Akademia Wychowania Fizycznego im. Eugeniusza Piaseckiego w Poznaniu

mgr Ewa Zarębska

Rozprawa doktorska

Zmiany osoczowego stężenia adenozynotrifosforanu u zawodników klasy mistrzowskiej w rocznym cyklu treningowym

Promotor:

prof. AWF dr hab. Jacek Zieliński

Promotor pomocniczy:

dr Łukasz Kruszyna

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Ewa Zarębska MSc

Doctoral dissertation

Changes in plasma ATP concentration in highly trained athletes in a one–year training cycle

Supervisor:

Assoc. Prof. Jacek Zieliński

Co–supervisor:

dr Łukasz Kruszyna

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- Oświadczenia współautorów
- Publikacja nr 1 pt.: „Plasma nucleotide dynamics during exercise and recovery in highly trained athletes and recreationally active individuals”
- Publikacja nr 2 pt.: „Alterations in exercise–induced plasma adenosine triphosphate concentration in highly trained athletes in a one–year training cycle”

I. AUTOREFERAT W JĘZYKU POLSKIM

Niniejszą rozprawę doktorską oparto o cykl publikacji pod wspólnym tytułem: „Zmiany osoczowego stężenia adenozynotrifosforanu u zawodników klasy mistrzowskiej w rocznym cyklu treningowym", na który składają się dwie publikacje przygotowane w ramach projektu naukowego nr 2013/09/B/NZ7/02556, sfinansowanego przez Narodowe Centrum Nauki:

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2. *Alterations in exercise–induced plasma adenosine triphosphate concentration in highly trained athletes in a one–year training cycle,* Metabolites, 2019, 9 (10 [230]), doi: 10.3390/metabo9100230; impact factor – 3.303, punktacja MNISW – 70pkt.

1. Wstęp

Adenozynotrifosforan (ATP), oprócz roli wewnątrzkomórkowego źródła energii, pełni równie ważną zewnątrzkomórkową funkcję jako cząsteczka sygnalizacyjna. Uwolnienie ATP do przestrzeni zewnątrzkomórkowej wpływa na procesy wazodylatacji (Ellsworth i wsp. 1995; Rosenmaier i wsp. 2004) i czynnościowej sympatykolizy (Kirby i wsp. 2008; Rosenmeier i wsp. 2004, 2008), które są współodpowiedzialne za zaopatrzenie mięśni szkieletowych w tlen podczas wysiłku fizycznego. Jednym z głównych źródeł ATP w przestrzeni zewnątrzkomórkowej jest erytrocyt, który uwalnia ATP w odpowiedzi na zmniejszenie ciśnienia parcjalnego tlenu we krwi (González–Alonso i wsp. 2002), wzrost temperatury (Kalsi i González–Alonso 2012; Kalsi i wsp. 2017), obniżone pH (Ellsworth i wsp. 1995), hiperkapnię (Bergfeld i Forrester, 1992) oraz wzmożoną deformację mechaniczną i siły ścinające (*shear stress*) działające na erytrocyt (Sprague i wsp. 1998; Wan i wsp. 2008). Wszystkie powyższe procesy przyczyniają się do odpowiedniej dystrybucji przepływu krwi, zwłaszcza podczas wysiłku fizycznego w obszarze mikrokrążenia aktywnych mięśni szkieletowych. W wysiłku fizycznym obserwuje się znaczny wzrost stężenia wewnątrzmięśniowego ATP, który jest ściśle związany ze wzrostem przepływu krwi. Sugeruje to, że mięśnie szkieletowe również mogą być źródłem ATP w osoczu (Hellsten i wsp. 2012; Mortensen i Saltin 2014).

González–Alonso (2012), analizując wazoaktywne i sympatykolityczne właściwości ATP, wykazał że wywiera on swój efekt bezpośrednio, a nie poprzez degradację do

adenozynodifosforanu (ADP) czy adenozynomonofosforanu (AMP). Funkcja ADP ogranicza się do współdziałania w szczytowej fazie przekrwienia (Olivecrona i wsp. 2004) oraz aktywacji negatywnego sprzężenia zwrotnego powodującego uwalnianie ATP z erytrocytów (Wang i wsp. 2005). Przejawem tego może być zmniejszone uwalnianie ATP z erytrocytu przy towarzyszącej mu podwyższonej wartości ADP w osoczu w trakcie wysiłku (Wang i wsp. 2005). Dotychczasowe prace dotyczące roli AMP w osoczu sugerują jego niewielki wpływ na mechanizmy prowadzące do wazodylatacji (Kirby i wsp. 2008).

Podczas wysiłku fizycznego dochodzi do zjawiska czynnościowej sympatykolizy, które ma kluczowe znaczenie dla prawidłowej regulacji dystrybucji krwi i tlenu do mięśni. Umożliwia ono zwiększenie perfuzji mięśni szkieletowych podczas wysiłków o wysokiej intensywności (Hearon i wsp. 2016). Mechanizm czynnościowej sympatykolizy zależy od wieku i stopnia wytrenowania (Mortensen i wsp. 2012). Sugeruje się, że ATP może odgrywać w nim ważną rolę, indukując lokalną wazodylatację, przezwyciężając lokalną wazokonstrykcję oraz stymulując odruch presyjny podczas wysiłku (Mortensen i Saltin 2014). Adaptacja do długotrwałego wysiłku fizycznego powoduje swoiste zmiany w metabolizmie mięśni i pochłanianiu tlenu w zależności od tego, jakie wymagania stawia przed zawodnikiem specyficzny trening (Mairbäurl 2013). Dotychczasowe badania wykazały, że trening fizyczny (szybkościowy i wytrzymałościowy) zwiększa zdolność do wazodylatacji w mięśniach podczas maksymalnych wysiłków (Calbet i Lundby 2012; Laughlin i Roseguini 2008, Walther i wsp. 2008). W szczególności zawodnicy wytrzymałościowi wykazują zwiększony potencjał wazodylatacyjny, przez który rozumieć należy zdolność do poszerzania naczyń krwionośnych (Boutcher i Bouchter 2005). Jednak trzeba pamiętać, że efekt wazodylatacyjny jest finalnym wynikiem wzajemnego oddziaływania między wieloma mechanizmami regulującymi przepływ krwi w łożysku naczyniowym aktywnych mięśni (Clifford i Hellsten, 2004), których względny udział zmienia się wraz ze wzrostem masy mięśniowej zaangażowanej w wysiłek fizyczny.

Dotychczasowe badania wykazały, że stężenie wewnątrznaczyniowych nukleotydów wzrasta podczas intensywnego wysiłku (Crecelius i wsp. 2013; González–Alonso i wsp. 2002, 2004, 2006; Kirby i wsp. 2012, 2013; Mortensen i wsp. 2007, 2011; Rosenmeier i wsp. 2004; Yegutkin i wsp. 2007). Jednak prace te skupiały się głównie na oznaczeniach wartości spoczynkowych i wysiłkowych (Dufour i wsp. 2010; González–Alonso i wsp. 2006; Rosenmeier i wsp. 2004), biorąc pod uwagę wysiłek lokalny, przeważnie krótkotrwały (Crecelius i wsp. 2013, Mortensen i wsp. 2009a). Niewiele jest prac, które charakteryzowałyby powysiłkowe zmiany stężenia ATP w osoczu (González–Alonso i wsp.

2002; Mortensen i wsp. 2011; Yegutkin i wsp. 2007). Badaniom poddano głównie osoby aktywne fizycznie (Dufour i wsp. 2010; González–Alonso i wsp. 2002, 2006; Pearson i wsp. 2011; Rosenmeier i wsp. 2004), w tym amatorsko uprawiające dyscypliny wytrzymałościowe (Mortensen i wsp. 2007, 2011), nie biorące udziału we współzawodnictwie sportowym. Wyżej wymienieni autorzy nie analizowali zmian pod wpływem wysiłku globalnego, ani wpływu długotrwałego, zaprogramowanego treningu, w tym także w odniesieniu do masy mięśniowej, u zawodników wyczynowo uprawiających sport o odmiennych profilach treningowych (szybkościowo–siłowym, wytrzymałościowym i mieszanym).

2. Cel badań

Celem badań było określenie zmian stężenia ATP w osoczu podczas testu wysiłkowego oraz w fazie restytucji u sportowców o odmiennych profilach treningowych i różnej masie mięśniowej (publikacja 1) w rocznym cyklu treningowym (publikacja 2).

Hipotezy badawcze:

1. Wyspecjalizowany trening sportowy (szybkościowo–siłowy, wytrzymałościowy oraz mieszany) determinuje profil zmian osoczowego stężenia ATP podczas wysiłku i restytucji powysiłkowej u wysokowytrenowanych sportowców (publikacja 1),
2. Osoczowe stężenie ATP jest uzależnione od masy mięśniowej. Wyższa masa mięśniowa będzie prowadziła do zwiększonego uwalniania ATP w osoczu (publikacja 1),
3. Osoczowe stężenie ATP zmienia się w rocznym cyklu treningowym, osiągając najwyższe wartości w okresie startowym (publikacja 2).

3. Metody badawcze

Procedury badań

Wszystkie badania wykonano w godzinach porannych, do 2–3 godzin po lekkim posiłku. Uczestnicy zostali poinformowani o celu i ryzyku badań oraz udzielili na nie pisemnej zgody. Zalecono im unikanie intensywnych i długotrwałych sesji treningowych co najmniej 24–48 godzin przed badaniem. Przed testem wysiłkowym przeprowadzono ocenę składu ciała. Badania wykonano pod koniec okresu przejściowego (publikacja 1) oraz czterokrotnie w rocznym cyklu treningowym (publikacja 2): (1) po okresie przejściowym, (2) po okresie przygotowania ogólnego, (3) po okresie przygotowania specjalnego oraz (4) po okresie bezpośredniego przygotowania startowego. Podczas wszystkich badań temperatura otoczenia wynosiła 20–21^{o}C.

Analiza składu ciała

Wysokość (cm) oraz masę ciała (kg) zmierzono za pomocą stadiometru (SECA 285; SECA, Hamburg, Niemcy). Ocenę składu ciała przeprowadzono metodą dwuenergetycznej absorbcjometrii rentgenowskiej (DXA) urządzeniem Lunar Prodigy Pro (GE Healthcare, Madison, WI, USA) zgodnie z *Best Practice Protocol* wg. Nana i wsp. (2015). Masę mięśni szkieletowych (SMM) obliczono zgodnie ze wzorem Kim i wsp. (2002).

Laboratoryjna próba wysiłkowa

Wszyscy badani wykonali test wysiłkowy na bieżni mechanicznej o wzrastającej intensywności do odmowy (H/P Cosmos Pulsar Sports & Medical, Nussdorf–Traunstein, Germany) w celu określenia maksymalnego pochłaniania tlenu (VO_{2max}). Przez pierwsze 3 min prędkość bieżni wynosiła 4 km·h^{-1}, następnie 8 km·h^{-1} przez kolejne 3 min; od tego momentu w każdym 3–minutowym etapie prędkość zwiększano o 2 km·h^{-1} aż do wolicjonalnego wyczerpania zawodnika. Główne parametry krążeniowo–oddechowe mierzono w każdym cyklu oddechowym przy użyciu ergospirometru Cortex MetaMax 3BR2 (Cortex Biophysik, Leipzig, Germany). Próg kompensacji oddechowej (RCP) określono według metody V–slope (Beaver i wsp. 1986). Częstość skurczów serca (ud·min^{-1}) mierzono w sposób ciągły za pomocą pulsometru Polar Bluetooth Smart H6 (Polar Electro Oy, Kempele, Finlandia).

Próbki krwi

Próbki krwi żylnej pobierano w spoczynku, na końcu każdego 3–minutowego etapu powyżej 10 km·h^{-1}, bezpośrednio po zakończeniu wysiłku oraz w 5, 10, 15, 20 i 30 minucie restytucji powysiłkowej. W celu pobrania próbek krwi zawodnikom zakładano kaniulę (1.3×32 mm, BD Venflon Pro, Becton Dickinson, Helsingborg, Sweden) do żyły odłokciowej lub odpromieniowej. Do analizy parametrów hematologicznych i stężeń nukleotydów w osoczu użyto probówek zawierających kwas etylenodiaminotetraoctowy (EDTA) (S–Monovette, 2.7 ml KE, Sarstedt, Nümbrecht, Germany). Do pomiaru mleczanu zastosowano jako antykoagulant heparynę litową (S–Monovette, 2.7 ml KE, Sarstedt, Nümbrecht, Germany).

Oznaczenia biochemiczne

Stężenie mleczanu we krwi pełnej oznaczono za pomocą spektrofotometrii enzymatycznej (Biosen C–line, EKF diagnostic GmbH, Barleben, Niemcy). Analizę hematologiczną przeprowadzono aparatem Mythic®18 (Orphée, Geneva, Switzerland).

Nukleotydy w osoczu oznaczono za pomocą wysokosprawnej chromatografii cieczowej (HPLC – *high–performance liquid chromatography*) z detekcją w ultrafiolecie według metodologii Smoleńskiego i wsp. (1990) oraz Smoleńskiego i Yacouba (1993).

Wyżej opisana procedura została przeprowadzona pod koniec okresu roztrenowania (publikacja 1) oraz w kolejnych okresach rocznego cyklu treningowego (publikacja 2): pierwszy pomiar przeprowadzono po okresie roztrenowania, drugi po okresie przygotowania ogólnego, trzeci po okresie przygotowania specjalnego oraz czwarty podczas bezpośredniego przygotowania startowego (BPS) przed okresem startowym.

Na badania uzyskano zgodę Komisji Bioetycznej przy Uniwersytecie Medycznym im. Karola Marcinkowskiego w Poznaniu: uchwała nr 1079/12 z dnia 6 grudnia 2012 r.

Analiza statystyczna

W celu określenia zmian stężenia nukleotydów w osoczu przeprowadzono jednoczynnikową ANOVA z powtarzanymi pomiarami. Do opisania związku między masą mięśni szkieletowych (SMM) a stężeniem ATP w osoczu wykorzystano współczynnik korelacji Pearsona (publikacja 1). Wszystkie analizy statystyczne przeprowadzono przy użyciu oprogramowania STATISTICA (Tibco Software Inc., Palo Alto, CA, USA).

4. Wyniki oraz ich omówienie

Publikacja 1

Plasma nucleotide dynamics during exercise and recovery in highly trained athletes and recreationally active individuals, BioMed Research International, 2018, doi: 10.1155/2018/4081802.

W badaniu uczestniczyły trzy grupy sportowców (41 mężczyzn): sprinterzy (SP; n=11; wiek 24.2±3.2 lat; staż treningowy 8.5±2.5 lat), sportowcy wytrzymałościowi uprawiający biegi długodystansowe i triathlon (EN; n=16; wiek 23.4±3.6; staż treningowy 8.7±1.9 lat) oraz futsaliści (FU; n=14; wiek 24.7±3.9 lat; staż treningowy 9.8±3.3 lat). Wszyscy zawodnicy w czasie badań byli członkami kadry narodowej. Grupa kontrolna składała się z mężczyzn aktywnych fizycznie 3 do 5 razy w tygodniu (CO; n=12; wiek 27.7±4.1 lat) bez udziału we współzawodnictwie sportowym.

W pracy analizowano stężenie nukleotydów w osoczu w odpowiedzi na wysiłek o wzrastającej intensywności oraz w okresie restytucji powysiłkowej. Jest to pierwsze badanie naukowe, które wykazało wpływ długotrwałego treningu na wzrost stężenia ATP w osoczu

podczas wysiłku fizycznego w zależności od profilu treningowego. Wyniki sugerują, że długofalowy trening ma wpływ na wielkość odpowiedzi ATP w osoczu, ponieważ u osób aktywnych fizycznie wzrost stężenia był niższy niż u wyczynowych sportowców (~31% vs. ~61%). Różnice te można częściowo wyjaśnić przebudową naczyń krwionośnych w mięśniach spowodowaną specyficzną adaptacją do wysiłków dominujących w danej dyscyplinie sportu. Wydaje się, że u wysokowytrenowanych zawodników na podobnym poziomie sportowym stężenie ATP w osoczu wiąże się z ilością masy mięśniowej. Po przeliczeniu ATP na kilogram masy mięśni szkieletowych, nie wykazano różnic pomiędzy wyczynowymi zawodnikami w spoczynku, przy maksymalnej intensywności oraz podczas restytucji powysiłkowej. Potwierdzono nagły wzrost stężenia ATP w osoczu przy intensywności 83–87% VO_2max u sportowców wyczynowych i 94% VO_2max w grupie kontrolnej. Temu gwałtownemu wzrostowi towarzyszyło osiągnięcie punktu kompensacji oddechowej, odzwierciedlające częściową niezdolność do dostarczania tlenu do mięśni podczas wysiłku.

Wysiłkowe stężenie nukleotydów

Wraz ze wzrostem intensywności wysiłku zaobserwowano zwiększenie osoczowego stężenia ATP, ADP i AMP. Nie podjęto dyskusji na temat zmian osoczowego stężenia ADP i AMP gdyż są one wypadkową zmian stężenia ATP w osoczu. Ponadto ich udział w mechanizmie wazodylatacji jest do tej pory nieznany. Najwyższe stężenie ATP w osoczu zanotowano w momencie uzyskania maksymalnej intensywności wysiłku. Różnica wysiłkowo–spoczynkowa osoczowego stężenia ATP wynosiła u sportowców średnio 60%, natomiast w grupie kontrolnej 30%. Specyficzny trening (szybkościowo–siłowy, wytrzymałościowy i mieszany) wpłynął na profil zmian stężenia ATP w osoczu. Zjawisko to można wyjaśnić w następujący sposób. Trening sprinterski oparty na wysiłkach o wysokiej intensywności i krótkim czasie trwania (Zieliński i Kusy 2012) wymaga zwiększonego stężenia ATP w osoczu, co może wskazywać na adaptację naczyń krwionośnych do szybkiej i wzmożonej wazodylatacji. Natomiast trening wytrzymałościowy, oparty na obciążeniach o niskiej intensywności i dłuższym czasie trwania (Esteve–Lanao i wsp. 2007), wymaga mniejszego wzrostu stężenia ATP w osoczu pod wpływem wysiłku niż trening sprinterski. Trening futsalistów z kolei charakteryzuje się wielokrotnie powtarzanymi krótkimi wysiłkami o wysokiej intensywności, co umiejscawia tą grupę pomiędzy krzywymi stężenia ATP charakterystycznymi dla sprinterów i zawodników wytrzymałościowych. Wynika stąd, że

charakter treningu wpływa na fizjologiczne mechanizmy uwalniania ATP i jego funkcję wazodylatacyjną podczas wysiłku o wzrastającej intensywności.

