10 sessions administered daily) combined with resistance training, modified BDNF concentration; however, changes in physical performance depended on the physical workload applied. At the same time, the frequency and duration of breaks in-between cold therapy sessions affect the change of BDNF concentration. For instance, no change in BDNF was noted in untrained students in response to 12 sessions of whole body cryostimulation performed 3 days per week, a day after a strength training session had been completed (Jaworska et al., 2020). IGF-1 is involved in muscle hypertrophy (Adams and McCue, 1998), reduces protein breakdown, and acts as a mediator of anabolic actions in skeletal muscles (Lee et al., 2017). Chronically low concentrations of IGF-1 may reflect an overreaching condition or impaired muscular adaptation to training (Lee et al., 2017). Moreover, IGF-1 is necessary to transform pro-BDNF to BDNF in the central nervous system (Ding et al., 2006). In our study applied procedure induced an increase of IGF-1 only in CRY group, which could have prevented a decline in muscle function.

The obtained data also show a slight increase of IL-15 concentration in blood serum. This myokine, released into the bloodstream in response to exercise (Bazgir et al., 2015), may have a beneficial effect on muscle adaptation by stimulating mitochondrial activity (Quinn et al., 2013). Its elevated level and intensified expression may in turn contribute to an enhanced

TABLE 3 | Amino acid profile at baseline and after 2-week of intervention.

Amino acid [μmol · l^{-1}]		PRE		POST		SMD	MBI	ANOVA p (η^2_p)
		$\bar{X} \pm$ SD	95% CI	$\bar{X} \pm$ SD	95% CI	d – Cohena	Practically worthwhile effect	Group × time interaction
AAs-after deamination form keto acid like alpha-ketoglutarate								
Proline	CON	149.25 ± 63	83.13 – 215.36	134.35 ± 32.52	100.23 – 168.47	−0,31	Very likely	**0.046 (0.34)**
	CRY	157.98 ± 51.39	104.04 – 211.91	90.99 ± 41.26*	47.69 – 134.29	−1,45		
AAs-after deamination form keto acid like acetyl-CoA								
Leucine	CON	85.79 ± 22.92	61.73 – 109.85	104.29 ± 37.37	65.07 – 143.50	0,61	Likely	**0.008 (0.53)**
	CRY	138.51 ± 37.25#	99.42 – 177.61	93.43 ± 24.47*	67.75 – 119.10	−1,46		
Isoleucine	CON	138.27 ± 62	73.20 – 203.34	121.73 ± 114.67	71.39 – 203.34	−0,19	Unclear	0.718 (0.01)
	CRY	108.10 ± 31.53	68.72 – 147.49	67.65 ± 41.34	24.27 – 111.03	−1,11		
Tryptophan	CON	39.62 ± 13.81	25.13 – 54.12	39.52 ± 7.71	31.43 – 47.61	−0,01	Unclear	0.051 (0.32)
	CRY	50.55 ± 8.93	38.16 – 62.94	32.33 ± 10.03	21.81 – 42.86	−1,82		
AAs-after deamination form keto acid like fumarate								
Valine	CON	113.53 ± 47.79	63.43 – 163.73	105.53 ± 46.33	56.91 – 154.16	−0,17	Trivial	0.379 (0.08)
	CRY	133.05 ± 35.75	95.54 – 170.56	95.48 ± 22.31	72.06 – 118.89	−1,29		

*PRE, before 2-week of special judo training program; POST, after 2-week of special judo training program; AAs, amino acids; CRY, cryostimulation group; CON, control group; 95%CI, confidence interval; η^2_p, effect size, partial eta-squared; SMD, standardized mean difference; MBI, magnitude-based inference; #, significant differences between groups; *, significant differences between measurements.*

muscle endurance. Besides the power and strength potential, a high level of aerobic capacity in judokas is particularly relevant for an efficient recovery in-between actions or for an ability to perform technical actions effectively in the last minute of a match (Franchini et al., 2011). Although cryostimulation did not change drastically IL-15 concentration, its level was statistically different after the second Special Judo Efficiency Test.

As evidenced by previous studies, not only exercise (Huh, 2018), but also whole body cryostimulation can be associated with an increase of irisin concentration in the blood (Ziemann et al., 2013). Irisin is considered to be a factor regulating metabolic muscle adaptation, including stimulation of glucose uptake and lipid metabolism (Lee and Jun, 2019). In this study, no statistical alternations in the resting level of irisin were recorded in response to the applied intervention. Similar results were registered by Sliwicka and co-workers (Sliwicka et al., 2020), who noted that whole body cryostimulation had not affected serum irisin concentration in subjects characterized by a high physical fitness level. An interesting correlation between the irisin level and ΔLA after the second SJET in the CRY group was still observed. Currently available data account for a positive impact of irisin on maintaining a balance between glucose uptake in peripheral tissues, including skeletal muscles, and hepatic glucose homeostasis (Arhire et al., 2019). Studies on animal models have shown that irisin stimulated glucose uptake in myocytes and reduced gluconeogenesis in hepatocytes (Mo et al., 2016; Xin et al., 2016). The significant correlation between irisin and ΔLA observed in this study suggest that an increased concentration of circulating irisin may have enhanced lactate removal from blood in response to a high-intensity SJET. During and after exercise, lactate can be utilized via gluconeogenesis, and metabolized to carbon dioxide and water substrate by tissues including brain, skeletal muscle and some others. The actions of irisin on the removal of circulating lactate via gluconeogenesis process is unequivocal (Mo et al., 2016; Xin et al., 2016). A lower concentration of lactate in the CRY group after the high-intensity SJET may indicate that its production decreased, while the utilization increased. Since the cryostimulation intervention was associated with slightly improved scores in the SJET, based mainly on anaerobic glycolysis, lower lactate production can likely be excluded. The role of irisin in lactate removal also cannot be ruled out. A recently published study by Kujach and co-workers (Kujach et al., 2019) demonstrated that a high cumulation of circulating lactate positively correlated with circulating BDNF. It thus cannot be dismissed that the changes in BDNF concentration noted in this study were associated with prefrontal cortex and muscular oxygenation in response to whole body cryostimulation. This observation corresponds with the data obtained by Douzi and co-workers (Douzi et al., 2020), using near-infrared spectroscopy. The authors noted a significant increase in the relative blood perfusion in subjects, who performed the exercise with a cooling vest during submaximal exercise.

Our study also aimed to verify if the applied intervention can affect the amino acid profile. To the best of our knowledge, this is the first study to show an influence of extremely cold temperature on the blood level of proline. The observed change can be explained by two mechanisms.

First, the exposure to cold induces sympathetic-mediated vasoconstriction followed by a reactive vasodilation response that cause the reduction first and the next consequent forced blood flow (Sieroń et al., 2010), resulting in limited availability and subsequently, better uptake of amino acids. Proline is known as the main amino acid component of collagen (Wu et al., 2011) and has an impact on the regeneration process of the articular cartilage matrix (de Paz-Lugo et al., 2018). Poor availability of this crucial amino acid disturbs collagen synthesis, which may increase proneness to osteoarthritis

(Wu et al., 2011). In the CRY group, cryostimulation caused a significant drop in the circulating level of this amino acid, whereas in the CON group, the opposite tendency was observed. This may suggest that cryostimulation enhanced the prolinase activity, which was found to recycle proline for collagen resynthesize (Zareba and Palka, 2016). The potential of long-term whole body cryostimulation to enhance collagen structure can make it a useful injury prevention treatment in judo. Permanent grip fight is a crucial part of competitive judo fights; hence, finger injuries are the most common chronic problem among judokas (Strasser et al., 1997). Repetitive and untreated damage to fingers can increase the risk of osteoarthritis, thus, enhanced uptake of proline in response to cold treatment can be considered a recovery method particularly pragmatic for judo players.

Second, the obtained changes can be linked with brown adipose tissue. It is known that exposure to low temperatures stimulates browning fat tissue and its activity (Cannon and Nedergaard, 2004). Recently, Marmol and co-workers (Marmol et al., 2020) revealed that stimulation of activin receptor ALK7 suppressed the expression of Kruppel Like Factor 15 (key regulator of amino acid metabolism) and proline dehydrogenase (amino-acid catabolizing enzyme) in both mouse and human brown adipocytes. This can account for the observed decrease in proline concentration. The applied whole body cryostimulation also caused a drop in the level of branched-chain amino acids in serum after the 2-week intervention. The inter-group difference was most pronounced for leucine, a potential stimulator of muscle protein synthesis and mammalian target of rapamycin complex 1 (mTORC1) signaling human skeletal muscle (Moro et al., 2016). Those findings suggest that cryostimulation may have enhanced the amino acid uptake, supporting the maintenance of motor abilities in judokas after the 2-week training program. Cholewka and co-workers (Cholewka et al., 2012) reported, using thermal imaging, that the extremely cold exposure had a significant effect on skin temperature changes, especially extremities. With a thermal vision camera, the authors demonstrated that one session of whole body cryostimulation (3 min at −120°C) caused a substantial reduction of skin temperature. This rapid body cooling stimulates circulation in the thermoregulation prosses, which affects the constriction of microcirculation vessels and in turn, is quickly followed by vasodilatation due to transmitter substances being released (Sieroń et al., 2010). In this study, whole body cryostimulation caused a significant decrease of proline and leucine levels as well as negative shifts in blood isoleucine and valine. Hence, it can be assumed that by stimulating microcirculation, the applied cold therapy encouraged the peripheral amino acid tissue uptake.

Our study is not without limitations, namely the small sample size of the study group. Our participants were belonged to members of Poland's national judo team, a selected group accustomed to training with a very high intensity. Extending this group to other, less advanced athletes was hardly possible without affecting the study groups' core characteristics. In order to credibly interpret the obtained results, we used statistical methods adjusted to the undersized sample size of the study. It is due to these sample size considerations that we could not organize a whole body cryostimulation group in parallel with an untrained group. Future studies, however, could aim to verify the effect of whole body cryostimulation on adaptation training markers in bigger groups of subjects. We would also recommend using in future research not only fitness tests, but also tests to assess the fatigue of the nervous system and the number of mistakes made in tactical tasks, and monitor sleep quality and patterns.

To the best of our knowledge, no reports in the past provided evidence of the effectiveness of whole body cryostimulation as a recovery method in judo. The applied specific training program, combined with 10 sessions of whole body cryostimulation, caused positive, significant changes in BDNF and IGF-1 concentrations as well as the amino acid pool in blood stream post intervention at rest and 1 h after training. The changes in growth factors could be deemed responsible for the muscle function being maintained (not dropping), while the amino acid profile improved. Whole body cryostimulation can thus be considered as a promising physiotherapy procedure for judokas, which can mitigate the overload and overtraining syndrome. Hence, we recommend the use of this treatment during both intense training and competition periods.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics commission in Gdańsk, KB 28/17 on 24 October 2017. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JJ, RL, EZ, and PZ designed the study. JJ, RL, EZ, GL, and PZ performed the research. JJ, EZ, KZ, JA, and PZ wrote the manuscript. RL, AJ, KZ, and GL reviewed and editioned the manuscript. All authors contributed to the article and approved the submitted version.

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A 2-Week Specific Volleyball Training Supported by the Whole Body Cryostimulation Protocol Induced an Increase of Growth Factors and Counteracted Deterioration of Physical Performance

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Potentially beneficial effects of cold therapies on training adaptation still remain unequivocal. We have, thus, decided to evaluate the effects of a 2-week volleyball training program supported by 10 sessions of whole body cryostimulation (WBC) on growth factors and physical performance. Twenty healthy college-aged men and women randomly assigned either to the cryostimulation group (CRY) or the control group (CON; executed passive rest). Both groups took part in the same 2-weeks training program. Additionally, the CRY group attended in 10 cryo-sessions (3 min, −110°C temperature, five times/week). Blood samples were collected at baseline, 1 h after the first cryo-session as well as before and 1 h after the last session of WBC to assess growth factors, myokines concentration and the amino acid profile. Motor abilities were tested before commencing the training program and 2 days after its completion. The applied intervention resulted in an increase of brain-derived neurotrophic factor and insulin-like growth factor 1 concentrations. The adjusted effect describing the difference between groups in response to applied procedures was for both growth factors large and very likely in the CRY, higher than in the CON group (113%; Coefficient Interval: 38–230%, 45%; Coefficient Interval: 17–79%, respectively). Physical performance dropped in both groups, yet in the CRY group, the magnitude of change was smaller. The fibroblast growth factor dropped significantly 1 h following the first cryo-session, yet irisin remained statistically unchanged. The similar tendency was maintained after the whole procedure, still the range of changes was smaller. In the CRY group, an elevated uptake of tryptophan and valine noted in response to the whole intervention, could have induced a significant decrease of fasting glucose

concentration (the adjusted effect small and very likely −6%; Coefficient Interval: −10 to −2%). Overall, a 2-week volleyball training program supported by the whole body cryostimulation protocol resulted in an increase of growth factors and offset a decline of physical performance. Thus these procedure can be applied in professional sport during competition period, especially among those disciplines focusing on an explosive power and ability to concentrate.

