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Wpływ jednorazowej hipoksji normobarycznej oraz wysiłku fizycznego na zmiany stężenia markerów biologicznych we krwi oraz funkcje poznawcze

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SPIS TREŚCI

- Publikacja pt. *Effect of Acute Normobaric Hypoxia Exposure on Executive Functions among Young Physically Active Males*

- Publikacja pt. *Acute Normobaric Hypoxia Lowers Executive Functions among Young Men despite Increase of BDNF Concentration*

- Publikacja pt. *Exercise-Induced Elevated BDNF Concentration Seems to Prevent Cognitive Impairment after Acute Exposure to Moderate Normobaric Hypoxia among Young Men*

1. Wykaz prac stanowiących rozprawę doktorską

Przedstawiona rozprawa doktorska pod tytułem: ***„Wpływ jednorazowej hipoksji normobarycznej oraz wysiłku fizycznego na zmiany stężenia markerów biologicznych we krwi oraz funkcje poznawcze”***, składa się z cyklu trzech prac opublikowanych w czasopismach zagranicznych o sumarycznej punktacji Impact Factor (IF) równej 4.964 oraz Ministerstwa Edukacji i Nauki (MEiN) równej 300 pkt.:

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Publikacja III: ***Exercise-Induced Elevated BDNF Concentration Seems to Prevent Cognitive Impairment after Acute Exposure to Moderate Normobaric Hypoxia among Young Men*** (2023)
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2. Wykaz skrótów

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

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

CBF – mózgowy przepływ krwi (*ang. cerebral blood flow)*

HIF-1 – czynnik indukowany hipoksją (*ang. hypoxia-inducible factor 1)*

OUN – ośrodkowy układ nerwowy (*ang. central nervous system)*

NOR – normoksja (*ang. normoxia)*

NH – hipoksja normobaryczna (*ang. normobaric hypoxia)*

NOR EX – wysiłek w normoksji (*ang. normoxia exercise)*

NH EX – wysiłek w hipoksji normobarycznej (*ang. normobaric hypoxia exercise)*

TrkB – receptor kinazy tyrozynowej B (*ang. tyrosine receptor kinase B)*

SpO_2 – saturacja krwi (*ang. oxygen saturation)*

n.p.m. – nad poziomem morza (*ang. above seal level – a.s.l.)*

FIO_2 – stężenie tlenu w mieszaninie oddechowej (*ang. fraction of inspired oxygen*)

VEGF – czynnik wzrostu śródbłonka naczyniowego (*ang. vascular endothelial growth factor)*

3. Wprowadzenie

Dostarczanie wystarczającej ilości tlenu do mózgu ma kluczowe znaczenie dla funkcji poznawczych [1]. Zwiększony mózgowy przepływ krwi (CBF) wynika ze wzrastającego zapotrzebowania na energię spowodowanego wzmożoną aktywnością mózgu podczas przetwarzania poznawczego [2]. Szacuje się, że podczas zadań wymagających poznawczo, zużycie energii przez tkankę neuronalną wzrasta o 15% [3–6]. Dlatego zmiany w metabolizmie tlenowym mózgu wywołane niedotlenieniem mogą objawiać się spadkiem funkcji poznawczych [2].

Osoby mieszkające lub pracujące na dużych wysokościach, takie jak przewodnicy górscy, personel wojskowy czy też sportowcy trenujący na dużych wysokościach nad poziomem morza (n.p.m.) narażone są na niedotlenienie, a co za tym idzie, upośledzenie funkcji poznawczych [7–9]. Nadal nie jest jasne, w jaki sposób ostra hipoksja wpływa na ośrodkowy układ nerwowy (OUN) i zdolności poznawcze wyższego rzędu, w tym procesy wykonawcze [10, 11]. Ze względu na spadek poziomu nasycenia tlenem obwodowych naczyń włosowatych (SpO_2), zmiany w ilości neuroprzekaźników i czynników hormonalnych/humoralnych, niedotlenienie może wpływać na OUN [12] i prowadzić do upośledzenia funkcji poznawczych, w tym może mieć wpływ na pamięć, widzenie kolorów, czy czas reakcji. Wydaje się, że reakcja organizmu może zależeć od wielkości niedotlenienia (np. od wysokości nad poziomem morza) i czasu trwania ekspozycji [13]. Na reakcję hipoksyjną silny wpływ może mieć tryb i protokół hipoksji (hipoksja hipobaryczna/normobarycza, przerywana/ciągła), a także wiek uczestników, poziom sprawności fizycznej, stan zdrowia oraz rodzaj zadania poznawczego czy czas wykonania testu kognitywnego [13–17]. Obserwuje się, że warunki niedotlenienia negatywnie wpływają na centralne zadania wykonawcze (takie jak funkcje wykonawcze) częściej niż na umiejętności niewykonawcze (takie jak percepcja, uwaga i zadania dotyczące pamięci krótkotrwałej) [18]. Jednak analiza meta-regresji McMorrisa i wsp. [13] nie wykazała różnic między czynnościami wykonawczymi i niewykonawczymi we wpływie niedotlenienia na funkcje poznawcze. Zgodnie z badaniem pozytronowej tomografii emisyjnej [19], zadania pamięci operacyjnej mają wpływ na grzbietowo-boczną korę przedczołową, przednią korę zakrętu obręczy, hipokamp, a być może także na jądra podstawne i móżdżek. W rezultacie uważa się, że niektóre zawody i prace są bardziej podatne na negatywne skutki zleconych im zadań.

Oprócz powodowania zaburzeń poznawczych, niedotlenienie może również wpływać na syntezę katecholamin, neurotrofin, takich jak neurotroficzny czynnik pochodzenia mózgowego (BDNF) lub czynnik wzrostu śródbłonka naczyniowego (VEGF), a także przyspieszać CBF. Ludzie mają co najmniej kilka systemów, które wspomagają myśleć bardziej klarownie. Zgodnie z badaniami obrazowania mózgu, zwiększony CBF, indukowany przez łagodną hipoksję, jest jednym z tych procesów, które mogą poprawić zdolności poznawcze [20, 21]. Badania wykazują, że kilka sesji

hipoksji normobarycznej (NH) wpływa na poprawę zdolności poznawczych [22]. W eksperymencie przeprowadzonym przez Loprinzi i wsp. [23], jednorazowa hipoksja normobaryczna wpłynęła korzystnie na zakłócenia/interferencję pamięci. Ze względu na zwiększoną dostępność glukozy, tlenu i hormonów wytwarzanych obwodowo do mózgu, zwiększony CBF (np. w tętnicy środkowej mózgu) może potencjalnie wpływać na tkankę mózgową i jej metabolizm [24]. Wiele z tych hormonów funkcjonuje jako związki neurochemiczne i pełnią ważną rolę w utrzymaniu plastyczności mózgu. BDNF jest jednym z najważniejszych. Poprzez kinazę receptorową B (TrkB), BDNF wyzwala szereg działań, które mogą zwiększyć strukturalną i funkcjonalną plastyczność mózgu [25]. Oprócz tego, białko to jest niezbędne do wzmacniania połączeń neuronalnych czy konsolidacji wspomnień [26–28]. Wzrost stężenia BDNF we krwi można zaobserwować, gdy ekspresja BDNF w mózgu jest podwyższona [29, 30]. Jest to możliwe dzięki zdolności tego białka do przechodzenia przez barierę krew-mózg w obu kierunkach według gradientu stężeń [31]. Ostatnie badania wskazują, że BDNF może być biomarkerem upośledzenia pamięci u ludzi [32]. Hipokamp, zakręt zębaty i kora okołowęchowa wykazują wyższy poziom ekspresji BDNF, co jednocześnie może poprawić funkcje poznawcze [33]. Poprawa tych zdolności może wynikać z wspomnianego wzrostu poziomu neurotroficznego czynnika pochodzenia mózgowego, który z kolei wpływa na neurogenezę mózgu [34–36].

Podczas gdy niektóre badania sugerują, że ćwiczenia o wysokiej intensywności zmniejszają sprawność psychomotoryczną [37, 38], inne pokazują, że ćwiczenia o niskiej lub umiarkowanej intensywności w normoksji poprawiają funkcje psychomotoryczne [39–41]. W rezultacie wydaje się, że to intensywność wysiłku decyduje o efektach, przynajmniej w warunkach normoksji. Wyniki badań dotyczących niedotlenienia są bardziej złożone i nie są tak jednoznaczne. Wykazano bowiem, że sama ekspozycja na niedotlenienie zwykle powoduje pogorszenie funkcji poznawczych i że jest to związane z czasem trwania ekspozycji, poziomem niedotlenienia lub obydwoma czynnikami [11, 42]. Z drugiej strony, funkcje poznawcze mogą się poprawić poprzez wzrost poziomu pobudzenia OUN, nawet przy niskiej intensywności ćwiczeń, gdyż pobudzenie OUN w efekcie ćwiczeń wykazuje wzrost aktywacji neuroelektrycznej [43]. Jest niewiele danych dotyczących wpływu niedotlenienia na sprawność psychomotoryczną badanych podczas ćwiczeń o niskiej i wysokiej intensywności. Nie jest pewne, czy niekorzystny wpływ niedotlenienia na OUN może wpływać na wyżej wymienione wyniki ćwiczeń. Główną hipotezą dotyczącą przyspieszenia prędkości przetwarzania w OUN jest zwiększone uwalnianie katecholamin w mózgu [44]. Obwodowe katecholaminy mogą promować wytwarzanie większej ilości hormonów stresu, podnosić tętno i powodować zróżnicowane zwężenie naczyń krwionośnych, które sprzyja przepływowi krwi do mięśni szkieletowych, a także podnosić poziom centralnego pobudzenia [45]. Każdy z tych wysoce funkcjonalnych wyników pomaga

zmaksymalizować sprawność neurologiczną, metaboliczną i mięśniowo-szkieletową, gdy organizm poddany jest bardziej wymagającym próbom. Kiedy te efekty łączą się, organizm szybko dostosowuje się do zwiększonych wymagań metabolicznych i funkcjonalnych ćwiczeń, a mózg przetwarza informacje szybciej i wydajniej [45]. Ostatnie badania wskazują, że BDNF ma kluczowe znaczenie dla tego zjawiska [10,11]. Ustalono, że obniżony poziom BDNF we krwi jest związany z upośledzeniem funkcji poznawczych w chorobach neurodegeneracyjnych [48]. Z drugiej strony zakłada się, że BDNF odgrywa znaczącą rolę w poprawie funkcji poznawczych w następstwie ćwiczeń fizycznych [49]. Wzrost koncentracji BDNF jest odwrotnie skorelowany z intensywnością ćwiczeń, a wskaźnik ten jest wrażliwy na jednorazowy wysiłek fizyczny [50]. Ponieważ BDNF może przekraczać barierę krew-mózg, zakłada się, że wspomniane zmiany stężenia BDNF w surowicy wskazują na wzrost BDNF w mózgu, a co za tym idzie, mają długoterminowe znaczenie dla zdrowia mózgu i poprawy funkcji poznawczych [51]. Zatem produkcja BDNF indukowana niedotlenieniem może wspomagać pamięć (tj. plastyczność) poprzez zwiększenie siły synaptycznej. BDNF wydaje się mieć kluczowe znaczenie w neuronalnej separacji wzorców porównywalnych informacji, co ma wpływ na minimalizowanie zakłóceń/interferencji pamięci, a także jest związane z poprawą funkcjonowania pamięci epizodycznej [52].

Wpływ wysiłku fizycznego jako inhibitora niepożądanych zmian kognitywnych, nie został jeszcze określony. Dlatego podejmowanie badań w tym obszarze jest zasadne. Biorąc pod uwagę powyższy stan wiedzy, w swojej pracy badawczej postanowiłem zweryfikować efekt wpływu pojedynczej sesji oddechowej w warunkach hipoksji normobarycznej, jak i wysiłku fizycznego na zmiany przystosowawcze u młodych mężczyzn w zakresie funkcji poznawczych i kluczowych markerów biologicznych.

4. Pytania i hipotezy badawcze

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

1. Czy pojedyncza sesja oddychania w warunkach hipoksji normobarycznej wpłynie na funkcje poznawcze oraz czy wielkość tych zmian będzie uzależniona od symulowanej wysokości nad poziomem morza?

2. Czy pojedyncza sesja oddychania w warunkach hipoksji normobarycznej wywoła ekspresję BDNF oraz czy istotnie wpłynie na stężenie tego białka we krwi obwodowej?

3. Czy pojedyncza jednostka wysiłku fizycznego w warunkach hipoksji normobarycznej wywoła zwiększoną ekspresję BDNF oraz czy zmiany te wpłyną na funkcje poznawcze?

Dodatkowo, sformułowałem poniższe hipotezy:

1. Pojedyncza sesja oddychania w warunkach hipoksji normobarycznej powoduje pogorszenie funkcji poznawczych a zmiany te uzależnione są od symulowanej wysokości nad poziomem morza.

2. Pojedyncza sesja oddychania w warunkach hipoksji normobarycznej zwiększa ekspresję BDNF i istotnie wpływa na stężenie tego białka we krwi obwodowej.

3. Pojedyncza jednostka wysiłku fizycznego w warunkach hipoksji normobarycznej wpływa na zwiększoną ekspresję BDNF i tym samym na poprawę funkcji poznawczych.

5. Materiał i metody

5.1. Charakterystyka osób badanych

Do badań zrekrutowano 76 młodych, zdrowych, mężczyzn prowadzących aktywny tryb życia. Z badań wykluczono 8 uczestników (przeziębienie, nieobecność, powody osobiste, uraz).