Piil i wsp. (2018) wykazali, że trening fizyczny wpływa na poprawę zdolności sympatykolitycznych i w konsekwencji na skuteczniejszą regulację dystrybucji krwi i tlenu do aktywnych mięśni szkieletowych. Wielkość odpowiedzi wazodylatacyjnej jest uzależniona od stężenia ATP w osoczu (Kirby i wsp. 2008). Na tej podstawie można przypuszczać, że wyższe wartości w końcowym etapie wysiłku mogą być spowodowane wydajniejszym dostarczaniem krwi do mięśni szkieletowych. Jednak dyskusyjne pozostaje stwierdzenie, czy przepływ krwi przez mięśnie jest rzeczywiście zmniejszony, czy też obserwuje się tylko jego redystrybucję w obrębie mięśni (Heinonen i wsp. 2013). Wysokowytrenowani zawodnicy wykazali znacznie wyższe stężenia ATP w osoczu pod wpływem wysiłku niż osoby aktywne fizyczne. Jedną z przyczyn może być wydajniejsza dystrybucja krwi i tlenu w pracujących mięśniach.

Powysiłkowe stężenie nukleotydów

Stężenie nukleotydów w osoczu malało w okresie restytucji powysiłkowej. Wartości uzyskane po 30 minutach różniły się od wartości spoczynkowych u sportowców, lecz nie w grupie kontrolnej. Sugeruje to, że potrzebna jest dłuższa faza restytucji, aby wywołane wysiłkiem wysokie osoczowe stężenie ATP wróciło do wartości spoczynkowych u sportowców wyczynowych.

Równocześnie, niezależnie od profilu treningowego, nie zaobserwowano różnic pomiędzy grupami wysokowytrenowanych zawodników w spoczynku oraz podczas restytucji powysiłkowej. Zbliżony przebieg reakcji powysiłkowych sugeruje taki sam kierunek adaptacji, niezależnie od profilu treningowego.

Stężenie ATP w odniesieniu do masy mięśni szkieletowych

Jednym z potencjalnych źródeł ATP w osoczu mogą być mięśnie szkieletowe (Hellsten i wsp. 1998; Mortensen i Saltin 2014). W związku z tym możliwy jest wpływ ich masy na zmiany osoczowego stężenia ATP. Sprinterzy, którzy odznaczali się najwyższą masą mięśniową, charakteryzowali się również wyższym osoczowym stężeniem nukleotydów w spoczynku niż zawodnicy wytrzymałościowi i grupa kontrolna. Spoczynkowe i maksymalne stężenie ATP w osoczu silnie korelowało (odpowiednio r=0.81 i r=0.75) z masą mięśni szkieletowych u wysokowytrenowanych zawodników, w przeciwieństwie do grupy kontrolnej, w której nie stwierdzono korelacji. Przeliczenie stężenia ATP na kilogram masy mięśni szkieletowych (ATP_{SMM}) wyeliminowało częściowo różnice pomiędzy

wysokowytrenowanymi zawodnikami w spoczynku, przy maksymalnym wysiłku i podczas restytucji powysiłkowej. Jednak utrzymujące się różnice pomiędzy zawodnikami w trakcie wysiłku sugerują, że nie tylko SMM, ale także ich specyficzna adaptacja do wyczerpujących wysiłków jest tu istotna. Należy sądzić, że zwiększona kapilaryzacja mięśni u sportowców wytrzymałościowych (Holloszy i Coyle 1984; Takada i wsp. 2012) wymaga niższych stężeń ATP w osoczu niż u zawodników dyscyplin szybkościowo–siłowych i mieszanych dla wywołania podobnego efektu przekrwiennego.

Publikacja 2

Alterations in exercise–induced plasma adenosine triphosphate concentration in highly trained athletes in a one–year training cycle, Metabolites, 2019, 9 (10 [230]). doi: 10.3390/metabo9100230.

W badaniu brało udział 33 wysokowytrenowanych sportowców (mężczyzn): sprinterów (SP, n=11) w wieku 24.1±3.3 lat o stażu treningowym 8.6±2.3 lat, zawodników wytrzymałościowych uprawiających biegi długodystansowe i triathlon (EN, n=11) w wieku 23.3±4.1 lat o stażu treningowym 8.5±1.9 lat oraz futsalistów (FU, n=11) w wieku 25.8±4.0 lat o stażu treningowym 10.1±3.9 lat. Grupa kontrolna (CO, n=11) składała się ze zdrowych, aktywnych mężczyzn w wieku 27.5±3.8 lat, bez wcześniejszego i aktualnego doświadczenia sportowego.

Wykazano, że stężenie ATP w osoczu wzrasta znacząco w kolejnych okresach rocznego cyklu treningowego u wysokowytrenowanych zawodników w trakcie wysiłku oraz restytucji powysiłkowej. Natomiast stężenie ATP w osoczu w grupie kontrolnej pozostawało na niezmienionym poziomie we wszystkich terminach badań. Trening sprinterski przyczynił się do wyższych maksymalnych stężeń ATP w osoczu pod wpływem wysiłku w porównaniu z treningiem wytrzymałościowym i mieszanym. Grupa kontrolna, amatorsko uprawiająca sport, osiągnęła najniższe stężenia ATP w osoczu pod wpływem wysiłku. Pomimo występowania różnic w wielkości odpowiedzi, każdy rodzaj zaprogramowanego treningu (szybkościowo–siłowego, wytrzymałościowego i mieszanego), obejmujący wystarczającą ilość wysiłków o wysokiej intensywności, doprowadził do takiego samego wzorca adaptacji. Kluczowym czynnikiem wydaje się udział obciążeń treningowych o wysokiej intensywności, które indukują wzrost stężenia ATP w osoczu w okresie startowym. Zmniejszenie lub brak takich obciążeń w innych okresach cyklu treningowego wiąże się ze spadkiem osoczowego stężenia ATP.

Wysiłkowe stężenie nukleotydów

Periodyzacja treningu w rocznym cyklu treningowym, w przeciwieństwie do niezmieniających się obciążeń w grupie kontrolnej, przyczyniła się do znacznie wyższych stężeń ATP w osoczu podczas wysiłku u wysokowytrenowanych zawodników. Podczas wysiłku we wszystkich badanych grupach sportowych wystąpił średni wzrost osoczowego stężenia ATP o 62% w okresie przejściowym i o 103% w okresie startowym. Największy wzrost zaobserwowano u sprinterów (odpowiednio 60% i 114%), a najmniejszy u zawodników wytrzymałościowych (odpowiednio 64% i 95%). Ponadto zanalizowano zmiany stężenia ADP i AMP w rocznym cyklu treningowym, które odzwierciedlały zmiany stężenia ATP jako produktów jego degradacji. Odstąpiono jednak od omówienia tych wyników ze względu na nieudowodniony wpływ ADP i AMP na mechanizmy regulujące przepływ krwi.

Osoczowe stężenie nukleotydów odgrywa kluczową rolę w procesach adaptacyjnych podczas wysiłku fizycznego. Wydaje się, że zwiększone stężenie ATP w osoczu pozwala na wzmocnienie efektu wazodylatacji, wspierając wykorzystanie metabolizmu oksydacyjnego do produkcji energii. Zwiększenie stężenia ATP w osoczu u zawodników szybkościowo–siłowych w porównaniu do zawodników o profilu wytrzymałościowym może być spowodowane wcześniejszym zwiększeniem udziału metabolizmu beztlenowego podczas wysiłku o wzrastającej intensywności. Ponadto zaobserwowano wzrost stężenia ATP w osoczu wraz ze wzrostem procentowego udziału wysiłków o średniej i wysokiej intensywności podczas rocznego cyklu treningowego. Z wykonanych badań wynika, że intensywnym wysiłkom fizycznym towarzyszy zwiększone stężenie ATP w osoczu, które może wiązać się z pokrywaniem zapotrzebowania mięśni na tlen. U wysokowytrenowanych sportowców, ze względu na specyficzną adaptację, istnieje możliwość skuteczniejszej regulacji przepływu krwi i dostarczania tlenu w stosunku do zapotrzebowania metabolicznego mięśni szkieletowych. Zdolność ta może zależeć od charakteru dominującego metabolizmu w różnych dyscyplinach sportu i/lub odmiennej struktury obciążeń treningowych (Zieliński i Kusy 2012). Specyficzne adaptacje mięśni szkieletowych do treningu sprinterskiego związane są z wysokimi wymaganiami metabolicznymi wysiłku o wysokiej intensywności, które mogły wpłynąć na wyraźny wzrost osoczowego stężenia ATP w kolejnych terminach badań. Również u futsalistów zaobserwowano wzrost stężenia ATP w osoczu, który najprawdopodobniej wynikał z wymogów dyscypliny, czyli długotrwałego wysiłku przeplatanego dużą ilością sprintów, szczególnie podczas rozgrywek wiosennych i jesiennych, charakteryzujących się dużą liczbą meczów i treningów. U zawodników

wytrzymałościowych duża objętość treningowa (liczba sesji treningowych i całkowity czas ich trwania) w zakresie od niskich do średnich intensywności może skutkować mniejszymi przyrostami stężenia ATP w osoczu.

Próg kompensacji oddechowej

We wszystkich badanych grupach (sportowych i kontrolnej) punkt kompensacji oddechowej (RCP) został osiągnięty w zakresie od 83% do 94% VO_2max, czemu towarzyszył nagły wzrost stężenia ATP w osoczu. W związku z tym wydaje się, że mechanizm odpowiedzialny za moment wyrzutu ATP do przestrzeni wewnątrznaczyniowej jest zmienną niezależną od profilu i statusu treningowego. Natomiast profil i status treningowy wpływają na osiągane bezwzględne stężenie ATP w osoczu podczas wysiłku fizycznego.

5. Wnioski

- Długotrwały trening i specjalizacja sportowa mają znaczący wpływ na osoczowe stężenie ATP w spoczynku, podczas wysiłku o wzrastającej intensywności oraz podczas maksymalnego wysiłku, natomiast w trakcie restytucji powysiłkowej takiego wpływu nie zaobserwowano.
- Gwałtownemu wzrostowi osoczowego stężenia ATP towarzyszy osiągnięcie punktu kompensacji oddechowej, co może odzwierciedlać częściową niezdolność do dostarczania tlenu do mięśni podczas wysiłku.
- Różnice w osoczowym stężeniu ATP między grupami sportowymi w spoczynku oraz podczas maksymalnego wysiłku są związane z masą mięśni szkieletowych.
- Osoczowe stężenie ATP u wysokowytrenowanych zawodników w czasie rocznego cyklu treningowego osiąga najniższe wartości w okresie przejściowym, a najwyższe w okresie startowym.

II. DISSERTATION SUMMARY

This doctoral dissertation is based on series of studies entitled: „Changes in plasma ATP concentration in highly trained athletes in a one–year training cycle.", which consists of two publications prepared as a part of scientific project No. 2013/09/B/NZ7/02556, funded by the National Science Center:

1. *Plasma nucleotide dynamics during exercise and recovery in highly trained athletes and recreationally active individuals*, BioMed Research International, 2018, doi: 10.1155/2018/4081802; impact factor – 2.197, Ministerial score – 70pts.
2. *Alterations in exercise–induced plasma adenosine triphosphate concentration in highly trained athletes in a one–year training cycle*, Metabolites, 2019, 9 (10 [230]), doi: 10.3390/metabo9100230; impact factor – 3.303, Ministerial score – 70pts.

1. Introduction

Adenosine triphosphate (ATP), apart from being intracellular energy source, plays an equally important extracellular function as a signaling molecule. Releasing ATP into the extracellular space affects vasodilatation (Ellsworth et al. 1995; Rosenmaier et al. 2004) and functional sympatholysis (Kirby et al. 2008; Rosenmeier et al. 2004, 2008), which are responsible for oxygen supply within skeletal muscles during exercise. One of the main sources of ATP in the extracellular space is erythrocyte which releases ATP in response to reduction in oxygen tension (González–Alonso et al. 2002), increased temperature (Kalsi and González–Alonso 2012; Kalsi et al. 2017), decreased pH (Ellsworth et al. 1995), hypercapnia (Bergfeld and Forrester, 1992), increased mechanical deformation and elevated shear stress (Sprague et al. 1998; Wan et al. 2008). All of the above processes contribute to the proper distribution of perfusion, especially during exercise in the area of microcirculation of the active skeletal muscle. There is a substantial increase in the interstitial ATP concentration during exercise, closely coupled with the increase in blood flow. This suggests that skeletal muscles are the source of plasma ATP (Hellsten et al. 2012; Mortensen and Saltin 2014).

González–Alonso (2012), analyzing the vasoactive and sympatholytic properties of ATP, showed that it exerts its effect directly, not through degradation to adenosine diphosphate (ADP) or adenosine monophosphate (AMP). The function of ADP is limited to contributing to peak hyperemia (Olivecrona et al. 2004) and activating a negative feedback reaction of ATP release from erythrocytes (Wang et al. 2005). This may be manifested by reduced ATP release from erythrocytes along with elevated plasma ADP during exercise

(Wang et al. 2005). Previous studies on plasma AMP role suggest a minor effect on the mechanisms leading to vasodilatation (Kirby et al. 2008).

During exercise, the phenomenon known as functional sympatholysis occurs, which is crucial in proper regulation of blood and oxygen distribution to the muscles. It allows skeletal muscle perfusion to increase during high–intensity efforts (Hearon et al. 2016). The mechanism of functional sympatholysis depends on age and training status (Mortensen et al. 2012). It is suggested that ATP may play an important role in inducing local vasodilatation, overriding local sympathetic vasoconstriction and stimulating pressor reflex during exercise (Mortensen and Saltin 2014). Long–term physical training can cause specific adaptations in muscle metabolism and oxygen uptake, depending on unique training demands (Mairbäurl 2013). Research done so far has shown that both speed and endurance training increase vasodilatory capacity during maximal effort (Calbet and Lundby 2012; Laughlin and Roseguini 2008, Walther et al. 2008). In particular, endurance athletes have shown increased vasodilatation capacity, by which the ability of blood vessels to widen is meant (Boutcher and Bouchter 2005). However, it must be remembered that vasodilatation is the result of the interaction between mechanisms regulating blood flow in the vascular bed of active muscles (Clifford and Hellsten, 2004), whose relative contribution changes with the increase in muscle mass involved in exercise.

Previous studies showed that intravascular nucleotide concentrations increase during intense exercise (Crecelius et al. 2013; González–Alonso et al. 2002, 2004, 2006; Kirby et al. 2012, 2013; Mortensen et al. 2007, 2011; Rosenmeier et al. 2004; Yegutkin et al. 2007). However, these studies focused mainly on resting and exercise measurements (Dufour et al. 2010; González–Alonso et al. 2006; Rosenmeier et al. 2004), taking into account local, usually transitory effort (Crecelius et al. 2013, Mortensen et al. 2009). Predominantly, physically active participants were examined (Dufour et al. 2010; González–Alonso et al. 2002, 2006; Pearson et al. 2011; Rosenmeier et al. 2004) including amateur endurance runners (Mortensen et al. 2007, 2011) not participating in sports competition. The above authors did not analyze changes in response to global effort or the impact of long–term and periodized training, including the relation with total–body skeletal muscle mass in athletes representing different sport specializations (speed–power, endurance and mixed).

2. Study Aim

The study aimed to determine the changes in plasma ATP concentration during exercise and recovery period in athletes representing different training profiles and different total–body skeletal muscle mass (publication 1) in a one–year training cycle (publication 2).

Research hypotheses:

1. Specialized sport training (speed–power, endurance and mixed) determines the profile of changes in plasma ATP concentration during exercise and recovery period in highly trained athletes (publication 1).
2. Plasma ATP concentration depends on total–body skeletal muscle mass. Higher total–body skeletal muscle mass will lead to increased ATP release in plasma (publication 1).
3. Plasma ATP concentration changes in the one–year training cycle, reaching the highest values in the competition period (publication 2).

3. Methods

Procedures

All tests were performed in the morning, up to 2–3 hours after a light breakfast. Participants were informed about the purpose and risk of research and they gave their written consent. They were advised to avoid intensive and prolonged training sessions at least 24–48 hours before the study. Before exercise, body composition was assessed. The exercise tests were performed at the end of the transition phase (publication 1) and four times during the annual training cycle (publication 2): (1) after the transition phase, (2) after the general preparation phase, (3) after the specific preparation phase and (4) after the tapering before the competition phase. During all tests, the ambient temperature was 20–21^{o}C.

Body Composition Analysis

Height (cm) and weight (kg) were measured with a stadiometer (SECA 285; SECA, Hamburg, Germany). Body composition was assessed by dual–X ray absorptiometry method (DXA) using the Lunar Prodigy Pro device (GE Healthcare, Madison, WI, USA) according to the *Best Practice Protocol* by Nana et al. (2015). Total–body skeletal muscle mass (SMM) was calculated according to the regression models proposed by Kim et al. (2002).