Keywords: myokines, training adaptations, branched amino acids, tryptophan, coldness

INTRODUCTION

Different studies have demonstrated the effectiveness of whole body cryostimulation (WBC) in supporting recovery processes. This procedure can be applied in the preparation phase of training (Wozniak et al., 2007), in the midseason break/regenerative period (Ziemann et al., 2012) and after the competition season (Selfe et al., 2014). As it was shown to lower the perceived level of fatigue, WBC can be a useful way to counteract the onset of the overreaching state (Schaal et al., 2015). Generally, WBC treatments involve a 3-min exposure in a special cryo-chamber set at −110 to −140°C, depending on the cooling system (electrical or nitrogen). Such an exposure repeated 10 times has been shown to be sufficient to induce the anti-inflammatory response (Lombardi et al., 2017).

WBC is often compared with cold water immersion (CWI) treatment. Changes in muscle and core temperatures induced by these two cold therapies may indeed appear mostly similar, though not for the skin (Costello et al., 2012). The response of these therapies is depended on an applied kind of exercise, its duration, frequency, intensity and other factors: such body composition, gender and environmental conditions (Costello et al., 2015; Stephens et al., 2017). Recently published papers revealed that these two cold therapies used after high-intensity exercise, may not always bring significant and beneficial changes in muscle damage markers compare to other recovery methods (Chan et al., 2016; Krueger et al., 2018). Still, the review written by Rose and colleagues has shown that the application of WBC following an intensive exercise had an analgesic effect and improved physical performance in 71% of the analyzed studies. (Rose et al., 2017) However, regular post-exercise CWI (10°C, 10 min) has been demonstrated to inhibit muscular adaptation when following a strength training program (12 weeks, twice a week). Authors have observed that training supported by CWI attenuated acute changes in satellite cell numbers and the activity of kinases that regulate muscle hypertrophy (Roberts et al., 2015). Consequently, a possibility that the effect of a cold treatment may not necessarily be beneficial for training adaptation, it should be considered, as it may weaken the anabolic response triggered by resistance training.

Extensive metabolic adaptations in skeletal muscle are induced through various molecular pathways and myokines (interleukins: IL-6, IL-10, IL-15, irisin, and others), which not only act on muscles via an autocrine/paracrine manner, but also mediate interaction of muscles with other organs through endocrine mechanisms (Egan and Zierath, 2013) The influence of physical training on myokines' concentrations is well documented, but data regarding the impact of cold treatments on their levels are still limited. The levels of irisin and fibroblast growth factor 21 (FGF21) have been shown to have shifted in response to cold exposure, leading to fat browning (Lee et al., 2014). At the same time, irisin changes have also been observed in response to resistance exercise (Knaepen et al., 2010) as well as after endurance training (Nygaard et al., 2015). It has also been revealed that irisin is crucial for training adaptation (Fatouros, 2018), while FGF21 predicts non-shivering thermogenesis response in humans (Lee et al., 2014). What is more, irisin can play a significant role in stimulating brain-derived neurotrophic factor (BDNF) (Zsuga et al., 2016) and this increase can affect human cognitive functions and their performance (Phillips et al., 2014). Among other myokines, which can modify an increase of anabolic signal in response to resistance training are interleukin 15 (IL-15) (Nielsen et al., 2007), insulin-like growth factor (IGF-1) (Fink et al., 2017) and myostatin (Huh, 2018).

Also, supplements (for example branched amino-acids /BCAA/), sometimes referred to as immuno-nutrients, often used in combination with resistance training, may reduce immunosuppression and excessive inflammation, regulating the anabolic signal as a result. The anabolic response to training is, thus, very complex as it involves adaptation over different levels. Importantly, this response is not limited to the skeletal muscle tissue, but depends on the integration of signals coming from the organs deputed to energy management, the immune system and the brain. Hence, the study of the adaptive response to training (i.e., damage, regeneration, anabolism) requires for at least the key factors involved in this crosstalk to be investigated as well (Giudice and Taylor, 2017). This is even more valid in the case when training (resistance) is combined with WBC; the combination which is known to induce whole-body homeostatic responses that somehow mimic the effects of exercise (Lombardi et al., 2017). Still, the influence of resistance training applied in conjunction with cold exposure on the anabolic response remains uncertain.

In light of the presented reasoning, our study was set out to investigate whether a combination of a 2-week resistance training program and 10 sessions of whole body cryostimulation would improve motor abilities and delay mental fatigue in college volleyball players. We have evaluated the effect of these two factors (training and cold exposure) on myokines' levels and amino acids concentrations, due to their crucial role in training adaptation.

MATERIALS AND METHODS

Study Design

The study design and timeline is presented in **Figure 1**. Two days before the main training program we performed a battery of tests. The same battery of test was repeated 2 days after the program's completion.

Subjects

A group of players from the university volleyball team took part in our experiment (n = 10 women, n = 10 men). The physical testing was performed before the randomization of participants into the subgroups. In order to avoid any effect on the final results, group allocation was done after the first physical performance assessment. The athletes were randomly assigned either to the experimental group (CRY; n = 8) or the control group (CON; n = 12). Two subjects in the CRY group had requested reallocation before the first WBC session due to hypersensitivity to low temperatures. With the two subjects transferred to the CON group, the groups became disproportionate. Subjects from the CON group did not participate in cryostimulation at any point of our study. In addition to basic parameters, the phase of menstrual cycles of female players was checked. We found that 70% of participating women were in the follicular phase, whereas the remaining female participants were in the luteal phase. Both groups participated in the same 2-week training program. All subjects lived in the same accommodation and followed the same training schedule and diet. Daily, energetic value of food offered in the menu did not exceed 3,800 kcal. The proposed protein dose varied from 1.2 to 1.4 g/kg of body mass (BM). Participants did not take any supplementation or prescription drugs during the study. The subjects were fully informed of the risks and stresses associated with the study and gave their written consent to participate.

Anthropometric Measurements

Body mass and body composition were assessed before the start of the training program and 2 days after the intervention, using a multi-frequency impedance plethysmograph body composition analyzer (In Body 720, Biospace Analyzer, Korea). This analyzer accurately measures the amount of body water and body composition, including fat mass, free fat mass, skeletal muscle mass and soft lean mass.

Physical and Mental Performance Assessment

Participants performed a 15-min standardized warm-up, including running, particular volleyball exercises and dynamic stretching exercises of upper and lower limbs. Afterward, athletes completed tests in the following order: a concentration test, explosive power assessment, a serve accuracy test, a single high-intensity interval bout of exercise and directly after its finishing the concentration test was repeated.

Concentration Test

Grid exercise was applied to evaluate concentration and focus abilities. The original grid exercise uses a 10-by-10 block. Each block contains a two-digit number ranging from 00 to 99. The aim of this exercise is to scan the grid, and within a given a time period, find and click (cross-out) as many numbers as possible in an ascending numerical sequence (Wilson et al., 2006). In the present study, participants had 2 min to connect numbers from 00 to 99. The test was performed twice, at rest and directly after interval volleyball exercise. A time countdown was visible during the exercise to build up the pressure experienced by each subject.

Explosive Power Measurements Test

Explosive power assessment consisted of series of countermovement jumps in two positions: a series of three countermovement jumps with arms rested on the hips and a series of three countermovement jumps with an arm swing with the shortest possible contact time between the feet and the surface. Subjects took a 3-min break between series. All jumps were performed on a tensometric mat (Smart

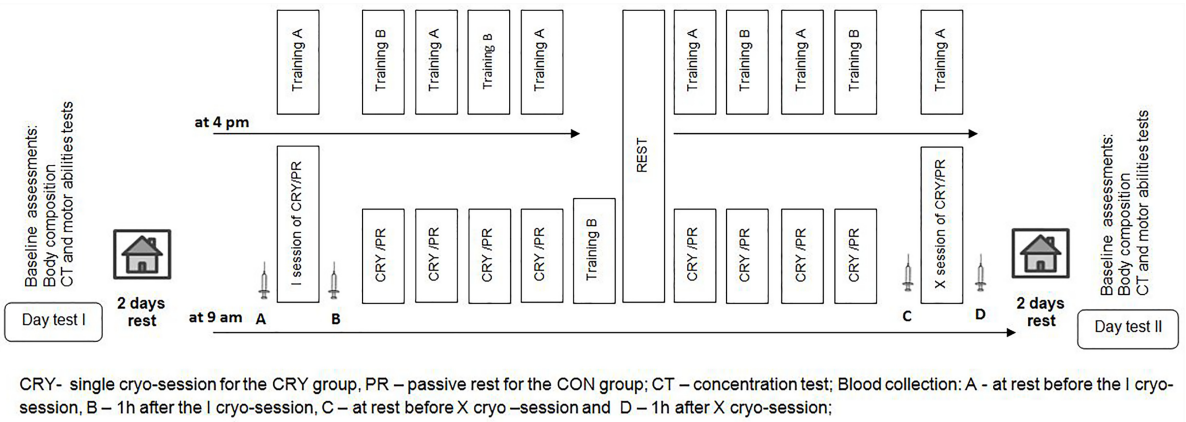

FIGURE 1 | Experiment schedule.

Jump Mat 120 cm × 120 cm—Fusion Sport, Cooper Plains, Australia).

Serve Accuracy Test

Serve accuracy was tested using Service Accuracy in resting conditions described by Lidor and colleagues. Players had to hit 10 consecutive serves into designated areas on the opposite side of the court (Lidor et al., 2007).

Interval Training Bout of Exercise

In order to induce fatigue similar to the one experienced during a professional match, a single high-intensity interval bout of exercise was performed. We decided to apply the work to rest ratio of 1:3 (10 s work and 30 s rest, repeated six times). We used a system of four double photoelectric cells (SMART SPEED PRO—Fusion Sport). We placed these cells round and opposite the player in the set-up of four gates. Subjects had to cross each lit up gate as soon as possible. The cells lit up randomly. Athletes began the test with one foot on the line in a standing starting position. All participants received standardized verbal instructions before the exercise and verbal encouragement throughout. Given the random and unrepeatable nature of the each test's sequence, we did not compare the results and reaction time achieved in this test.

Training Program

At the onset of the training program, a briefing session was organized to familiarize all participants with planned exercises. During the 2-week program, training was conducted once a day (including Sundays) with the total of 11 sessions. Players participated in six power training sessions at the gym (∼60 min per session) and five volleyball training sessions at the sports hall (∼120 min per session). Training details are presented in **Table 1** and **Figure 1**. Each power training session also included a 10-min warm-up and 10 min of plyometric exercises. The volleyball training focused on discipline-specific performance. Participants worked on improving technical and tactical skills such as blocking, attacking, serving in simulated volleyball matches. All training sessions were supervised by players' coaches.

Whole-Body Cryostimulation

Along with following the training program, the CRY group attended 10 sessions of whole-body cryotherapy in a cryogenic chamber at the Pomeranian Rheumatologic Center in Sopot, Poland. Each cryo-session lasted 3 min at a temperature of −110°C. The procedure of this cold exposure was similar to the one commonly recommended and applied by our research team (Ziemann et al., 2014). The control group followed only the training program without using any recovery methods.

Blood Collection and Analysis

Blood samples were taken by professional medical staff from the antecubital vein into vacutainer tubes with K2EDTA (Becton Dickinson & Co., Franklin Lakes, NJ, United States) on the first and last day of the training program, before breakfast and 1 h after the cryo-session. Immediately following blood collection, one sample was transferred into a centrifuge tube with aprotinin to undergo further irisin level assessment. We used tubes from Phoenix Pharmaceuticals Inc. (catalog no RK-APRO). The final concentration of aprotinin was 0.6 Trypsin Inhibitor Unit/1 mL of blood. The samples were centrifuged at 2,000 × *g* for 10 min at 4°C and the serum was stored at −80°C until later analysis. Concentrations of serum IL-15, IL-6, myostatin and BDNF were assessed using sandwich ELISA kit according to the manufacturers' instructions (R&D Systems, United States, catalog no D1500, HS600B, DGDF80 and DBD00, respectively). The average intra-assay coefficient of variability (CV) for IL-15, myostatin and BDNF was 5%. The inter-assay coefficient and detection sensitivity were as follows: 9.1% and 2 pg mL^{-1} for IL-15; 6% and 5.32 pg mL^{-1} for myostatin and 11.3% and 20 pg mL^{-1} for BDNF. The detection limit for BDNF was <20 pg mL^{-1} and 2.25 pg mL^{-1} for myostatin. The samples of myostatin were diluted prior to analysis to an assay 1:4. The maximal intra-assay CV for IL-6 was 7.8% and the maximal inter-assay CV was 9.6%. Serum IGF-1 and FGF21 were also evaluated via a sandwich ELISA (R&D Systems, United States, catalog no DG100 and DF2100). Average intra-assay CV reported by the manufacturer was 4% for both. The detection sensitivity of IGF-1 and FGF21 was 0.056 ng mL^{-1} and 8.69 pg mL^{-1}, respectively. The maximal inter-assay CV was 8.3% for IGF-1 and 10.9% for FGF21. Concentrations of serum irisin were determined using competitive enzyme immunoassay kits from Phoenix Pharmaceuticals Inc (catalog no EK 067-16). Intra-assay CV and inter-assay CV were 4–6 and 8–10%, respectively.