W poszczególnych eksperymentach wzięło udział

1. Publikacja I: 19 uczestników

   24 młodych mężczyzn (średnia wieku 23.1 ± 2.1) zakwalifikowano do dwóch eksperymentów, z czego 19 uczestników zostało poddanych analizie. W pierwszym eksperymencie pilotażowym wzięły udział 4 osoby. Badani zostali poddani pojedynczym sesjom oddechowym w warunkach hipoksji normobarycznej na różnych symulowanych wysokościach nad poziomem morza (3500m n.p.m., 4500m n.p.m. i 5500m n.p.m.). Na podstawie otrzymanych wyników przeprowadzono drugi eksperyment, w którym wzięło udział 20 uczestników. Eksperyment ukończyło 15 osób. Badani wykonywali pojedyncze sesje oddechowe w warunkach hipoksji normobarycznej na jednej, wybranej symulowanej wysokości (3500 m n.p.m.) oraz wykonywali testy poznawcze.

2. Publikacja II: 32 uczestników

   W badaniach wzięło udział 32 mężczyzn (średnia wieku 20.4 ± 0.6 lat). Uczestnicy zostali poddani dwóm sesjom oddychania w dwóch różnych warunkach środowiskowych: normoksji (NOR) i hipoksji normobarycznej (NH). Badani wykonali również testy poznawcze, a także pobrano od nich próbki krwi.

3. Publikacja III: 17 uczestników

   W badaniach wzięło udział 20 uczestników, z czego 17 (średnia wieku 20.6 ± 0.7 lat) zostało poddanych analizie. Uczestnicy wzięli udział w badaniu randomizowanym w układzie krzyżowym (cross-over). Zostali oni poddani sesji oddychania połączonej z jednorazowym wysiłkiem fizycznym o umiarkowanej intensywności (50% VO_{2max}) na cykloergometrze rowerowym w warunkach normoksji (NOR EX) i takim samym wysiłku w warunkach hipoksji normobarycznej (NH EX). Wykonano również analizę próbek krwi oraz testy poznawcze.

5. 2. Metody

Badania przeprowadzone były w oparciu o zgodę komisji bioetycznej (KB-9/16), a w badaniach zastosowano następujące metody i narzędzia badawcze:

5.2.1. Metody pomiarów antropometrycznych i wydolności fizycznej

- Analiza komponentów składu ciała została wykonana za pomocą metody spektroskopowej bio-impedancji przy użyciu analizatora TBF-300 Tanita Body Fat Monitor/Scale Analyzer (Japonia) [53].

- Ocena pułapu tlenowego (VO_{2max}) wykonana została w oparciu o test o progresywnie wzrastającej intensywności na cykloergometrze rowerowym (884E Sprint Bike Sweden, Monark) z użyciem analizatora gazów MetaMAx3B (Cortex) [54].

5.2.2. Metody oznaczeń biochemicznych we krwi

- Do oznaczenia stężeń wybranych biomarkerów we krwi użyto metodę immunoenzymatyczną ELISA przy użyciu gotowych zestawów z firm: R&D Systems, Minneapolis, MN, USA, catalogue no. DBNT00; Ray Biotech Inc., Cambridge, UK do oznaczenia białka BDNF oraz Demeditec Diagnostics GmbH, catalog no. DEH3388, Kiel, Germany do oznaczenia kortyzolu [55].

- Krew pobierana była przed oraz bezpośrednio po ukończeniu danego eksperymentu.

5.2.3. Metody hipoksji normobarycznej

- Generator powietrza hipoksycznego/hiperoksycznego GO2Altitude ERA II firmy Biomedtech (Australia) został wykorzystany do stworzenia odpowiednich warunków niedotlenienia podczas testów. Proponowane wysokości (n.p.m.) symulowano poprzez zmniejszenie zawartości tlenu w mieszaninie wdechowej zgodnie z zaleceniami producenta Biomedtech Australia Pty. Ltd. Biomedical Research and Development opisanymi w instrukcji obsługi [56].

5.2.4. Metody oceny funkcji poznawczych

- Test funkcji poznawczych przeprowadzony został z wykorzystaniem komputerowej wersji Wiedeńskiego systemu Testów firmy SCHUHFRIED (Austria), mianowicie: test interferencji Stroopa [57].

6. Wyniki

6.1. Wyniki badań opublikowane w pracy pt. *Effect of Acute Normobaric Hypoxia Exposure on Executive Functions among Young Physically Active Males.*

W badaniu pilotażowym analiza wyników wykazała spadek saturacji krwi (między warunkami $F(1.197, 7.183) = 29.77$; $p = 0.0007$)), a zmiany te były adekwatne i skorelowane ze wzrostem symulowanej wysokości. Podobną zmianę ($t = 18.5$; $p < 0.0001$; d Cohena = 1.186634) zaobserwowałem w drugim badaniu eksperymentalnym na wybranej symulowanej wysokości (3500 m n.p.m. ; $FIO_2 = 13\%$).

Pojedyncza sesja oddychania w warunkach hipoksji normobarycznej nie wykazała różnic statystycznych w teście Stroopa w badaniu pilotażowym, zarówno w wartościach interferencji „czytania" (interakcja $F(2, 12) = 0.1374$; $p = 0.8729$; czas $F(2, 12) = 0.5171$; $p = 0.6090$), jak i w wartościach interferencji w „nazywaniu" (interakcja $F(2, 12) = 1.647$; $p = 0.2334$; czas $F(2, 12) = 0.8909$; $p = 0.4358$), jednak liczebność grupy była bardzo mała. Po zwiększeniu liczby uczestników i ponowynym przeprowadzeniu analizy nie odnotowałem istotnych statystycznie różnic w wartościach interferencji w „czytaniu" (interakcja $F(1, 28) = 0.007749$; $p = 0.7828$; czas $F(1, 28) = 0.03697$; $p = 0.8489$). Zaobserwowałem jednak statystycznie istotne zmiany w wartościach interferencji w„nazywaniu" (interakcja $F(1, 28) = 5.404$; $p = 0.0276$; czas $F(1, 28) = 11.73$; $p = 0.0019$; $\eta^2 = 0.16765$). Następnie przeprowadziłem analizę kontrastu między NOR (po-przed) a NH (po-przed). Delta z interferencji w „czytaniu" nie była istotna: $t = 0{,}9252$; $p = 0{,}3705$, natomiast delta w interferencji w „nazywaniu" była istotna statystycznie między grupami: $t = 2.392$; $p = 0.0314$; d Cohena = 0.878572.

6.2. Wyniki badań opublikowane w pracy pt. *Acute Normobaric Hypoxia Lowers Executive Functions among Young Men despite Increase of BDNF Concentration.*

Analiza wyników wykazała spadek saturacji krwi w warunkach NH ($t = 12$; $df = 29$; $p < 0.001$), a zmiany te były adekwatne do symulowanej wysokości n.p.m..

Pojedyncza ekspozycja na warunki NH nie wykazała statystycznych różnic w interferencji w "czytaniu" (interakcja $F (1, 60) = 0.006$, $p = 0.939$; czas $F (1, 60) = 0.0047$, $p = 0.946$). Natomiast druga część testu Stroopa, dotycząca interferencji w "nazywaniu" różniła się znacząco (interakcja $F (1, 60) = 4.644$, $p = 0.035$; czas $F (1, 60) = 6.375$, $p = 0.014$).

Średnie wartości stężenia BDNF nie zmieniły się w warunkach NOR w pomiarach przed-po, aczkolwiek zaobserwowano znaczący wzrost stężenia BDNF po warunkach NH (interakcja F (1, 62) = 3.893, p = 0.053; współczynnik grup F (1, 62) = 7.358, p = 0.009; czas F (1, 62) = 3.999, p = 0.0499).

Ponadto analiza wyników ukazała istotne zmiany w stężeniu kortyzolu w warunkach NOR w pomiarach przed-po, chociaż nie zaobserwowano tego samego efektu po symulowanych warunkach NH. Wystąpiły zmiany międzygrupowe (interakcja F (1, 62) = 35.9, p < 0.001; współczynnik grup F (1, 62) = 11.71, p = 0.001; czas F (1, 62) = 16.61, p = 0.001).

6.3. Wyniki badań opublikowane w pracy pt. *Exercise-Induced Elevated BDNF Concentration Seems to Prevent Cognitive Impairment after Acute Exposure to Moderate Normobaric Hypoxia among Young Men.*

Saturacja krwi zmniejszyła się podczas jednorazowej ekspozycji na hipoksję normobaryczną (t = 10,51; p < 0.0001), a efekty te były adekwatne do symulowanej wysokości n.p.m..

Pojedyncza ekspozycja na warunki hipoksji normobarycznej połączonej z wysiłkiem fizycznym o umiarkowanej intensywności (50% VO_{2max}) nie wpłynęła istotnie na zmiany w teście Stroopa. Wartości zarówno interferencji "czytania" (interakcja F(1, 32) < 1, p = 0.49; czas F(1, 32) < 1, p = 0.85, η^2 = 0.001), jak i interferencji "nazywania" (interakcja F(1, 32) = 1.7, p = 0.20; czas F(1, 32) = 1.15, p = 0.29, η^2 = 0.012), pomimo znacznego spadku nasycenia w warunkach niedotlenienia, nie okazały się istotne statystycznie.

Dodatkowo analiza krwi ukazała istotny wzrost stężenia BDNF po wysiłku fizycznym niezależnie od warunków (normoksja/hipoksja normobaryczna) (interakcja F(1, 32) < 1, p = 0.72; czas F(1, 32) = 59.45, p < 0.0001, η^2 = 0.553). Interesujący był fakt, iż zwiększone stężenie BDNF po wysiłku fizycznym mogło przyczynić się do zahamowania negatywnego wpływu ostrej hipoksji, co obserwowaliśmy już w poprzednich pracach.

7. Podsumowanie

Badania ostatnich lat wykazały, że białka uwalniane do krwiobiegu w odpowiedzi na wysiłek fizyczny mogą być wykorzystywane w zapobieganiu wielu schorzeniom, a także wpływać na funkcje poznawcze [58]. Geny wrażliwe na niedotlenienie regulują czynnik indukowany hipoksją 1 (HIF-1), który reguluje ekspresję wielu genów, w tym BDNF. Białko BDNF poprzez przyłączenie się do określonych receptorów w jądrze komórkowym lub błonie komórkowej, działa endokrynnie, autokrynnie i parakrynnie [59]. Produkcja BDNF indukowana niedotlenieniem może ułatwić funkcjonowanie pamięci poprzez poprawę siły połączeń synaptycznych. Również powysiłkowa synteza BDNF może wpływać na zwiększenie funkcjonowania poznawczego, a białko to swoje działanie realizuje poprzez kontrolę wielu procesów, w tym rozwoju neuronów i ich fizjologicznych funkcji, stymulacji połączeń dendrytycznych czy też neurogenezy [59]. Dlatego celem powyższej rozprawy doktorskiej była weryfikacja efektu zastosowania pojedynczej sesji oddychania w warunkach hipoksji normobarycznej, jak i również sesji połączonej z wysiłkiem fizycznym na funkcje poznawcze u młodych mężczyzn.

Dane z badań wskazują, że po pojedynczej sesji samego oddychania w warunkach NH, nastąpiło znaczne pogorszenie funkcji poznawczych [2, 60, 61]. Zwiększona interferencja w teście Stroopa w moich badaniach odzwierciedlała to pogorszenie. Metadane potwierdzające te wyniki, wykazują, że niedotlenienie ma selektywny wpływ na funkcje poznawcze, poprawiając przetwarzanie informacji, ale upośledzając funkcje poznawcze oparte na uwadze, funkcje wykonawcze i pamięć [62]. Efekt reperfuzji, omówiony na organizmach ludzkich [63, 64], może w tym czasie wpływać na poprawę funkcji poznawczych. Następstwem reperfuzji jest lepsze dotlenienie tkanek, które prawdopodobnie ma związek z odnotowaną poprawą po ekspozycji [65]. Ponadto stres oksydacyjny wywołany niedotlenieniem może wpływać na produkcję i/lub uwalnianie BDNF, utrudniając tym samym upośledzenie hipokampa [66]. Jako neuroregulator i czynnik wpływający na neurogenezę mózgu, wzrost mózgowego BDNF może prowadzić do polepszenia funkcji poznawczych [67].

Badania przedstawiające zastosowanie pojedynczego wysiłku fizycznego na zmiany funkcji poznawczych, wykazują korzystny wpływ na proces przetwarzania informacji, czy koncentrację [50, 54]. Wpływ ten był widoczny zarówno podczas ćwiczeń o umiarkowanej (40% VO_{2max}) [68], jak i wyższej intensywności wysiłku (70-80% VO_{2max}) [50, 69]. Wysiłek fizyczny o umiarkowanej intensywności może również wpływać na zwiększenie CBF, co pomaga kompensować spadek SpO_2 po ekspozycji na niedotlenienie [70]. Efekt zastosowania połączonej sesji hipoksji normobarycznej z wysiłkiem fizycznym na zmiany w funkcjonowaniu mózgu, nie jest do końca poznany. Wyniki badań przedstawione w powyższej rozprawie doktorskiej pokazują, że dobroczynny wpływ wysiłku może

zmniejszyć negatywne skutki hipoksji i znacząco wpłynąć na funkcje poznawcze. Zarówno sama sesja oddychania, jak i wysiłek fizyczny powodowały istotny wzrost stężenia BDNF mierzonego w surowicy krwi. Za wzrost poziomu BDNF we krwi prawdopodobnie odpowiedzialne są płytki krwi lub przechodzenie tego czynnika przez barierę krew-mózg. Nie stwierdzono bowiem do tej pory, że BDNF może pochodzić z tkanki mięśniowej.