Laboratory Exercise Tests

All subjects performed an incremental treadmill exercise test until exhaustion (H/P Cosmos Pulsar Sports & Medical, Nussdorf–Traunstein, Germany) to determine maximum oxygen uptake (VO_{2max}). For the first 3 minutes, the treadmill speed was 4 $km \cdot h^{-1}$, then 8 $km \cdot h^{-1}$(next 3 min), from that moment in each 3–minute stage the speed was increased by 2 $km \cdot h^{-1}$ until volitional exhaustion. The main circulatory and respiratory parameters were measured breath by breath using the MetaMax 3BR2 ergospirometer (Cortex Biophysik, Leipzig, Germany). The respiratory compensation point (RCP) was determined according to the V–slope method (Beaver et al. 1986). Heart rate (HR) was measured continuously using the Smart H6 heart rate monitor (Polar Electro Oy, Kempele, Finland).

Blood Sampling

Venous blood samples were taken at rest, at the end of each 3–minute stage above 10 $km \cdot h^{-1}$, immediately after exercise and 5, 10, 15, 20 and 30 minutes into the recovery period. In order to collect blood samples, the participants wore a catheter (1.3×32 mm, BD Venflon Pro, Becton Dickinson, Helsingborg, Sweden) inserted into basilica or cephalic vein. For the analysis of hematological parameters and plasma nucleotide concentrations, tubes containing ethylenediaminetetraacetic acid (EDTA) were used (S–Monovette, 2.7 ml KE, Sarstedt, Nümbrecht, Germany). Heparin was used as lithium anticoagulant to measure lactate concentration (S–Monovette, 2.7 ml KE, Sarstedt, Nümbrecht, Germany).

Biochemical Analysis

Lactate concentration in whole blood was determined by enzymatic spectrophotometry (Biosen C–line, EKF diagnostic GmbH, Barleben, Germany). Hematological analysis was performed using the Mythic®18 device (Orphée, Geneva, Switzerland). Plasma nucleotides were determined by high–performance liquid chromatography (HPLC) with UV detection according to the methodology by Smoleński et al. (1990) and Smoleński and Yacoub (1993).

The procedures described above were carried out at the end of the transition phase (publication 1) and in consecutive phases of the one–year training cycle (publication 2): the first measurement was carried out after the transition phase, the second after the general preparation phase, the third after the specific preparation phase and the fourth during tapering before the competition phase.

The project was approved by the Ethics Committee at the Poznan University of Medical Sciences: resolution No. 1079/12, 6th of December 2012.

Statistical Analysis

To determine the changes in plasma nucleotide concentrations, the one–way repeated–measures ANOVA was performed. To describe the relationship between total–body skeletal muscle mass (SMM) and plasma ATP concentration, the Pearson correlation coefficients were used (publication 1). All statistical analyses were performed using the STATISTICA software (Tibco Software Inc., Palo Alto, CA, USA).

4. Results and their analysis

Publication 1

Plasma nucleotide dynamics during exercise and recovery in highly trained athletes and recreationally active individuals, BioMed Research International, 2018, doi: 10.1155/2018/4081802.

The study included three athletic groups (41 men) i.e. sprinters (SP; n=11; age 24.2±3.2 yr; training experience 8.5±2.5 yr), endurance athletes, i.e. long–distance runners and triathletes (EN; n=16; age 23.4±3.6; training experience 8.7±1.9 yr) and futsal players (FU; n=14; age 24.7±3.9 yr; training experience 9.8±3.3 yr). All athletes were members of the Polish national teams. The control group (CO; n=12; age 27.7±4.1 yr) consisted of physically active men exercising 3–5 times per week without any competitive sport experience.

The study analyzed plasma nucleotide concentrations in response to incremental exercise test and during post–exercise recovery period. This is the first study to investigate the effect of long–term training on the increase in plasma ATP concentration during exercise depending on sport specialization. The results suggest that long–term training affects the magnitude of plasma ATP response, since moderately physically active subjects have lower increments in ATP concentration than highly trained athletes (~31% vs. ~61%). These differences can be partially explained by the remodeling of blood vessels within the skeletal muscle due to specific training adaptations dominating in different sport disciplines. It seems that in highly trained athletes at similar sport level plasma ATP concentration is associated with the total–body skeletal muscle mass. After expressing ATP as concentration per kilogram of total–body skeletal muscle mass, there were no differences between competitive athletes at rest, at maximum intensity and during post–exercise recovery. A rapid increase in plasma ATP concentration at intensities of 83–87% of VO_2max in competitive athletes and at 94% of VO_2max in the control group was revealed. This rapid increase was concomitant with

the occurrence of the respiratory compensation point, the latter reflecting partial inability to supply oxygen to the muscles during exercise.

Nucleotide concentration during exercise

Along with increases in exercise intensity, an increase in plasma ATP, ADP and AMP concentrations was observed. However, changes in plasma ADP and AMP concentration were not discussed as they are the resultant change of plasma ATP concentration. Furthermore, their involvement in vasodilatation mechanism is still unknown. The highest plasma ATP concentration was observed at maximum exercise intensity. The difference between exercise and resting plasma ATP concentration was at ~60% in highly trained athletes, while ~30% in controls. Specific training (speed–power, endurance and mixed) altered the profile of changes in plasma ATP concentration. This phenomenon can be explained as follows. Sprint training consists in high–intensity short–duration efforts (Zieliński and Kusy 2012) and requires increased plasma ATP concentration, suggesting an adaptation of blood vessels enabling them to cope with rapid and increased vasodilatation. On the contrary, endurance training based on low–intensity loads and longer duration (Esteve–Lanao et al. 2007) requires less increase in plasma ATP concentration in response to exercise compared to sprint training. The futsal–specific training is characterized by repeated short–time high–intensity efforts, which places this group between the ATP concentration curves characteristic of sprinters and endurance athletes. In view of the above, it can be assumed that the training structure affects the physiological mechanisms of ATP release and its vasodilatory properties during incremental exercise.

Piil et al. (2018) have shown that physical training improves sympatholityc capacity and, consequently, more effectively regulates blood and oxygen distribution to the active skeletal muscles. The amount of vasodilatation response depends on the plasma ATP concentration (Kirby et al. 2008). On this basis, it can be assumed that higher concentrations in the final stage of exercise may be caused by more efficient blood flow distribution to skeletal muscles. However, it remains debatable whether the blood flow to the skeletal muscles is actually reduced or its redistribution within the skeletal muscles is only observed (Heinonen et al. 2013). Highly trained athletes showed significantly higher plasma ATP concentrations during exercise than physically active subjects. One of the reasons may be more efficient distribution of blood and oxygen to the working muscles.

Nucleotide concentration during recovery

Plasma nucleotide concentrations decreased during post–exercise recovery period. Values reported after 30 minutes differed from resting values in competitive athletes, but not in the control group. This suggests that longer restitution periods are needed for exercise–induced high plasma ATP concentration to return to resting values in highly trained athletes.

At the same time, regardless on the sport specialization, no differences were observed between groups of highly trained athletes at rest and during recovery period. A similar pattern suggests the same direction of adaptation, regardless of training profile.

Changes in ATP normalized to skeletal muscle mass

Skeletal muscles may be one of potential sources of plasma ATP (Hellsten et al. 1998; Mortensen and Saltin 2014). Therefore, it is possible that total–body skeletal muscle mass influences changes in plasma ATP concentration between competitive athletes. Sprinters had the highest SMM and were also characterized by higher plasma nucleotide concentration at rest than endurance athletes and control group. Resting and maximum plasma ATP concentrations strongly correlated (r=0.81 and r=0.75, respectively) with SMM in highly trained athletes, in contrast to the control group where no correlation was found. The conversion of ATP concentration per kilogram of skeletal muscle mass (ATP_{SMM}) partially eliminated the differences between the groups of highly trained athletes at rest, maximum exercise and during recovery. However, the differences between competitive athletes, that persisted during exercise, suggest that not only SMM, but also specific training adaptations to exhaustive efforts are essential. The conclusion is that increased muscle capillarization in endurance athletes (Holloszy and Coyle 1984; Takada et al. 2012) requires lower plasma ATP concentration increments than in speed–power and mixed athletes to evoke a similar vasodilatory effect.

Publication 2

Alterations in exercise–induced plasma adenosine triphosphate concentration in highly trained athletes in a one–year training cycle, Metabolites, 2019, 9 (10 [230]), doi: 10.3390/metabo9100230.

The study involved 33 highly trained athletes (men): sprinters (SP, n=11) aged 24.1±3.3 yr, training experience 8.6±2.3 yr, endurance athletes (EN, n=11) represented by long–distance runners and triathletes aged 23.3±4.1 yr, training experience 8.5±1.9 yr, and futsal players (FU, n=11) aged 25.8±4.0 yr, training experience 10.1±3.9 yr. The control

group (CO, n=11) consisted of healthy physically active men aged 27.5±3.8 yr, without previous and current competitive sport experience.

The study revealed that plasma ATP concentration increased in consecutive phases of the annual training cycle in highly trained athletes during exercise and post–exercise recovery period. In the control group, plasma ATP concentration remained unchanged in all examinations. Sprint training contributed to higher maximum plasma ATP concentration in response to exercise compared to endurance and mixed training. The recreationally active control group had the lowest plasma ATP concentration in response to exercise. Despite the differences in response magnitude, each type of structured training program (speed–power, endurance and mixed), involving a sufficient amount of high–intensity efforts, led to the same pattern of adaptation. The key factor seems to be the contribution of high–intensity training loads that induce the increase in plasma ATP concentration during the competition phase. Reduction or lack of high–intensity loads during other training phases is associated with lower increases in plasma ATP concentration.

Nucleotide concentration during exercise

Training periodization during the annual training cycle, in contrast to unchanging loads in the control group, contributed to significantly higher plasma ATP concentrations during exercise in highly trained athletes. During exercise, in all groups of competitive athletes, there was an average increase in plasma ATP concentration by 62% during the transition phase and by 103% during the competition phase. The highest increase was observed in sprinters (by 60% and 114%, respectively), and the lowest in endurance athletes (by 64% and 95%, respectively). Furthermore, annual changes in plasma ADP and AMP concentration were analyzed, reflecting changes in ATP concentration as its degradation products. However, these results were not discussed due to the unconfirmed effect of ADP and AMP on the mechanisms regulating blood flow.

Plasma nucleotide concentration plays the key role in adaptation processes induced by exercise. It seems that the increased plasma ATP concentration allows to enhance vasodilatation, promoting energy production via oxidative metabolism in energy production. The greater increase in plasma ATP concentration in speed–power compared to endurance–trained athletes may be due to earlier increase in anaerobic metabolism contribution during incremental exercise. Furthermore, an increase in plasma ATP concentration during the annual training cycle was observed with an increase in the percentage of medium– and high–intensity exercises. The obtained results show that intense physical activity is accompanied by

the increased plasma ATP concentration, which may be associated with covering the oxygen demand in skeletal muscles. In highly trained athletes, due to their specific training adaptation, there is the possibility of more effective regulation of blood flow and oxygen supply in relation to metabolic demands of skeletal muscles. This ability may depend on the nature of the dominant metabolism in particular sports disciplines and/or the different structure of training loads (Zieliński and Kusy 2012). Specific skeletal muscle adaptations to sprint training are associated with high metabolic requirements of high–intensity exercise which could have affected the substantial increase in plasma ATP concentration in consecutive training phases. Likewise, futsal players had an increased plasma ATP concentration, which most likely resulted from the requirements of the discipline, i.e. prolonged effort interspersed by multiple repetitions of sprint bouts, especially during spring and autumn seasons characterized by a large number of matches and training sessions. In endurance athletes, a high training volume (number of training sessions and their total duration) and low to medium intensity may have resulted in lower increases in plasma ATP concentration.

Respiratory compensation point

The respiratory compensation point (RCP) was achieved in the range of 83% to 94% of VO_2max in all examined groups (both athletic and control) and was concomitant with a rapid increase in plasma ATP concentration. Therefore, it seems that the mechanism responsible for the moment of ATP efflux into the intravascular space is a variable independent of profile and training status.

5. Conclusions

- Long–term training and sports specialization have a significant impact on plasma ATP concentration at rest, during incremental exercise and during maximal effort, while such effect was not observed during recovery period.
- A rapid increase in plasma ATP concentration is concomitant with respiratory compensation point, which may reflect the partial inability to supply oxygen to muscles during exercise.
- Differences in plasma ATP concentration between the specific groups of competitive athletes at rest and during maximal effort are related to total–body skeletal muscle mass.
- Plasma ATP concentration in highly trained athletes during annual training cycle reaches the lowest values in the transition phase and the highest values in the competition phase.

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Streszczenie

Wewnątrznaczyniowy adenozynotrifosforan (ATP) jest w stanie regulować lokalny przepływ krwi i podaż tlenu do mięśni szkieletowych, wywołując wazodylatację podczas wysiłku. Celem badań była ocena wpływu treningu na stężenie ATP w osoczu u wysokowytrenowanych sportowców o odmiennej specjalizacji sportowej (publikacja 1) w rocznym cyklu treningowym (publikacja 2). Postawiono hipotezę, że (1) wyspecjalizowany trening sportowy wpływa na stężenie nukleotydów w osoczu podczas wysiłku i restytucji powysiłkowej, (2) osoczowe stężenie ATP zależy od masy mięśniowej oraz (3) stężenie ATP w osoczu zmienia się w rocznym cyklu treningowym, osiągając najwyższe wartości w okresie startowym.

Cztery grupy sportowe składające się ze sprinterów (SP), zawodników wytrzymałościowych (EN), zawodników futsalu (FU) oraz osób umiarkowanie aktywnych (CO) przebadano w charakterystycznych okresach rocznego cyklu treningowego. Pomiary zmiennych fizjologicznych i osoczowego stężenia nukleotydów wykonano czterokrotnie w odpowiedzi na test wysiłkowy o wzrastającej intensywności na bieżni mechanicznej do odmowy. Próbki krwi żylnej pobrano w spoczynku, podczas wysiłku oraz w okresie restytucji powysiłkowej.

U wysokowytrenowanych sportowców zaobserwowano znacznie wyższe wysiłkowe i powysiłkowe stężenia ATP w osoczu, przy czym najwyższe wartości osiągnięto po okresie startowym. Nie stwierdzono różnic w stężeniach ATP w osoczu w grupie kontrolnej we wszystkich badanych terminach. Sprinterzy wykazali większy wzrost stężenia ATP w osoczu podczas wysiłku w kolejnych okresach cyklu treningowego niż futsaliści i zawodnicy wytrzymałościowi. Wykazano gwałtowny wzrost osoczowego stężenia ATP przy intensywności wysiłku w zakresie 83–94% VO_2max. Osoczowe stężenie ATP po 30 minutach restytucji powysiłkowej różniło się od stężeń spoczynkowych u wszystkich sportowców, lecz nie w grupie kontrolnej.

Podsumowując, specjalizacja sportowa wpływa na osoczowe stężenie ATP w spoczynku, podczas wysiłku i restytucji powysiłkowej. Zwiększone stężenie ATP w osoczu może wpływać na procesy wazodylatacji podczas wysiłku u wysokowytrenowanych zawodników. Najszybszy wzrost osoczowego stężenia ATP związany był z osiągnięciem punktu kompensacji oddechowej, odzwierciedlającym częściową niezdolność do dostarczania tlenu do mięśni podczas wysiłku fizycznego. W badaniach wykazano, że stężenie ATP w osoczu znacząco zmieniało się u wysokowytrenowanych zawodników w trakcie rocznego cyklu treningowego. Wyniki sugerują, że stężenie ATP w osoczu zależy od udziału wysiłków o średniej i wysokiej intensywności w określonej fazie rocznego cyklu treningowego. Nie zaobserwowano różnic między grupami wyczynowych sportowców w okresie restytucji powysiłkowej, co sugeruje podobny wzorzec reakcji, niezależnie od specjalizacji sportowej. Jednym z najciekawszych wniosków wynikającym z przeprowadzonych badań jest to, że tylko speriodyzowany trening z dominacją wysiłków o średniej i wysokiej intensywności wywołuje zmiany osoczowego stężenia ATP, co podkreśla rolę ATP w adaptacji naczyń do zwiększonej wazodylatacji podczas wysiłku.

Abstract

Circulating plasma adenosine triphosphate (ATP) is capable of regulating local skeletal muscle blood flow and oxygen delivery causing vasodilatation during exercise. This study aimed to assess the effect of training on plasma ATP concentration in highly trained athletes of different sport specializations (study 1) in a 1–yr training cycle (study 2). It was hypothesized that (1) specific long–term training stimuli have an impact on plasma nucleotide concentrations during exercise and recovery period, (2) plasma ATP concentration depends on total–body skeletal muscle mass and (3) plasma ATP concentration changes over the annual training cycle, reaching the highest values during the competition phase.

Four athletic groups consisting of sprinters (SP), endurance–trained athletes (EN), futsal players (FU) and active individuals (CO) were studied in characteristic phases of the annual training cycle. Four–time measurements of physiological variables and plasma nucleotide concentrations were performed in response to an incremental treadmill test until exhaustion. Venous blood samples were collected at rest, during an incremental exercise and recovery.

Considerably higher exercise and post–exercise plasma ATP concentrations were observed in consecutive training phases in highly trained athletes, with the highest values reached after the competition period. No differences in plasma ATP concentrations were found in the control group during all examinations. Sprinters showed a higher increase in plasma ATP concentration during exercise in consecutive training phases than futsal players and endurance athletes. A rapid increase in plasma ATP concentration at exercise intensities of 83–94% of VO_2max was demonstrated. Plasma ATP concentrations measured after 30 minutes of recovery were different from those obtained before exercise in competitive athletes but not in controls.

