UNIVERSIDADE FEDERAL DE UBERLÂNDIA

INSTITUTO DE GENÉTICA E BIOQUÍMICA

PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

O EXERCÍCIO COMO MODELO PARA ESTUDO DO METABOLISMO DE AMINOÁCIDOS E AMÔNIA

Adriana Bassini

Prof. Dr. Luiz-Claudio Cameron

UBERLÂNDIA – MG

2008

UNIVERSIDADE FEDERAL DE UBERLÂNDIA

INSTITUTO DE GENÉTICA E BIOQUÍMICA

PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

O EXERCÍCIO COMO MODELO PARA ESTUDO DO METABOLISMO DE AMINOÁCIDOS E AMÔNIA

Adriana Bassini

Prof. Dr. Luiz-Claudio Cameron

Tese apresentada à Universidade Federal de Uberlândia como parte dos requisitos para obtenção do Título de Doutor em Genética e Bioquímica (Área Bioquímica)

UBERLÂNDIA – MG

2008

Dados Internacionais de Catalogação na Publicação (CIP)

B321e Bassini, Adriana, 1978-
O exercício como modelo para estudo do metabolismo de aminoácidos e amônia / Adriana Bassini. - 2008.
240 f. : il.

Orientador:.Luiz-Claudio Cameron.
Tese (doutorado) - Universidade Federal de Uberlândia, Programa de Pós-Graduação em Genética e Bioquímica.
Inclui bibliografia.

1. Metabolismo - Teses. 2.Aminoácidos - Teses. 3. Cafeína - Teses. 4. Bioquímica do exercício. I. Cameron, Luiz-Claudio. II. Universidade Federal de Uberlândia. Programa de Pós-Graduação em Genética e Bioquímica. III. Título.

CDU: 612.015.3

Elaborado pelo Sistema de Bibliotecas da UFU / Setor de Catalogação e Classificação

UNIVERSIDADE FEDERAL DE UBERLÂNDIA

INSTITUTO DE GENÉTICA E BIOQUÍMICA

PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

O EXERCÍCIO COMO MODELO PARA ESTUDO DO METABOLISMO DE AMINOÁCIDOS E AMÔNIA

Aluna: Adriana Bassini

COMISSÃO EXAMINADORA

Presidente:

Prof. Dr. Luiz-Claudio Cameron

Examinadores:

Prof. Dr. Mauro Sola-Penna

Prof. Dr. Jose Roberto Mineo

Prof. Dr. Julio Alberto Mignaco

Prof. Dr. Nilson Penha-Silva

Data da Defesa: 10 de dezembro de 2008.

As sugestões da Comissão Examinadora e as Normas PGGB para o formato da Tese foram contempladas

_____

Prof. Dr. Luiz-Claudio Cameron

DEDICATÓRIA

Dedico esta parte da minha História ao meu marido que sempre esteve presente e nunca me deixou desistir.

A Família Laboratório de Bioquímica de Proteínas pelo apoio, trabalho, sofrimento, lágrimas, amadurecimento, discussões, brigas, felicidade e paz. Enfim, todo equilíbrio que um Doutor precisa ter.

A Deus...

A minha mãe (Maria Fardin) e irmã (Alice Bassini) que sempre souberam dizer 'EU TE AMO" quando eu precisava ouvir.

As Famílias Fardin e Bassini que do seu próprio jeito me incentivaram a continuar subindo os degraus, mesmo que isso significasse muita saudade.

AGRADECIMENTOS

Ao Mestre Cameron por compartilhar toda sua humildade e sabedoria com seus alunos.

A meus colaboradores nos artigos.

Aos atletas que doaram seu sangue literalmente por estes trabalhos.

A Antônio Carlos Jobim, Vinícius de Moraes e Ney Matogrosso.

SUMÁRIO

LISTA DE FIGURAS

CAPÍTULO I

CAPÍTULO IV

CAPÍTULO V

CAPÍTULO VI

LISTA DE TABELAS

LISTA DE ABREVIAÇÕES

AAs- aminoácidos

ADA- adenosina desaminase

ADC- arginina descarboxilase

ADP- adenosina difosfato

AGMase- agmatinase

ALA- alanina

ALT=TGP- alanina aminotransferase

AMP- adenosina monofosfato

AP- fosfatase alcalina

APAP- acetaminafen

AR- receptor de adenosina

Arg- arginina

AsL- arginosuccinato liase

Asp- aspartato

AsS- arginosuccinato sintase

AST=GOT- aspartato aminotransferase

AT- L-arginina glicina amidinotransferase

ATCase- aspartato transcarbamilase

ATP- adenosina trifosfato

BCAAs- aminoácidos de cadeia ramificada

BCCA- alfacetoácido de cadeia ramificada

CF- fadiga central

CHO- grupo carboidrato

CK- creatinacinase

CPS- carbamoil fosfato sintetase

CrP- creatinofosfato

CTP- carbamoil fosfato

Cys- cisteína

FFA- ácidos graxos livres

GABA- ácido gama amino-butírico

GDH- glutamato desidrogenase

GDP- guanosina difosfato

GFPA- *glial fibrillarly acidic protein* (proteína glial fibrilar ácida)

GGT- gama glutamil transferase

Gln- glutamina

Glu- glutamato

GTP- guanosina trifosfato

HIU- *high intensity ultraendurance* (alta intensidade *ultraendurance*)