Podsumowując, wyniki moich badań wykazują, że zarówno pojedyncza sesja oddychania w warunkach hipoksji normobarycznej, jak i wysiłek fizyczny wpływa na zwiększoną ekspresję BDNF, a tym samym na jego wyższą koncentrację we krwi obwodowej. Działanie tego czynnika neurotroficznego może poprawiać/przeciwdziałać obniżeniu funkcji kognitywnych wywołanych przez niedotlenienie, które wydają się być jednym z kluczowych elementów podczas podejmowania decyzji przy presji czasu. Należy podkreślić, że zaproponowany model sesji oddechowo-wysiłkowej jest oszczędny czasowo, a przystępna metodyka umożliwia wykorzystanie go nie tylko w warunkach laboratoryjnych.

8. Wnioski

Wyniki przeprowadzonych przeze mnie badań dowodzą, że:

1. Pojedyncza sesja oddychania w warunkach hipoksji normobarycznej wpływa istotnie na pogorszenie funkcji poznawczych, a zmiany te nie różniły się niezależnie od symulowanej wysokości.

2. Pojedyncza sesja zarówno oddychania w warunkach NH, jak i wysiłku fizycznego o umiarkowanej intensywności wpływa na zwiększenie stężenia BDNF we krwi obwodowej.

3. Wykonanie pojedynczej sesji wysiłku fizycznego o umiarkowanej intensywności w warunkach NH wpływa pozytywnie na funkcje poznawcze, powodując zahamowanie negatywnego wpływu hipoksji.

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10. Streszczenie

Mimo iż wiedza na temat prozdrowotnego wpływu wysiłku fizycznego jest coraz bardziej obszerna to mechanizmy związane ze zmianami adaptacyjnymi indukowanymi przez różne formy aktywności fizycznej nie są dostatecznie zweryfikowane i wyjaśnione. Dużym zainteresowaniem cieszą się badania dotyczące wpływu wysiłku fizycznego na funkcje poznawcze. Liczne badania pokazują, że wysiłek fizyczny może poprawiać pamięć, koncentrację, umiejętności rozwiązywania problemów, a przez to wpływać na lepszą decyzyjność. Stan, w którym organizm nie otrzymuje wystarczającej ilości tlenu, znany jako niedotlenienie, może mieć głęboki wpływ na funkcje poznawcze. Wysiłek fizyczny wydaje się mieć kluczowe znaczenie dla zmniejszenia negatywnego wpływu niedotlenienia na funkcje poznawcze.

Celem przeprowadzonych przeze mnie badań była ocena wpływu pojedynczej sesji oddychania w warunkach hipoksji normobarycznej, jak również wysiłku fizycznego w takich warunkach na poziom BDNF oraz zweryfikowanie, czy wpłynie to zmiany w funkcjach poznawczych.

W badaniach wzięło udział 76 młodych mężczyzn. Uczestnicy zostali poddani pojedynczej sesji oddychania w warunkach hipoksji normobarycznej (NH), jak również wysiłkowi fizycznemu w takich warunkach (NH EX). Grupa kontrolna oddychała normoksyjną mieszanką gazową (NOR) oraz wykonywała ćwiczenia w takich warunkach (NOR EX). Przed rozpoczęciem i bezpośrednio po każdej z sesji, od uczestników pobrano krew w celu zmierzenia stężenia wybranych markerów oraz poproszono o wykonanie testów kognitywnych.

Wyniki przeprowadzonych badań wykazały, że zaproponowana sesja oddechowa, a także wysiłek fizyczny wpływają znacząco na funkcje poznawcze. Pojedyncza sesja NH spowodowała istotne obniżenie funkcji poznawczych, równolegle podnosząc znacząco stężenie neurotroficznego czynnika pochodzenia mózgowego. Z kolei pojedynczy wysiłek fizyczny nie powodował zmian w funkcjach poznawczych, aczkolwiek powodował istotne zwiększenie BDNF. Co ciekawe, połączenie tych dwóch czynników spowodowało zahamowanie negatywnego wpływu niedotlenienia, uwidaczniając to w wynikach testu Stroopa.

Podsumowując, wyniki przedstawione w tej rozprawie dowodzą, że pojedyncza sesja NH, jak również wysiłek fizyczny wpływa na zmiany w stężeniu BDNF. Czynnik ten może przyczyniać się do przeciwdziałania negatywnym konsekwencjom niedotlenienia. Zaproponowany model sesji oddechowo-wysiłkowej jest oszczędny czasowo i wpływa pozytywnie na funkcje poznawcze.

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Effects of an acute normobaric hypoxia and exercise on changes in biological markers blood levels and cognitive function

Ph.D. thesis under the supervision of:

Professor Radosław Laskowski

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11. Summary

Current knowledge of the health-promoting effects of exercise is growing. However, the mechanisms involved in the adaptive changes induced by various forms of exercise are not sufficiently verified and explained. Of great interest is research on the effects of physical exertion on cognitive function, which in many situations is a key element in daily life. Numerous studies show that physical exercise can improve memory, concentration, problem-solving skills, and thus make better decisions. A condition in which the body does not receive enough oxygen, also known as hypoxia, can have a profound effect on cognitive function. Physical exercise appears to be key to reducing the negative effects of hypoxia on cognitive function.

The purpose of my study was to evaluate the effect of a single breathing session under normobaric hypoxia, as well as physical exercise under such conditions, on BDNF levels and to verify whether this would affect cognitive functions.

The study included 76 young men. The participants were subjected to a single session of breathing under normobaric hypoxia (NH), as well as physical exercise under such conditions (NH EX). The control group breathed a normoxic gas mixture (NOR) and performed exercise under such conditions (NOR EX). Before and immediately after each session, blood was drawn from the participants to measure the concentration of selected markers and they were asked to perform cognitive tests.

The results of the study showed that the proposed breathing session, as well as physical exercise, significantly affected cognitive function. A single NH session caused a significant decrease in cognitive function, in parallel, significantly raising the concentration of brain-derived neurotrophic factor. A single exercise session induced no change in cognitive function, although it did significantly increase BDNF. Interestingly, the combination of these two components resulted in an inhibition of the negative effect of hypoxia, making this apparent in the Stroop test results.

In conclusion, the results presented in this dissertation demonstrate that a single session of NH, as well as exercise session, affects changes in BDNF concentration. This factor may contribute to counteracting the negative consequences of hypoxia. The proposed breathing-exercise session model is time-saving and has a positive effect on cognitive function.

12. Oświadczenia współautorów

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OŚWIADCZENIE WSPÓŁAUTORÓW PUBLIKACJI

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Niniejszym oświadczamy, że indywidualny wkład w powstanie ww publikacji jest następujący:

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* **A** – przygotowanie projektu badania, **B** – przeprowadzanie badań, **C** – analiza statystyczna, **D** – interpretacja wyników, **E** – przygotowanie publikacji, **F** – opracowanie piśmiennictwa, **G** – pozyskanie funduszy

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Effect of Acute Normobaric Hypoxia Exposure on Executive Functions among Young Physically Active Males

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Abstract: Background: On the one hand, hypoxic exposure may result in progressive brain metabolism disturbance, causing subsequent cognitive impairments. On the other hand, it might also enhance neurogenesis and brain vascularization as well as accelerate cerebral blood flow, leading to cognitive function improvement. The aim of this study was to investigate whether progressive stages of normobaric hypoxia (NH) (FIO_2 = 13%, FIO_2 = 12%, and FIO_2 = 11%) differentially affect post-exposure cognitive performance. Methods: Fifteen physically active men (age = 23.1 ± 2.1) participated in the study. The Stroop test (ST) was applied to assess cognitive function. To generate NH conditions, a hypoxic normobaric air generator was used. Results: We observed an executive function impairment ("naming" interference $p < 0.05$) after NH exposure (FIO_2 = 13%). After exposure at FIO_2 = 12% and FIO_2 = 11%, no changes were observed in the Stroop test. Also, changes in SpO_2 during subsequent NH exposure were observed. Conclusions: The current investigation shows that executive functions deteriorate after acute NH exposure and this post-exposure deterioration is not proportional to the normobaric hypoxia stages among young physically active males.

Keywords: cognitive function; physical exercise; altitude

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1. Introduction

Cognitive functions are highly dependent on adequate oxygen delivery to the brain [1]. Elevated brain activity during cognitive processing causes a rise in energy demand, leading to an increase in cerebral blood flow (CBF) [2]. It is assumed that the energy demand of neuronal tissue increases by 15% during tasks that require cognitive functioning [3–6]. Therefore, disturbances in cerebral aerobic metabolism, caused by hypoxia, could manifest as a cognitive function impairment [2].

Cognitive impairment can negatively affect residents or workers staying at high altitudes, e.g., mountain guides, militaries, athletes at altitude training camps, mountaineers or skiers, as well as exposed-to-hypoxia aircraft pilots or flight personnel [7–9]. Moreover, cognitive decline is observed commonly among patients with severe forms of hypoxic–ischemic brain injury such as stroke, sleep apnea, or chronic obstructive pulmonary disease (COPD) [10–17]. Additionally, reduced vascularization and blood flow in

aging also diminish the ability to supply oxygen to the brain, leading to cognitive function impairment [18–21].

Hypoxic exposure, therefore, impairs memory, color vision, reaction time, and executive functions [22]. The deterioration of cognitive abilities seems to be also dependent on the magnitude of hypoxia and exposure time [22]. It also appears that hypoxic exposure response is largely dependent on hypoxic mode and protocol (hypobaric/normobaric, intermittent/continuous), the participants' age, fitness level, and health status, cognitive task type, post-cognitive test timing, and other confounding factors [22–26]. Numerous human studies have revealed that normobaric hypoxia at various exposure times (from 16 to 30 min) and at different simulated altitudes have a detrimental influence on reaction time and error rate during cognitive performance [27,28]. Nevertheless, Pavlicek et al. (2005) and Taylor et al. (2015) did not observe changes in human cognition (word fluency, word association task) after 30–45 min exposure to simulated altitudes (2440 m–4500 m a.s.l.) [29,30].

However, mild hypoxic exposure can also have a neuroprotective effect in improving cognitive performance; therefore, a right dosage or time of exposure/measurement could be a key factor [31,32]. Moreover, a combination of mild hypoxia and aerobic training seems to enhance cognitive function among the elderly [33]. Furthermore, some NH protocols show enhancement of cognitive functions; for example, intermittent hypoxia exerts a beneficial impact on protective mechanisms [34]. In a study conducted by Loprinzi et al. (2019), a positive effect of acute normobaric hypoxia on memory interference was observed [35]. Although hypoxia may lead to progressive disturbance in brain metabolism, causing subsequent cognitive impairments, it might also induce the synthesis of catecholamines and neurotrophins such as brain-derived neurotrophic factor (BDNF) or vascular endothelial growth factor (VEGF) as well as accelerate CBF, positively affecting neurogenesis and brain vascularization [22,30,36]. Hypoxia can induce the release of BDNF, relevant for memory formation and potentiation; therefore, a positive effect might be expected [37].

The aim of this study was to investigate whether cognitive abilities among young physically active males could be facilitated after normobaric hypoxia exposure. We hypothesized that progressive stages of normobaric hypoxia (FIO_2 = 13%; FIO_2 = 12% and FIO_2 = 11%) affect human cognition differently.

2. Materials and Methods

2.1. Participants

Twenty-four healthy, non-obese young adults were enrolled to the experiment. At the beginning, four subjects participated in Experiment 1 (a pilot study), and after its completion, the experimental group was enlarged to up to 20 participants taking part in the main study—Experiment 2. At the end, 15 physically active men finished the main experiment (5 of them did not complete it due to several reasons, e.g., cold, absence, or personal reasons). All participants were Polish native speakers. Before the start of the experiment, they were introduced to the procedures to which they had volunteered. Exclusion criteria included a history of alpine expedition, dyslexia, daltonism, and blurred vision. All participants were university students and were physically active. The participants did not have any medical contraindications. No participant stated a history of neurological, psychiatric, or respiratory disorders or had a disease that required medical care. Additionally, they were required to refrain from consumption of caffeine 24 h prior to the testing session. All participants gave their written consent after they had been informed about the purpose of the study and the procedures. The study was approved by the local Ethics Committee and the Bioethical Committee of the Regional Medical Society (KB-9/16) according to the Helsinki Declaration. Detailed anthropometric characteristics of the participants are presented in Table 1.

Table 1. Anthropometric characteristics of the participants.

$N = 15$	X	SD
Age [years]	23.1	2.1
Height [cm]	181	2.7
Weight [kg]	76.7	1.5
FAT [%]	13.9	1.4
FAT [kg]	11.2	1.6
FFM [kg]	67.1	1.5
BMI [kg·m^{-2}]	22.8	0.9

X—mean average; SD—standard deviation; FAT—adipose tissue; FFM—free fat mass or lean body mass; BMI—body mass index.

2.2. Measures

2.2.1. Anthropometric Measurements

To measure body height in a standing position, an anthropometer from a GPM measuring set—Skinfold Caliper User's Manual (Poland)—was used. Body mass and body composition: body fat (FAT) and fat-free mass (FFM), were measured using the TBF-300 Tanita Body Fat Monitor/Scale Analyzer (Japan) with the use of the bioelectrical impedance method. The body mass index was also used to assess overall body build: relative body mass (BMI) [kg·m^{-2}]. The participants were asked to arrive at the laboratory fasted, with voided bladders and bowels [38].