In conclusion, sport specialization is significantly related to plasma ATP concentration at rest, during exercise and postexercise recovery. Increased plasma ATP concentration may affect vessel dilatation during exercise in highly trained athletes. The most rapid increase in plasma ATP concentration was associated with the respiratory compensation point reflecting the partial inability to supply oxygen to muscles during exercise. In this study, it was demonstrated that plasma ATP concentration significantly changes in highly trained athletes over the annual training cycle. The results suggest that plasma ATP concentration depends on the contribution of moderate– and high–intensity exercise to the training loads applied in the preceding training phase. No differences between groups of competitive athletes were observed during the post–exercise recovery period, suggesting a similar pattern of response after exercise regardless of sport specialization. One of the most interesting findings of this study is that only moderate– and high–intensity periodized training is able to induce plasma ATP changes, highlighting its role in vascular adaptation to increased exercise–induced vasodilation.

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mgr Ewa Zarębska

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Potwierdzenie współautorów:

Kusy Krzysztof .....

Słomińska Ewa Maria .....

Kruszyna Łukasz .....

Zieliński Jacek .....

Poznań, 22.10.2019r.

mgr Ewa Zarębska

Akademia Wychowania Fizycznego im. Eugeniusza Piaseckiego w Poznaniu

Wydział Wychowania Fizycznego, Sportu i Rehabilitacji

Zakład Lekkiej Atletyki i Przygotowania Motorycznego

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Potwierdzenie współautorów:

Kusy Krzysztof .....

Słomińska Ewa Maria .....

Kruszyna Łukasz .....

Zieliński Jacek .....

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Research Article

Plasma Nucleotide Dynamics during Exercise and Recovery in Highly Trained Athletes and Recreationally Active Individuals

Ewa A. Zarębska,[1] **Krzysztof Kusy**,[1] **Ewa M. Słomińska**,[2] **Łukasz Kruszyna**,[3] **and Jacek Zieliński**[1]

[1]*Department of Athletics, Strength and Conditioning, Poznan University of Physical Education, Królowej Jadwigi 27/39, 61-871 Poznań, Poland*
[2]*Department of Biochemistry, Medical University of Gdansk, Dębinki 1, 80-211 Gdańsk, Poland*
[3]*Department of General and Vascular Surgery, Poznan University of Medical Sciences, Długa 1/2, 61-848 Poznań, Poland*

Correspondence should be addressed to Jacek Zieliński; jacekzielinski@wp.pl

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Circulating plasma ATP is able to regulate local skeletal muscle blood flow and 0_2 delivery causing considerable vasodilatation during exercise. We hypothesized that sport specialization and specific long-term training stimuli have an impact on venous plasma [ATP] and other nucleotides concentration. Four athletic groups consisting of sprinters (n=11; age range 21–30 yr), endurance-trained athletes (n=16; age range 18–31 yr), futsal players (n=14; age range 18–30 yr), and recreationally active individuals (n=12; age range 22–33 yr) were studied. Venous blood samples were collected at rest, during an incremental treadmill test, and during recovery. Baseline [ATP] was 759±80 nmol·l^{-1} in competitive athletes and 680±73 nmol·l^{-1} in controls and increased during exercise by ~61% in competitive athletes and by ~31% in recreationally active participants. We demonstrated a rapid increase in plasma [ATP] at exercise intensities of 83–87% of VO_2max in competitive athletes and 94% in controls. Concentrations reported after 30 minutes of recovery were distinct from those obtained preexercise in competitive athletes ($P < 0.001$) but not in controls ($P = 0.61$). We found a correlation between total-body skeletal muscle mass and resting and maximal plasma [ATP] in competitive athletes (r=0.81 and r=0.75, respectively). In conclusion, sport specialization is significantly related to plasma [ATP] at rest, during exercise, and during maximal effort. Intensified exercise-induced plasma [ATP] increases may contribute to more effective vessel dilatation during exercise in highly trained athletes than in recreational runners. The most rapid increase in ATP concentration was associated with the respiratory compensation point. No differences between groups of competitive athletes were observed during the recovery period suggesting a similar pattern of response after exercise. Total-body skeletal muscle mass is indirectly related to plasma [ATP] in highly trained athletes.

1. Introduction

The interest in skeletal muscle blood flow regulation has long and rich history but, recently, there has been a growing interest in this field, especially regarding exercise [1–7]. Several mechanisms controlling skeletal muscle blood flow were reviewed [8, 9]. Studies in this field focused on the contribution of purines and nitric oxide (NO) metabolites on vasodilatation. Additionally, adenosine has been proposed as a potent vasodilator and potential regulator of muscle blood flow [10, 11] although its role might not be as important in skeletal muscle substrate metabolism as its regulating blood flow properties [12, 13].

Optimizing O_2 delivery to skeletal muscle is caused by release of both ATP and O_2 from erythrocytes in regions of low O_2 tension [14]. The released ATP binds to P2Y receptors on the endothelium and releases NO, endothelium-derived hyperpolarization factor (EDHF), and prostacyclins. As a result, local dilatation occurs, leading to increased blood flow to regions supplied by the vessel [2, 4].

ATP, in addition to functioning as an intracellular energy source, is equally important as an extracellular signalling

molecule matching oxygen delivery with demand under physiological stress such as exercise [2]. In the skeletal muscle interstitium, there is a marked increase in ATP concentration, tightly coupled with the increase in blood flow. Skeletal muscle and endothelial cells are thought to be possible sources of interstitial ATP [5, 15]. ATP is also likely derived from red blood cells as they become deoxygenated and mechanically deformed when passing through the microcirculation of contracting skeletal muscle [16]. During exercise, the interstitial ATP concentration increases in direct proportion to the workload [17]. Moreover, a connection was noticed between exercise hyperaemia and an increase in venous [ATP], draining active muscle in proportion to exercise intensity [4, 18]. It was also noted that ATP increases in the feed artery and the vein that drains contracting muscle, which suggests that erythrocytes are the main origin of venous ATP [19]. ATP is released from the erythrocyte simultaneously with the offloading of O_2 from the hemoglobin molecule. On the other hand, shear stress release of ATP from endothelial cells is suggested to be the main source of arterial ATP [4, 19, 20]. Notably, it has been shown *in vitro* that vasodilation of isolated resistance vessels in response to reduced O_2 requires erythrocyte presence [2, 21].

During exercise, there is a dynamic interaction between vasoconstriction and vasodilation mediated by purine stimulation. Experimental studies performed on the vascular effects of ATP had crucial importance in revealing the complexities of this system [4, 22]. Extracellular ATP released from erythrocytes into the extracellular space in response to a variety of stress conditions acts as an important regulator of vascular homeostasis. *In vitro* data demonstrates that red blood cells release ATP when exposed to hypoxia in the presence of hypercapnia, hypoxia alone [4], and increased mechanical deformation [23]. Additionally, red blood cells release ATP in response to reductions in oxygen tension and pH [2, 4], elevated shear stress [24], and increased temperature [25]. These are typical conditions occurring during physical exercise, especially within the active skeletal muscle microcirculation. The physiological stimuli for *in vivo* ATP release in the vasculature of human muscle remain unclear, nonetheless recent data confirms that ATP release from erythrocytes is temperature dependent [26].

Nucleotide concentration plays a crucial role in adaptive processes during exercise [4]. However, there are still controversies concerning resting venous plasma ATP concentrations. Likewise, nucleotide concentrations during exercise still remain debatable. It has been shown that extracellular [ATP], [ADP], and [AMP] increase during submaximal and maximal intensity exercise [27]. González-Alonso et al. reported an increase in plasma ATP concentration during incremental knee-extensor exercise [4]. Other studies reported unchanged or slightly elevated plasma ATP concentrations during exercise [22, 28, 29]. Only a few studies showed changes in plasma ATP concentration during recovery after incremental [4, 19, 27] or exhaustive maximal exercise [20]. Cycle-ergometer or one-legged knee-extensor exercise protocol was used in those studies. To our knowledge, there are no studies showing changes in [ATP] during and after whole body exercise, e.g., treadmill exercise. Whole body exercise may provide additional information on changes in [ATP] that cannot be obtained using small muscle mass exercise protocols [4, 22, 29]. Moreover, there is no study concerning the effect of long-term training programs on plasma nucleotide concentration.

During aerobically dominant exercise, skeletal muscle blood flow increases to ensure appropriate supply of O_2 sustaining the contractile activity of active skeletal muscle [30]. Skeletal muscle blood flow and oxygen delivery are strong predictors of aerobic exercise capacity [31]. During local maximal exercise, the skeletal muscle vascular bed is completely vasodilated [32] while during maximal whole body exercise, less active muscle fibers are under high vasoconstrictor influence of the sympathetic nervous system [30]. In trained humans during maximal whole body exercise, heart has insufficient capacity to supply oxygen and blood flow to exercising muscles [33]. Circulating ATP is able to regulate local skeletal muscle blood flow and O_2 delivery by causing considerable vasodilatation and overcoming increased sympathetic vasoconstrictor activity [22]. Long-lasting physical training program can cause specific adaptations in response to specific demands of training type, especially muscle metabolism and O_2 uptake. Previous studies have shown that among endurance-trained athletes an increased vasodilatation capacity during maximal exercise is mainly due to enhanced vasodilatory capacity [32, 34].

The main purpose of this study is to assess the effect of long-term training program on plasma nucleotide concentration and total-body skeletal muscle mass (ATP_{SMM}) among highly trained athletes of different sport specializations in response to incremental treadmill test until exhaustion. We hypothesize that (1) training type has an impact on magnitude of ATP response during exercise and recovery among highly trained athletes and that (2) ATP efflux will depend on skeletal muscle mass.

2. Materials and Methods

2.1. Subjects. The study included 41 highly trained male athletes from different sport disciplines. The athletic groups consisted of sprinters (SP; n=11; age range, 21-30 yr), endurance-trained subjects including long-distance runners and triathletes (EN; n=16; age range, 18–31 yr), and futsal players (FU; n=14; age range, 18–30 yr). All athletes were members of the Polish national team. The control group consisted of 12 healthy recreationally active men aged 22–33 yr without previous and current competitive sport experience. The controls were invited through announcements via local mass media to participate in this study. More detailed characteristics of the study participants are presented in Table 1.

The project was approved by the Ethics Committee at the Karol Marcinkowski Poznan University of Medical Sciences and has been performed according to the ethical standards laid down in the Declaration of Helsinki. The participants were fully informed of the purpose and risks of the study before giving their written consent to participate. They were also recommended to avoid high-intensity and long-duration training sessions 24–48 h before the tests. All tests were conducted at the Human Movement Laboratory of the Poznan

Table 1: Basic characteristics of the athletic groups and controls.

	Sprinters (n=11)	Futsal players (n=14)	Endurance athletes (n=16)	Control group (n=12)	ANOVA*
Age (yr)	24.2±3.2	24.7±3.9	23.4±3.6	27.7±4.1†	0.03
Training experience (yr)	8.5±2.5	9.8±3.3	8.7±1.9	-	0.38
Height (cm)	186.2±4.6	181.7±5.6	181.8±6.1	180.4±5.6	0.09
Body mass (kg)	81.6±5.5†	77.0±6.7	73.4±7.2	76.7±7.7	0.03
BMI (kg/m^2)	23.5±1.0	23.4±2.2	22.2±2.1	23.7±2.6	0.24
Total-body SMM (kg)	39.1±3.7	33.8±3.0§	32.4±3.2a	33.1±3.1a	<0.001
Total Tissue Fat (%)	12.56 ±2.22	17.46±2.67§	15.72 ±2.44	18.32 ±3.77a	<0.001
LA_{rest} ($mmol \cdot l^{-1}$)	1.4±0.6	1.4±0.4	1.2±0.3	1.4±0.3	0.36
LA_{max} ($mmol \cdot l^{-1}$)	11.0±1.4	11.4±2.0	11.6±1.9	10.1±1.1	0.15
HR_{max} ($beats \cdot min^{-1}$)	188±10	187±10	192±9	187±8	0.40
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.27±3.63‡	56.66±2.62‡	66.86±4.98	56.34±2.70‡	<0.001
VO_2max ($ml \cdot kg\ SMM^{-1} \cdot min^{-1}$)	111.54±8.83‡	129.44±8.97‡§	151.65±13.49	130.46±7.04‡a	<0.001
RBC ($10^{12} \cdot l^{-1}$)	5.11±0.29	4.91±0.25	4.81±0.43	4.92±0.14	0.12
Hb ($mmol \cdot l^{-1}$)	8.94±0.56	8.84±0.4	9.05±0.52	9.10±0.44	0.52
Hct ($l \cdot l^{-1}$)	0.42±0.03	0.42±0.02	0.42±0.03	0.42±0.02	0.59
MCV (fl)	83.16±2.19†	85.02±1.76	87.25±4.10	86.40±2.82	0.006
MCH (fmol)	1.75±0.04‡	1.80±0.04†	1.89±0.10	1.85±0.06§	<0.001
MCHC ($mmol \cdot l^{-1}$)	20.93±0.29	21.24±0.40	21.66±0.62	21.54±0.64	0.07
RDW (%)	11.67±0.69	11.70±0.40	11.58±0.58	11.51±0.52	0.82

*One-way ANOVA. Values are means ± SD. † $P < 0.05$, ‡ $P < 0.001$, significantly different from endurance athletes, § $P < 0.01$, a $P < 0.001$, significantly different from sprinters. Abbreviations. BMI: body mass index; SMM: skeletal muscle mass; LA_{rest}: resting lactate concentration; LA_{max}: maximal lactate concentration; HR_{max}: maximal heart rate; VO_2max: maximal oxygen uptake; RBC: red blood cell; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red blood cell distribution width.

University of Physical Education at the end of the transition phase of the annual training cycle. The measurements were performed in the morning, 2 h after light breakfast (bread and butter, water, without coffee or tea). Prior to an exercise test, body composition assessment was performed. Then, subjects performed an incremental treadmill test until exhaustion. During all examinations, the ambient temperature remained unchanged at 20–21°C.

2.2. Body Composition Analysis. The subjects' body mass and height were measured using digital stadiometer (SECA 285, SECA, Hamburg, Germany). To evaluate body composition (total-body fat and appendicular lean soft tissue) the dual X-ray absorptiometry method (DXA, Lunar Prodigy; GE Lunar Healthcare, Madison, WI, USA) was used. Each day, prior to measurements, the DXA device was calibrated using a phantom, according to manufacturer guidelines. All DXA scans were performed and analyzed by the same trained technician according to manufacturer's protocols using enCORE 16 SP1 software. During the examination, subjects were wearing only their underwear, without any jewelry or metal objects to minimize measurement error. Total-body skeletal muscle mass (SMM) was calculated using Kim's et al. regression model [35].

2.3. Respiratory Parameters. All athletes underwent an incremental running treadmill test (H/P Cosmos Pulsar, Sports & Medical, Nussdorf-Traunstein, Germany) in order to determine maximal oxygen uptake (VO_2max). The initial speed was set up at 4 $km \cdot h^{-1}$ and was increased after 3 min to 8 $km \cdot h^{-1}$. After that point, the moving strip was progressively increased by 2 $km \cdot h^{-1}$ every 3 min until an athlete reached voluntary exhaustion. After 10 $km \cdot h^{-1}$, blood samples were drawn at the end of each 3-minute stage. Main cardiorespiratory variables (minute ventilation, V_E; oxygen uptake, VO_2; carbon dioxide production, VCO_2) were measured constantly (breath by breath) using MetaMax 3B-R2 ergospirometer and analyzed using MetaSoft Studio 5.1.0 Software (Cortex Biophysik, Leipzig, Germany). Maximal oxygen uptake was considered achieved if at least three of the following criteria were met: (i) a plateau in VO_2 despite an increase in speed; (ii) cutoff blood lactate concentration ≥ 9 $mmol \cdot l^{-1}$; (iii) respiratory exchange ratio ≥ 1.10; and (iv) heart rate ≥ 95% of the age-predicted HR_{max} [36]. The respiratory compensation point (RCP) was determined from breath-by-breath data automatically at the inflection of the V_E versus VCO_2 slope (V-slope method) [37]. A nonlinear increase in V_E/VCO_2 and a point where $PETco_2$ begins to fall were determined when the V-slope method was insufficient and visual assessment was needed [38]. Before each test, the system was calibrated according to the manufacturer's instructions. Heart rate was measured continuously with Polar Bluetooth Smart H6 monitors (Polar Electro Oy, Kempele, Finland).

2.4. Blood Sampling. Venous blood samples were obtained at rest, at the end of each 3-min stage above 10 km·h^{-1}, immediately after exercise and in the 5th, 10th, 15th, 20th, and 30th min of postexercise recovery. The catheter (1.3 × 32 mm, BD Venflon Pro, Becton Dickinson, Helsingborg, Sweden) was inserted retrogradely into the antecubital vein which was kept patent with isotonic saline (0.9% NaCl) during the whole procedure. Syringes comprising EDTA (S-Monovette, 2.7 ml KE, Sarstedt, Nümbrecht, Germany) were used for hematological parameters and plasma nucleotide concentrations analyses. For lactic acid measurement, lithium heparin as an anticoagulant (S-Monovette, 2.7 ml KE, Sarstedt, Nümbrecht, Germany) was used.

2.5. Lactic Acid and Hematological Measurements. Lactate in whole blood (20 μl) was immediately assayed using the spectrophotometric enzymatic method (Biosen C-line, EKF Diagnostics, Barleben, Germany). 10 μl of blood was used for hematological analysis carried out on an 18-parametric automated hematology analyzer Mythic®18 (Orphée, Geneva, Switzerland).

2.6. Plasma ATP, ADP, and AMP Measurements. For plasma nucleotide concentration analyses, 2 ml of blood was pipetted into a test vial (Eppendorf, Wesseling-Berzdorf, Germany) and immediately centrifuged (Universal 320R, Hettich Lab Technology, Tuttlingen, Germany) for 30 seconds at 14 000 rpm at 4°C. Then, plasma (200 μl) was pipetted into 1.5 ml vials in duplicate and frozen down in liquid nitrogen. All samples were stored in -80°C until further analysis. Nucleotide degradation in blood is rapid; therefore the start of centrifugation was within 5 sec from collection, and plasma was deproteinized immediately after 30 sec centrifugation. The duration of the whole procedure from blood withdrawal to plasma separation did not exceed 45 seconds. The method of blood sampling for plasma ATP was tested for hemolysis that was absent.