TABLE 1 | Characteristics of training sessions.

Training	Number of sessions	Time (min)	Load
Volleyball	5	120	Volleyball training improved technical and tactical performers: fife actions: serve, reception, setting, spike, block; high-intensity intervals 2:1 (8 min work and 4 min break) 85–90% MHR; 60 min Small games (simulated volleyball matches): 4 × 12 min games (with 4 min break); high intensity 90–95% MHR; 60 min
Power	6	60	Power training at the gym; six exercises for volleyball players, each at 35% of 1 RM, involving arms and legs muscles; three sets of six repetitions of: clean and jerk, snatch, explosive bench press, continuous squat jump (with barbell on the back), bound, split squat jump alternating; the break between sets lasted >2 min; each session was preceded 10 min of warm-up and 10 min of plyometric exercises

MHR, maximal heart rate; RM, repetition maximum.

The serum concentrations of total cholesterol (TCH), high density lipoprotein cholesterol (HDL) and triglycerides (TG) were determined with the use of commercial kits based on enzymatic methods (Alpha Diagnostics, Poland). Glucose was assessed using *Cobos 6000analyzer*.

Quantification of serum leucine, isoleucine, valine and tryptophan was based on the ion-pair reversed phase high performance liquid chromatography combined with the tandem mass spectrometry IP-RP HPLC-MS/MS (TSQ Vantage Thermo Scientific). It was executed following the same procedure as in our previous study (Gmiat et al., 2018).

Statistical Analysis

All measurements were compiled in a spreadsheet for the analysis of parallel-group trials and the effects were interpreted using magnitude-based inferences (Hopkins, 2006). All data were log-transformed to reduce bias arising from the non-uniformity of error; means of change scores in both groups, SDs of change scores and effects (variations of change in both the means and their confidence intervals: CI) were all back-transformed to percent units. Mean changes and effects were adjusted to the overall mean baseline values in both groups, by including the baseline value as a covariate in the analysis. Magnitudes of the effects were evaluated with the log-transformed data by standardizing the deviation of the baseline values. Threshold values for assessing magnitudes of standardized effects were 0.20, 0.60, 1.2 and 2.0 for small, moderate, large and very large effect, respectively. The uncertainty in the effects was expressed as 90% CI and as a probability of whether the true value of the effect was substantially positive (an increase) or negative (a decrease). For a non-clinical inference, the effect was deemed unclear if the CI overlapped thresholds for substantial increases and decreases (>5% in both cases as equivalent of 90% CI overlapping thresholds for a substantial increase and decrease). All other effects were deemed clear and were evaluated probabilistically. The scale for interpreting the probabilities was as follows: 25–75%, possible; 75–95%, likely; 95–99.5%, very likely; >99.5%, most likely.

RESULTS

All players completed our study with no injuries or adverse events being reported. Baseline anthropometric characteristics are presented in **Table 2**. Obtained results showed that no significant changes were recorded in body composition in response to training and coldness therapy.

The Effect of the Applied Training Program and Coldness Therapy/Passive Recovery Procedures on Physical Performance

Results obtained in volleyball tasks indicated that the training program applied with either of the recovery procedures did not affect significant participants' physical performance. However, most of the measured parameters reflected attenuation of its performance. Changes recorded in the concentration test results were accompanied by changes in the serve accuracy test results. Given differing norms for performance in physical activity tests between men and women, we opted to present data according to participants' sex. Still, when comparing the CRY and CON groups, the trend of change proved to be similar. In the CRY group, women achieved slightly better results in the concentration and serve accuracy tests than females in the CON group, whereas men exhibited poorer performance with the deterioration of the serve

TABLE 2 | Participants' body composition before and after the 2-week training program supported with the whole body cryostimulation protocol.

	Group	Baseline mean ± SD	Observed change mean ± SD (%)	Adjusted change[a] mean ± SD (%)	Adjusted effect[b]	
					mean;CI (%)	Inference[c]
Female						
Body weight (kg)	CON	64.2 ± 9.7	−1 ± 2	−1 ± 2	2	Trivial
	CRY	64.0 ± 10.3	0 ± 1	0 ± 1	−2 to 5	
SMM (kg)	CON	27.3 ± 3.3	2 ± 4	2 ± 4	2	Trivial
	CRY	27.8 ± 3.7	8 ± 3	4 ± 3	4–9	
Body fat (%)	CON	22.9 ± 3.8	−8 ± 8	−8 ± 9	6	Small
	CRY	21.6+4.0	−4 ± 9	−2 ± 10	−8 to 21	
Male						
Body weight (kg)	CON	79.9 ± 8.1	−1 ± 1	−1 ± 1	0	Trivial
	CRY	79.7+8.1	−1 ± 1	−1 ± 1	−2 to 2	
SMM (kg)	CON	40.5 ± 2.6	0 ± 1	0 ± 1	1	Trivial
	CRY	40.8 ± 3.0	1 ± 2	1 ± 2	−2 to 5	
Body fat (%)	CON	10.9 ± 5.1	−3 ± 12	−2 + 14	−5	Trivial
	CRY	10.6 ± 4.5	−8 ± 11	−7 + 13	−23 to 19	

SMM, skeletal muscle mass; CON, control; CRY, cryotherapy. CI, 90% confidence interval. All data are percentages, with the exception of baseline values express measurement units. [a]*Adjusted to overall mean of in baseline CON and CRY group.* [b]*Adjusted mean change of CRY group minus adjusted mean CON group.* [c]*Magnitude thresholds (for difference in means divided by SD of CRY group): <0.20, trivial; 0.20–0.59, small; 0.60–1.19, moderate.*

TABLE 3 | Changes in motor abilities and concentration test after the 2 week training program combined with two different recovery methods (cryostimulation or passive rest).

	Group	Baseline mean ± SD	Observed change mean ± SD (%)	Adjusted change[a] mean ± SD (%)	Adjusted effect[b]	
					mean;CI (%)	Inference[c]
Female						
CT 1 (points)	CON	19.7 ± 8.0	−14 ± 86	−14 ± 96	14	Small
	CRY	20.0 ± 8.0	−3 ± 76	−2 ± 55	−44 to 130	
CT 2 (points)	CON	19.7 ± 8.1	5 ± 28	10 ± 11	21	Small
	CRY	16.5 ± 6.6	46 ± 51	33 ± 23	−14 to 70	
SAT test (points)	CON	30.3 ± 6.5	−9 ± 110	−15 ± 122	6	Small
	CRY	36.5 ± 11.1	−10 ± 2	−10 ± 4	−50 to 121	
CMJ 1 (cm)	CON	35.4 ± 4.2	2 ± 9	2 ± 10	0	Trivial
	CRY	31.5 ± 5.3	7 ± 16	2 ± 14	−17 to 22	
CMJ 1 ($W \cdot kg^{-1}$)	CON	46.9 ± 4.6	−25 ± 7	−25 ± 8	30	**Large ↑******
	CRY	55.3 ± 3.4	−3 ± 6	−3 ± 7	17–45	
CMJ 2 (cm)	CON	33.1 ± 4.1	3 ± 10	3 ± 11	−2	Trivial
	CRY	31.7 ± 3.7	4 ± 12	2 ± 9	−13 to 11	
CMJ 2 ($W \cdot kg^{-1}$)	CON	63.2 ± 8.0	−12 ± 54	−6 ± 18	8	**Small ↑***
	CRY	48.7 ± 5.3	3 ± 10	2 ± 11	−5 to 22	
Male						
CT 1 (points)	CON	18.5 ± 3.0	−2 ± 23	7 ± 24	6	Trivial
	CRY	14.8 ± 5.8	22 ± 23	14 ± 7	−20 to 42	
CT 2 (points)	CON	17.5 ± 2.5	8 ± 35	16 ± 34	−22	Moderate
	CRY	17.2 ± 6.5	−6 ± 88	−9 ± 85	−59 to 51	
SAT test (points)	CON	28.0 ± 9.0	16 ± 37	14 ± 13	−30	**Moderate ↓*****
	CRY	36.0 ± 6.0	−9 ± 23	−21 ± 16	−44 to −13	
CMJ 1 (cm)	CON	53.4 ± 4.6	−6 ± 4	−6 ± 5	0	Trivial
	CRY	45.9 ± 3.2	2 ± 14	−6 ± 15	−22 to 28	
CMJ 1 ($W \cdot kg^{-1}$)	CON	61.0 ± 3.6	−18 ± 5	−19 ± 5	8	**Small ↑****
	CRY	55.3 ± 4.8	−5 ± 13	−12 ± 1	1–16	
CMJ 2 (cm)	CON	51.0 ± 6.0	0 ± 5	2 ± 4	−4	Small
	CRY	44.2 ± 4.2	7 ± 19	−2 ± 17	−24 to 21	
CMJ 2 ($W \cdot kg^{-1}$)	CON	59.0 ± 4.5	0 ± 3	2 ± 3	−3	Small
	CRY	52.2 ± 3.8	6 ± 12	−2 ± 10	−17 to 13	

*CT1, concentration test at rest; CT2, concentration test after interval; SAT, service accuracy test; CMJ 1, countermovement jump with arms on the hips; CMJ 2, countermovement jump with arms swing; CON, control; CRY, cryotherapy. CI, 90% confidence interval. All data are percentages, with the exception of baseline values expressed in measurement units. Inferences shown in bold are clear at the 98% level of confidence. [a]Adjusted to overall mean of in baseline CON and CRY group. [b]Adjusted mean change of CRY group minus adjusted mean CON group. [c]Magnitude thresholds (for difference in means divided by SD of CRY group): <0.20, trivial; 0.20–0.59, small; 0.60–1.19, moderate; >1.20, large. ↑ – increase; ↓ – decrease. Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial: * – possible, ** – likely, *** – very likely, **** – most likely.*

accuracy recorded as a statistically significant change. Changes in motor abilities and concentration test are shown in **Table 3**.

Outcomes achieved in countermovement jumps with arms on the hips revealed significant shifts in maximal power. In both groups, the training program resulted in a drop of power achieved in this drill. Still, this decrease was much more pronounced in the CON group participants, who had been subject to a passive recovery procedure. The adjusted effect was large and most likely in female participants and small and likely in male subjects. We have not presented the data obtained through interval drills as comparing results between players proved difficult. Gates used for interval drills were lighting up in random sequences in each drill; thus, each athlete completed a different sequence of moves, covering a different distance. We have, therefore, only presented cognitive-concentration test results (**Table 3**).

The Effect of the First Single Session of WBC

The single session of WBC did not alter significantly the concentration of irisin but induced a significant drop of IL-6 and BDNF levels in the CRY group. The adjusted effects for these changes ranged from small and likely for IL-6 to moderate and very likely for BDNF (**Table 4**). Interestingly, in CRY group, a pronounced drop of BDNF concentration (−44% ± 80) was noted in men, whereas in women, a significant increase (87% ± 183) was observed (data not shown).

TABLE 4 | The effect of first exposure to whole body cryostimulation or passive rest on immunological response and amino acid profile among all volleyball athletes.