2.2.2. Normobaric Hypoxia (NH)

The GO2Altitude ERA II Hypoxic/hyperoxic air generator from Biomedtech (Australia) was used to create the appropriate hypoxic conditions during the tests. The proposed altitudes (a.s.l.) were simulated by reducing the oxygen content of the inspiratory mixture according to the recommendations of the manufacturer Biomedtech Australia Pty. Ltd. Biomedical Research and Development described in GO2 Altitude ERA II Hypoxicator System Operational Manual and in [39]. To produce a hypoxic mixture constituting a simulation of altitude at 3500 m, the oxygen level in the mixture (FIO_2 = 13%) was used, at 4500 m (FIO_2 = 12%) and 5500 m (FIO_2 = 11%), respectively. The participants were not aware of the simulated altitude. When performing tests in normoxia (NOR), the participants also wore masks connected to the generator and pulse oximeters simulating hypoxic conditions; however, at that time, the air generator produced a breathing mixture occurring naturally at sea level. Additionally, the oxygen saturation (SpO_2) was measured using a BEURER PO60 pulse oximeter during the whole experimental procedure.

2.3. Cognitive Functions

2.3.1. Stroop Interference Test (ST)

To measure cognitive control, the computer version of the ST test from the Vienna Test System database was used. The first part involves giving "names" of colors. Part two is about "reading" color names. The third part requires giving the name of the font color with which each word was written instead of reading the written word. For example, the "blue" stimulus should be reacted with the word "red," suppressing the natural tendency to read "blue." Such a task requires constant control and suppression of a natural automatic response in favor of a task consciously managed and subordinated to the rules. The result usually contains several elements, including the time of each test, the difference between the time of the first and the third test and the number of errors in the third test [40].

2.3.2. Design and Procedures

All participants were asked to refrain from exercise and the consumption of alcohol and caffeine for at least 24 h prior to each experiment to control the outside factors that could affect cardiovascular and executive functions. The participants underwent a familiarization to all the equipment needed to conduct the experiment as well as anthropometric

examinations. The experimental protocol was performed on five non-consecutive days (familiarization on day one and each altitude on a different day with a one-week break in between to avoid the learning effect). All hypoxic measurements were determined in four different conditions: normoxia and FIO_2 = 13%, FIO_2 = 12%, and FIO_2 = 11%, which correspond to simulated altitudes of 3500 m, 4500 m, and 5500 m, respectively. Experiment consisted of cognitive tests and gas mixture breathing. The participants underwent cognitive testing before and immediately after a 30 min acute exposure to the mentioned conditions.

A single blind protocol was used (the participants did not know under what conditions or on which day of testing they would be evaluated). During the testing, SpO_2 was monitored continuously using a BEURER PO60 pulse oximeter. Before performing the tests, the subjects were examined by a medical doctor.

2.3.3. Statistical Analysis

All data were collected to create a single data sheet for statistical analysis. Microsoft Excel v.10.0 for Windows was used for initial archiving and statistical processing of results. Statistical analysis was performed using the tools of GraphPad Prism 7. Arithmetic means, standard deviation, and significance levels of differences between means were calculated. Then, descriptive statistics were used, where a non-parametric paired version of the Student's *t*-test was used to examine the distribution of each variable. Then, we used two-way analysis of variance (ANOVA), with repeated measures, to investigate the significance of differences between groups and time. Significant main effects were further analyzed using the Bonferroni post hoc test. Significance for all analyses was assumed at $p < 0.05$.

3. Results

3.1. Cognitive Functions

Experiment 1

Deterioration of ST results at all implemented oxygen concentrations was observed, although these changes did not correspond to the increase in simulated altitude values. Noticeable changes were observed at the simulated altitude of 3500 m a.s.l. The analysis revealed no statistical differences either in the reading interference values (interaction F(2, 12) = 0.1374; $p = 0.8729$; time F(2, 12) = 0.5171; $p = 0.6090$) or in the naming interference values (interaction F(2, 12) = 1.647; $p = 0.2334$; time F(2, 12) = 0.8909; $p = 0.4358$), but a noticeable trend in naming was noticed (Figure 1).

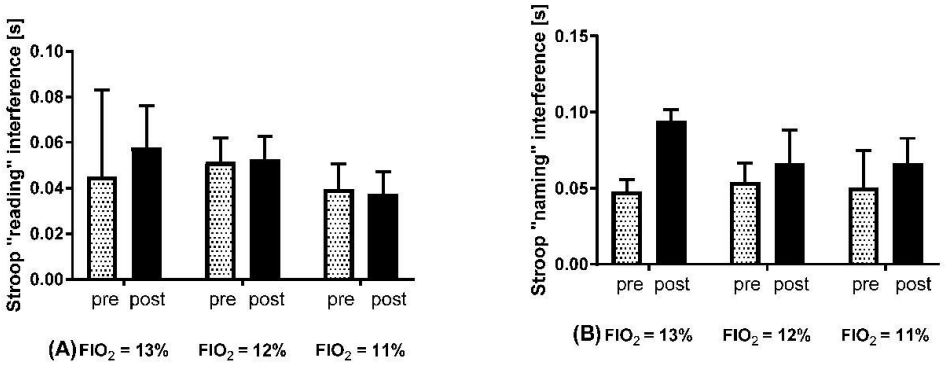

Figure 1. Effect of acute normobaric hypoxia at various simulated altitudes (a.s.l.) on post-exposure interference values in reading (**A**) and in naming (**B**). Values are means. Error bars indicate ± SEM (standard error of the mean).

Experiment 2

As a result of Experiment 1 tests, we enlarged our group and performed cognitive tests at simulated 3500 m a.s.l. (FIO_2 = 13%). There were no statistical differences in reading interference values (interaction F(1, 28) = 0.007749; p = 0.7828; time F(1, 28) = 0.03697; p = 0.8489) (Figure 2A). However, statistically significant changes were observed in naming interference values (interaction F(1, 28) = 5.404; p = 0.0276; time F(1, 28) = 11.73; p = 0.0019; $\eta 2$ = 0.16765) (Figure 2B). Next, contrast analysis between NOR (post–pre) versus NH (post–pre) was performed. The delta reading interference was not significant: t = 0.9252; p = 0.3705 (Figure 2C). Nevertheless, the delta in naming interference was significantly different between groups: t = 2.392; p = 0.0314; Cohen's d = 0.878572, paired t-test (Figure 2D).

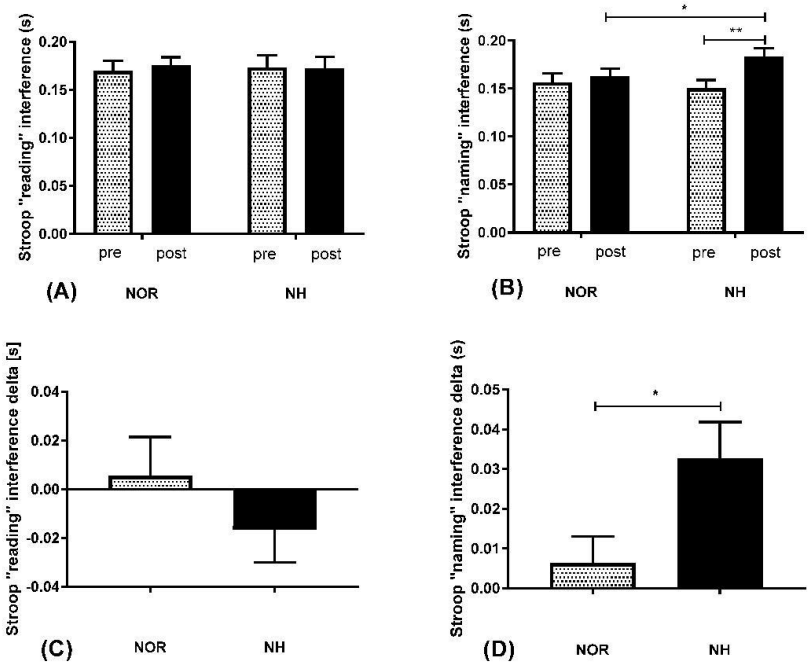

Figure 2. Effect of normoxia and acute normobaric hypoxia at FIO_2 = 13% on post-exposure interference values in reading (**A**) and in naming (**B**). Subsections (**C**,**D**) represent their deltas. Values are means. Error bars indicate SEM (standard error of the mean). * $p < 0.05$; ** $p < 0.01$. NOR—normoxia; NH—normobaric hypoxia.

3.2. Blood Saturation

Experiment 1

The tests revealed a decrease in blood saturation (between conditions F(1.197, 7.183) = 29.77; p = 0.0007)), and these changes were adequate and correlated with the reduction in oxygen concentration, which occurred with the increase in the simulated altitude (Figure 3).

Experiment 2

The saturation measurement at simulated 3500 m a.s.l. (FIO_2 = 13%) for an enlarged experimental group decreased (t = 18.5; $p < 0.0001$; Cohen's d = 1.186634), and it is shown in Figure 4.

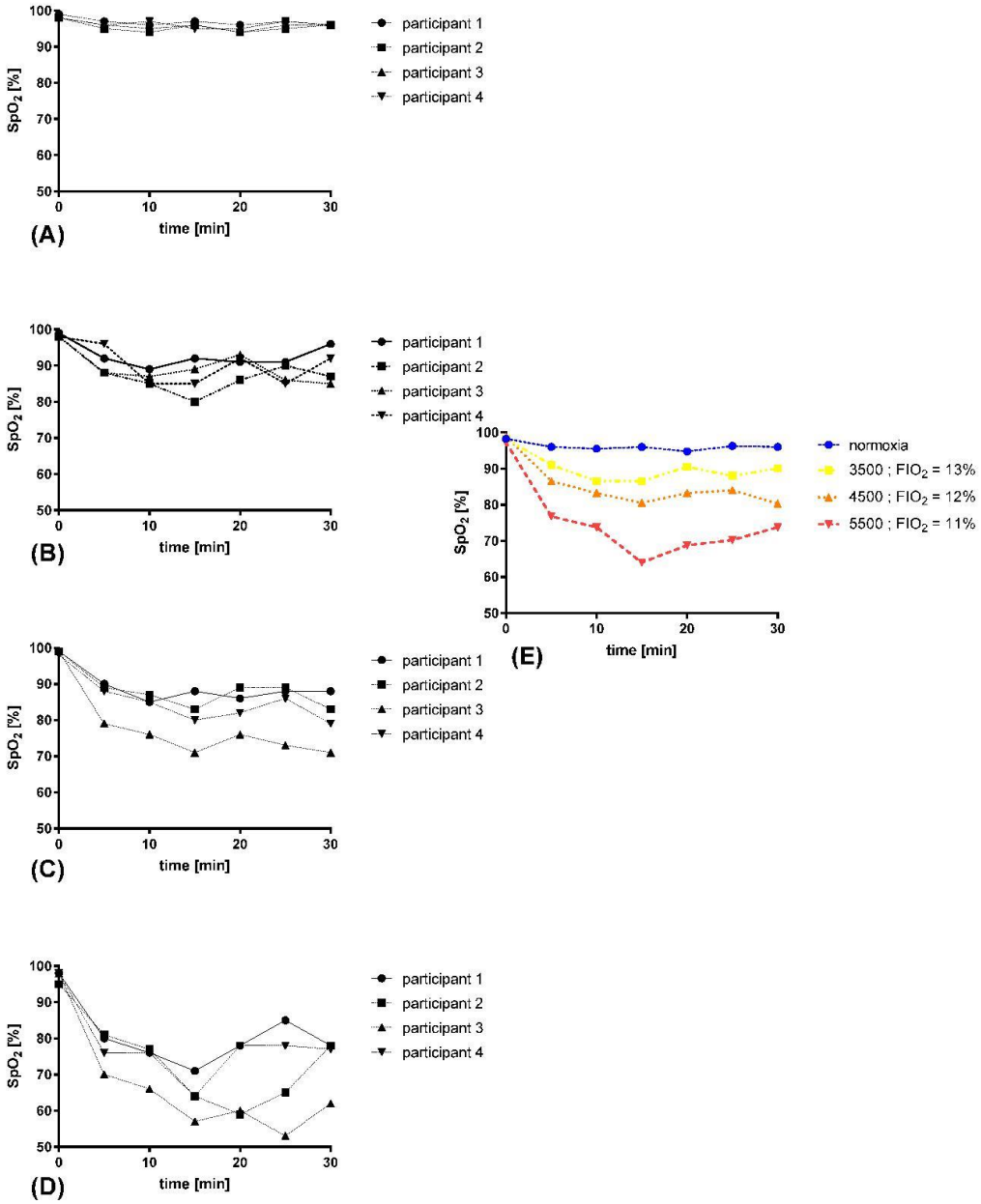

Figure 3. Effect of acute normobaric hypoxia exposure at various simulated altitudes (a.s.l.) on blood saturation: (**A**) saturation under normoxia; (**B–D**) saturation at FIO_2 = 13%, FIO_2 = 12%, and FIO_2 = 11%, respectively; (**E**) mean values at various simulated altitudes.