2.7. Chromatography Method. Nucleotides in plasma were determined by high performance liquid chromatography (HPLC) with UV detection according to the methodology of Smolenski et al. [39] and Smolenski & Yacoub [40]. In brief, samples were extracted using perchloric acid (2.4 mol·l^{-1}) on ice at the ratio of 1:0.25 for 15 min and then centrifuged at 13 000 rpm for 3 min at 4°C. Supernatant was collected and neutralized using 3 mol·l^{-1} K_3PO_4 before being centrifuged at 13 000 rpm for 3 min at 4°C.

The samples were left on ice for 30 min to ensure complete precipitation of potassium perchlorate. After that, supernatants were collected, transferred to another test tube, and stored at -80 before analysis. The analyses were performed using Spectra HPLC system (Thermo, USA) equipped with 10 cm path flow cell to increase sensitivity. Separation was achieved with analytical column BDS Hypersil C18 (150 mm x 4.6 mm x 3 μm; Thermo, USA) placed in a thermostat (18°C) protected by precolumn 20 mm x 4 mm (Phenomenex, type SecurityGuard, USA). The mobile phase consisted of A: 122 mM KH_2PO_4, 150 mM KCL, and 28mM K_2HPO_4 and B: 15% (v/v) acetonitrile in A. The percentage of B changed from 0% to 100% in several linear steps during analysis and then returned to 0% B for reequilibration. The separation time with reequilibration was 13.5 min and was conducted at 0.9 ml/min flow rate. The sample injection volume was 40 μl. The quantitative analyses were performed based on external calibration of the signal at 254 nm. Data acquisition and processing were managed by the Xcalibur™ software (v. 2.1, Thermo Scientific™, Waltham, MA, USA). The above described method provides good recoveries (>95%) and an acceptable coefficient of variation (<10%). Detection limit with high injection volume and 10 cm path flow cell in a detector used here was 50 nmol·l^{-1} for plasma ATP.

2.8. Statistical Analyses. To test changes in plasma ATP, ADP, and AMP concentration between measurement points during exercise and recovery within each group of participants, a one-way repeated-measures ANOVA was performed. We also used one-way repeated-measures ANOVA to test differences at the same measuring point between groups. If a significant difference was found ($P < 0.05$), post hoc Scheffe tests were conducted. The effect sizes for ANOVA analyses were small to very large for descriptive characteristics (η^2 = 0.02–0.68). At the same measuring point, the effect sizes of ANOVA analyses between groups were very large for plasma ATP (η^2 = 0.39–0.89), ATP_{SMM} (η^2 = 0.15–0.68), ADP (η^2 = 0.41–0.88), and AMP (η^2 = 0.41–0.87). The effect sizes of ANOVA values were very large within groups between measurement points (η^2 = 0.82–0.96). The statistical power of most observed ANOVA analyses for plasma ATP, ATP_{SMM}, ADP, and AMP was 1.00. Only in the case of three variables, statistical power ranged from 0.66 to 0.97. Pearson correlation coefficients were used to describe the relationship between total-body skeletal muscle mass and plasma ATP concentrations at rest or maximal exercise. All statistical analyses were performed using STATISTICA 12.0 software (StatSoft, Tulsa, OK). Significance level was set at $P < 0.05$. All values were presented as means ± SD.

3. Results

3.1. Subjects Description. All athletes did not differ significantly with regard to height, body mass index (BMI), resting and maximal lactate concentrations, and maximal heart rate (Table 1). Sprinters had significantly smaller percent of total tissue fat than futsal players and controls; in addition, sprinters were characterized by significantly larger total-body skeletal muscle mass than compared groups. Competitive athletes had statistically similar training experience. Subjects from control group were engaged in endurance type recreational sports activities (3–5 times a week) but none were competitive athletes. Endurance athletes presented a significantly higher $\dot{V}O_2$max than others. In addition to that, VO_2max per unit of total-body skeletal muscle mass was higher in futsal players and controls compared to sprinters. No significant differences in RBC, Hb, Hct, MCHC, and RDW (%) between compared groups were found. Sprinters had significantly lower MCV and MCH than endurance athletes who had also higher MCH values compared to futsal players.

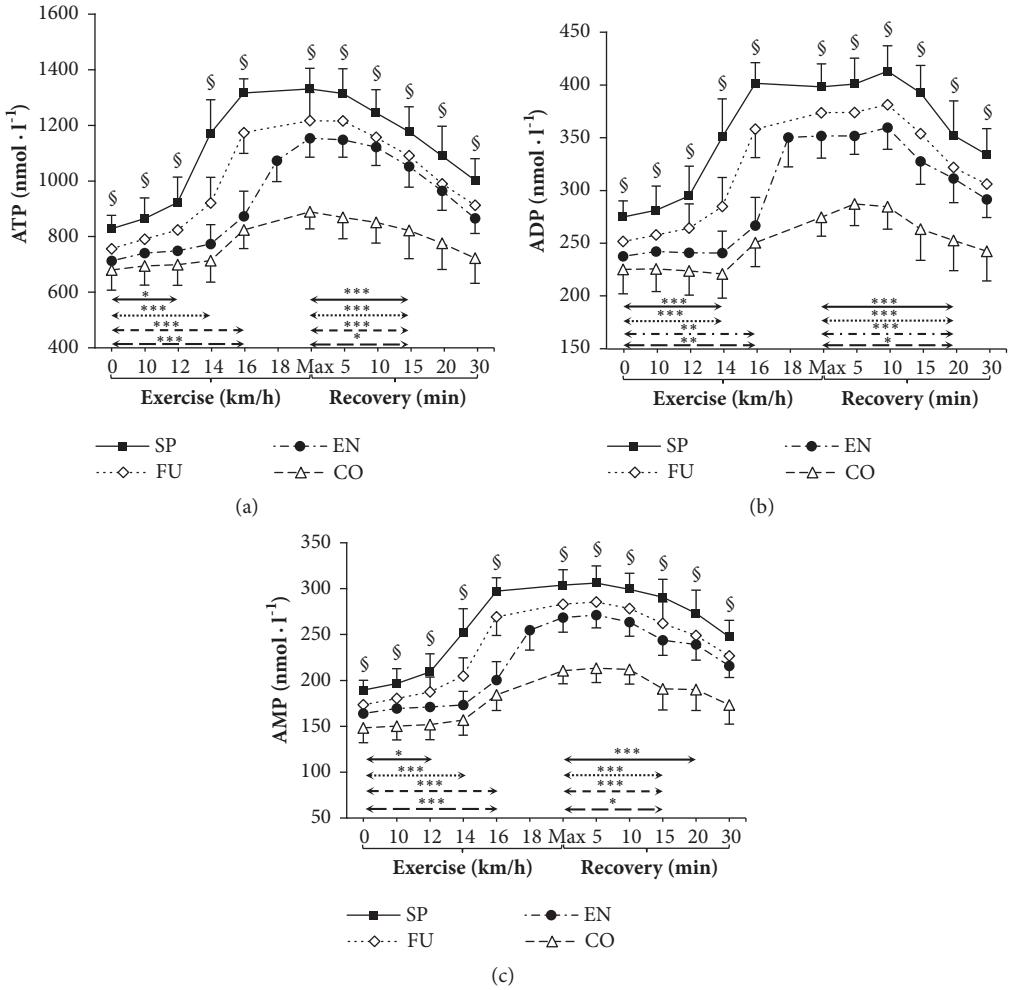

Figure 1: Venous plasma ATP, ADP, and AMP levels before exercise, during incremental treadmill test until exhaustion, and postexercise recovery in sprinters (n=11), futsal players (n=14), endurance athletes (n=16), and control group (n=12). Data are means ± SD. Arrows indicate the first significant differences from samples taken at rest and maximal exercise within groups. Significant differences between blood sampling points: $***P < 0.001$, $**P < 0.01$, $*P < 0.05$. Significant differences between groups at the same measurement point: §$P < 0.001$.

3.2. Preexercise Nucleotide Concentration. Resting venous plasma [ATP], [ADP], and [AMP] differed between groups. Sprinters had significantly higher plasma [ATP], [ADP], and [AMP] than endurance athletes and controls ($P < 0.001$ both). Preexercise plasma [ADP] and plasma [AMP] were also significantly higher in futsal players than in control group ($P < 0.05$) (Figure 1).

3.3. Nucleotide Concentration during Exercise. Our data showed significant increases in plasma [ATP], [ADP], and [AMP] during exercise (Figure 1). During incremental exercise plasma [ATP] and [AMP] increased progressively, being significantly higher after 12 km·h^{-1} in sprinters (11.3% and 22.4% increase, respectively), 14 km·h^{-1} in futsal players (21.8% and 18.1% increase, respectively), and 16 km·h^{-1} in endurance athletes (22.5% and 22.4% increase, respectively) and in control group (21.2% and 21.2% increase, respectively) compared with rest (Figure 1). First significant increase in plasma [ADP] was observed after 14 km·h^{-1} in sprinters (27.6%) and futsal players (13.2%) compared to resting values. In the endurance and control groups, the first observed significant increase was after 16 km·h^{-1} (12.2% and 11.1%, respectively). It should be noted that only endurance athletes reached 18 km·h^{-1} during their test. Moreover, venous plasma [ATP], [ADP] and [AMP] at a given workload tended to be significantly higher in sprinters than endurance athletes and controls ($P < 0.001$ for v = 10 km·h^{-1}) and all other groups beginning with 12 km·h^{-1} ($P < 0.05$ for FU; $P < 0.001$ for EN and CO), 14 km·h^{-1}, and 16 km·h^{-1} ($P < 0.001$). All athletes reached their peak plasma [ATP] at the end of the test. The absolute average net increase in plasma [ATP] was 502 nmol·l^{-1} in sprinters (60.6%, 1.61-fold), 461 nmol·l^{-1} in futsal players (60.9%, 1.61-fold), 441 nmol·l^{-1} in endurance athletes (61.9%, 1.62-fold), and 209 nmol·l^{-1} in the control group (30.8%, 1.31-fold). None of the athletic groups reached

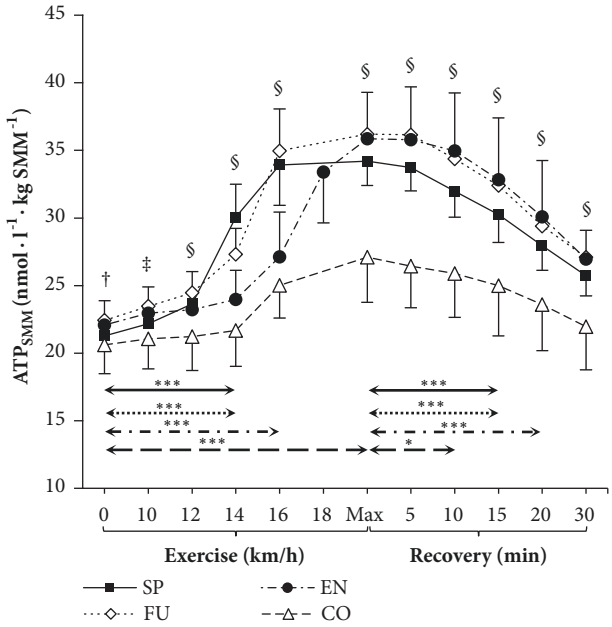

Figure 2: Venous plasma ATP concentration per kilogram of skeletal muscle mass (ATP_{SMM}) before, during, and after incremental treadmill test until exhaustion in sprinters (n=11), futsal players (n=14), endurance athletes (n=16), and control group (n=12). Data are means ± SD. Arrows indicate the first significant differences from samples taken at rest and maximal exercise within groups. Significant differences between blood sampling points: $***P < 0.001$, $*P < 0.05$. Significant differences between groups at the same measurement point: §$P < 0.001$, ‡$P < 0.01$, †$P < 0.05$.

peak plasma [ADP] and [AMP] at maximum intensity at the end of exercise.

3.4. Nucleotide Concentration during Recovery. During recovery there was a significant decrease in venous plasma nucleotide concentration in all groups. The first significant plasma [ATP] decrease compared to peak value was observed after 15 minutes from test completion in sprinters (11.5%), futsal players (10.4%), endurance athletes (11.5%), and control group (7.6%). The first observed significant plasma [ADP] decrease was observed after 20 minutes in sprinters (11.5%), futsal players (13.9%), endurance-trained group (11.4%), and controls (8.0%). The first significant decrease compared to peak value in plasma [AMP] was observed within 15 minutes of recovery in futsal players (7.4%), endurance athletes (9.1%), and control group (8.8%). In sprinters, the first significant plasma [AMP] decrease was detected after 20 minutes (10.2%). The absolute average net decrease of plasma [ATP] was 329 nmol·l^{-1} in sprinters (24.7%, 1.33-fold), 304 nmol·l^{-1} in futsal players (25.0%, 1.33-fold), 288 nmol·l^{-1} in endurance athletes (25.0%, 1.33-fold), and 167 nmol·l^{-1} in control group (18.8%, 1.23-fold). During recovery, venous plasma [ATP], [ADP], and [AMP] differed in all groups compared to controls (Figure 1.). Additionally, values reported after 30 minutes of recovery were distinct from those obtained preexercise in competitive athletes ($P < 0.001$), but not in controls ($P = 0.61$).

3.5. Changes in ATP Normalized to Skeletal Muscle Mass. Plasma [ATP] in relation to skeletal muscle mass before, during, and after exercise is expressed graphically in Figures 2 and 3. At rest, at peak exercise, and during the recovery period, venous plasma [ATP] per kilogram of skeletal muscle mass did not differ between groups of competitive athletes. During incremental exercise plasma [ATP_{SMM}] increased progressively, being significantly higher after 14 km·h^{-1} in sprinters and futsal players, after 16 km·h^{-1} in endurance athletes, and immediately after maximal exercise in control group compared to rest (Figure 2). Resting and maximal plasma [ATP] was positively correlated with total-body skeletal muscle mass among competitive athletes, most strongly in sprinters (r=0.89; $P < 0.001$ both) (Figure 3) while no correlations were found in the control group.

3.6. Respiratory Compensation Point. Respiratory compensation point (RCP) expressed as a percent of $\dot{V}O_2$max occurred within 83–88% in competitive athletes and at 94% in control group. RCP occurred between 12 and 14 km·h^{-1} in futsal players, sprinters, and controls. Endurance athletes obtained RCP between 16 and 18 km·h^{-1}. A rapid increase in plasma [ATP] occurred concomitantly with RCP.

4. Discussion

In this study, we analyzed venous plasma [ATP] in response to incremental exercise. The results showed that distinct sport specializations have different effects on plasma [ATP] in highly trained competitive athletes. However, no differences between the groups of competitive athletes were observed during recovery. Our study suggests that long-term training has an impact on plasma [ATP] responsiveness since recreationally active subjects showed significantly lower increments in response to exercise (~61% in competitive athletes versus ~31% in healthy recreationally active participants). These differences can be partly explained by vascular remodelling within muscle due to specific training adaptations resulting from sport type [41]. In highly trained athletes at similar sport level, absolute skeletal muscle mass seems to affect plasma [ATP], because after expressing [ATP] per kilogram of total-body skeletal muscle mass there were no differences between competitive athletes at rest, at maximal intensity, and during recovery. In our study, we confirmed a rapid increase in plasma [ATP] at exercise intensities of 83–87% of VO_2max in competitive athletes and at 94% of VO_2max in controls. We noticed that this sharp rise in plasma [ATP] is concomitant with the respiratory compensation point which reflects the partial inability to supply O_2 to muscles during exercise.

Skeletal muscle blood flow regulation and oxygen delivery during exercise is complex and involves the mechanical effects of muscle contraction, presence of red blood cell and endothelium-derived substances, and sympathetic nervous system activity. Initially, this effect was attributed to the muscle pump effect, but it is now firmly accepted that the temporary increase in blood flow is mainly due to local vasodilatory response [42]. To date, there have been relatively few studies investigating the vasodilatation mechanisms in

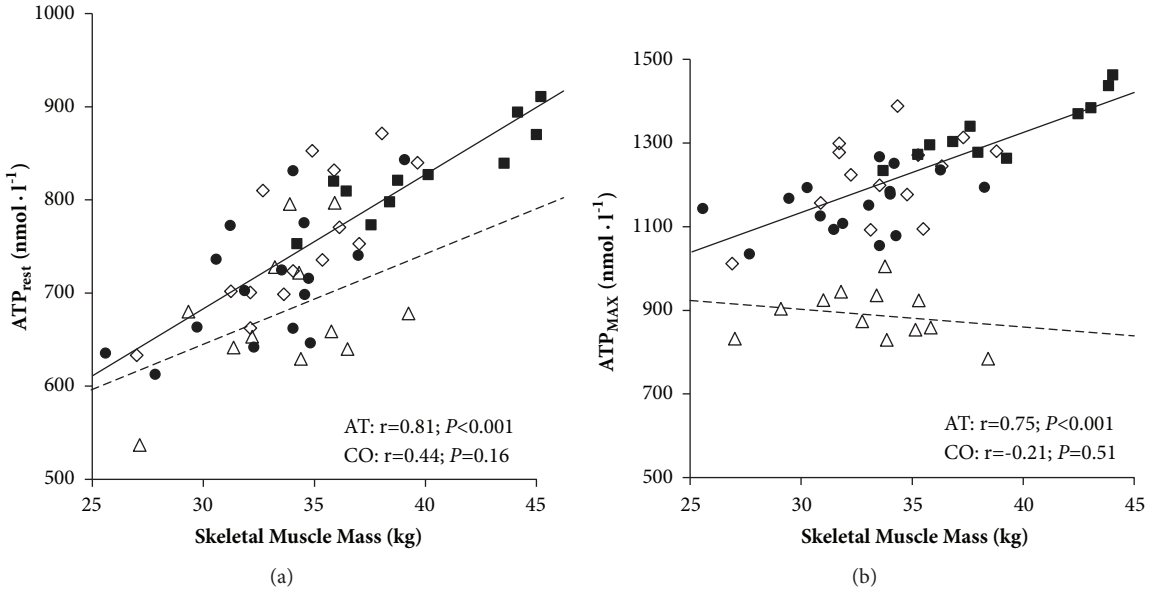

Figure 3: Relationship between skeletal muscle mass and plasma ATP concentration at rest (a) and maximal exercise (b) in competitive athletes (AT, solid line; including sprinters SP, n=11, ■; futsal players FU, n=14 ◊, and endurance athletes EN, n=16, ●) and control group (CO, n=12, dashed line, △).

humans. The major part of our understanding regarding the mechanisms of blood flow regulation is derived from studies using steady-state submaximal exercise.