	Group	Baseline mean ± SD	Observed change mean ± SD (%)	Adjusted change[a] mean ± SD (%)	Adjusted effect[b]	
					mean;CI (%)	Inference[c]
Irisin (ng·mL^{-1})	Control	9.3 ± 2.0	8 ± 40	6 ± 39	−7	Small
	Cryotherapy	9.7 ± 1.1	−5 ± 27	−2 ± 22%	−24 to 13	
IL-6 (pg·mL^{-1})	Control	1.1 ± 0.9	−38 ± 118	−37 ± 104%	−30	**Small ↓****
	Cryotherapy	1.0 ± 0.8	−55 ± 69	−56 ± 66	(57 to 13%	
BDNF (ng·mL^{-1})	Control	20.5 ± 6.4	31 ± 52	36 ± 51	−37	**Moderate ↓****
	Cryotherapy	20.9 ± 13.2	−4 ± 169	−14 ± 69	−57 to −7%	
FGF-21 (pg·mL(1)	Control	103.0 ± 124.7	−28 ± 101	−19 ± 92	(56%	**Moderate ↓*****
	Cryotherapy	202.0 ± 147.6	−66 ± 43	−64 ± 43%	−71 to −34	
IL-15 (pg·mL^{-1})	Control	2.1 ± 0.8	−28 ± 41	−25 ± 30	(10%	Small
	Cryotherapy	1.6 ± 0.9	−29 ± 64	−32 ± 53	−32 to 18%	
Myostatin (ng·mL^{-1})	Control	27.9 ± 11.3	16 ± 14	17 ± 15	−6	Trivial
	Cryotherapy	33.8 ± 8.5	13 ± 21	10 ± 22	−19 to 10	
IGF-1 (pg·mL^{-1})	Control	188.8 ± 34.5	−5 ± 107	−6 ± 106	9	Small
	Cryotherapy	199.4 ± 83.7	2 ± 32	2 ± 25	−28 to 64	
Valine (μmol·L^{-1})	Control	100.9 ± 25.9	5 ± 50	8 ± 25	27	**Moderate ↑****
	Cryotherapy	98.3 ± 29.9	41 ± 59	37 ± 44	2–59	
Leucine (μmol·L^{-1})	Control	101.8 ± 26.5	32 ± 57	26 ± 58	−3	Trivial
	Cryotherapy	125.8 ± 43.3	12 ± 42	23 ± 26	−29 to 33	
Isoleucine (μmol·L^{-1})	Control	117.5 ± 62.2	33 ± 51	33 ± 44	0	Trivial
	Cryotherapy	111.5 ± 41.0	33 ± 32	33 ± 28	−20 to 25	
Tryptophan (μmol·L^{-1})	Control	40.9 ± 11.2	31 ± 31	26 ± 30	−2	Trivial
	Cryotherapy	49.1 ± 14.3	18 ± 28	24 ± 24	−18 to 18	

*CI, 90% confidence interval. All data are percentages, with the exception of baseline values expressed in measurement units. Inferences shown in bold are clear at the 98% level of confidence. [a]Adjusted to overall mean of in baseline control and cryotherapy group. [b]Adjusted mean change of Cryotherapy group minus adjusted mean Control group. [c]Magnitude thresholds (for difference in means divided by SD of Cryotherapy group): <0.20, trivial; 0.20–0.59, small; 0.60–1.19, moderate; >1.20, large. ↑ – increase; ↓ – decrease. Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial: * – possible, ** – likely, *** – very likely, **** – most likely.*

The applied WBC protocol also affected the level of FGF21concentration. We recorded a significant drop in FGF21 1 h after the first, single WBC exposure. The adjusted effect was moderate and very likely (**Table 4**). We did not observe any significant changes in myostatin, IGF-1 or IL-15 in response to the first cryo-session. The amino acid profile also remained unchanged.

The Effect of the Whole Training Program Supported by Whole Body Cryostimulation or Passive Rest

The applied nine sessions of WBC resulted in a significant increase of the BDNF concentration in the CRY group, while in the CON group the opposite tendency was noted. The adjusted effect was moderate and likely (**Table 5**). Moreover, the adjusted effect for the shift in the level of BDNF in women athletes amounted to 97% (effect large and very likely), while in men, only a 20% rise was observed. Thereby, the effect of first session of WBC was maintained after the whole period of intervention (data not shown). The applied training program did not cause the concentration of irisin to shift regardless of the recovery procedure applied. A slight decrease of irisin was noted in both groups. No relationship was recorded between the level of irisin and BDNF.

The training program supported by WBC caused a rise of IGF-1 concentration, whereas in the CON group, a 22% drop was recorded. The adjusted effect for those changes was large and very likely. The intervention induced the drop of serum concentration of IL-15 in the CON group, yet caused the rise in the CRY group (**Table 5**). Still, when sex-dependent differences were considered, the level of IL-15 was found to have significant increased in men in the CRY group. The adjusted effect was moderate and very likely (data not shown in table). Interestingly, we have observed an inverse correlation between irisin and myostatin before and after the last session of WBC ($r = -0.40$ and $r = -0.42$, respectively). The observed changes were not statistically significant, yet in the CON group, a similar relationship had an opposite tendency and became more pronounced and significant ($r = 0.66$ and $r = -0.72$, respectively; $p = 0.05$ at the same time-points of blood collection).

Together with growth factors, we have evaluated the influence of training supported by two different recovery procedures on the amino acid profile. The combination of volleyball training and WBC protocol resulted in a significant drop of valine and tryptophan. The adjusted effect for these changes was moderate small and likely, respectively. The remaining amino acids assessed were not affected (**Table 5**).

Noteworthily, in the CRY group, the resting glucose level decreased significantly after the whole intervention. The adjusted

TABLE 5 | The effect of nine sessions of whole body cryostimulation or passive rest on immunological response and amino acid profile among all athletes.

	Group	Baseline mean ± SD	Observed change mean ± SD (%)	Adjusted change[a] mean ± SD (%)	Adjusted effect[b]	
					mean;CI (%)	Inference[c]
Irisin (ng·mL^{-1})	Control	9.3 ± 2.0	−6 ± 26	−6 ± 27	−2	Trivial
	Cryotherapy	9.7 ± 1.1	−11 ± 25	−8 ± 22	−17 to 15	
IL-6 (pg·mL^{-1})	Control	1.1 ± 0.9	−41 ± 71	−40 ± 44	32	Small
	Cryotherapy	1.0 ± 0.8	−18 ± 228	−21 ± 219	−38 to 180	
BDNF (ng·mL^{-1})	Control	20.5 ± 6.4	−32 ± 74	−33 ± 78	113	**Large ↑*** **
	Cryotherapy	20.9 ± 13.2	57 ± 112	43 ± 30	38–230	
FGF-21 (pg·mL^{-1})	Control	103.0 ± 124.7	−16 ± 125	−22 ± 125	7	Trivial
	Cryotherapy	202.0 ± 147.6	−37 ± 101	−17 ± 86	−42 to 100	
IL-15 (pg·mL^{-1})	Control	2.1 ± 0.8	−8 ± 41	−6 ± 40	13	Small
	Cryotherapy	1.6 ± 0.9	4 ± 43	6 ± 40	−15 to 50	
Myostatin (ng·mL^{-1})	Control	27.9 ± 11.3	15 ± 32	13 ± 33	−5	Trivial
	Cryotherapy	33.8 ± 8.5	4 ± 28	8 ± 28	−24 to 20	
IGF-1 (pg·mL^{-1})	Control	188.8 ± 34.5	−22 ± 22	−22 ± 23	45	**Large ↑*** **
	Cryotherapy	199.4 ± 83.7	14 ± 50	14 ± 35	17–79	
Valine (μmol·L^{-1})	Control	100.9 ± 25.9	3 ± 61	10 ± 36	−18	**Moderate ↓****
	Cryotherapy	98.3 ± 29.9	−7 ± 35	−10 ± 24	−34 to 1	
Leucine (μmol·L^{-1})	Control	101.8 ± 26.5	2 ± 56	−8 ± 38	−13	Small
	Cryotherapy	125.8 ± 43.3	−28 ± 64	−20 ± 53	−38 to 23	
Isoleucine (μmol·L^{-1})	Control	117.5 ± 62.2	−7 ± 66	−6 ± 46	2	Trivial
	Cryotherapy	111.5 ± 41.0	−4 ± 64	−5 ± 69	−31 to 51	
Tryotophan (μmol·L^{-1})	Control	40.9 ± 11.2	10 ± 27	5 ± 27	−14	**Small ↓****
	Cryotherapy	49.1 ± 14.3	−20 ± 41	−10 ± 23	−29 to 4	

*CI, 90% confidence interval. All data are percentages, with the exception of baseline values expressed in measurement units. Inferences shown in bold are clear at the 98% level of confidence. [a]Adjusted to overall mean of in baseline control and cryotherapy group. [b]Adjusted mean change of Cryotherapy group minus adjusted mean Control group. [c]Magnitude thresholds (for difference in means divided by SD of Cryotherapy group): <0.20, trivial; 0.20–0.59, small; 0.60–1.19, moderate; >1.20, large. ↑ – increase; ↓ – decrease. Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial: * – possible, ** – likely, *** – very likely, **** – most likely.*

change was equal −6% in the CON group, whereas in the CRY group, the shift was twofold- equal −12%. Alternations in glucose were not depended on sex. In addition, training supported by WBC induced changes in the lipid profile. The levels of HDL and TC dropped in the CON group, while in the CRY group, an increase of the level of HDL and a significant drop of the level of TC were noted. The adjusted effect for these shifts was trivial and possible for HDL and small and likely for TC (**Table 6**).

DISCUSSION

This study demonstrates that specific volleyball training supported by WBC caused a significant increase of the levels of growth factors like IGF-1 and BDNF. Still, physical performance became attenuated in response to the intervention, though the extent of the drop was smaller in the CRY group. The concentration of BDNF is known to grow in response to a single session of exercise as well as regular training. This neurotrophic factor improves cognitive functions; it can also act as a modifying factor enhancing glucose and fat uptake (Huh, 2018). Although in our group of participants a small deterioration of motor abilities was recorded, the volleyball training applied in conjunction of WBC resulted in a significant elevation of BDNF concentration. With the range of change more pronounced among women, it can explain the less pronounced deterioration of the serve accuracy as well as better results obtained in the concentration test in comparison to the CON group. Based on reports showing that extremely low temperatures cause the concentration of irisin to grow (Dulian et al., 2015) as well as that the skeletal muscle-derived irisin may be the link between physical activity and reward-related processes and motivation (Zsuga et al., 2016), we have evaluated changes of irisin and BDNF levels among our athletes. We assumed that low amount of fat tissue among our athletes would induce a bigger thermogenic effect, muscle shivering and finally, lead to an increase of irisin. Contrary to our expectations, we have not observed any changes in irisin concentration as a result of the applied intervention. Moreover we evaluated the impact of applied procedure on second myokine: IL-6 concentration. In both groups a decline of this myokine was recorded. Still, obtained results, in opposite to previous observations (Ziemann et al., 2012) indicated on ambiguous changes, thus further investigations are needed. A recently published review has summarized the particular role of irisin in glucose homeostasis. Data show that the elevated concentration of irisin enhanced glucose and fatty acid uptake by 30–40%. This increase in glucose uptake resulted from the upregulation of glucose transporter type 4 (GLUT4) expression, without significant changes in the expression of genes encoding insulin receptors (Perakakis et al., 2017). Results obtained in

TABLE 6 | Changes in glucose and lipid profile in response to 2 weeks of specific volleyball training supported by different recovery procedures.

	Group	Baseline mean ± SD	Observed change mean ± SD (%)	Adjusted change[a] mean ± SD (%)	Adjusted effect[b]	
					mean;CI (%)	Inference[c]
TCH ($mg \cdot dL^{-1}$)	Control	166.3 ± 20.3	−5 ± 5	−5 ± 5	−7	**Small ↓****
	Cryotherapy	165.5 ± 30.3	−9 ± 16	−11 ± 12	−13 to 0	
HDL ($mg \cdot dL^{-1}$)	Control	75.2 ± 22.4	−3 ± 9	−4 ± 9	5	**Trivial ↑***
	Cryotherapy	56.4 ± 11.6	0 ± 12	1 ± 13	−5 to 17	
LDL ($mg \cdot dL^{-1}$)	Control	76.0 ± 19.2	−5 ± 13	−6 ± 12	0	Trivial
	Cryotherapy	89.4 ± 26.5	−8 ± 28	−6 ± 19	−12 to 13	
TG ($mg \cdot dL^{-1}$)	Control	75.2 ± 21.8	−20 ± 16	−22 ± 15	−4	Trivial
	Cryotherapy	98.7 ± 55.4	−30 ± 79	−24 ± 40	−23 to 21	
Glucose ($mg\ dL^{-1}$)	Control	87.3 ± 5.7	−4 ± 8	−6 ± 5	−6	**Small ↓*****
	Cryotherapy	91.0 ± 5.1	−12 ± 5	−12 ± 5	−10 to −2	

*TCH, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride level. CI, 90% confidence interval. All data are percentages, with the exception of baseline values expressed in measurement units. Inferences shown in bold are clear at the 98% level of confidence. [a]Adjusted to overall mean of in baseline control and cryotherapy group. [b]Adjusted mean change of Cryotherapy group minus adjusted mean Control group. [c]Magnitude thresholds (for difference in means divided by SD of Cryotherapy group): <0.20, trivial; 0.20–0.59, small; ↑ – increase; ↓ – decrease. Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial: * – possible, ** – likely, *** – very likely.*

the current study revealed that although no direct correlation between irisin and glucose was observed, the applied training program supported by WBC treatment led to a significant drop of glucose level and affected the lipid profile. These results correspond with data published in our previous paper, which reported reduced blood cholesterol in young college men only in response to cold treatment (the same schedule of procedure) (Ziemann et al., 2014). We have, thus, concluded that the positive impact of our intervention on glucose level was most likely the effect of cold exposures. In addition, we also observed the significant drop of FGF21 in response to the first session of WBC treatment and the same tendency (still slightly less) was maintained after whole intervention. This myokine is also regulated by coldness therapy (Lee et al., 2014). Lee et al. (2014) noted that a cold treatment like water-infused thermoblankets (from 27°C cooled to 18°C and cooled further by 2°C every 3 min until 12°C temperature was reached) was found to have stimulated irisin secretion and a drop of FGF21. A drop of the FGF21 concentration implies activation of the sympathetic nervous system in the response to cold treatment. Therefore, changes in its level, in our opinion, have contributed to a better glucose uptake at the end of the intervention.