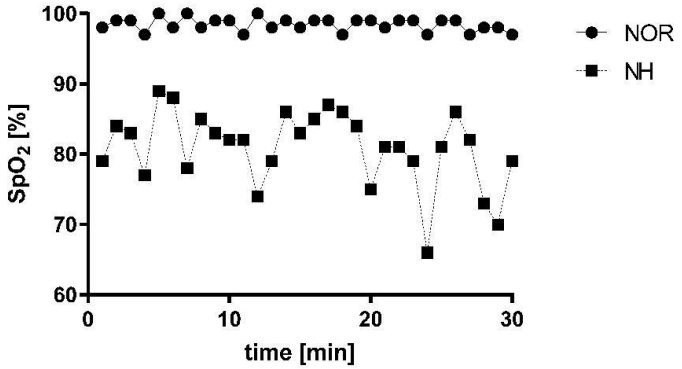

Figure 4. Effect of acute normobaric hypoxia exposure at FIO2 = 13% on blood saturation. Data are shown as the mean for the whole experimental group. NOR—normoxia; NH—normobaric hypoxia.

4. Discussion

In the present study, acute moderate NH led to a decline in post-exposure cognitive performance. In contrast to our initial hypothesis, the hypoxic magnitude did not affect differentially post-exposure cognitive functions.

It has been shown that a decrease in reaction time occurred at a simulated altitude of 3600 m a.s.l. and above [27]. Moreover, Kourtidou-Papadeli et al. (2008) showed that 16 min exposure at simulated 8000 ft (approx. 2440 m a.s.l.) was enough to increase error rate and decrease tracking performance [28].

The deterioration in human cognitive function in response to NH has been observed in previous studies. However, cognitive testing was carried out during NH exposure [27,28,41,42]. We observed a decline in cognitive performance after 30 min exposure to NH at simulated 3500 m (FIO_2 = 13%). Even if the oxygen level in the mixture was lowered to 10% O_2, a decline in cognitive functions was observed [2,27].

Based on our results, we can only speculate on the lack of potential impact of hypoxia on increased CBF, which could compensate/eliminate the destructive effect of hypoxia [22], since we did not measure it directly.

Moreover, we observe a post NH exposure decline in ST naming conditions. Color naming is presumed to be a more controlled response than reading. In the incongruent conditions in which the controlled naming response must be selected over the more habitual reading response, response should be slower [43]. In the Stroop paradigm, inhibition is thought to prevent the allocation of attention to the irrelevant stimulus dimension, allowing the participants to focus on the relevant dimension (i.e., not the name of the word, but the color of the ink in which the word is written) [43]. A decline in inhibitory control would therefore produce greater Stroop interference. This explanation is consistent with both behavioral and electrophysiological findings [44]. According to Bugg et al. (2007), the decline in naming differs depending on age [43]; however, it is not relevant in the age group that has been tested in our study [45]. It is possible that the NH exposure and thus desaturation contributed to these changes, as has also been shown in a recent study [46].

In the first experiment, we observed a tendency indicating a cognitive ability to decline in response to NH, but we did not observe any changes between the consecutive stages of hypoxia and executive function. Furthermore, the lack of difference between the executive function performance and hypoxia severity may arise from the cognitive testing time. Cognitive testing was carried out immediately after 30 min of exposure to hypoxia, under normoxic conditions, where the observed level of blood saturation corresponded to NOR. Since we did not notice significant differences between the various stages of hypoxia, for safety reasons, we decided to increase participant enlargement in the NH (simulated

altitude 3500 m a.s.l) group (Experiment 2). After increasing the number of subjects, we observed a significant executive function impairment as a result of NH exposure.

However, attention should be paid to the previously described large individual response to hypoxia exposure [47,48] and the small sample of participants in the first phase of the study (Experiment 1).

Furthermore, in our study, cognitive tests were performed immediately after hypoxic exposure, where we could expect the reperfusion effect and thus improvement of cognitive functions, as has already been described in animal models [49] as well as in humans [50–53]. The mechanism of post-exposure cognitive improvement could be a result of increased nervous tissue supply in oxygen, energy, and/or hormones as a result of increased blood flow [54,55]. Furthermore, the moderate oxidative stress and inflammation induced by hypoxic exposure could stimulate/modulate the synthesis and/or release of BDNF and prevent hippocampal impairment [31,32,56]. Despite the lack of similar results in the literature on the executive functions following NH exposure, it seems that the changes in reaction time are associated with increased growth factor production, including upregulation of BDNF, which plays a critical role in sustaining memory function [57]. Hypoxia-sensitive genes regulate hypoxia-inducible factor 1, which regulates BDNF. Hypoxia-induced BDNF production can facilitate memory function by improving synaptic strength.

A few limitations of this study must be mentioned. The small sample size could have disturbed detection of significant differences in Experiment 1 group. Secondly, the study involved a specific group that could be enlarged to evaluate, e.g., age or sex difference. Thirdly, the cerebral blood flow and BDNF concentration were not measured. Thus, further investigations are needed.

In conclusion, the current findings indicate that NH exposure impairs cognition among healthy, young, physically active men even if cognitive tests are performed in NOR conditions immediately after exposure.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local Ethics Committee and the Bioethical Committee of the Regional Medical Society in Gdansk (KB-9/16).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Article

Acute Normobaric Hypoxia Lowers Executive Functions among Young Men despite Increase of BDNF Concentration

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Abstract: Background: Decreased SpO_2 during hypoxia can cause cognitive function impairment, and the effects of acute hypoxia on high-order brain functions such as executive processing remain unclear. This study's goal was to examine the impact of an acute normobaric hypoxia breathing session on executive function and biological markers. Methods: Thirty-two healthy subjects participated in a blind study performing two sessions of single 30 min breathing bouts under two conditions (normoxia (NOR) and normobaric hypoxia (NH), $FIO_2 = 0.135$). The Stroop test was applied to assess cognitive function. Results: No significant difference was observed in the Stroop interference in the "reading" part of the test in either condition; however, there was a significant increase in the "naming" part under NH conditions ($p = 0.003$), which corresponded to a significant decrease in SpO_2 ($p < 0.001$). There was a significant increase ($p < 0.013$) in the brain-derived neurotrophic factor (BDNF) level after NH conditions compared to the baseline, which was not seen in NOR. In addition, a significant drop ($p < 0.001$) in cortisol levels in the NOR group and a slight elevation in the NH group was noticed. Conclusions: According to these findings, acute hypoxia delayed cognitive processing for motor execution and reduced the neural activity in motor executive and inhibitory processing. We also noted that this negative effect was associated with decreased SpO_2 irrespective of a rise in BDNF.

Keywords: cognitive function; physical exercise; altitude; cortisol

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1. Introduction

Hypoxia is often linked with cognitive decline. Hypoxia SpO_2 levels decrease when ascending to high altitudes, which, in turn, can cause cognitive impairment. However, it is still unknown how acute hypoxia affects higher-order brain functions including executive processing and how it affects the central nervous system (CNS) [1,2]. Hypoxia can cause perturbations in the CNS due to alterations in hormonal/humoral factor levels as well as neurotransmitters [3].

On the other hand, humans have at least several mechanisms responsible for improving cognitive function. Brain imaging studies have shown that one of these mechanisms that can improve cognitive performance is increased cerebral blood flow (CBF). Increased CBF (e.g., in the middle cerebral artery) has the potential to affect brain tissue and its metabolism due to enhancing the brain's supply of glucose, oxygen, and peripherally produced hormones [4].

Many of these hormones act as a neurochemical and play a sufficient role in brain plasticity. One of the more crucial ones is brain-derived neurotrophic factor (BDNF). BDNF induces a cascade of events via receptor kinase B (TrkB), which can promote the functional

and structural plasticity of the brain [5]. It is currently considered one of key proteins that can modulate cognitive functions [6]. It is also crucial for memory consolidation and for creating additional neural connections, etc. [7–9]. This may be explained by the ability of BDNF to cross the blood–brain barrier bidirectionally according to a concentration gradient [10]. Additionally, BDNF can be a biomarker for impaired memory in humans, as shown in a recent study [11]. On the other hand, it can cause cognitive enhancements, which are connected to higher BDNF expression in the hippocampus, dentate gyrus, and perirhinal cortex [12]. The data show that, when BDNF expression in the brain is increased, its blood level can also be observed [13,14]. The mentioned increase of the cerebral origin neurotrophic factor level influences brain neurogenesis. Improvement in cognitive functions has also been detailed [15–17]. In turn, decreased levels of neurotrophin may lead to an impaired ability to remember assigned tasks, mainly through exposure to stress or depressive conditions [18].

The hypothalamic–pituitary–adrenal axis is responsible for a whole symphony of actions when the body encounters so-called stressors and environmental differences, and can be counted as one. They can significantly affect cognitive functions due to the stress reaction, and determining the level of cortisol could help estimate the stress level [19,20]. Cortisol is one of the main glucocorticoids, produced in the adrenal gland cortex, and has a wide metabolic effect [21–23]. Some studies show cortisol rises after hypoxic exposure [24,25], although on the other hand, there are some studies showing no effect after such exposure [26,27].

The purpose of this study was to answer the question of whether acute normobaric hypoxia exposure will affect executive functions, peripheral BDNF concentrations, and stress level through cortisol. It was hypothesized that executive functions will improve after intervention and that this improvement will appear due to rise of BDNF concentration independent of changes in the cortisol level. It was examined how executive function was affected after acute exposure to hypoxic gas mixtures with FIO_2 = 13% oxygen concentration.

2. Materials and Methods

Information about the experiment was provided by members of the research team through posters and short presentations. The study involved Gdansk University of Physical Education and Sport students (n = 32, all men, aged 20.4 ± 0.6). The subjects participated in a single blind study where they performed two separate sessions of 30 min breathing bouts, under two conditions (normoxia (NOR) and normobaric hypoxia (NH)) on different days. One week prior to the intervention, the participants underwent familiarization with all laboratory equipment and performed trial tests. On the next visit, the experiment began. The first session consisted of ambient air breathing. On the second session, the participants breathed hypoxic air (fraction of inspired oxygen (FIO_2) = 0.135), which corresponded to an altitude of 3500 m. The breathing sessions were separated by a two-week break. Before and after both sessions, the participants performed a color-word Stroop task (which lasted an average of 5–6 min) and immediately afterwards were subjected to a blood draw. Two participants were tested at one time, with a staggered time allowing for cognitive testing and blood draws to avoid queuing and to standardize the testing conditions. During inhalation, one research team member per participant observed their condition and the level of SpO_2. To assess BDNF serum concentrations and estimate cortisol levels, the ELISA method was applied (a description is provided in the 'Blood Sampling' section). Cognitive testing included a Stroop interference test (cognitive control). Written consent was obtained from all participants before executing the experimental protocol. The participants were verbally informed of the possibility of opting out of the study. The research was approved by the Local Ethics Committee and the Bioethical Committee of the Regional Medical Society (KB-9/16) according to the Helsinki Declaration. The participants did not have any medical contraindications. Detailed participant characteristics are shown in Table 1.

Table 1. Anthropometric characteristics of the participants.

N = 32	X	SD
Age (years)	20.4	0.6
Height (cm)	178	7.1
Weight (kg)	76.6	11.4
FAT (%)	18.6	4.1
FAT (kg)	14.4	5.0
FFM (kg)	62.1	7.9
BMI ($kg \cdot m^{-2}$)	23.9	2.4

X—mean average; SD—standard deviation; FAT—adipose tissue; FFM—free fat mass; BMI—body mass index.

2.1. Anthropometric Measurements

To measure body height, an anthropometer from a GPM measuring set was used (Skinfold Caliper User's Manual—Poland). A TBF-300 Tanita Body Fat Monitor/Scale Analyzer (Japan) was used to assess body mass and body composition (body fat (FAT); fat-free mass (FFM)). To evaluate overall body build, body mass index (BMI) ($kg \cdot m^{-2}$) was used.

2.2. Normobaric Hypoxia

The hypoxic gas mixture for the trials was produced by the GO2Altitude ERA II Hypoxic/Hyperoxic Air Generator from Biomedtech (Australia). Height above sea level (a.s.l.) was simulated by reducing the oxygen content of the inspiratory gas mixture according to the manufacturer's recommendations (Biomedtech Australia Pty Ltd., Biomedical Research and Development, Belmore, Australia) and described in GO2Altitude ERA II Hypoxicator System Operational Manual [28]. The FIO_2 = 13% oxygen level in the mixture was used to create a hypoxic mixture that replicated an altitude of 3500 m a.s.l. While breathing, the participants were unaware of the gas mixture. When conducting testing under normoxia conditions, they also had on pulse oximeters and used masks; however, at that time, the air generator only produced a sea-level breathing mixture.

2.3. Cognitive Functions

Executive Functions: Stroop Test

To measure cognitive control, the abbreviated version of the Stroop interference test from the Vienna Test System database was used. The first part involves giving "names" of colors. Part two is about "reading" color names. The third part requires giving the name of the font color with which each word was written instead of reading the written word. For example, the "blue" stimulus should be reacted with the word "red", suppressing the natural tendency to read "blue". Such a task requires constant control and suppression of a natural automatic response in favor of a task consciously managed and subordinated to the rules. The result usually contains several elements, including the time of each test, the difference between the time of the first and third test, and the number of errors in the third test [29]. The cognitive test was the same as in a previously published study [30].