In this study, we tested the effect of exhaustive whole body incremental exercise on venous plasma [ATP]. Comparison of previously reported data shows heterogeneity in physiological plasma [ATP] levels indicating that accurate estimation of this compound is currently difficult to perform. Also, ATP response during exercise remains controversial as plasma [ATP] has been reported to remain unchanged [22, 28, 29] or to increase rapidly at intensities above 60% of maximal workload [4]. In the present study, a continuous increase in venous plasma [ATP] from rest to maximal workload was observed. This pattern demonstrates that the rise in plasma [ATP] in response to exercise can be both large and rapid. Mortensen et al. demonstrated that ATP is released locally into both arterial and venous blood during exercise and that compression of the vasculature as well as hypoxia stimulates ATP release into plasma [19]. The present results provide evidence that ATP is released into venous plasma of contracting muscle and its concentration increases progressively with exercise intensity.

Determining plasma [ATP] is crucial for understanding its role in the skeletal muscle blood flow adjustments. ATP has been suggested to contribute to the local regulation of skeletal muscle blood flow by causing local vasodilatation [9]. It was previously shown that an arterial infusion of ATP in a human leg can cause vasodilatation similar to that observed during maximal exercise [4, 22]. Unfortunately, direct evidence for the role of ATP in blood flow regulation is lacking because of the absence of a selective receptor antagonist for human use [5]. An interesting observation is that both physical training [43] and chronic hypoxia [44] reduce the vasodilator response to arterially infused ATP, suggesting that purinergic P2 receptor sensitivity and/or ATP degradation in plasma is altered with training and hypoxia. Type of training can influence the physiological mechanisms of ATP release and its influence on muscle vessel dilatation during incremental exercise. We hypothesized that distinctive training types in highly trained sprinters, futsal players, and endurance athletes result in different responses of plasma [ATP]. Considerably higher plasma [ATP] in sprinters than in endurance athletes and futsal players, demonstrated during incremental treadmill test, indicates training-specific adaptations. Sprint training is mainly based on frequent stimulation in short time periods, contrary to endurance athletes whose training reflects the ability to maintain relatively low intensity workloads for longer periods of time. Futsal requires prolonged exercise characterized by frequent high-intensity efforts.

During exercise, skeletal muscle perfusion increases in direct proportion to the metabolic demand. Skeletal muscle blood flow can increase 100-fold from resting conditions up to 300–400 ml·min^{-1}·kg^{-1} [45]. Several vasoactive compounds cooperate in synergy in regulation of skeletal muscle blood flow [15]. Skeletal muscles are one of the potential sources of extracellular ATP in venous blood. To explain the changes in plasma [ATP], we assumed that they are partially caused by total-body skeletal muscle mass. Sprinters, characterized by high SMM, obtained the highest absolute plasma [ATP] despite the lowest VO_2max. Plasma [ATP] per kilogram of skeletal muscle mass eliminated differences between the groups of competitive athletes at rest, during maximal effort, and during recovery, which indirectly shows the influence of SMM in highly trained individuals. After normalizing ATP into 1 kilogram of skeletal muscle mass,

differences between competitive athletes still exist during exercise, which suggests that not only skeletal muscle mass but also its specific adaptation to exhaustive exercise is important.

During exercise, the magnitude of ATP-induced vasodilatation may reflect increased demand for oxygen among athletes and healthy recreational runners. Speed-power training containing large proportion of anaerobic exercise results in adaptation to both rapid and intensified response of vessel dilatation. In contrast, endurance training, mainly based on aerobic exercise [46], seems to lead to moderate increase in ATP levels, spread over exercise time as their training is mainly focused on maintaining moderate intensities exercise for longer periods. In the control group, lower ATP_{SMM} suggests that training status and muscle adaptation to exercise are also crucial. In our opinion increased muscle capillarization in endurance athletes requires lower ATP increments than in speed-power athletes. Also, in speed-power athletes during exercise, increased hypoxia due to higher muscle mass requires an increase of vasodilatation to sustain exercise ability. Our finding about sport specialization impact appears to be in agreement with Laughlin & Roseguini [34] who demonstrated mode-specific training adaptive changes in vascular smooth muscle and endothelium as interactions of muscle fiber-type composition and muscle fiber recruitment patterns during exercise. However, the differences disappeared among our highly trained athletes if expressed per kilogram of SMM; thus we concluded that long-term intensive training of any kind leads to similar [ATP] response at rest and during recovery. Our results should be supported by further research.

Some substances have the ability to inhibit the local vasoconstrictor effect of increased sympathetic nerve activity. This phenomenon, termed functional sympatholysis, is also known to occur during exercise [22], but its importance involved in regulation is still under debate [47]. The ability of contracting skeletal muscle to blunt sympathetic vasoconstriction is critical for the proper regulation of tissue blood flow distribution and oxygen delivery. It allows increasing the skeletal muscle perfusion during high-intensity and/or large muscle mass exercise. "Functional sympatholysis" describes the ability of contracting skeletal muscle to block sympathetic vasoconstrictor activity. This is crucial to ensure proper blood flow distribution and O_2 delivery to metabolically active skeletal muscle. The exact signalling mechanism responsible for sympatholysis in healthy humans is unknown, but its role varies between individuals, in particular differences in age and training status. Moreover, the compounds mediating functional sympatholysis in healthy humans are still unknown. It was proposed that ATP can play an important role by inducing local vasodilatation, overriding local sympathetic vasoconstriction, and stimulating the exercise pressor reflex [5].

To the best of our knowledge, the only vasoactive substance that has been shown to independently cause sympatholysis in humans is ATP. Local intra-arterial infusion of ATP showed a significant blunting of tyramine-induced vasoconstriction in the leg, similarly during moderate knee-extensor exercise [22]. ATP has a dose-dependent ability to attenuate sympathetic vasoconstriction during exercise and hypoxia. Low levels of ATP did not impact α_1-mediated vasoconstriction while increasing ATP concentration gradually restricted α_1-mediated vasoconstriction [48]. These observations are quite analogous to the intensity-dependent character of functional sympatholysis within contracting skeletal muscle [8]. One recent study provides evidence that α-adrenergic responsiveness, thus functional sympatholysis, can be improved by physical training [49]. With reference to what was indicated above, we assume that long-term training will lead to improvement in sympatholytic capacity. This assumption is consistent with our results as competitive athletes had significantly higher plasma [ATP] during exercise compared to healthy controls. Since ATP is capable of independently overriding sympathetic vasoconstriction in a dose-dependent manner, higher reported values at the end of exercise seem to be related to more efficient blood flow distribution to skeletal muscles. However, it is debatable whether the blood flow to exercising muscle is indeed constricted or only redistribution within muscle will be observed [50].

The balance between O_2 delivery and its utilization during exercise depends on the specific needs of muscles which are influenced by the intensity, duration, and mode of exercise. Not only cardiac output increases during exercise but also blood flow is redistributed in such a way that over 80–90% of it can be directed to skeletal muscles [51]. The breakpoints observed during incremental exercise may reflect sudden changes in adjusting local oxygen demand according to its requirements; it is expected that oxygenation pattern changes might be reflected in whole body physiological responses [52]. The breakpoints of locomotor muscle oxygenation responses range between 75 and 90% of VO_2 max and are closely related to the traditionally determined threshold in pulmonary gas exchange, i.e., RCP [52]. There is currently a strong controversy as to whether these breakpoints in oxygenated and deoxygenated hemoglobin and myoglobin response in distinct regions of the body are corresponding to these thresholds in whole body responses and whether they can be used alternatively [52]. Nevertheless, erythrocytes are known to serve as oxygen sensors releasing vasoactive ATP in regions of low O_2 tension. We suppose that low muscle oxygenation results in enhanced blood flow distribution to these regions to sustain muscle contractions. However, a further increase in exercise intensity causes an accumulation of metabolic by-products signalled by exercise-induced lactic acidosis and hyperventilation [53], a phenomenon known as RCP. We suggest that a rapid increase in plasma ATP curve is accompanied by RCP or even precedes this point. The main observation of our study was that highly trained athletes demonstrated remarkably increased plasma [ATP] during exercise compared to recreational participants. This may be caused by more optimal tissue oxygen delivery and vascularization of the muscle capillary bed. This study is the first attempt to prove that long-term training per se may result in considerable increases in plasma levels of the powerful vasoactive molecule ATP. Further research should focus on training-related plasma [ATP] alterations in the whole annual training cycle in relation to the predominant training type.

5. Conclusions

In summary, the obtained results show a significant impact of long-term training and sport specialization on plasma [ATP] at rest, during incremental treadmill exercise, and during maximal effort but not during the recovery period. The differences between athletic groups appear to be related to total-body skeletal muscle mass. A rapid increase in plasma [ATP] curve is concomitant with respiratory compensation point reflecting the partial inability to supply O_2 to muscles during exercise.

Data Availability

The raw data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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metabolites

MDPI

Article

Alterations in Exercise-Induced Plasma Adenosine Triphosphate Concentration in Highly Trained Athletes in a One-Year Training Cycle

Ewa Anna Zarębska [1,*], Krzysztof Kusy [1], Ewa Maria Słomińska [2], Łukasz Kruszyna [3] and Jacek Zieliński [1,*]

1 Department of Athletics, Strength and Conditioning, Poznan University of Physical Education, Królowej Jadwigi 27/39, 61-871 Poznan, Poland; kusy@awf.poznan.pl
2 Department of Biochemistry, Medical University of Gdansk, Dębinki 1, 80-211 Gdansk, Poland; eslom@gumed.edu.pl
3 Department of General and Vascular Surgery, Poznan University of Medical Sciences, Długa 1/2, 61-848 Poznan, Poland; lukaszkruszyna@poczta.onet.pl
* Correspondence: zarebska@awf.poznan.pl (E.A.Z.); jacekzielinski@wp.pl or jzielinski@awf.poznan.pl (J.Z.); Tel.: +48-61-83-13-55-333 (E.A.Z.); +48-61-83-55-270 (J.Z.)

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Abstract: This study aimed to assess the effect of training loads on plasma adenosine triphosphate responsiveness in highly trained athletes in a 1 y cycle. Highly trained futsal players (11 men, age range 20–31 y), endurance athletes (11 men, age range 18–31 y), sprinters (11 men, age range 21–30 y), and control group (11 men, age range 22–34 y) were examined across four characteristic training phases in response to an incremental treadmill test until exhaustion. A considerably higher exercise and post-exercise plasma adenosine triphosphate concentrations were observed in consecutive training phases in highly trained athletes, with the highest values reached after the competitive period. No differences in plasma adenosine triphosphate concentrations were found in the control group during the 1 y cycle. Sprinters showed a higher absolute and net increase in plasma adenosine triphosphate concentration by 60–114% during exercise in consecutive training phases than futsal players (63–101%) and endurance athletes (64–95%). In this study, we demonstrated that exercise-induced adenosine triphosphate concentration significantly changes in highly trained athletes over an annual training cycle. The obtained results showed that high-intensity but not low- to moderate-intensity training leads to an increased adenosine triphosphate response to exercise, suggesting an important role of ATP for vascular plasticity.

Keywords: annual training cycle; ATP release; plasma nucleotides; training adaptation; incremental exercise test

1. Introduction

Exercise-related shear stress, local hemoglobin desaturation, and increased temperature in the working muscle are typical phenomena that induce adenosine triphosphate (ATP) release, predominantly from erythrocytes, to improve local blood flow [1–3]. The vasodilatory response during prolonged dynamic exercise is due to thermal and metabolic rate-sensing mechanisms within skeletal muscle, presumably through signaling pathways that regulate the intravascular concentration of ATP [4]. Plasma ATP levels are increased in the venous effluent from exercising muscle [5]. Intravascular ATP can independently attenuate α_1-adrenergic vasoconstriction, which further supports the potential blood flow regulative role of ATP during exercise in humans [6–8]. Furthermore, ATP can elicit prolonged vasodilatation for up to 3 h [9]; therefore, it is an attractive mediating signal because of

its sympatholytic and skeletal muscle blood flow regulation properties. Adenosine diphosphate (ADP), an ATP derivative, has been proposed to mediate the middle phase of reactive hyperemia via endothelial $P2Y_1$ receptors [10]. ADP also activates a negative feedback pathway of ATP release from erythrocytes via $P2Y_{13}$ receptors [11]. Therefore, an increased ADP concentration during exercise [12] may be of great physiological importance to diminishing ATP release. Plasma adenosine monophosphate (AMP), alongside from ATP and ADP, has also been shown to increase during submaximal and maximal exercise intensity [13]. However, its importance to the vasodilatory response is minor [7].

Repeated elevated shear stress improves the flow-mediated dilatation of large conduit arteries as well as enhancing vasodilatory capacity during isolated exercise in trained muscles [14]. Long-lasting physical training causes specific adaptations in response to the unique demands of different types of training, i.e., enhanced oxygen (O_2) efflux through increased maximal cardiac output, improved blood flow resulting from vascularization, and improved erythrocyte deformability [15]. It appears that high-volume, low-intensity training is crucial to providing a platform for specific adaptations that are developed in response to high-intensity exercise [16]. For example, endurance training programs promote skeletal muscle capillary supply and muscle fiber oxidative capacity with little increase in either muscle strength and muscle size [17,18]. In particular, aerobically trained athletes show enhanced vasodilatory and venous capacity in their muscles [19]. Well-trained endurance athletes perform ~80% of their training at intensities below the lactate threshold (low-intensity exercise zone), despite competing at intensities reaching maximal oxygen consumption (VO_2max) [20]. The speed–power training used by sprinters is qualitatively different. The relative anaerobic energy system contribution is estimated at around 70–80% [21]. Speed–power training promotes the maximization of speed–strength abilities relative to body mass, in addition to modest increases in both capillary supply and oxidative capacity [18]. Specific muscle adaptations to sprint training are associated with the high metabolic demands of high-intensity muscle contractions. A mixed training regime, e.g., in futsal players, involves multiple high-intensity intermittent exercise bouts during training sessions and matches, which induce significant muscle fatigue [22]. Such a specific training program is aimed at developing intermittent endurance capacity, repeated maximal sprint ability, and power maximization [23]. In the above-mentioned training profiles, most of the total body muscle mass is activated, but the stress placed on the central circulation to suddenly provide blood flow seems to be much more marked in resistance than endurance training. Futsal training requires, in turn, multiple moments of high blood flow delivery during prolonged exercise. A recent study showed the impact of sport specialization on exercise-induced plasma ([ATP]) in highly trained athletes, and indicated that total-body skeletal muscle mass is an important factor [12]. We believe there is a need for further research addressing the effect of long-term whole-body training on vascular function, and in particular, its influence on ATP and its derivatives. We presume that these results will improve the understanding of metabolic adaptation to long-term structured training programs. Possible future applications of this knowledge include applications in the fields of exercise medicine, sport, and public health.

To the best of our knowledge, no previous studies have encompassed the changes in plasma nucleotide concentration during an annual training cycle, taking into account the amount and type of training load. Thus, the plasma ATP exercise-induced response within consecutive phases of periodized endurance, speed–power, or mixed training is still unknown.

2. Results

2.1. Training Characteristics

The exercise loads between training subphases in competitive athletes were precisely monitored. Every exercise was assigned to the one of "energy zones", simplified for the needs of this article, that corresponded with estimates of energy sources for ATP resynthesis [24]. In control subjects, the levels of training loads remained unchanged during the whole study period. They recreationally practiced running at moderate intensity 3–5 times per week. During a 1 month transition phase,

competitive athletes focused on physical and psychological regeneration and recovery from injuries. The low-intensity training loads mainly consisted of activity forms other than those typical of athletes' primary sports disciplines. The aim of the general subphase (12 weeks) was to develop general endurance based on low-intensity training and to increase general fitness. The specific subphase lasted 12 weeks and was focused on the development of specific endurance and physical fitness, and was mostly based on high-intensity interval exercise and speed runs. The competition phase (10 weeks) was characterized by increased intensity and decreased volume of training. Athletes competed in their specialized distances, reaching peak performance. More detailed training characteristics of the consecutive examinations are presented in Table 1.

Table 1. Typical structure of training loads in tested futsal players, endurance athletes, and sprinters in successive examinations in a 1 year cycle.