Together with irisin, we have evaluated changes of myostatin in response to our intervention. To the best of our knowledge, there is no study showing the effect of exposure to cold temperature on myostatin serum level in humans. In our experiment, we did not register any significant shifts in myostatin concentration in response to a single or to 10 sessions of WBC. However, the range of changes was smaller in the CRY group. Interestingly, the higher the increase of irisin noted, the lower the level of myostatin was. It is also worth of noting that although these correlations were not significant in the CRY group, the tendency was opposite and significant in the CON group. It has been shown that not only strength training but also aerobic training lead to a decrease in the production of myostatin in rat muscles (Heinemeier et al., 2007), yet contradictory results have been reported for humans. Walker et al. (2004) have demonstrated a decrease in plasma myostatin after 10 weeks of resistance training. On the other hand, some studies have found that a single bout of resistance exercise as well as a long-term strength training can lead to an increase in serum myostatin level in young males (Willoughby, 2004).

The rise of BDNF concentration was accompanied by the significant increase of IGF-1. As a result, in the CRY group, volleyball motor abilities deteriorated to a smaller extent than in the CON group. The elevated level of IGF-1 could have positively influenced muscle hypertrophy. Another myokine, IL-15, increased slightly in the CRY group, whereas the tendency in the CON group was opposite. In the male part of the CRY group, the effect was statistically significant. Rinnov et al. (2014) have observed up-regulation of IL-15 in response to endurance training and reported data suggesting that increased production of IL-15 may be involved in mediating training-induced muscle adaptation through mitochondrial biogenesis. Also, data presented by Tamura et al. (2011) have revealed a rise of IL-15 in response to 30-min treadmill running at 70% HR (max). Finally, a recently published set of data has shown that serum IL-15 was increased ∼5.3-fold immediately after resistance exercise, contributing to myofibrillar protein synthesis (Perez-Lopez et al., 2018). The volleyball training program applied in our study enhanced muscle endurance strength, while relying on rather low resistance workload.

Any application of diverse coldness methods with the intention of supporting recovery processes, especially in conjunction with specific training, should precisely monitor different effects observed throughout the treatment due to possible attenuation of adaptation training process. Such effect was registered by Roberts et al. (2015), who have revealed that resistance training supported by CWI attenuated adaptive changes in response to exercise. On the other hand, athletes who underwent CWI (10 min at 8°C) together with resistance training exhibited greater expression of PGC-1α and angiogenic

vascular endothelial growth factors (Joo et al., 2016). In our intervention, we implemented a training program focusing mainly on explosive strength. This program relied on low resistance and fast repetition to induce athletes' explosive power. The timing of training sessions was very demanding, and their intensity was very similar to the preparatory camps organized before main tournaments. On the one hand, participants complained about fatigue; on the other hand, the subjective range of experienced fatigue was smaller in the CRY group. General weariness translated into poorer results recorded in volleyball drills performed at the end of the intervention, yet the magnitude of this drop was lower in the CRY group. A longer training period, e.g., 6 or 16 weeks (Verma et al., 2015), which would have allowed for some recovery breaks, could have potentially proven more effective in boosting explosive power, however, further research would be required to verify this observations. Still, our study has limitations that warrant mention. We have examined the influence of cryostimulation only on short term effects on athletes' motor abilities. Future investigations should pursue studying long term outcomes of similar procedures. The same battery of tests should be executed >4 weeks after the training program combined with WBC or passive rest. Levels of growth factors would be also worth checking in order to establish whether cryostimulation can affect long term benefits in adaptation markers and hence, inhibit accumulated fatigue and reduced performance.

The additional aim of our study was to investigate the effect of the applied procedure on the amino acid profile. We decided to assess the concentration of branched amino acids according to their crucial role in muscle synthesis and tryptophan. We have observed that the tryptophan concentration dropped in response to training supported by WBC. The program combined with cryo-sessions led to an increase in the uptake of tryptophan in the CRY group, especially among women. This can provide an explanation for their better results in the serve accuracy and concentration tests compared to men. Kaluzna-Czaplinska et al. (2017) have elaborated extensively on tryptophan and its metabolites, emphasizing the role of tryptophan as a precursor of biologically active compounds such as serotonin and melatonin and showing that better uptake resulted in improved cognitive functions. Among other amino acids, we have noted a significant decrease of valine blood concentrations, however, this shift could have had a double meaning. On the one hand, just like other BCAAs, valine is associated with hyperglycaemia or insulin resistance (Lynch and Adams, 2014), thus, a decrease in valine could be associated with a boost of the overall metabolic profile—the change we recorded in our athletes. On the other hand, cryostimulation is known to be associated, at least as far as the first adaptation phase goes, with a mild haemolysis (Lombardi et al., 2013). Given that valine is essential for hematopoietic stem cell self-renewal (Taya et al., 2016), its mild decrease can be associated with an increased request from the bone marrow to restore haematopoiesis. The remaining branched amino acids exhibited the same tendency, with changes more pronounced in the CRY group. Although the effect was small, better uptake of those amino acids could have counteracted fatigue and deterioration of physical performance. As a result, in the CRY group physical capability was less affected than in the CON group.

Overall, our findings indicate that a specific volleyball training program induced fatigue, leading to a decline in the results achieved in the applied drill tests. Still, the magnitude of the drop was smaller in the CRY group compared to the CON group. At the same time, the applied protocol of recovery stimulated a higher increase of growth factors in the CRY group, which possibly counteracted further deterioration of physical performance. Our results characterize the response to the applied procedure as sex-dependent, however, the number of participants in our groups can be considered small. Therefore, we used a statistical model based on the effect size to validate our observations. Further research would be recommended to confirm our conclusions. To the best of our knowledge, most of the published papers up to date, evaluated the effect of WBC when associated to an exercise modality or middle intensity training, in particular endurance training. Our investigation considers the association of WBC with specific resistance-volleyball training and specific volleyball physical workload, what is rarely treated in literature.

ETHICS STATEMENT

The experiment was approved by the Bioethical Committee of the Regional Medical Society in Gdańsk (KB-25/14).

AUTHOR CONTRIBUTIONS

JJ, KM, MK, KW, LR, and JS designed the study and performed the research. JJ, AB, GL, and EZ performed the research and wrote the paper. ER, GL, KW, and JS performed the research.

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Article

Short-Term Resistance Training Supported by Whole-Body Cryostimulation Induced a Decrease in Myostatin Concentration and an Increase in Isokinetic Muscle Strength

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Abstract: The study aimed to determine whether combining cryostimulation with resistance training would effectively increase muscle strength, and if so, whether this adaptation would be related to changes in circulating levels of exerkines (i.e., mediators of systemic adaptation to exercise). Twenty-five students completed 12 sessions of resistance training, each followed by either cryostimulation (n = 15, 3 min exposure at −110 °C) or passive recovery (n = 10). Prior to and post this intervention, participants performed two eccentric cycling bouts (before and after training). At these points, serum concentrations of muscle damage marker (myoglobin), exerkines (interleukin 6 (IL-6), interleukin 15 (IL-15), irisin, brain-derived neurotrophic factor), hypertrophy-related factors (myostatin, insulin-like growth factor 1), and muscle strength were measured. The applied procedure reduced the physiological burden of the second eccentric cycling bout and myoglobin concentrations only in the group subject to cryostimulation. The same group also exhibited decreased levels of myostatin (from 4.7 ± 1.7 to 3.8 ± 1.8 ng·mL^{-1}, $p < 0.05$). A significant and large interaction between the group × time was noted in IL-15 concentration ($p = 0.01$, $\eta_p^2 = 0.27$). Training and cryostimulation induced a positive and likely significant improvement of isokinetic muscle strength. Altogether, obtained results support the claim that resistance training combined with cold exposure modified muscle strength through modulation of myostatin and IL-15 concentrations.

Keywords: adaptation; growth factors; myokines; irisin; recovery

1. Introduction

Resistance training is a form of physical activity particularly popular among exercise novices. Compared to running or cycling, however, it is more technically complex and risky for individuals unaccustomed to exercise. A lack of experience, limited motor control, or incorrect exercise technique runs the risk of injury when left unsupervised. At the same time, individuals may experience exercise-induced muscle damage (EIMD) [1], which can hinder exercising for several days, possibly discouraging beginners to maintain physical activity.

However, EIMD is an integral part of the high-intensity and resistance training process [2], which may be associated with delayed-onset muscle soreness (DOMS), muscle weakness, and a decreased range of motion. The terms EIMD and DOMS are often, although erroneously, used interchangeably. Indeed, EIMD can be, although not necessarily, the cause of DOMS when the intensity of exercise exceeds the level to which the subject is accustomed. On the other hand, muscle damage induced by low- and moderate-intensity exercise triggers the regenerative response that is not associated with soreness [3]. Ultimately, EIMD can affect physical performance with its effects lasting even for several days [4]; however, many studies showed that excessive EIMD, associated with eccentric work (also called negative muscle actions) [5] in resistance training protocols, did not promote additional gains in muscle strength [6]. EIMD is associated with high levels of circulating myoglobin, creatine kinase, and interleukin 6 (IL-6) [4]. This pro-inflammatory state in skeletal muscles stimulates expression and signaling of cytokines and growth factors, which, in turn, may trigger proliferation of satellite cells [7].

Among several interventions (including heat, manual therapy, and compression garments), cold treatment is a popular strategy to reduce EIMD symptoms [8]. Easily accessible, cold therapies such as ice bags, cold water immersion (CWI) [9], winter swimming [10], partial- and whole-body cryostimulation (CRY) [11,12] are particularly popular among professional sportsmen and late sport enthusiasts [13–15]. Nevertheless, recent reports have put forward a concept that resistance training combined with cold treatment may impair muscle adaptation to exercise. For instance, CWI (20 min at 10 °C) applied immediately after each session of moderate-intensity resistance training resulted in significantly lower gains in muscle strength and muscle thickness compared to passive rest [16]. The applied protocol lasted 6 weeks and consisted of 18 training sessions with a workload of about 70–80% of single-repetition maximum (1-RM) [16]. Furthermore, Roberts et al. reported attenuated muscle adaptation to long-term resistance training (12 weeks; 24 sessions; workload 8, 10, and 12 RM) [17], when training and CWI were combined (10 min at 10 °C). Nevertheless, data on how to use different cold procedures interchangeably are inconsistent. Costello and co-workers compared effects of a single CWI (4 min at 8 °C) and CRY session (3.40 min at −110 °C), and found that their impacts on muscle and core temperatures were similar, differing only in the aspect of skin cooling [18].

Recent reports indicated that cold therapies are not only effective in reducing inflammation and muscle pain after EIMD [19] but also modulate expression and release of a plethora of circulating growth factors [20]. What is more, cold exposure may change concentrations of amino acids, proteins released from muscle mass-myokines [20,21], and other proteins stimulated by exercise called “exerkines”, which are considered as the main mediators of systemic adaptation to exercise [22]. Broatch et al. reported neither positive nor negative effects of CWI combined with resistance training on the adaptation process, strongly emphasizing the importance of investigating different protocols of cold therapy during real-world resistance training [23]. The range of changes in growth factors and muscle functions in response to resistance training combined with cold treatment in beginners remains an open question.

In light of this background, the aim of this study was to investigate whether combining resistance training with cold treatment applied one day post the training session, would have an additional beneficial effect on muscle strength, muscle damage, and blood exerkine profile (such as interleukins (IL-6, IL-15), brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), myostatin, irisin) as they are the main mediators of systemic adaptation to exercise [24].

2. Materials and Methods

In our study, we assessed the effects of different recovery strategies during one month of strength training program among young, healthy participants, who had never undergone strength training. In the experimental group, we used whole-body cryostimulation (CRY), a day after each training unit, while the control group (CON) recovered passively. Muscle strength, adaptation to EIMD (based on the eccentric muscle actions), and blood exerkines concentration were tested at baseline and after completion of the training program. We considered the necessary number of training units in order to induce adaptive changes [6]. The study design is provided in Figure 1.