2.4. Blood Sampling

Before and after the intervention, blood samples from the antecubital vein into vacutainer tubes were taken to measure the serum concentrations of BDNF and cortisol. At 4 °C and 2000× *g*, the samples were centrifuged for 10 min. Following separation, the serum samples were frozen and stored at 70 °C for further analysis. Since the most popular technique for evaluating how human growth factors in blood relate to individual variations in neuropsychiatric, cognitive, and exercise aspects, serum analysis was conducted [31]. The sample was diluted 1:5 before use. The manufacturer reported 4–6% for the intra-assay coefficients of variability (CVs) and 8–10% for the inter-assay CVs, respectively. Both BDNF and cortisol were determined via an enzyme immunoassay method using commercial kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA, catalog no. DBD00; Ray Biotech Inc., Cambridge, UK; Demeditec Diagnostics GmbH,

Kiel, Germany, catalog no. DEH3388, respectively). Based on our prior experiences and recommendations from the literature, a 1 h clotting period was permitted for the proper serum BDNF dosage [32,33].

2.5. Statistical Analysis

The initial storage of the results and their statistical analysis were done using Microsoft Excel version 10.0 for Windows. Utilizing GraphPad Prism 7's tools, a statistical analysis was conducted. Calculated were arithmetic means, standard deviation, and levels of significance for variances between means. The distribution of each variable was then examined using descriptive statistical techniques, including a parametric paired Student's *t*-test. To further examine the significance of variations across groups and over time, a two-way analysis of variance (ANOVA) with repeated measurements was performed. The Bonferroni post hoc test was used to further examine significant effects. The significance for all analyses was assumed at $p < 0.05$.

3. Results

3.1. Blood Saturation under Normoxia and Acute Normobaric Hypoxia Conditions

Acute exposure to normobaric hypoxia conditions revealed a decrease in blood saturation ($t = 12$, $df = 29$; $p < 0.001$). These changes were appropriate to the simulated altitude above sea level (Figure 1).

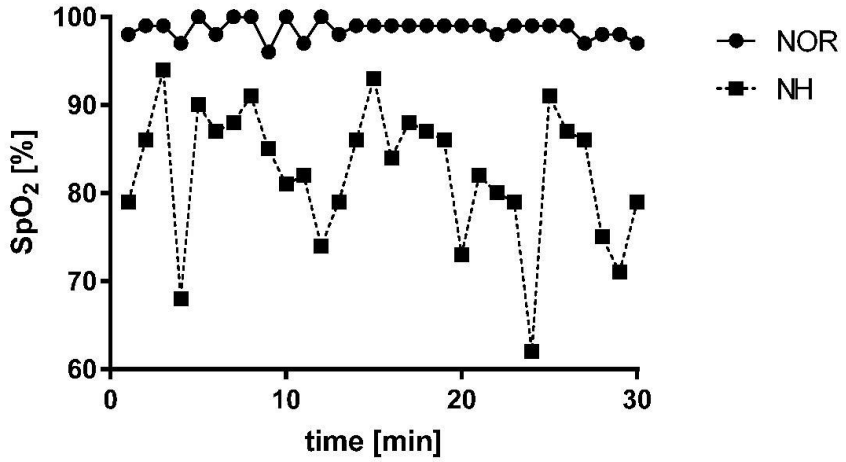

Figure 1. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at $FIO_2 = 13\%$ conditions on peripheral blood saturation. NOR—normoxia; NH—normobaric hypoxia. Data expressed as mean of the entire group at particular time points.

3.2. Stroop Test under Conditions of Normoxia and Acute Normobaric Hypoxia

The participants performed cognitive tests after exposure to normobaric hypoxic conditions simulating 3500 m a.s.l. There were no statistical differences in "reading" interference (interaction F (1, 60) = 0.006, $p = 0.939$; time F (1, 60) = 0.0047, $p = 0.946$) (Figure 2A). On the contrary, "naming" interference was significantly changed (interaction F (1, 60) = 4.644, $p = 0.035$; time F (1, 60) = 6.375, $p = 0.014$) (Figure 2B), which corresponded with a significant decrease in the peripheral level of SpO_2. Next, contrast analysis between NOR versus NH (post vs. pre) was performed (Figure 2C,D).

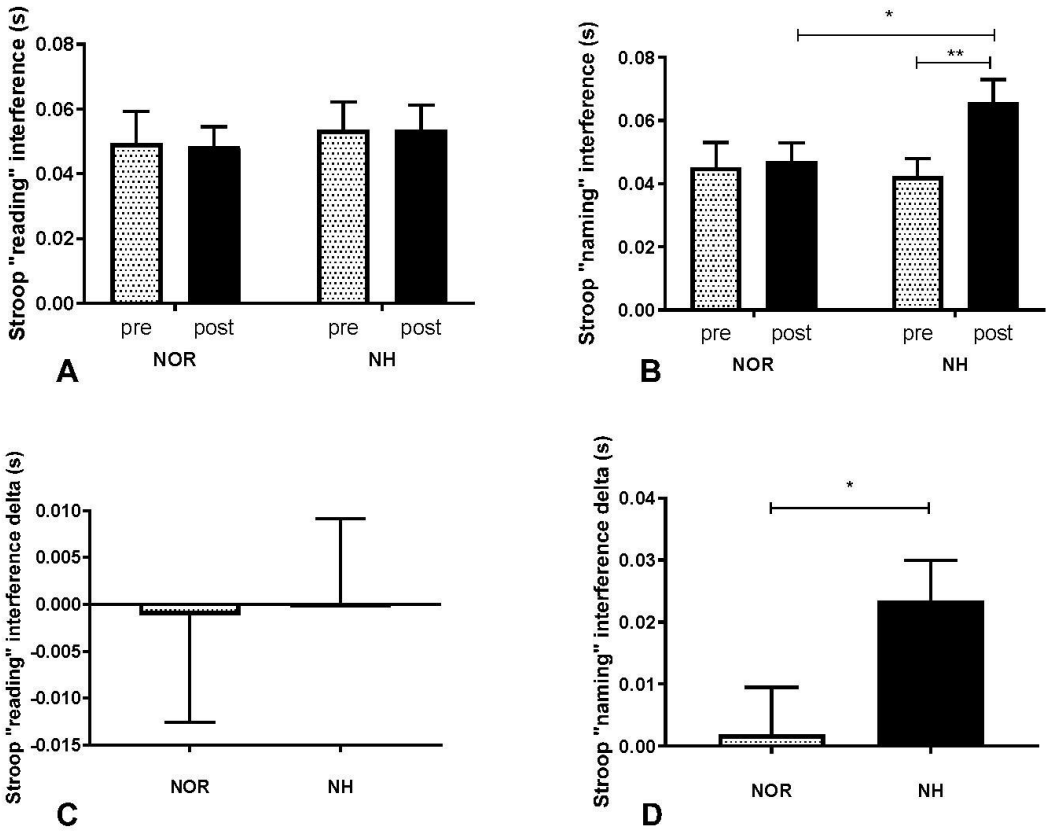

Figure 2. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at FIO_2 = 13% conditions on post-exposure interference values in "reading" (**A**) and in "naming" (**B**) and their deltas (**C**,**D**). Values are means. Error bars indicate SEM (standard error of the mean). * $p < 0.05$; ** $p < 0.01$.

3.3. Blood Analysis

The mean values of BDNF concentration did not change in the NOR conditions in pre-post measurements, but a significant rise in the concentration of BDNF after the simulated NH conditions was observed (interaction F (1, 62) = 3.893, $p = 0.053$; row factor F (1, 62) = 7.358, $p = 0.009$; time F (1, 62) = 3.999, $p = 0.0499$) (Figure 3).

The test analysis revealed significant changes in the cortisol values under NOR conditions in the pre-post measurements, although the same effect after simulated NH conditions was not observed. Interactions between groups occurred (interaction F (1, 62) = 35.9, $p < 0.001$; row factor F (1, 62) = 11.71, $p = 0.001$; time F (1, 62) = 16.61, $p = 0.001$), and the delta was also shown (Figure 4). Mixed model ANOVA showed the same results and they are available in the supplementary materials.

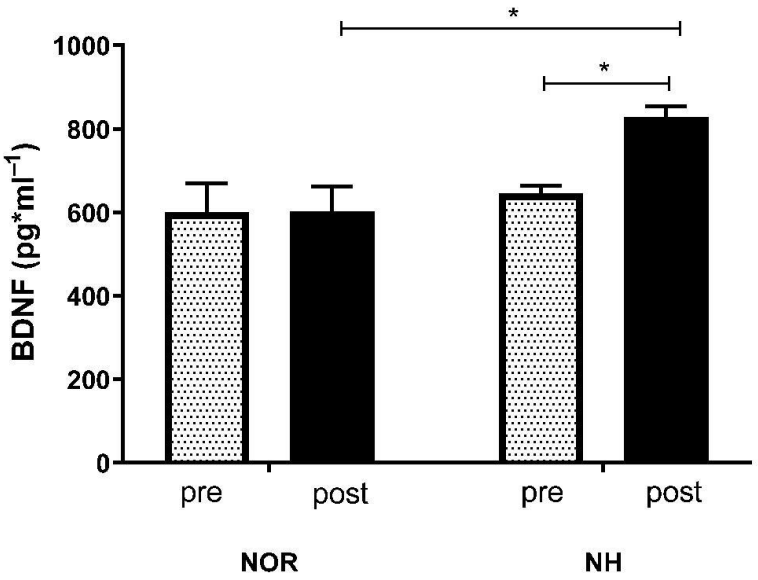

Figure 3. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at FIO_2 = 13% conditions on post-exposure serum BDNF concentration. Values represent means. Error bars indicate SEM. * $p < 0.05$.

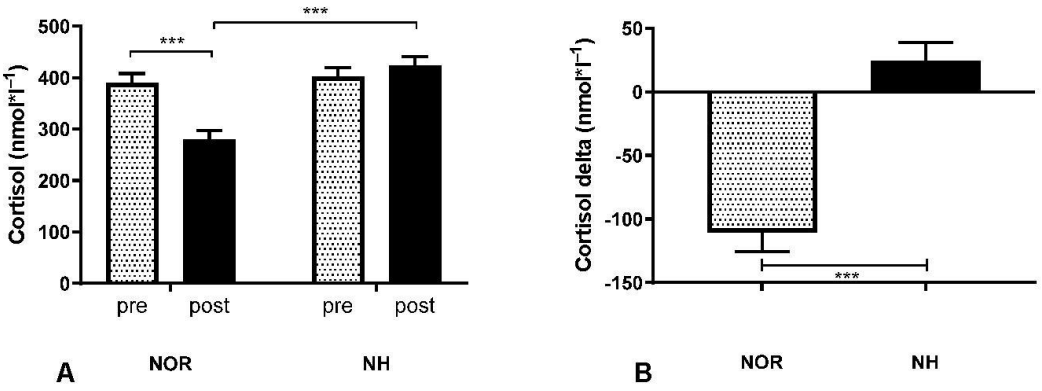

Figure 4. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at FIO_2 = 13% conditions on post-exposure serum cortisol concentration (**A**) and its delta (**B**). Values represent means. Error bars indicate SEM (standard error of the mean). *** $p < 0.001$.

4. Discussion

The main goal of the present study was to examine the impact of a single session of simulated acute normobaric hypoxia on the BDNF concentration and relate to it executive functions, as well as the cortisol level in young adults.

Significant cognitive decline after a single session of acute NH breathing was noticed, which is in line with previous research [34–36]. This deterioration was reflected by increased interference in the Stroop test.

The timepoint of cognitive performance, which in our study occurred immediately after hypoxia exposure, is not without relevance. The reperfusion effect, which has been described in both animal models [37] and in humans [38–40], could improve cognitive

functions at that time. This post-exposure improvement is most likely due to this reperfusion and, thus, better tissue oxygenation [41,42]. Furthermore, hypoxic-induced oxidative stress may alter BDNF production and/or its release, preventing hippocampal impairment [43–45]. As a neuro-regulator and factor influencing brain neurogenesis, an increase in the level of the cerebral neurotrophic factor and, consequently, an improvement in cognitive functioning has also been reported [15–17].

Although an increase in BDNF concentration was observed, a decline in cognitive function after 30 min of acute exposure to normobaric hypoxia at a simulated altitude of 3500 m (FIO_2 = 13%) was also noticed. This decrease is consistent with earlier research [36,46]. Cerebral hypoxia, which can be caused by arterial oxygen deficiency and decreased cerebral blood flow, is thought to play a role in executive function impairment. This impairment is related to the level of SpO_2, which drops proportionally due to the severity of the hypoxic conditions, as we have shown in a previous study [30]. In addition, since it is strongly connected with prefrontal oxygenation, it can have an impact [47,48]. This is consistent with earlier research showing that cognitive function declines as the severity of hypoxic conditions increases [34], and that SpO_2 can alter prefrontal oxygenation and result in CBF restriction through cerebral vasoconstriction [49].

Furthermore, our findings seem to confirm earlier studies that hypoxia had little or no impact on the post-exposure increase in CBF, which could counteract or minimize the adverse effects of hypoxia [50]. This is only speculation, since we did not measure it directly. This effect is seen in the results of the cognitive tests used in our study. Reading is thought to be a more instinctive response than naming because it is less controlled. When circumstances require that the controlled naming response prevails over the automatic reading response, it slows down [51]. Inhibition is thought to prevent participants in the Stroop paradigm from directing their attention to the irrelevant stimulus component, allowing them to concentrate on the important dimension (i.e., the color of ink in which the word is written, rather than the name of the word) [51]. Therefore, a decrease in inhibitory control would result in greater Stroop interference. Both behavioral and electrophysiological data seem to support such an interpretation [52].