	2nd Examination *			3rd Examination **			4th Examination **		
	General Preparation			Specific Preparation †			Competition Phase #		
	FU	EN	SP	FU	EN	SP	FU	EN	SP
Training sessions (no.)	71	181/122	80	62	132/96	61	63	179/120	87
Competitions (no.)	10	–/–	–	11	4/5	6	13	6/9	8
Net exercise time (hours)									
total	84.3	225.5/151.3	92.6	70.1	201/142.4	67.3	70.4	212.2/140.3	100.1
per one training session	1.19	1.25/1.24	1.16	1.13	1.52/1.48	1.10	1.12	1.18/1.17	1.15
Total training distance (km)									
running	–	1975/–	–	–	501/–	–	–	589/–	–
swimming	–	251/–	–	–	162/–	–	–	204/–	–
cycling	–	865/–	–	–	2655/–	–	–	2875/–	–
Exercise zones (% of total time)									
low-intensity	74.3	83.9/83.6	70.6	67.4	81.3/80.9	82.5	67.5	78.0/75.6	73.3
moderate-intensity	18.3	14.4/14.5	19.7	19.2	13.8/14.1	7.1	19.6	11.8/13.9	23.0
high-intensity	7.4	1.7/1.9	9.7	13.4	4.9/5.0	10.4	12.9	10.2/10.5	3.7

Abbreviations: FU, futsal players; EN, endurance athletes (triathletes/long distance runners); SP, sprinters. * Data encompass the period between the beginning of the training cycle and the 2nd examination. ** Data encompass the period between the preceding and the present examination. † Spring round of the competitive season in futsal players. # Autumn round of the competitive season in futsal players.

2.2. Pre-Exercise Nucleotide Concentration

Resting venous plasma [ATP], [ADP] and [AMP] significantly differed between training phases in sprinters (specific preparation and competition phase differed from both transition and general preparation phase) (Figures 1–3). Pre-exercise [AMP] after the transition phase differed from other phases in futsal players (Figure 3).

2.3. Nucleotide Concentration during Exercise

Our data showed significant increases in plasma [ATP], [ADP], and [AMP] during exercise (Figures 1–3) in each athletic group throughout the whole 1 y training cycle, but not in controls. All athletes reached their peak plasma [ATP] at the end of the test, except sprinters. None of the athletic groups reached peak plasma [ADP] and [AMP] at maximum intensity at the end of the exercise. The level of ATP, ADP, and AMP during exercise was considerably higher in all competitive athletes in each consecutive examination. In sprinters, the exercise-induced [ATP] net increase above the baseline after the transition and competition phase was 60% and 114%, respectively. In futsal players, exercise [ATP] values changed significantly ($p < 0.001$), causing a net increase of 63% after the transition period and 101% after the competition period. A similar pattern of change was noted in endurance athletes; however, the change was smaller in this group. In endurance athletes, ATP concentration increased by 64% after the transition period, and 95% after the competition period.

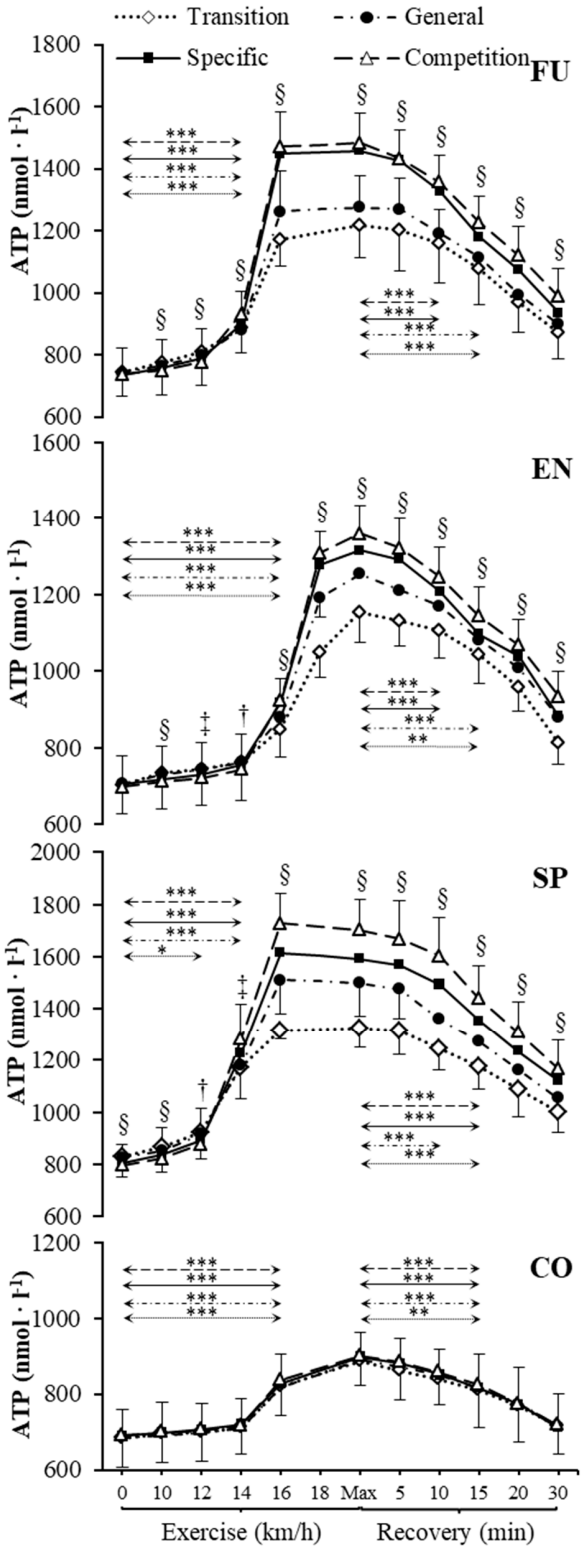

Figure 1. Venous plasma [ATP] before exercise, during incremental treadmill test until exhaustion, and post-exercise recovery in futsal players (FU; $n = 11$), endurance athletes (EN; $n = 11$), sprinters (SP; $n = 11$), and control group (CO; $n = 11$) in four consecutive training phases. Arrows indicate the first significant differences from samples taken at rest and maximal exercise within examinations. Significant differences between blood sampling points: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Significant. differences between examinations at the same sampling point: § $p < 0.001$, ‡ $p < 0.01$, † $p < 0.05$. Data are presented as means ± SD.

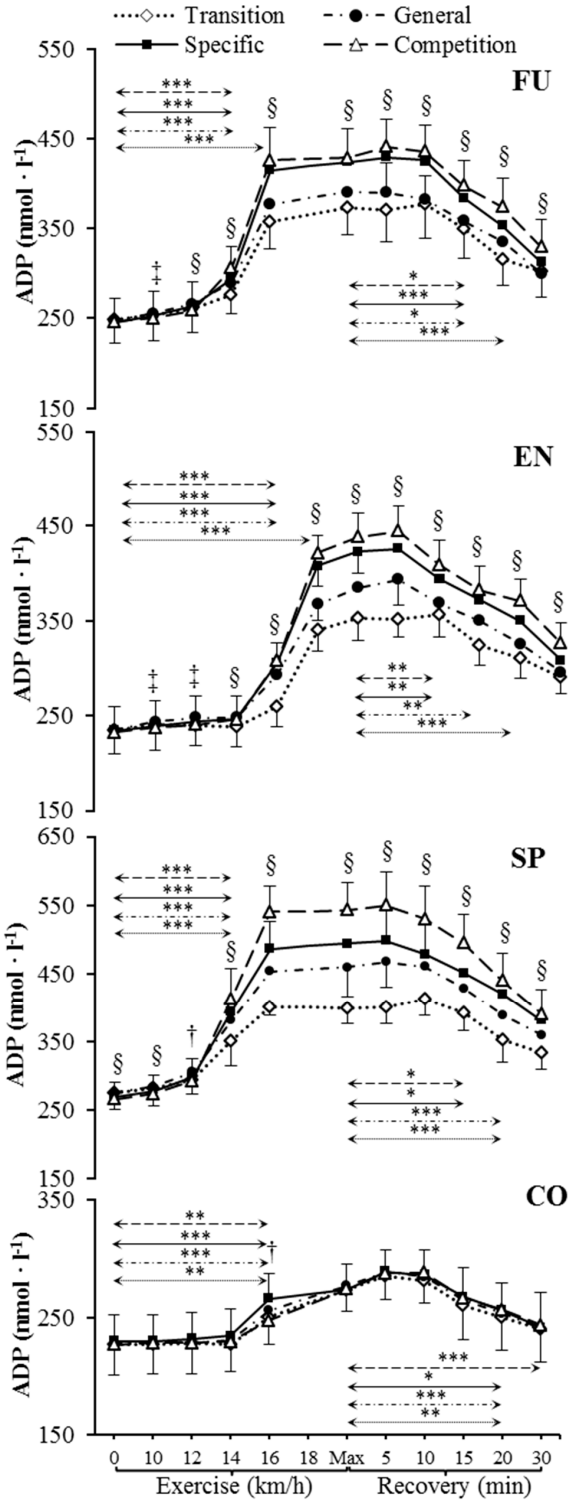

Figure 2. Venous plasma [ADP] before exercise, during incremental treadmill test until exhaustion, and post-exercise recovery in futsal players (FU; n = 11), endurance athletes (EN; n = 11), sprinters (SP; n = 11), and control group (CO; n = 11) in four consecutive training phases. Arrows indicate the first significant differences from samples taken at rest and maximal exercise within examinations. Significant differences between blood sampling points: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Significant differences between examinations at the same sampling point: § $p < 0.001$, ‡ $p < 0.01$, † $p < 0.05$. Data are presented as means ± SD.

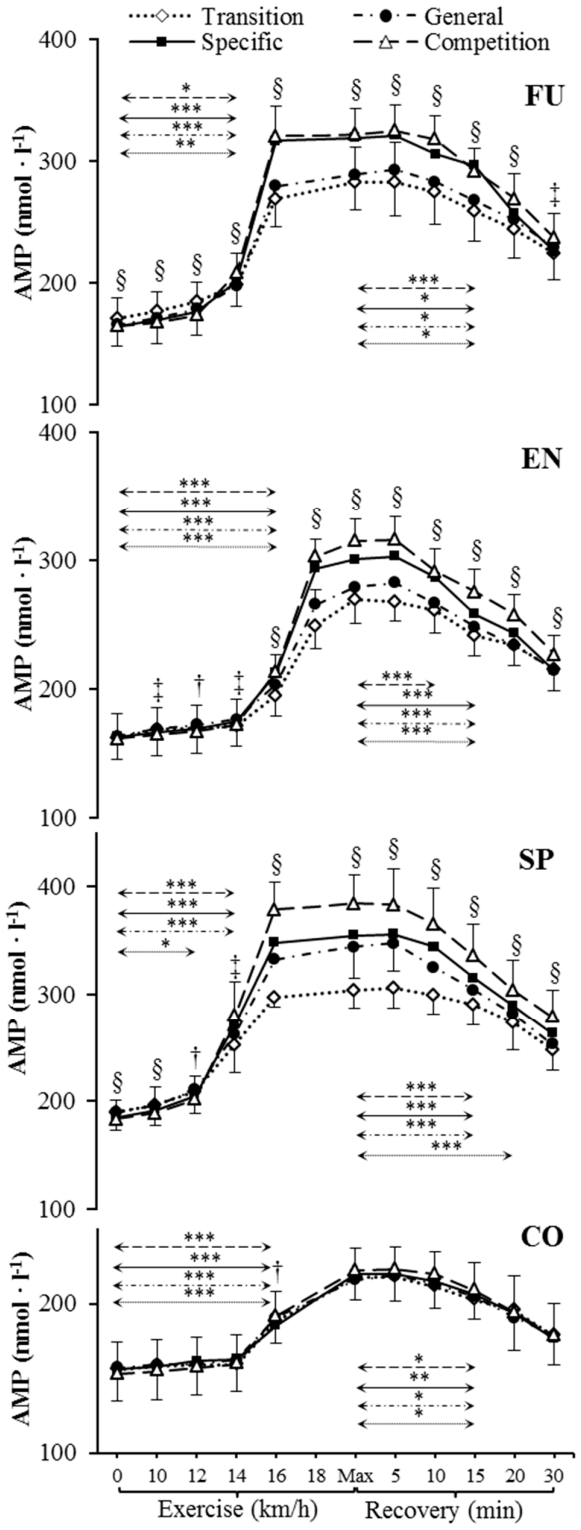

Figure 3. Venous plasma [AMP] before exercise, during incremental treadmill test until exhaustion, and post-exercise recovery in futsal players (FU; $n = 11$), endurance athletes (EN; $n = 11$), sprinters (SP; $n = 11$), and control group (CO; $n = 11$) in four consecutive training phases. Arrows indicate the first significant differences from samples taken at rest and maximal exercise within examinations. Significant differences between blood sampling points: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Significant differences between examinations at the same sampling point: § $p < 0.001$, ‡ $p < 0.01$, † $p < 0.05$. Data are presented as means ± SD.

2.4. Nucleotide Concentration at Maximal Intensity

[ATP], [ADP], and [AMP] at maximal exercise differed between groups in consecutive training phases ($p < 0.001$). Between all groups, except for futsal players and endurance athletes, maximal [ATP] differed after transition and general preparation phase ($p < 0.001$). After the specific preparation and competition phases, [ATP] differed between all athletic groups ($p < 0.001$). Sprinters presented higher [ATP] at maximal intensity than futsal players and endurance athletes throughout the whole training cycle. After the transition phase, maximal [ADP] and [AMP] in the control group were lower than in other groups ($p < 0.001$). Additionally, higher peak [ADP] and [AMP] were observed in sprinters than in endurance athletes ($p < 0.001$). In consecutive training phases, [ADP] and [AMP] at maximal exercise controls and sprinters varied compared to other groups ($p < 0.001$). Sprinters presented the highest maximal [ADP] and [AMP], starting from the general preparation phase.

2.5. Nucleotide Concentration during Recovery

During recovery, there was a significant decrease in venous plasma nucleotide concentration in competitive athletes in each training period. In controls, no significant changes in recovery plasma [ATP], [ADP], and [AMP] during the 1 y training cycle were observed. The first significant plasma [ATP], [ADP], and [AMP] decrease compared to the peak value and differences between examinations at the same sampling point are presented in Figures 1–3. Additionally, [ATP], [ADP], and [AMP] values reported after 30 min of recovery were significantly different from those obtained pre-exercise in competitive athletes, but not in controls, except for [AMP].

2.6. Respiratory Compensation Point

Respiratory compensation point (RCP) expressed as a percentage of VO_2max occurred within the range of 85% to 94% in all groups. As regards the running speed, the RCP occurred between 16 and 18 km·h^{-1} in endurance athletes and between 14 and 16 km·h^{-1} in sprinters in all training phases. Futsal players reached RCP at between 12 and 14 km·h^{-1} after the transition phase and general subphase of the preparatory phase. After the specific subphase of the preparatory phase and the competition phase, RCP was reached at between 14 and 16 km·h^{-1} in futsal players. In the control group, the RCP occurred between 14 and 16 km·h^{-1} during all examinations, except for the 2nd examination where RCP was reached after 16 km·h^{-1}.

3. Discussion

This was the first study to investigate the changes in plasma nucleotide concentration in response to an annual training cycle in highly trained athletes from distinct sports disciplines. The primary novel findings were as follows: (1) exercise-induced plasma [ATP] significantly changed over the annual training cycle in highly trained athletes (increases from transition to competition phase), (2) sprint training brought about higher absolute exercise-induced plasma [ATP] than endurance and mixed training or recreational non-periodized activity, and (3) in spite of differences in magnitude, each kind of structured training program (sprint, endurance, or mixed) incorporating a sufficient amount of high-intensity exercise led to the same adaptation pattern.

The key factor seems to have been the proportion of high-intensity training loads that were related to increased plasma [ATP] in the competition period. The reduction or lack of high-intensity exercise in other training phases was associated with a decrease in plasma [ATP]. In competitive athletes, the sudden increase in plasma [ATP] during exercise was concurrent with the occurrence of the respiratory compensation point. In controls, RCP preceded a statistically significant increase in plasma [ATP]. Therefore, it seems that the mechanism responsible for the moment of plasma ATP outflow is a variable that is independent of training type, and irrelevant to the training status (competitive athletes vs. controls). However, it affected the magnitude of the exercise response. Programmed training resulted in a much higher plasma [ATP] during exercise including maximum effort, contrary

to the effects of recreational activity where the exercise-induced [ATP] increase was poorly visible. Furthermore, the annual changes in plasma [ADP] and [AMP] reflected the changes in [ATP] as its degradation products.

Exercise training has been shown to lower blood flow to the exercising leg at a given submaximal power output. Training adaptations lead to increased capillarization, optimized blood flow distribution, and higher O_2 extraction within skeletal muscle [25,26]. Additionally, previous studies have shown an enhanced vasodilatory capacity in endurance-trained athletes during maximal effort [27,28]. We presumed that increased [ATP] has to occur to provide a comparable vasodilation effect and O_2 delivery allowing for enhanced energy production from oxidative metabolism. Increased [ATP] in speed–power compared to endurance athletes during the exercise test was likely caused by a more rapid increase in anaerobic–aerobic metabolism ratio. We concluded that the relationship between [ATP] and the percentage ratio of low-to-high exercise intensity is altered in highly trained athletes. Vasodilation during exercise may require higher [ATP] to cover muscle demands during more intense efforts. However, during maximal and supramaximal whole-body exercise, cardiac function limitation and muscle vasoconstriction contribute to the incapability of the circulatory system to meet the increasing skeletal muscle metabolic demands [29]. Collectively, these observations suggest an inability to meet the increased metabolic demands during intense whole-body exercise. Nonetheless, it would be reasonable to suggest that well-trained subjects, due to specific training stimuli, can more effectively and precisely match blood flow and O_2 delivery with demand, and as a result, delay the inability to cover the metabolic requirements of the skeletal muscle. As mentioned above, this specific feature may depend on the predominant metabolism type in different sport disciplines. Another reason might be that the structure of training loads brings about specific adaptations [30]. We assume that short sprint bouts (predominance of anaerobic metabolism), dominant in sprinters, accounted for a pronounced increase in plasma [ATP] during exercise. In futsal players, [ATP] also increased in consecutive phases of the annual training cycle around the exercise test. However, this was most likely attributed to the demands of high-intensity intermittent efforts and a large number of matches and training sessions during the spring and autumn round in the competition period. In endurance athletes, a much higher volume of training (number of sessions and total net time) in the low-intensity and moderate-intensity energetic zone could result in lower needed plasma [ATP].