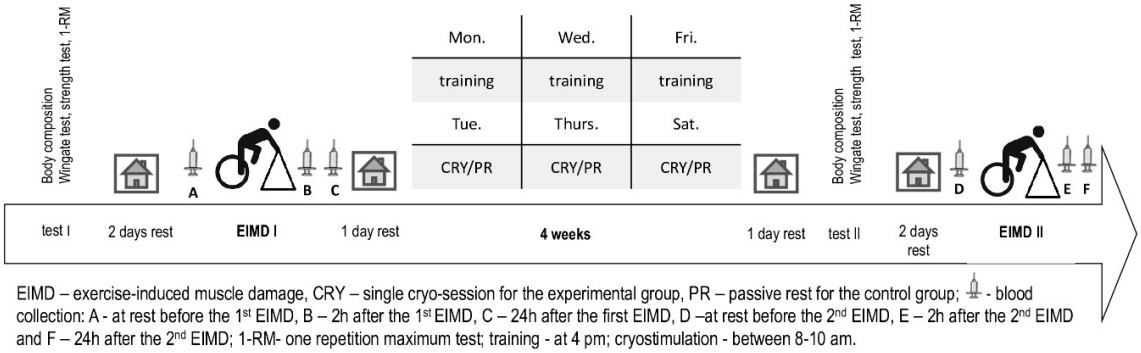

Figure 1. The schedule of the experiment.

2.1. Subjects

A total of 30 healthy (17 women and 13 men), untrained students, from the University of Physical Education, volunteered to take part in the investigation (age 20.3 ± 1.1, body weight 73.7 ± 11.7 kg). Twenty-five participants completed the whole intervention (5 students did not finish the experiment due to personal reasons). 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 consent to participate and underwent a medical examination. After performing the first EIMD, participants were randomly assigned to one of the two groups: the experimental group, that was treated with whole-body cryostimulation (CRY; n = 15, body mass index 23.7 ± 3.2 kg·m^{-2}) and the passively recovering control group (CON; n = 10, body mass index 23.2 ± 2 kg·m^{-2}). None of the participants had previous experience with CRY in the year preceding the study. The participants were asked to eat in the same place (open-access canteen in the dorm, where they were living) as well as not to take any supplementation during the study. Additionally, they were instructed by the professional dietitian to eat 1.2 g·kg^{-1} of protein per day. During the study, none of the subjects was involved in any organized or recreational physical activity, other than the protocol.

2.2. Ethics Statement

This study was approved by the Bioethical Committee of the Regional Medical Society in Gdansk KB-28/17. Before the study initiation, the participants received a verbal description of the experiment, and informed consent was obtained from all participants.

2.3. Test-Day Design

The subjects had to successively participate in the following tests, three days prior to commencing the experiment: anthropometric measurements, a muscle strength test, a 1-RM test (leg extension test, hamstring curl, and leg press). Additionally, in order to establish the individual intensity of eccentric cycling exercise on Cyclus2, the maximal anaerobic power (Wingate Anaerobic Test (WAnT)) was determined. WAnT was performed on a mechanical cycle ergometer (Monark Ergomedic 884E Sprint Bike, Sweden) The protocol started with the standardized 5 min warm-up (1.0 W·kg^{-1} of body mass) including two all-out sprints (of approximately 5 s each). After the warm-up, the participants

took 2 min of rest before performing the 30 s all-out supramaximal concentric work. The applied external load equaled 7.5% of the individual body mass. The subjects were instructed to pedal at their maximum rate throughout the test [25]. After two days of rest, our participants performed the single bout of eccentric work on a cycling ergometer (negative work) [5]. Then, all of them participated in training program and underwent the specific recovery method. The day after the last training session, the participants repeated the mentioned measurements (Figure 1).

2.4. Anthropometric Measurements

Body mass and composition were estimated using a multi-frequency impedance plethysmography body composition analyzer (In Body 720, Biospace, Korea). Body mass was measured after an overnight fast, 12 h after the last meal and drink [26].

2.5. Muscle Strength Assessment

Isometric and isokinetic knee muscle functions were measured using the Biodex System 4 dynamometer (Biodex Medical System, Inc. Shirley, NY, USA). All tests were conducted in the sitting position (with the hip joint at an angle of 90°) with the arms folded across the chest, or the hands clasped in front of the body, and the trunk and lower limbs stabilized with a belt. The participants were asked to hold their tongue extended up to the palatine spot during the execution of all tests [27]. The subjects received standardized verbal instructions before the test and verbal encouragement throughout. Measurements of the peak torque were obtained for the extension of the knee joints (of both legs, separately) in conditions of a 5 s isometric contraction. The subjects were asked to contract "as hard as possible" (to the maximum) to obtain their maximal peak torque. Each peak torque measurement was obtained 3 times with a 1 min break between them. The extension of the knee was measured during isometric contractions at 90° of knee flexion. After completing the isometric strength test on both legs, the participants immediately proceeded to the isokinetic strength testing of the knee extensors. This test was performed at a velocity of $90°{\cdot}s^{-1}$ (Nm) and repeated 3 times. Measurements of the peak torque and average power were analyzed in isokinetic conditions

2.6. Muscle Adaptation Assessment

In order to assess muscle adaptation to resistance training and evaluate the tolerance to EIMD, both groups completed the two sessions on an eccentric cycle ergometer (Cyclus2 Eccentric Trainer, Germany) (Figure 1). Two months before the main experiment, we had established the exercise protocol (intensity and duration) to induce muscle damage. To avoid muscle adaptation to eccentric contraction, a different group of volunteers (with similar anthropometric features and physical abilities and in the same age range) participated in the eccentric cycling trail sessions. They also received the information about the experiment and they signed an informed consent. Based on subjective feedback of feeling pain, values of serum creatine kinase activity and myoglobin concentrations (and mutual colorations between them), 50% of maximal anaerobic power was chosen for the main experimental session as workload.

In this study, eccentric cycling began with 1 min of initiation/induction at a workload of $1.5\ W{\cdot}kg^{-1}$, followed by 10 min of exercise with a pedaling cadence ranging between 50 and 65 $rev{\cdot}min^{-1}$, at an individual workload of 50% of the values of maximal anaerobic power output. To assess the physiological cost of exercise, the heart rate (HR) was monitored throughout the exercise (Garmin, Olathe, KS, USA). Muscle pain in the lower limbs was assessed using the visual analog scale (VAS), in which 0 represents the feeling of no pain and 10 represents the feeling of maximal pain. The participants gave their subjective rating of muscle soreness immediately, 2 h, and 24 h after performing the bouts of EIMD on the Cyclus2.

2.7. Training Program

The short-term strength training program, focused on the lower limbs, was based on the latest guidelines of the American College of Sports Medicine [28]. All subjects attended 12 training sessions

at the gym, during the 4-week program. Training took place 3 times a week (on Monday, Wednesday, and Friday); each session started at 4 pm, lasted approximately 50 min and included an individual 15 min warm-up: 5 min on a cycle ergometer (XC530 York Fitness, Cardiofit 220 p, Daventry, UK), and 10 min of dynamic stretching; the main part (individual workload); and stretching (5 min of static stretching exercises for the lower limbs). The 1-RM strength test of a leg extension, hamstring curl, and leg press was determined by the use of the protocol from Ammar et al. [29], and it was assessed before, half-way through, and at the end of the program, in order to determine/update the individual load for subjects. This exercise mainly targeted the hamstrings, quadriceps, and gluteus maximus muscles. The leg press exercise was executed with a protocol according to Padulo et al. [30]. Leg extension, hamstring curl, and leg press were performed in the same order, during each training session. All training sessions were performed under the supervision of an experienced trainer, specialized in motor preparation.

The training exercise load during the first two weeks was 70% of 1-RM. The participants performed 3 sets in the first week and 4 sets in the second one. Each set consisted of 8 repetitions, a 3 min rest between sets in the first week and a 2.5 min break in the second week. The last two weeks of the training protocol consisted of 80% of 1-RM load, 3 sets × 6 repetitions in the third week (3 min rest between sets) and 4 sets × 6 repetition in the fourth week (2.5 min rest between sets).

2.8. Whole-Body Cryostimulation

The CRY group participated in 12 sessions of cold exposure in a cryogenic chamber at the Pomeranian Rheumatology Center in Sopot, Poland. Cryostimulation was conducted 3 times per week (on Tuesday, Thursday, and Saturday) between 8 and 10 a.m., under medical supervision. All sessions lasted 3 min at the temperature of −110 °C. Each entry to the cryogenic chamber was preceded by an adaptation period in the vestibule, at the temperature of −60 °C (for approximately 30 s). Participants wore shorts, socks, and gloves, and hats were covering their auricles [11].

2.9. Blood Samplings and Biochemical Assays

Blood samples were taken at rest, 2 h, and 24 h after each EIMD bout (Figure 1). Samples were collected from the antecubital vein into tubes for single use. All samples were immediately placed at 4 °C (after clotting, for serum-separating tubes); they were centrifuged at 2000× *g* for 10 min. Serum aliquots were stored at −80 °C. Serum concentrations of IL-6, IL-15, BDNF, IGF-1, and myostatin were measured using sandwich ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol (catalog no. HS600B, D1500 DBD00, DG100, and DGDF80, respectively). Serum irisin concentration was determined using a competitive enzyme immunoassay kit from Phoenix Pharmaceuticals, Inc. (Burlingame, CA, USA, catalog no. EK 067-29). To establish muscle damage extent after EIMD bouts, we decided to measure the serum myoglobin level [31], which was determined by ELISA (Wuhan EIAab Science Co. Wuhan, China, catalog no. E048h).

2.10. Statistical Analysis

Statistical analysis was performed using the Statistica 13.1 software (StatSoft, Tulsa, OK, USA). All values are expressed as mean ± standard deviation (SD) and 95% confidence intervals (95% CI). The Shapiro–Wilk test was applied to the data to assess the homogeneity of dispersion from the normal distribution. The Brown–Forsythe test was used to evaluate the homogeneity of variance. Then, separate 2 (group: CRY, CON) × 3 (time: PRE, after 2 h, and 24 h) repeated analyses of variances (ANOVA) were performed. In case of a significant time × group interaction for variables that did violate the normality assumption, ANOVA for repeated measurements and Tukey's post hoc honest significant difference test for unequal sample sizes were performed to identify significantly different results; for variables that did not violate the normality assumption, the ANOVA Friedman test and 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 [32]. Standardized mean differences (SMD) were calculated for pairwise comparison. The magnitude of the SMD was classified according to the following scale: 0–0.19 = negligible effect; 0.20–0.49 = small effect; 0.50–0.79 = moderate effect; and ≥0.80 = large effect. The probability for an effect being practically worthwhile was calculated following the magnitude-based inference approach, using the following scale: 25–75%, possibly; 75–95%, likely; 95–99.5%, very likely; >99%, most likely. Default probabilities for describing the effect as practically beneficial were set at <0.5% (most unlikely) for harm and >25% (possibly) for benefit.

3. Results

3.1. Anthropometric Measurement

No relevant differences between groups, at baseline, in anthropometric features: body weight 73.7 ± 11.7 kg (95% CI: 68.8–78.6); skeletal muscle mass 32.9 ± 7.5 kg (95% CI: 29.8–36.1); or fat mass 15.3 ± 6.9 kg (95% CI: 12.4–18.2) were noted. Those parameters remained unchanged during the entire observation. The only exception was the visceral fat area. This parameter dropped significantly, among all subjects (from 55.2 ± 24.4 cm^2, 95% CI: 44.9–65.5 to 51.5 ± 22.5 cm^2, 95% CI: 42.0–61.0).

3.2. Physiological Cost of EIMD, Performed before and after the Intervention

All participants performed the first bout of EIMD with an average workload of 331 ± 88 W. At that time, the average pedaling force reached 290 ± 78 N. The main task for subjects was to maintain the exercise intensity. The physiological response, expressed as the HR, gradually increased from 148 ± 37 bpm in the second minute of the exercise, to 162 ± 49 bpm at the end of the bout. After 4 weeks of resistance training and cryostimulation (or passive recovery), the second bout of EIMD was performed with an average workload of 337 ± 85 W. The average HR recorded at the beginning of EIMD was lower (135 ± 36 bpm); yet, changes depended on the group. Differences between the groups' physiological cost of exercise were especially visible in the eighth minute of EIMD, when the CRY group maintained their HR at 157 ± 12 bpm, and the CON group at 171 ± 9 bpm (Figure 2). The average pedaling force in the CRY group increased to 348 ± 93 N, whereas in the CON group, the average force dropped to 294 ± 93 N (Figure 2).