Decreases in cognitive function induced by environmental changes noted as a stressor may also be reflected in biological markers. Hypoxia can activate the gland cortex to release cortisol, which has been identified as a presumed endocrinological mediator that can affect brain function [21]. Nonetheless, the results of hypoxia investigations are inconsistent in terms of the observed reactions. The most common response to these environmental stimuli is a rise in cortisol levels [53], although no changes have been documented [54]. This difference could be due to the different research designs (i.e., normobaric vs. hypobaric hypoxia). The participants' cortisol levels were considerably lower following exposure to NOR than under NH conditions in our study. Due to the lack of difference at baseline and the non-significant increase in concentrations after exposure to NH conditions, we can speculate that the reduction in the NOR group may have been due to calming down/entering a state of relaxation by breathing calmly. On the other hand, as this was still at the start of the study, perhaps a defense mechanism to an impending unknown stimulus triggered significant changes at the biochemical level. This seems to effectively counteract the sudden cortisol level increase in the NH group, which is a possibility, considering that a stress trigger could result in such a response, especially when this hormone has the potential to impair the prefrontal cortex [22]. Some may argue that because this area of the brain regulates so many of our cognitive activities, the negative influence of cortisol on psychomotor performance is negligible, yet it seems to be one of the elements that can be thoroughly investigated in future research.

A few limitations of this study must be mentioned. Future research groups could be enlarged to assess, for example, age or sex differences. To make the experimental conditions less predictable, the sequence of NOR and NH could be more randomized. Alternately, a control group that received the NH intervention twice in a row might be used. Further studies should include brain tissue oxygenation measurement to investigate the potential

mechanism responsible for these changes. This study revealed that a single session of simulated acute normobaric hypoxia leads to a significant impact on BDNF concentrations as well as cognitive impairment in the "naming" aspect of the Stroop test. Moreover, this attenuation can be likely linked to cortisol levels, although this requires further research.

5. Conclusions

The consequences of acute hypoxia on cognitive function are still debatable. However, hypoxia has the potential to impair cognition. The effects of a single acute exposure to simulated moderate normobaric hypoxia conditions were investigated and impairments in executive functions were observed. A reduced SpO_2 level was associated with this detrimental effect irrespective of the rise in BDNF. Although higher BDNF levels can induce positive changes through improvements in human cognition, further research about acute hypoxia, exercise/training in such conditions, and its effect on cognition need to be conducted. According to these findings, acute hypoxia delayed cognitive processing for motor execution and reduced the neural activity in motor executive and inhibitory processing.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijerph191710802/s1, Table S1: Results of the mixed-model ANOVA.

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Article

Acute Normobaric Hypoxia Lowers Executive Functions among Young Men despite Increase of BDNF Concentration

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Abstract: Background: Decreased SpO_2 during hypoxia can cause cognitive function impairment, and the effects of acute hypoxia on high-order brain functions such as executive processing remain unclear. This study's goal was to examine the impact of an acute normobaric hypoxia breathing session on executive function and biological markers. Methods: Thirty-two healthy subjects participated in a blind study performing two sessions of single 30 min breathing bouts under two conditions (normoxia (NOR) and normobaric hypoxia (NH), $FIO_2 = 0.135$). The Stroop test was applied to assess cognitive function. Results: No significant difference was observed in the Stroop interference in the "reading" part of the test in either condition; however, there was a significant increase in the "naming" part under NH conditions ($p = 0.003$), which corresponded to a significant decrease in SpO_2 ($p < 0.001$). There was a significant increase ($p < 0.013$) in the brain-derived neurotrophic factor (BDNF) level after NH conditions compared to the baseline, which was not seen in NOR. In addition, a significant drop ($p < 0.001$) in cortisol levels in the NOR group and a slight elevation in the NH group was noticed. Conclusions: According to these findings, acute hypoxia delayed cognitive processing for motor execution and reduced the neural activity in motor executive and inhibitory processing. We also noted that this negative effect was associated with decreased SpO_2 irrespective of a rise in BDNF.

Keywords: cognitive function; physical exercise; altitude; cortisol

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1. Introduction

Hypoxia is often linked with cognitive decline. Hypoxia SpO_2 levels decrease when ascending to high altitudes, which, in turn, can cause cognitive impairment. However, it is still unknown how acute hypoxia affects higher-order brain functions including executive processing and how it affects the central nervous system (CNS) [1,2]. Hypoxia can cause perturbations in the CNS due to alterations in hormonal/humoral factor levels as well as neurotransmitters [3].

On the other hand, humans have at least several mechanisms responsible for improving cognitive function. Brain imaging studies have shown that one of these mechanisms that can improve cognitive performance is increased cerebral blood flow (CBF). Increased CBF (e.g., in the middle cerebral artery) has the potential to affect brain tissue and its metabolism due to enhancing the brain's supply of glucose, oxygen, and peripherally produced hormones [4].

Many of these hormones act as a neurochemical and play a sufficient role in brain plasticity. One of the more crucial ones is brain-derived neurotrophic factor (BDNF). BDNF induces a cascade of events via receptor kinase B (TrkB), which can promote the functional

and structural plasticity of the brain [5]. It is currently considered one of key proteins that can modulate cognitive functions [6]. It is also crucial for memory consolidation and for creating additional neural connections, etc. [7–9]. This may be explained by the ability of BDNF to cross the blood–brain barrier bidirectionally according to a concentration gradient [10]. Additionally, BDNF can be a biomarker for impaired memory in humans, as shown in a recent study [11]. On the other hand, it can cause cognitive enhancements, which are connected to higher BDNF expression in the hippocampus, dentate gyrus, and perirhinal cortex [12]. The data show that, when BDNF expression in the brain is increased, its blood level can also be observed [13,14]. The mentioned increase of the cerebral origin neurotrophic factor level influences brain neurogenesis. Improvement in cognitive functions has also been detailed [15–17]. In turn, decreased levels of neurotrophin may lead to an impaired ability to remember assigned tasks, mainly through exposure to stress or depressive conditions [18].

The hypothalamic–pituitary–adrenal axis is responsible for a whole symphony of actions when the body encounters so-called stressors and environmental differences, and can be counted as one. They can significantly affect cognitive functions due to the stress reaction, and determining the level of cortisol could help estimate the stress level [19,20]. Cortisol is one of the main glucocorticoids, produced in the adrenal gland cortex, and has a wide metabolic effect [21–23]. Some studies show cortisol rises after hypoxic exposure [24,25], although on the other hand, there are some studies showing no effect after such exposure [26,27].

The purpose of this study was to answer the question of whether acute normobaric hypoxia exposure will affect executive functions, peripheral BDNF concentrations, and stress level through cortisol. It was hypothesized that executive functions will improve after intervention and that this improvement will appear due to rise of BDNF concentration independent of changes in the cortisol level. It was examined how executive function was affected after acute exposure to hypoxic gas mixtures with FIO_2 = 13% oxygen concentration.

2. Materials and Methods

Information about the experiment was provided by members of the research team through posters and short presentations. The study involved Gdansk University of Physical Education and Sport students (n = 32, all men, aged 20.4 ± 0.6). The subjects participated in a single blind study where they performed two separate sessions of 30 min breathing bouts, under two conditions (normoxia (NOR) and normobaric hypoxia (NH)) on different days. One week prior to the intervention, the participants underwent familiarization with all laboratory equipment and performed trial tests. On the next visit, the experiment began. The first session consisted of ambient air breathing. On the second session, the participants breathed hypoxic air (fraction of inspired oxygen (FIO_2) = 0.135), which corresponded to an altitude of 3500 m. The breathing sessions were separated by a two-week break. Before and after both sessions, the participants performed a color-word Stroop task (which lasted an average of 5–6 min) and immediately afterwards were subjected to a blood draw. Two participants were tested at one time, with a staggered time allowing for cognitive testing and blood draws to avoid queuing and to standardize the testing conditions. During inhalation, one research team member per participant observed their condition and the level of SpO_2. To assess BDNF serum concentrations and estimate cortisol levels, the ELISA method was applied (a description is provided in the 'Blood Sampling' section). Cognitive testing included a Stroop interference test (cognitive control). Written consent was obtained from all participants before executing the experimental protocol. The participants were verbally informed of the possibility of opting out of the study. The research was approved by the Local Ethics Committee and the Bioethical Committee of the Regional Medical Society (KB-9/16) according to the Helsinki Declaration. The participants did not have any medical contraindications. Detailed participant characteristics are shown in Table 1.

Table 1. Anthropometric characteristics of the participants.

N = 32	X	SD
Age (years)	20.4	0.6
Height (cm)	178	7.1
Weight (kg)	76.6	11.4
FAT (%)	18.6	4.1
FAT (kg)	14.4	5.0
FFM (kg)	62.1	7.9
BMI ($kg \cdot m^{-2}$)	23.9	2.4

X—mean average; SD—standard deviation; FAT—adipose tissue; FFM—free fat mass; BMI—body mass index.

2.1. Anthropometric Measurements

To measure body height, an anthropometer from a GPM measuring set was used (Skinfold Caliper User's Manual—Poland). A TBF-300 Tanita Body Fat Monitor/Scale Analyzer (Japan) was used to assess body mass and body composition (body fat (FAT); fat-free mass (FFM)). To evaluate overall body build, body mass index (BMI) ($kg \cdot m^{-2}$) was used.

2.2. Normobaric Hypoxia

The hypoxic gas mixture for the trials was produced by the GO2Altitude ERA II Hypoxic/Hyperoxic Air Generator from Biomedtech (Australia). Height above sea level (a.s.l.) was simulated by reducing the oxygen content of the inspiratory gas mixture according to the manufacturer's recommendations (Biomedtech Australia Pty Ltd., Biomedical Research and Development, Belmore, Australia) and described in GO2Altitude ERA II Hypoxicator System Operational Manual [28]. The FIO_2 = 13% oxygen level in the mixture was used to create a hypoxic mixture that replicated an altitude of 3500 m a.s.l. While breathing, the participants were unaware of the gas mixture. When conducting testing under normoxia conditions, they also had on pulse oximeters and used masks; however, at that time, the air generator only produced a sea-level breathing mixture.

2.3. Cognitive Functions

Executive Functions: Stroop Test

To measure cognitive control, the abbreviated version of the Stroop interference test from the Vienna Test System database was used. The first part involves giving "names" of colors. Part two is about "reading" color names. The third part requires giving the name of the font color with which each word was written instead of reading the written word. For example, the "blue" stimulus should be reacted with the word "red", suppressing the natural tendency to read "blue". Such a task requires constant control and suppression of a natural automatic response in favor of a task consciously managed and subordinated to the rules. The result usually contains several elements, including the time of each test, the difference between the time of the first and third test, and the number of errors in the third test [29]. The cognitive test was the same as in a previously published study [30].

2.4. Blood Sampling

Before and after the intervention, blood samples from the antecubital vein into vacutainer tubes were taken to measure the serum concentrations of BDNF and cortisol. At 4 °C and 2000× *g*, the samples were centrifuged for 10 min. Following separation, the serum samples were frozen and stored at 70 °C for further analysis. Since the most popular technique for evaluating how human growth factors in blood relate to individual variations in neuropsychiatric, cognitive, and exercise aspects, serum analysis was conducted [31]. The sample was diluted 1:5 before use. The manufacturer reported 4–6% for the intra-assay coefficients of variability (CVs) and 8–10% for the inter-assay CVs, respectively. Both BDNF and cortisol were determined via an enzyme immunoassay method using commercial kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA, catalog no. DBD00; Ray Biotech Inc., Cambridge, UK; Demeditec Diagnostics GmbH,

Kiel, Germany, catalog no. DEH3388, respectively). Based on our prior experiences and recommendations from the literature, a 1 h clotting period was permitted for the proper serum BDNF dosage [32,33].

2.5. Statistical Analysis

The initial storage of the results and their statistical analysis were done using Microsoft Excel version 10.0 for Windows. Utilizing GraphPad Prism 7's tools, a statistical analysis was conducted. Calculated were arithmetic means, standard deviation, and levels of significance for variances between means. The distribution of each variable was then examined using descriptive statistical techniques, including a parametric paired Student's *t*-test. To further examine the significance of variations across groups and over time, a two-way analysis of variance (ANOVA) with repeated measurements was performed. The Bonferroni post hoc test was used to further examine significant effects. The significance for all analyses was assumed at $p < 0.05$.

3. Results

3.1. Blood Saturation under Normoxia and Acute Normobaric Hypoxia Conditions

Acute exposure to normobaric hypoxia conditions revealed a decrease in blood saturation ($t = 12$, $df = 29$; $p < 0.001$). These changes were appropriate to the simulated altitude above sea level (Figure 1).

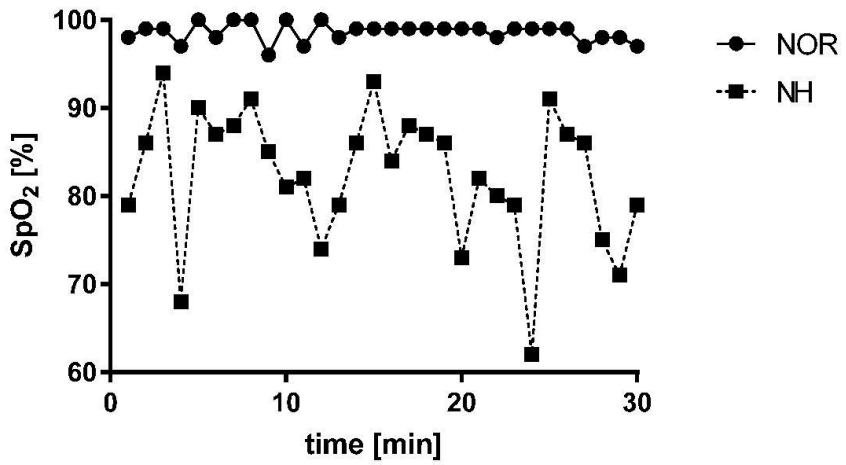

Figure 1. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at $FIO_2 = 13\%$ conditions on peripheral blood saturation. NOR—normoxia; NH—normobaric hypoxia. Data expressed as mean of the entire group at particular time points.