In trained individuals, there might be a greater increase in the arterial–venous O_2 difference [15], which suggests enhanced O_2 extraction in the active muscle capillary beds. Although ATP is released from the erythrocytes together with the O_2 off-loading [5], training adaptations may to some extent explain enhanced [ATP] among highly trained athletes compared to controls. This emphasizes the training status as a significant variable. The differences between [ATP] curves around exercise over the annual training cycle also indicated that endurance, speed–power, and mixed training had a comparable effect on vascular response during exercise. However, the absolute maximal [ATP] was different between competitive athletes depending on sports discipline. Selection for a particular sport and predispositions to specific efforts may be relevant. Specific requirements of a training type result in the magnitude of response to an exhaustive treadmill test. The reasons for such discrepancies are unknown, but could be related to differences in exercise type (endurance vs. resistance training). Physical activities such as futsal and sprinting encompass both endurance and speed–power components, whereas triathlon/endurance athletes predominantly rely on aerobic energy sources. Further studies will be needed to better understand training-induced vascular function adaptations in highly trained athletes.

It has been proposed that inadequate cardiac output and peripheral vasoconstriction substantially limit skeletal muscle blood flow during severe whole-body exercise, despite increased peripheral blood flow and O_2 demand [29]. The sympathetic nervous system is strongly involved in local vasoconstriction. It limits blood flow once a certain point is reached, which indicates that metabolic vasodilatation does not override sympathetic vasoconstriction activity in intense whole-body exercise [29]. In our study, exercise [ATP] sharply increased when metabolic demand started to be increasingly covered by anaerobic sources (85–95% VO_2max). Attenuated vasodilatory activity due to increased contribution of

anaerobic metabolism during incremental whole-body exercise may explain the diverse ATP response. Shepherd et al. [9] observed significant additional vasodilatation during exercise with simultaneous exogenous ATP infusion. This suggests that other substances are responsible for dilatation and/or additional endogenous ATP is released during exercise, causing further vasodilatation. This is in line with our results that showed that sports disciplines containing greater high-intensity training loads (sprinters and futsal players) required enhanced plasma [ATP] during exercise. Furthermore, a year-long cycle resulted in increased exercise and post-exercise [ATP] in all competitive athletes. An increase in [ATP] during exercise may result in additional vasodilatation to meet the increased blood flow and O_2 demand. Previous research has shown that [ATP] increased in proportion to workload at higher intensities [2,31]. Considering all the above, we presume that the magnitude of [ATP] increase during exercise can be modulated by structured training, especially when high-intensity load predominates.

It has been suggested that high-intensity training leads to a reduction in the α-adrenergic responsiveness and improves functional sympatholysis at rest [32,33]. Kruse et al. [34] concluded that faster compared to slower volume-matched muscle contractions led to improved functional sympatholysis muscle contractions in humans. The question is whether increased [ATP] during exercise in consecutive phases of a one-year training cycle affects the ability to override sympathetic vasoconstriction, or simply that larger [ATP] is required for an adequate response to exercise. It has been demonstrated that intravascular [ATP] draining active skeletal muscle increases progressively with exercise intensity in young healthy adults [2,31], and has an intensity-dependent ability to limit α_1-mediated vasoconstriction [8]. Importantly, training-induced lowering of the α-adrenergic responsiveness in humans [32] facilitates the increases in muscle blood flow in trained leg muscles during exercise. However, exercise training reduces the vasodilatory response to arterially infused ATP, suggesting that physical activity may alter purinergic P_2 receptor sensitivity and/or ATP degradation in plasma [32,35]. Based on our results showing increased exercise and post-exercise [ATP] during an annual training cycle, we assume that the type of training may influence the physiological mechanisms of ATP release and/or degradation and its influence on muscle vessel dilatation during incremental exercise. However, the effect of programmed specific exercise training on intravascular ATP signaling, and thus on sympatholytic capacity, especially in highly trained individuals, needs to be investigated.

4. Materials and Methods

4.1. Subjects

Thirty-three highly trained male athletes from different sporting disciplines were studied in the Human Movement Laboratory at the Poznan University of Physical Education. Eleven male sprinters aged 24.1 ± 3.3 y, body height 186.2 ± 4.6 cm, having practiced competitive sport for 8.6 ± 2.3 y, and with a maximum heart rate (HR_{max}) of 189 ± 9 beat/min, participated in the study. Eleven male endurance athletes (long-distance runners and triathletes) and 11 male futsal players aged 23.3 ± 4.1 y and 25.8 ± 4.0 y, body height 182.0 ± 5.6 cm and 181.3 ± 6.1 cm, having practiced competitive sport for 8.5 ± 1.9 y and 10.1 ± 3.9 y, and with a HR_{max} of 192 ± 7 and 187 ± 11 beat/min, respectively, participated in the study. All athletes competed at the international and Olympic level. The control group consisted of 11 healthy male recreationally active runners aged 27.5 ± 3.8 y, body height 180.0 ± 5.6 cm, without previous and current competitive sports experience, HR_{max} 189 ± 8 beat/min. All participants were healthy during the whole study period, having all hematological variables in the normal range. More detailed descriptive and exercise characteristics are presented in Table 2.

Table 2. Descriptive and exercise characteristics in four consecutive training phases in futsal players (n = 11), endurance athletes (n = 11), sprinters (n = 11), and control group (n = 11).

	Transition	General	Specific	Competition	ANOVA *
Body Mass (kg)					
Futsal Players	75.8 ± 6.9	76.9 ± 7.0	77.6 ± 7.8 [a]	77.9 ± 7.4 [a]	0.006
Endurance	74.6 ± 8.1	73.1 ± 7.6 [†]	73.7 ± 6.7 [†]	73.2 ± 7.3 [†]	0.078
Sprinters	81.6 ± 5.5	82.8 ± 5.4	82.7 ± 5.6	83.3 ± 6.1 [a]	0.009
Control group	77.2 ± 7.9	77.8 ± 8.0	76.9 ± 7.6	76.8 ± 7.0	0.295
ANOVA **	0.125	0.022	0.039	0.014	
Total-body SMM (kg)					
Futsal Players	33.0 ± 3.0 [†]	33.9 ± 3.6 [†,a]	34.1 ± 3.3 [†,a]	34.2 ± 3.2 [†,a]	0.001
Endurance	32.9 ± 3.6 [†]	32.8 ± 3.3 [†]	33.1 ± 3.3 [†]	33.0 ± 3.2 [†]	0.672
Sprinters	39.1 ± 3.7	40.5 ± 3.6	40.4 ± 3.8	41.4 ± 4.6 [a]	0.002
Control group	33.3 ± 3.1 [†]	33.5 ± 3.2 [†]	33.0 ± 3.6 [†]	33.5 ± 3.6 [†]	0.335
ANOVA **	0.000	0.000	0.000	0.000	
Total-body fat (%)					
Futsal Players	17.4 ± 3.0 [†]	16.4 ± 2.1 [†]	16.7 ± 2.7 [†]	17.1 ± 2.3 [†]	0.391
Endurance	16.1 ± 2.6	14.0 ± 2.7 [a]	14.5 ± 2.3 [†,a]	14.2 ± 2.1 [†,a]	0.002
Sprinters	12.6 ± 2.2	11.0 ± 2.0 [a]	10.8 ± 1.9 [a]	10.6 ± 1.8 [a]	0.000
Control group	18.4 ± 3.9 [†]	18.5 ± 4.2 [‡,†]	18.2 ± 3.7 [‡,†]	17.2 ± 4.2 [†]	0.315
ANOVA **	0.000	0.000	0.000	0.000	
LA_{rest} (mmol·L^{-1})					
Futsal Players	1.4 ± 0.4	1.2 ± 0.2	1.0 ± 0.2 [a]	0.8 ± 0.2 [a,b]	0.000
Endurance	1.2 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.1 [a]	0.006
Sprinters	1.4 ± 0.6	1.4 ± 0.5	1.2 ± 0.4	0.9 ± 0.2	0.023
Control group	1.3 ± 0.3	1.4 ± 0.3	1.2 ± 0.3	1.1 ± 0.2 [§,‡,b]	0.015
ANOVA **	0.652	0.033	0.057	0.002	
LA_{max} (mmol·L^{-1})					
Futsal Players	11.6 ± 2.2	11.2 ± 2.9	10.8 ± 2.4	9.9 ± 1.5	0.065
Endurance	11.2 ± 1.8	9.9 ± 2.1	10.2 ± 1.9	10.1 ± 1.5	0.135
Sprinters	10.7 ± 1.9	10.8 ± 2.2	9.6 ± 1.9	10.0 ± 1.4	0.026
Control group	10.7 ± 1.4 [b]	11.6 ± 1.8	10.2 ± 1.9 [b]	10.6 ± 2.1 [b]	0.001
ANOVA **	0.543	0.319	0.575	0.784	
VO_2max (ml·kg^{-1}·min^{-1})					
Futsal Players	55.81 ± 3.94 [‡]	55.57 ± 2.81 [‡]	57.04 ± 2.18	58.47 ± 2.06 [‡,†]	0.063
Endurance	64.58 ± 3.52	65.26 ± 7.81	67.72 ± 3.15	66.81 ± 4.66	0.392
Sprinters	52.53 ± 4.32 [‡]	53.01 ± 4.19 [‡]	52.88 ± 3.92 [‡]	52.91 ± 3.92 [‡]	0.932
Control group	57.92 ± 3.42 [‡,†]	57.38 ± 4.25 [‡]	56.66 ± 3.08 [‡]	55.96 ± 3.47 [‡]	0.128
ANOVA **	0.000	0.000	0.007	0.000	

Abbreviations: SMM, skeletal muscle mass; LA_{rest}, resting lactate concentration; LA_{max}, maximal lactate concentration; VO_2max, maximal oxygen uptake. Values are means ± SD. * one-way ANOVA between examinations within group; ** one-way ANOVA between groups at the same examination period. [§] Significantly different from FU. [‡] Significantly different from EN. [†] Significantly different from SP. [a] Significantly different from transition phase. [b] Significantly different from general preparation phase. [c] Significantly different from specific preparation phase.

4.2. Study Design

An incremental running treadmill test until voluntary exhaustion, as described below, was used to assess the changes in exercise and post-exercise variables between training subphases. For all subjects, the same criteria for achieving maximal values were established. Each testing session was preceded by two days of reduced training volume and intensity. The study procedure was adapted to the training phases of the annual cycle: the first measurement was performed after the transition phase, second after the general subphase, third after the specific subphase of the preparatory phase, and the fourth, final examination was performed before the tapering period during the competition phase. Biochemical parameters were measured at rest, 4–5 times during the incremental exercise, and up to 30 min after exercise. During the incremental test, cardio-respiratory characteristics were monitored. All procedures and potential risks were explained and informed consent was obtained from each participant. The study was approved by the Local Bioethical Committee at the Karol Marcinkowski Poznan University of Medical Sciences. During all examinations, the ambient temperature remained unchanged at 20–21 °C.

4.3. Somatic and Physiological Variables

Weight and height were measured using a digital stadiometer (SECA 285, SECA, Hamburg, Germany). Body composition evaluation was performed using the dual X-ray absorptiometry method (DXA; Lunar Prodigy device; GE Lunar Healthcare, Madison, WI, USA) and analyzed using enCORE 16 SP1 software. All DXA scans were performed and analyzed following the best practice protocol proposed by Nana et al. [36]. Total-body skeletal muscle mass was calculated according to Kim et al. [37]. Heart rate was measured with Polar Bluetooth Smart H6 monitors (Polar Electro Oy, Kempele, Finland). An incremental running test (H/P Cosmos Pulsar, Sports & Medical, Nussdorf-Traunstein, Germany) was performed after 3 min of standing on the treadmill, the initial speed was 4 km·h^{-1} for the first 3 min, then increased to 8 km·h^{-1} and increased by 2 km·h^{-1} every 3 min until volitional exhaustion. VO_2max was considered to be achieved if the test met at least three of the following criteria: (i) a plateau in VO_2 despite an increase in workload; (ii) cutoff blood lactate concentration ≥9 mmol·L^{-1}; (iii) RER ≥ 1.10; and (iv) heart rate ≥95% of the age-predicted HRmax [38]. The respiratory compensation point was determined based on the breaking point in the VE/VO_2 and VE/VCO_2 curve [39]. Athletes had their VO_2max and main cardiorespiratory variables determined using MetaMax 3BR2 ergospirometer and analyzed by MetaSoft Studio 5.1.0 Software (Cortex Biophysik, Leipzig, Germany).

4.4. Hematological and Lactic Acid Measurements

Blood samples for hematological parameters were carried out as described elsewhere [40]. For lactic acid measurement, lithium heparin was used as an anticoagulant (S-monovette, 2.7 mL KE, Sarstedt, Nümbrecht, Germany). Lactate in whole blood (20 µL) was immediately assayed using the spectrophotometric enzymatic method (Biosen C-line, EKF Diagnostics, Barleben, Germany).

4.5. Plasma Nucleotide Measurements

Plasma nucleotide concentration analyses were performed using high-performance liquid chromatography (HPLC) with UV detection, according to the methodology of Smolenski et al. [41]. The catheter (1.3 × 32 mm, BD Venflon Pro, Becton Dickinson, Helsingborg, Sweden) was placed into the antecubital vein. Blood samples (2 mL) were drawn at rest, during, and after exercise using syringes containing ethylenediamine tetraacetic acid (EDTA) (S-monovette, 2.7 mL KE, Sarstedt, Nümbrecht, Germany). Samples were immediately centrifuged (Universal 320R, Hettich Lab Technology, Tuttlingen, Germany) for 30 s at 14.000 rpm in 4 °C. Subsequently, 200 µL of plasma was frozen down in liquid nitrogen in duplicate and stored at −80 °C until analysis. Samples were extracted using perchloric acid (2.4 mol·L^{-1}) on ice at the ratio of 1:0.25 for 15 min and then centrifuged at 13.000 rpm for 3 min in 4 °C. The collected supernatant was neutralized using 3 mol·L^{-1} K_3PO_4 and centrifuged at 13.000 rpm for 3 min in 4 °C. The samples were left on ice for 30 min to ensure complete precipitation of potassium perchlorate. Then, supernatants were collected and stored at −80 °C before analysis. The analyses were performed using a Specta HPLC system (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a 10 cm path flow cell to increase sensitivity. The separation was achieved with an analytical column BDS Hypersil C18 (150 mm × 4.6 mm × 3 µm; Thermo, Waltham, MA, USA) placed in a thermostat (18 °C) supported by a precolumn 20 mm × 4 mm (Phenomenex, type SecurityGuard, Torrance, CA, USA). The mobile phase consisted of A: 122 mM KH_2PO_4, 150 mM KCL, and 28 mM K_2HPO_4 and B: 15% (*v*/*v*) acetonitrile in A. The percentage of B changed from 0% to 100% in several linear steps during analysis and then returned to 0% B for equilibration. The whole separation time was 13.5 min and was conducted at 0.9 mL·min^{-1} flow rate with a sample injection volume of 40 µL. The quantitative analyses were performed based on the external calibration of the signal at 254 nm. Data acquisition and processing were managed by the Xcalibur™ software (v. 2.1, Thermo Scientific™, Waltham, MA, USA). The above-described method provided coefficients of variation <5% at different ATP concentrations.

4.6. Statistical Analyses

A one-way repeated measures ANOVA was performed to assess the differences in measured variables between consecutive examinations and between measurement points during exercise and recovery within each group of participants. Furthermore, a one-way ANOVA was performed to estimate the differences in nucleotide concentrations between groups at maximal exercise in the same training phase. If a significant difference was found ($p < 0.05$), post hoc Scheffe tests were performed. The effect size for ANOVA analyses was small to large for descriptive characteristics ($\eta^2 = 0.01–0.68$), and statistical power was 0.07–1.00. The effect sizes for ANOVA analyses for plasma [ATP], [ADP], and [AMP] were large within groups between measurement points ($\eta^2 = 0.84–0.98$). The statistical power for ANOVA at $\alpha = 0.05$ for plasma [ATP], [ADP], and [AMP] between measurement points was 1.00. The effect sizes for ANOVA at the same measuring point between four consecutive examinations were large for plasma [ATP] ($\eta^2 = 0.29–0.93$), ADP ($\eta^2 = 0.24–0.94$) and [AMP] ($\eta^2 = 0.24–0.92$), except for the control group where effect sizes for ANOVA were small to large for plasma [ATP] ($\eta^2 = 0.02–0.14$), [ADP] ($\eta^2 = 0.01–0.23$), and [AMP] ($\eta^2 = 0.04–0.24$). The statistical power for ANOVA at $\alpha = 0.05$ for plasma [ATP], [ADP], and [AMP] between four consecutive examinations within competitive athletes were 0.66–1.00, except for controls (0.07–0.68). The effect size and statistical power for ANOVA between groups at maximal exercise in each training phase were 0.77–0.92 and 1.00, respectively. All calculations were performed using STATISTICA 13.1 software (StatSoft, Tulsa, OK). The significance level was set at $p < 0.05$. All values are presented as means ± SD.

5. Conclusions

In this study, we demonstrated that ATP concentration significantly changed over consecutive training phases in highly trained athletes in an annual training cycle. Sprint training brought about adaptations resulting in higher maximal exercise-induced plasma ATP levels compared to endurance and mixed training, and especially compared to recreational non-periodized activity. In spite of differences in magnitude, each kind of structured training program (sprint, endurance, or mixed) incorporating a sufficient amount of high-intensity exercise led to the same adaptation pattern. The key factor seems to be the proportion of high-intensity training loads that are related to an increased exercise-induced plasma [ATP] in the competition period, whereas the reduction or lack of high-intensity exercise in other training phases is associated with a decrease in plasma [ATP].

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