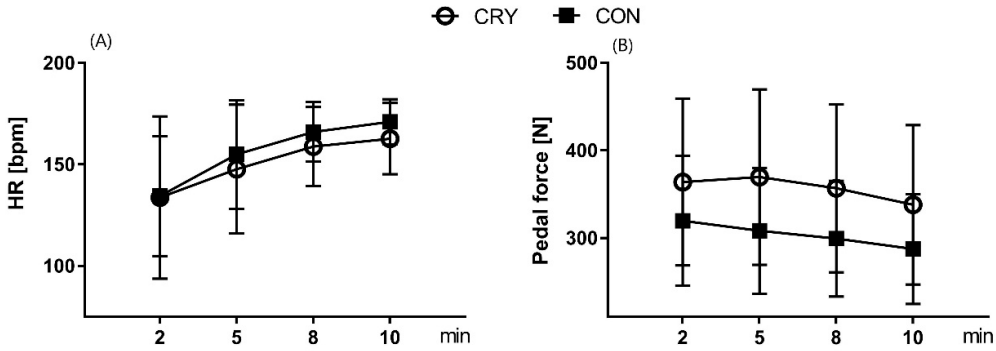

Figure 2. Changes of heart rate (HR) (**A**) and pedal force (**B**) during the second bout of exercise-induced muscle damage. Values between groups were significant different ($p < 0.05$), CRY—cryotherapy group, CON—control group.

3.3. Exercise-Induced Muscle Damage

The first bout of eccentric cycling induced muscle damage in all participants. This observation was based on a high level of pain sensation and muscle stiffness as well as on serum myoglobin levels, which were noticed 2 h after the exercise and persisted for 24 h after finishing the bout. Subjects in both groups assessed their pain using the VAS scale as 3 ± 2, 2 h directly after the exercise and 6 ± 2 24 h after finishing the bout. The recorded pain sensation did not correspond to an average increase of the serum myoglobin level. The first bout of EIMD induced a significant rise of myoglobin among all

participants (baseline vs. 2 h post $p = 0.05$, baseline vs. 24 h post $p = 0.00$) (Figure 3). After 4 weeks of training in the CON group, a significant increase of myoglobin was observed (baseline vs. 2 h post $p = 0.02$, baseline vs. 24 h post $p = 0.01$), whereas the CRY group was characterized by low myoglobin, which remained unchanged (Figure 3). The second bout of EIMD did not trigger a similar perception of pain. Compared to the first bout, both groups experienced a lower perceived pain 24 h post-exercise. However, on the VAS scale, perceived pain was still higher in the CON group (5 ± 2) compared to that in the CRY group (2 ± 3). Differences were significant ($p = 0.05$).

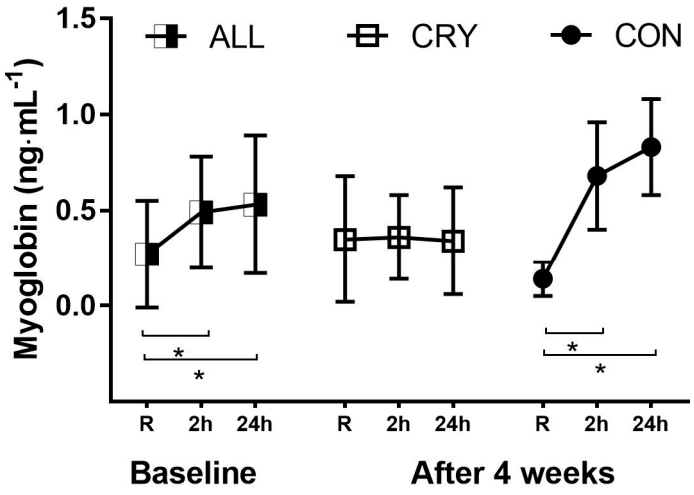

Figure 3. Myoglobin serum concentration at rest and recorded 2 and 24 h post the second bout of exercise-induced muscle damage. Differences between measurements were significant ($p < 0.05$), CRY—cryotherapy group, CON—control group.

3.4. Exerkine Response to Training and Treatment

The first bout of eccentric exercise did not cause significant changes in the concentration of assayed myokines and growth factors. Due to the fact that cryotherapy sessions were introduced just one day after performing the first EIMD, those values are not presented.

The applied training response varied between the CRY and CON groups expressed in resting values of measured indicators (Figure 4). There was a significant and large interaction between group × time after 4 weeks of training for myostatin ($p = 0.03$, $\eta_p^2 = 0.20$) and IL-15 ($p = 0.01$, $\eta_p^2 = 0.27$), (Figure 4b,c). A similar tendency was registered for irisin (Figure 4e), but the effect was not significant ($p = 0.72$, $\eta_p^2 = 0.00$). The IGF-1 level decreased in both groups; however, the magnitude of change was more pronounced in the CON group ($p = 0.57$, $\eta_p^2 = 0.01$) (Figure 4f). Levels of IL-6 and BDNF remained unchanged across the observation ($p = 0.10$, $\eta_p^2 = 0.11$; $p = 0.61$, $\eta_p^2 = 0.01$), respectively) (Figure 4a,d).

The correlations among the changes in muscle function and IGF-1 were investigated. The growth factor IGF-1 positively correlated with the peak torque maximal isokinetic $90°{\cdot}s^{-1}$ (Nm) (r = 0.62, $p = 0.04$), and the average power isokinetic $90°{\cdot}s^{-1}$ (Nm) (r = 0.54, $p = 0.04$). In the CON group, the correlations had an opposite tendency and were not significant (r = −0.32, $p = 0.37$; r = −0.28, $p = 0.37$, respectively).

The second bout of EIMD triggered different responses in exerkines and growth factors, depending on the recovery strategy applied. This effort induced a significant increase of myostatin concentration in the CON group (Table 1), while in the CRY group, myostatin was unaffected over the entire post-exercise observation (the difference between groups was significant, $p = 0.27$, $\eta_p^2 = 0.05$). The second bout of EIMD triggered a significant drop of BDNF in all participants (noticed 2 h as well 24 h post exercise), still the magnitude of this change was greater in the CON group in comparison to that in CRY ($p = 0.00$, $\eta_p^2 = 0.16$). A similar trend was noted in IGF-1 concentration, but in this case, the magnitude of change was not that noticeable ($p = 0.86$, $\eta_p^2 = 0.01$). A different IL-15 response was noted among two

groups. In the CRY group, IL-15 concentration increased only 2 h after the last eccentric bout, whereas its concentration decreased in the CON group and remained low, until 24 h after EIMD ($p = 0.21$, $\eta_p^2 = 0.07$). Changes in the irisin levels were not significant ($p = 0.57$, $\eta_p^2 = 0.02$) (Table 1).

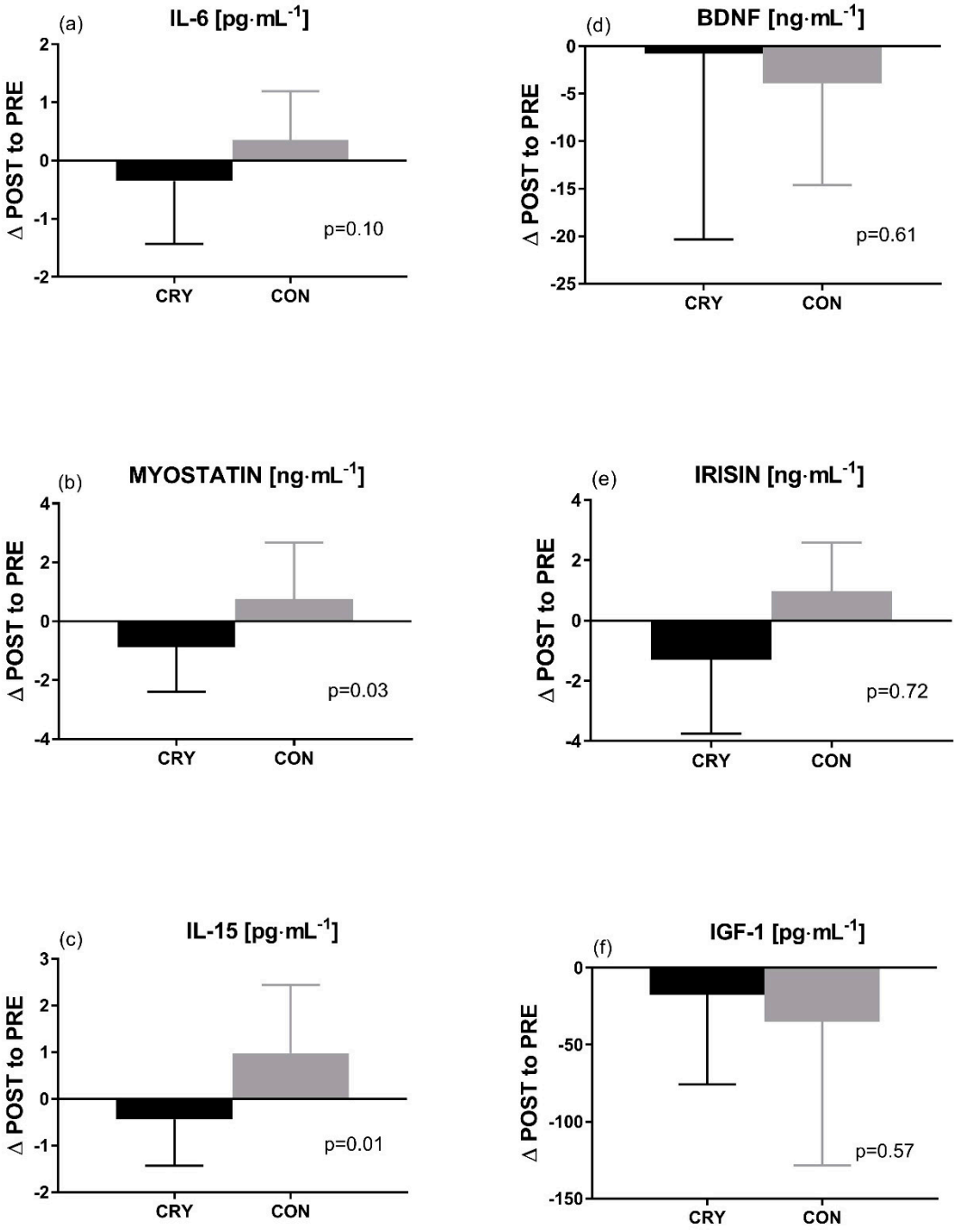

Figure 4. Delta changes (Δ POST to PRE) in serum myokine and growth factor concentrations at rest: before and after 4 weeks of resistance training combined with whole-body cryostimulation or passive rest; (**a**) interleukin 6 (IL-6), (**b**) myostatin, (**c**) interleukin 15 (IL-15), (**d**) brain-derived neurotrophic factor (BDNF), (**e**) irisin, (**f**) insulin-like growth factor (IGF-1); POST—post-intervention values, PRE—pre-intervention values, CRY—cryotherapy group, CON—control group; $p < 0.05$. The probability of an effect being practically worthwhile was calculated according to the magnitude-based inference method.

Table 1. Exerkines concentration before and after the second bout of exercise-induced muscle damage performed after 4 weeks of resistance training combined with cryostimulation or passive rest.

Exerkines	Group	PRE Second EIMD		POST After 2 h		SMD	MBI	POST After 24 h		SMD	MBI	ANOVA p (η_p^2)
		$\overline{X} \pm SD$	95% CI	$\overline{X} \pm SD$	95% CI	Cohen's d	Practically Worthwhile Effect	$\overline{X} \pm SD$	95% CI	Cohen's d	Practically Worthwhile Effect	Group × Time Interaction
IL-6	CON	1.3 ± 0.6	0.9–1.7	1.1 ± 0.5	0.7–1.5	0.36	possible	0.9 ± 0.4	0.6–1.2	**0.80**	unclear	0.42 (0.04)
(pg·mL^{-1}) †	CRY	1.4 ± 1.1	0.8–2.0	1.4 ± 1.1	0.8–2.0	0.00		0.9 ± 0.7	0.5–1.3	**0.56**		
Myostatin	CON	5.1 ± 1.8	3.8–6.4	6.2 ± 1.2	5.3–7.1 *	**0.73**	likely	5.7 ± 1.4	5.0–6.4	0.38	likely	0.27 (0.05)
(ng·mL^{-1}) †	CRY	3.8 ± 1.8	2.8–4.8	3.8 ± 2.2	2.6–5.0	0.00		3.9 ± 1.1	3.3–4.5	0.07		
IGF-1	CON	173.6 ± 69.6	123.8	167.8 ± 68.7	118.7–216.9	0.08	unclear	162.2 ± 77.2	107.0–217.4	0.16	unclear	0.86 (0.01)
(pg·mL^{1}) †	CRY	122.2 ± 50.7	94.1–150.3	118.8 ± 42.9	95.0–142.6	0.07		115.8 ± 44.4	91.2–140.4	0.13		
IL-15	CON	1.7 ± 1.4	0.7–2.7	1.1 ± 0.9	0.5–1.7	**0.52**	possible	0.9 ± 0.8	0.3–1.5	**0.73**	unclear	0.21 (0.07)
(pg·mL^{-1}) †	CRY	1.2 ± 0.6	0.9–1.5	1.6 ± 1.7	0.7–2.5	0.35		1 ± 0.6	0.7–1.3	0.33		
BDNF	CON	42.6 ± 14.6	32.2–53.0	25.4 ± 9.6 *	18.5–32.3	**1.42**	unclear	37.3 ± 6.1 *	32.9–41.7	**0.51**	likely	**0.00 (0.16)**
(ng·mL^{-1})	CRY	40.4 ± 9.6	35.1–45.7	32.8 ± 13.4 *	25.4–40.2	**0.66**		32.1 ± 12.9 *	25.0–39.2	**0.74**		
Irisin	CON	3.8 ± 4.5	0.6–7.0	3.6 ± 4	0.7–6.5	0.05	possible	3.2 ± 3.2	0.9–5.5	0.16	possible	0.57 (0.02)
(ng·mL^{-1}) †	CRY	1.8 ± 1.7	0.9–2.7	1.9 ± 1.7	0.9–2.8	0.06		1.8 ± 1.7	0.9–2.7	0.00		

Note: Values are presented as mean ± SD; † nonparametric analysis; 95% CI—confidential interval, CON—control group, CRY—cryotherapy group; EIMD—exercise-induced muscle damage; * significant differences from pre-second EIMD level, $p < 0.05$. Moderate to large standardized mean differences (SMD) have been highlighted in bold. Eta-squared effects (η^2_p) for interaction are presented in brackets. The probability of an effect being practically worthwhile was calculated according to the magnitude-based inference (MBI) method. Unclear effect means that more data are needed to assess the intervention effect.