3.2. Stroop Test under Conditions of Normoxia and Acute Normobaric Hypoxia

The participants performed cognitive tests after exposure to normobaric hypoxic conditions simulating 3500 m a.s.l. There were no statistical differences in "reading" interference (interaction F (1, 60) = 0.006, $p = 0.939$; time F (1, 60) = 0.0047, $p = 0.946$) (Figure 2A). On the contrary, "naming" interference was significantly changed (interaction F (1, 60) = 4.644, $p = 0.035$; time F (1, 60) = 6.375, $p = 0.014$) (Figure 2B), which corresponded with a significant decrease in the peripheral level of SpO_2. Next, contrast analysis between NOR versus NH (post vs. pre) was performed (Figure 2C,D).

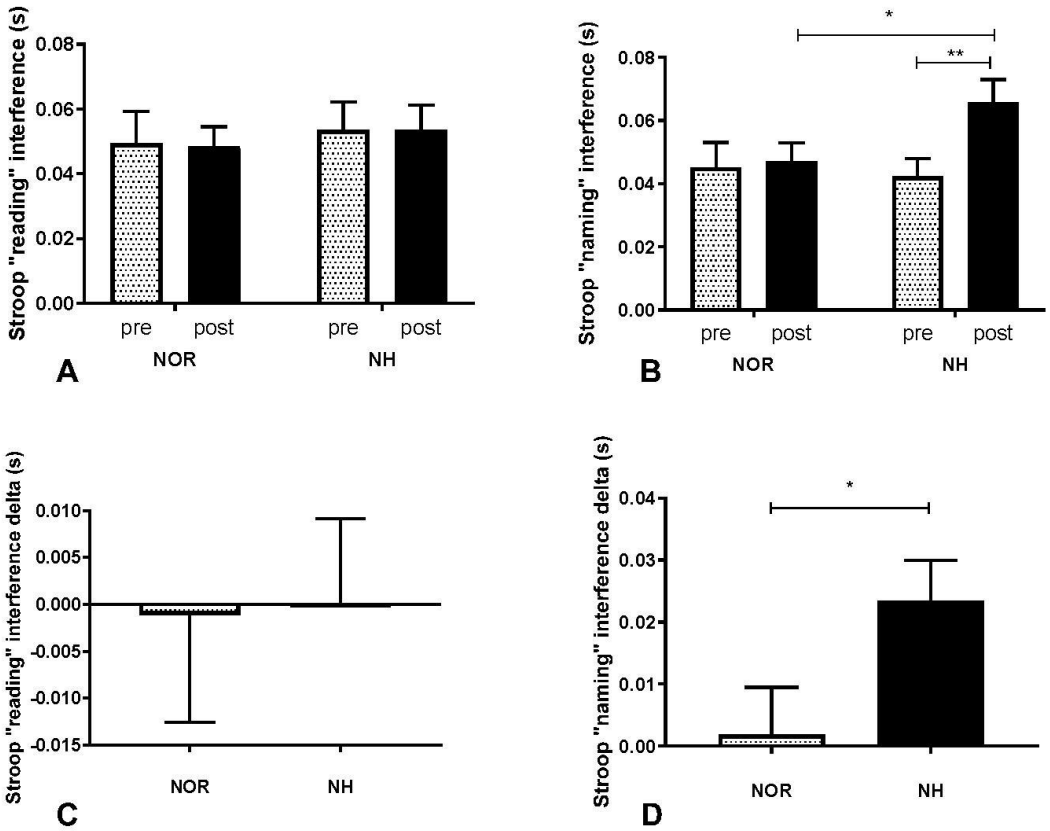

Figure 2. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at FIO_2 = 13% conditions on post-exposure interference values in "reading" (**A**) and in "naming" (**B**) and their deltas (**C**,**D**). Values are means. Error bars indicate SEM (standard error of the mean). * $p < 0.05$; ** $p < 0.01$.

3.3. Blood Analysis

The mean values of BDNF concentration did not change in the NOR conditions in pre-post measurements, but a significant rise in the concentration of BDNF after the simulated NH conditions was observed (interaction F (1, 62) = 3.893, $p = 0.053$; row factor F (1, 62) = 7.358, $p = 0.009$; time F (1, 62) = 3.999, $p = 0.0499$) (Figure 3).

The test analysis revealed significant changes in the cortisol values under NOR conditions in the pre-post measurements, although the same effect after simulated NH conditions was not observed. Interactions between groups occurred (interaction F (1, 62) = 35.9, $p < 0.001$; row factor F (1, 62) = 11.71, $p = 0.001$; time F (1, 62) = 16.61, $p = 0.001$), and the delta was also shown (Figure 4). Mixed model ANOVA showed the same results and they are available in the supplementary materials.

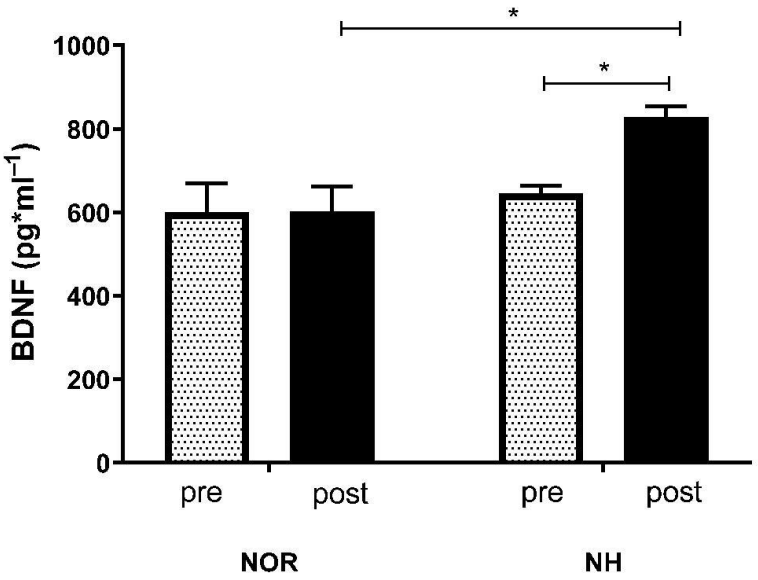

Figure 3. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at FIO_2 = 13% conditions on post-exposure serum BDNF concentration. Values represent means. Error bars indicate SEM. * $p < 0.05$.

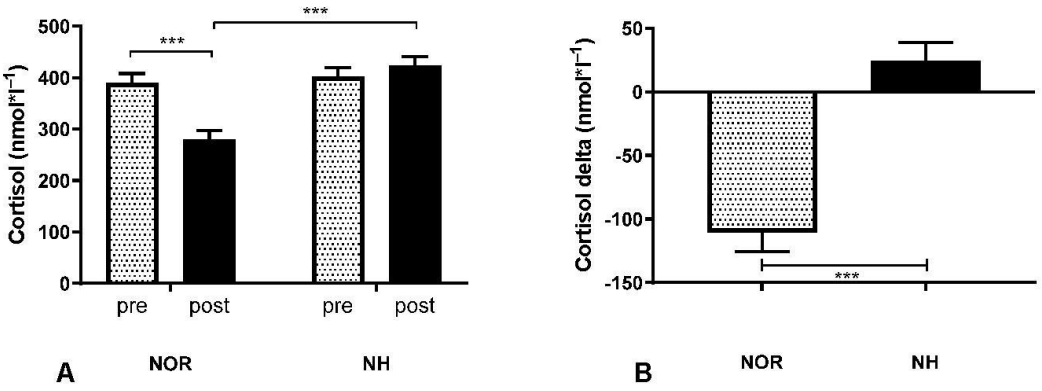

Figure 4. Effect of a single 30 min exposure under normoxia and normobaric hypoxia at FIO_2 = 13% conditions on post-exposure serum cortisol concentration (**A**) and its delta (**B**). Values represent means. Error bars indicate SEM (standard error of the mean). *** $p < 0.001$.

4. Discussion

The main goal of the present study was to examine the impact of a single session of simulated acute normobaric hypoxia on the BDNF concentration and relate to it executive functions, as well as the cortisol level in young adults.

Significant cognitive decline after a single session of acute NH breathing was noticed, which is in line with previous research [34–36]. This deterioration was reflected by increased interference in the Stroop test.

The timepoint of cognitive performance, which in our study occurred immediately after hypoxia exposure, is not without relevance. The reperfusion effect, which has been described in both animal models [37] and in humans [38–40], could improve cognitive

functions at that time. This post-exposure improvement is most likely due to this reperfusion and, thus, better tissue oxygenation [41,42]. Furthermore, hypoxic-induced oxidative stress may alter BDNF production and/or its release, preventing hippocampal impairment [43–45]. As a neuro-regulator and factor influencing brain neurogenesis, an increase in the level of the cerebral neurotrophic factor and, consequently, an improvement in cognitive functioning has also been reported [15–17].

Although an increase in BDNF concentration was observed, a decline in cognitive function after 30 min of acute exposure to normobaric hypoxia at a simulated altitude of 3500 m (FIO_2 = 13%) was also noticed. This decrease is consistent with earlier research [36,46]. Cerebral hypoxia, which can be caused by arterial oxygen deficiency and decreased cerebral blood flow, is thought to play a role in executive function impairment. This impairment is related to the level of SpO_2, which drops proportionally due to the severity of the hypoxic conditions, as we have shown in a previous study [30]. In addition, since it is strongly connected with prefrontal oxygenation, it can have an impact [47,48]. This is consistent with earlier research showing that cognitive function declines as the severity of hypoxic conditions increases [34], and that SpO_2 can alter prefrontal oxygenation and result in CBF restriction through cerebral vasoconstriction [49].

Furthermore, our findings seem to confirm earlier studies that hypoxia had little or no impact on the post-exposure increase in CBF, which could counteract or minimize the adverse effects of hypoxia [50]. This is only speculation, since we did not measure it directly. This effect is seen in the results of the cognitive tests used in our study. Reading is thought to be a more instinctive response than naming because it is less controlled. When circumstances require that the controlled naming response prevails over the automatic reading response, it slows down [51]. Inhibition is thought to prevent participants in the Stroop paradigm from directing their attention to the irrelevant stimulus component, allowing them to concentrate on the important dimension (i.e., the color of ink in which the word is written, rather than the name of the word) [51]. Therefore, a decrease in inhibitory control would result in greater Stroop interference. Both behavioral and electrophysiological data seem to support such an interpretation [52].

Decreases in cognitive function induced by environmental changes noted as a stressor may also be reflected in biological markers. Hypoxia can activate the gland cortex to release cortisol, which has been identified as a presumed endocrinological mediator that can affect brain function [21]. Nonetheless, the results of hypoxia investigations are inconsistent in terms of the observed reactions. The most common response to these environmental stimuli is a rise in cortisol levels [53], although no changes have been documented [54]. This difference could be due to the different research designs (i.e., normobaric vs. hypobaric hypoxia). The participants' cortisol levels were considerably lower following exposure to NOR than under NH conditions in our study. Due to the lack of difference at baseline and the non-significant increase in concentrations after exposure to NH conditions, we can speculate that the reduction in the NOR group may have been due to calming down/entering a state of relaxation by breathing calmly. On the other hand, as this was still at the start of the study, perhaps a defense mechanism to an impending unknown stimulus triggered significant changes at the biochemical level. This seems to effectively counteract the sudden cortisol level increase in the NH group, which is a possibility, considering that a stress trigger could result in such a response, especially when this hormone has the potential to impair the prefrontal cortex [22]. Some may argue that because this area of the brain regulates so many of our cognitive activities, the negative influence of cortisol on psychomotor performance is negligible, yet it seems to be one of the elements that can be thoroughly investigated in future research.

A few limitations of this study must be mentioned. Future research groups could be enlarged to assess, for example, age or sex differences. To make the experimental conditions less predictable, the sequence of NOR and NH could be more randomized. Alternately, a control group that received the NH intervention twice in a row might be used. Further studies should include brain tissue oxygenation measurement to investigate the potential

mechanism responsible for these changes. This study revealed that a single session of simulated acute normobaric hypoxia leads to a significant impact on BDNF concentrations as well as cognitive impairment in the "naming" aspect of the Stroop test. Moreover, this attenuation can be likely linked to cortisol levels, although this requires further research.

5. Conclusions

The consequences of acute hypoxia on cognitive function are still debatable. However, hypoxia has the potential to impair cognition. The effects of a single acute exposure to simulated moderate normobaric hypoxia conditions were investigated and impairments in executive functions were observed. A reduced SpO_2 level was associated with this detrimental effect irrespective of the rise in BDNF. Although higher BDNF levels can induce positive changes through improvements in human cognition, further research about acute hypoxia, exercise/training in such conditions, and its effect on cognition need to be conducted. According to these findings, acute hypoxia delayed cognitive processing for motor execution and reduced the neural activity in motor executive and inhibitory processing.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijerph191710802/s1, Table S1: Results of the mixed-model ANOVA.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Prof. Dr hab. Radosław Laskowski