3.5. Changes in Muscle Strength after the Intervention

The applied procedure of resistance training resulted the improvement of workload at 1-RM in all participants (20% for hamstring-curl, 19% for leg-extension, and 11% for leg press).

The effect of the training program associated with different recovery strategies on strength in isometric and isokinetic conditions is shown in Table 2. Training combined with CRY induced significant increase of maximal average power in the knee isokinetic extension strength test, SMD: 0.42 (left leg), 0.32 (right leg), while in the control group the level of strength has remained stable, SMD: 0.11 (left leg), 0.03 (right leg). Similar, but not significant, changes have been observed in maximal peak torque in the knee isokinetic extension strength test, SMD: 0.18 (left leg), 0.15 (right leg), where at the same time, in the control group, a decrease has been noted, SMD: 0.06 (left leg), 0.22 (right leg). An adverse tendency was registered in isometric strength.

Table 2. The effect of 4 weeks of resistance training combined with different recovery procedures on extension maximal isometric and isokinetic measurements.

		Pre Intervention Level		Post Intervention Level		SMD	ANOVA p (η_p^2)	MBI
		$\overline{X} \pm SD$	95% CI	$\overline{X} \pm SD$	95% CI	Cohen's d	Group × Time Interaction	Practically Worthwhile Effect
PT max isometric [Nm] extension								
Left leg	CON	207 ± 56	167–247	208 ± 59	166–250	0.02	0.08 (0.13)	possible
	CRY	222 ± 71	183–261	208 ± 63 *	173–243	0.21		
Right leg	CON	201 ± 60	158–244	203 ± 63	158–248	0.03	0.69 (0.01)	unclear
	CRY	217 ± 58	185–249	215 ± 59	182–248	0.03		
PT max isokinetic 90° s^{-1} [Nm] extension								
Left leg †	CON	144 ± 32	121–167	142 ± 31	120–164	0.06	0.17 (0.08)	likely
	CRY	167 ± 46	142–192	175 ± 41	152–198	0.18		
Right leg	CON	156 ± 39	128–184	148 ± 33	124–172	0.22	0.05 (0.16)	likely
	CRY	166 ± 46	141–191	173 ± 45	148–198	0.15		
AP max isokinetic 90° s^{-1} [W] extension								
Left leg †	CON	129 ± 27	110–148	132 ± 27	113–151	0.11	0.13 (0.10)	very likely
	CRY	153 ± 53	124–182	173 ± 43 *	149–197	0.42		
Right leg	CON	141 ± 31	119–163	142 ± 29	121–163	0.03	0.10 (0.11)	likely
	CRY	157 ± 51	129–185	173 ± 50 *	145–201	0.32		

Note: Values are presented as mean ± SD; † nonparametric analysis; 95% CI—confidential interval, PT max—peak torque maximal, AP max—average power maximal, CON—control group, CRY—cryotherapy group; * significant differences from pre-intervention level, $p < 0.05$. Moderate-to-large standardized mean differences (SMD) have been highlighted in bold. Eta-squared effects (η^2_p) for interaction are presented in brackets. The probability of an effect being practically worthwhile was calculated according to the magnitude-based inference (MBI) method. Unclear effect means that more data are needed to assess the intervention effect.

4. Discussion

To the best of our knowledge, this is the first study to evaluate the impact of short-term resistance training combined with a whole-body cryostimulation protocol, on muscle strength and circulating biomarkers related to muscle damage and recovery. Data recorded after the 4-week intervention demonstrated improvement in average power and isokinetic extension muscle strength. These changes were accompanied by shifts in exerkines concentrations: a drop in myostatin and a growth in IL-15 concentration in the participants subject to both resistance training and cold treatment. Morton et al. found recently that an acute response of circulating levels of growth factors did not correlate with functional changes in muscles (i.e., strength) following resistance training [33]. This suggests other factors must have contributed to the training-induced adaptations. The current study, for the first time, revealed that the effectiveness of whole-body cryostimulation is manifested in a lower circulating level of myostatin, a negative regulator of muscle hypertrophy [34].

Burd et al. have reported [35] that a workload of as little as 30% and up to 90% of 1-RM played a minimal role in stimulating muscle protein synthesis and altered greater motor unit recruitment. Conversely, low temperatures are known to result in lower nerve conduction velocities [36]. In our study, isokinetic muscle strength increased. Since no changes in muscle mass were recorded, this increase can be connected with better motor unit recruitment.

At the same time, positive changes in muscle strength were accompanied by a drop of myostatin. The effects of this protein are mediated by inhibition of the Akt kinase activity, which can lead to the FOXO3a transcription factor being activated, which in turn induces expression of atrogin-1 encoding for a protein strongly linked to muscle atrophy [37]. A decline in myostatin was recorded after 4 weeks of strength training and cold treatment as well as within 2 and 24 h after the second bout of EIMD. Literature has characterized the influence of exercise on myostatin concentration as ambiguous. Kazemi et al. reported that a single session of high-volume circuit resistance training resulted in a significant decrease in plasma myostatin 24 h after exercise [38]. Contrarily, according to Willoughby et al., a single session of eccentric muscle actions caused an increase in serum myostatin concentration with peak values recorded 24 h post exercise [39]. Such divergence of effects was also reported in response to regular resistance training. A total of 4 weeks of whole-body resistance training caused a significant decline in myostatin levels, independently of the administered creatine supplementation [40]. At the same time, Willoughby documented increased myostatin concentrations after 12 weeks of lower-body resistance training [41]. A recently published paper indicated that a 3 h exposure to 7 °C temperature did not affect myostatin gene expression [42]. In a separate study, the aforementioned protocol of whole-body cryostimulation, combined with specific volleyball training, had no impact on myostatin concentration [20]. Therefore, we concluded that the specific response to resistance training and cold treatment depended on the presence of a specific exercise stimulus. Alternatively, a drop in myostatin concentration could have been connected with the discovery by Kong et al., who showed that skeletal muscle and brown adipose tissue (BAT) are functionally interlinked [43]. They described an intriguing role of the transcription factor interferon regulatory factor 4 (IRF4) in BAT, whereby it had mediated BAT-muscle crosstalk through myostatin. Thermoneutrality or loss of IRF4 were found to have resulted in elevated serum myostatin levels and decreased exercise capacity [43]. Although, we did not measure BAT, exposure coldness is known to considerably affect its metabolic activity [44].

Previously published data revealed that myostatin can also modulate metabolic homeostasis by regulating adipose tissue function [45]. In his review, Huh argued that myostatin modified irisin concentration, acting through adipocyte browning and subsequent induction of energy expenditure [24]. In our investigation, irisin was not affected in response to cryostimulation, contrarily to previous observations [46]. These results may be linked to the lower levels of adipose tissue exhibited by the subjects of the current study, or even the frequency of whole-body cryostimulation (3 times per week) in combination with the training program.

In the present study, a decrease in myostatin levels in the CRY group was accompanied by a decline in IGF-1. In a previous study, an increased level of IGF-1 was recorded after a single session of acute resistance exercise as well as in response to 8 weeks of resistance training [47]. According to recent findings, myostatin and IGF-1 regulate skeletal muscle size and myofiber type expression through different mechanisms [48]. In response to the applied intervention, these two hormones followed opposite trends of change, ascending and descending, respectively, depending on the recovery strategy applied. In myostatin null mice, a decreased *Igf-1* mRNA expression in skeletal muscles was observed, with the difference being greater in younger than in older mice [49]. Based on these findings, it was suggested that myostatin regulated hyperplasia, while IGF-1 regulated hypertrophy of myofibers, with the two processes being temporarily separated [49]. It is worth considering that this decrease of IGF-1 occurred due to an increase of IGF-binding proteins (IGFBPs). The same researchers demonstrated that myostatin knock-out caused expression of *Igfbp5* mRNA to grow [49] despite previous studies reporting contradictory results [50]. In the context of whole-body cryostimulation, the treatment is associated with an increase of blood catecholamine levels [51], which in turn is

associated with an increase of IGFBP-1 in humans [50]. The lack of IGFBP-1 in blood analysis can, thus, be considered as a limitation of this study and should be included in future investigations.

Although muscle mass was not affected in response to the applied training program, significant changes were noted in IL-15 concentration among the CRY subjects. IL-15 was shown to have an impact on muscle metabolism and hypertrophy [24]. Perez-Lopez et al. demonstrated that a single session of 4 sets of leg press and leg extension at 75% 1-RM stimulated the IL-15/IL-15Rα signaling pathway together with an elevated serum concentration of IL-15. They also observed that the activation of this signaling pathway supported myofibrillar protein synthesis [52]. Nevertheless, this was only the effect of a single unit of exercise without any recovery-supporting method. In a previous investigation, a specific volleyball training program combined with cryostimulation did not significantly affect the concentration of IL-15 [20]. In our study, 12 units of regular resistance training with a workload comparable to the Perez-Lopez study, but combined with cryostimulation, caused a significant increase of IL-15 resting concentration. The obtained results may indicate that cold treatment did not attenuate synthesis of IL-15.

We also measured BDNF concentration—an exercise-induced growth factor with a major impact on the nervous system including a protective role for the central and peripheral neurons [53]. Some studies, however, highlighted the metabolic role of BDNF in regulating energy homeostasis [54], which may have a positive effect on skeletal muscle adaptation to resistance training. Despite this, resistance training was reported not to have affected the basal circulating level of BDNF in comparison to aerobic training [55]. A recently published investigation confirmed this observation [56]. Until now, only one study observed an increase of BDNF concentration in response to volleyball training combined with whole-body cryostimulation [20]. Results obtained in this study indicate that resistance training combined with whole-body cryostimulation did not affect the BDNF level, which can suggest that neither resistance training alone nor in combination with cold treatment affects BDNF concentration.

Previous studies showed that submaximal-intensity eccentric training, performed for 4–8 weeks in between acute EIMD bouts, contributed to an ameliorated systemic response to the second EIMD bout in young active participants [57,58]. Our results show that resistance training may have had a similar effect. The increased muscle strength would have also contributed to a reduced physiological cost of the second bout of EIMD. The CRY group exhibited lower values of myoglobin concentration than those recorded in the CON group, which agrees with previously published studies showing that whole-body cryostimulation may reduce circulating enzymes related to muscle damage, such as creatine kinase and lactate dehydrogenase [59,60]. Our study examined 12 sessions of high-intensity resistance training in combination with either whole-body cryostimulation or passive recovery. According to a review by Damas, the applied number of training units could have been insufficient to induce muscle hypertrophy [6]. However, our data suggest that cryostimulation was effective in lowering the concentration of myostatin in the early phase of resistance training and, hence, allow to conclude that the number of training units applied may have been sufficient to induce muscle remodeling and to trigger the process of muscle adaptation to training.

In the context of the reported results and findings, limitations of the study should be discussed. Firstly, the analyses' week's training program only showed the first step of adaptation to a training process; hence, the impact of long-term resistance training combined with whole-body cryostimulation should still be investigated further. Secondly, the applied resistance training program started shortly after the first bout of EIMD. Thus, measuring the size of muscle damage may not have been justified even though we were able to measure muscle damage at 48 and 72 h after the first bout.

5. Conclusions

The whole-body cryostimulation combined with resistance training can positively modify concentrations of growth factors among untrained subjects by reducing myostatin concentration. This type of cold treatment, applied a day after a high-intensity resistance training session, did not inhibit muscle adaptation to resistance training among beginners. Thus, the time difference in treatment

application should be taken into consideration in future investigations to formulate precise conclusions regarding combining cold therapy with resistance training.

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