IMP- inosina monofosfato

Km- constante de Michaelis-Menten

LDH- lactato desidrogenase

LPS- lipopolissacarídeo de membrana

Met- metionina

NAC- N-acetil cisteína

NADH- nicotinamida adenina dinucleotídeo reduzida

NADPH- nicotinamida adenina dinucleotídeo fosfato reduzida

NH_3- amônia

NH_4^+ - íon amônio

NMDA- receptor para N-metil-D-aspartato

NO- óxido nítrico

NOS- óxido nítrico sintase

NSAIDs- *non-steroidal anti-inflammatory drugs* (droga anti-inflamatória derivada de não esteróide)

OAT- ornitina aminotransferase

ODC- ornitina descarboxilase

OKT- ornitina cetoácido transcarbamilase

Orn- ornitina

OTC- ornitina transcarbamilase

PBMCs- *peripheral blood mononuclear cells* (células sanguíneas mononucleares periféricas)

PCR- proteína C reativa

PDH- fosfodiesterase do AMPc

PFK- fosfofrutokinase 1

PLP- piridoxal fosfato

PNC- ciclo das purinas nucleotídeos

SNC- sistema nervoso central Tau- taurina

TCA- ciclo do ácido tricarboxílico

TNF- fator de necrose tumoral

TNFα- fator de necrose tumoral alfa

α-KG- alfacetoglutarato

α–KGDH - α-cetoglutarato desidrogenase

RESUMO GERAL

A produção de amônia durante o exercício submáximo ocorre principalmente pela quebra da adenosina monofosfato (AMP) via AMP deaminase produzindo inosina monofosfato (IMP) e amônia. Na tentativa de auxiliar a manutenção das concentrações de ATP constantes e sinérgico a desaminação do AMP, os aminoácidos (AAs) são utlizados como doares de carbono para o ciclo dos ácidos tricarboxílicos (TCA) com consequênte liberação de amônia. Nestas situações a amonemia pode aumentar em até 400% acima dos valores considerados normais para o indivíduo sadio em repouso.

A hiperamoniemia está associada à alteração na regulação de neurotransmissores podendo ser suficiente para causar excitotoxidade neural e/ou morte. Por isso, propõe-se que a produção de amônia relacionada ao exercício físico pode ser um dos fatores responsável pela diminuição da capacidade cognitivo-física em atletas saudáveis, afetando o processo de continuidade da atividade e produzir queda da performance. Estes distúrbios momentâneos no funcionamento do sistema nervoso central (SNC) são semelhantes àqueles encontrados em fases iniciais de diversas doenças relacionadas à hiperamoniemia e/ou doenças neuro-degenerativas.

Pode-se assim, postular que o exercício intenso e prolongado seja capaz de induzir um estado tóxico de amonemia agudo e subclínico. Podendo ser suficientemente severo a regiões críticas do SNC afetando a realização de atividades coordenadas.

Nesta tese avaliaremos exercícios de diferentes intensidades combinado a modificações metabólicas induzidas por dieta e/ou suplementação para estudo do metabolismo de amônia em humanos, buscando estabelecer um modelo experimental.

Palavras-chave: Hiperamoniemia transitória, dieta cetogênica, suplementação de cafeína, suplemetação de aminoácidos, atividade imunomodulatória.

Abstract

Intracelular increase of AMP during sub maximal exercise leads to an activation of AMP deaminase following production of inosine monophosphate and ammonia. In the same direction amino acids (AAs) are used as a carbon donors for the tricarboxylic acid cycle to maintain the ATP concentration in the cell. Both metabolic pathways lead to an increase in intracellular and blood ammonia concentration. In these events, the blood ammonia concentration can raise up to 400% the resting levels.

Hyperammonemia is linked with lack in neurotransmitter regulation and can be associated with neuronal excitotoxicity and/or death. Raise in ammonia synthesis during exercise is related to decrease in neuro-physical capacity in health athletes and can affect the performance. The temporary disturbances in the central nerve system caused by exercise are similar to the observed in hepatic disease and neurodegenerative disorders.

Here we evaluate different exercises intensities associated with metabolic modifications induced by diet and/or supplementation to understand ammonia metabolism. We showed the blood appearance kinetics of muscle injury markers and some metabolites. We suggested that the increase in these enzymes came primarily from muscle damage instead of liver and that white blood cells are selectively mobilized independently of hemoconcentration. We also had shown the early appearance of muscle injury markers in different kinds of exercise. Our results suggest that we are able to use exercise as a general model to study ammonia metabolism in humans without requiring external ammonia exposure.

Keywords: transitory hyperammonemia, ketogenic diet, caffeine supplementation, amino acids supplementation, Immunomodulatory properties of exercise

OBJETIVOS

GERAL

Utilizar exercício em diferentes intensidades combinado a dieta e/ou suplementação como agente modificador do metabolismo de amônia em humanos.

ESPECÍFICOS

1. Analisar em exercício de alta intensidade a cinética de aparecimento de enzimas marcadoras de injúria muscular e compostos nitrogenados
2. Estabelecer um modelo de exercício indutor de hiperamoniemia para humanos.
3. Utilizar intermediários metabólicos como sondas para o entendimento da hiperamoniemia em exercícios de moderada-alta intensidade.

Para responder ao Objetivo 1:

O modelo mais óbvio para o estudo de hiperamônemia em humanos seria a utilização de sais de amônia em infusão parenteral o que tornaria a investigação inviável. Por isso, precisamos ter uma forte correlação entre o binômio intensidade do exercício e producão de amônia. Não há descrição sistematizada da cinética de marcadores de lesão muscular em função da intensidade e tempo da atividade. Apesar da grande variedade de protocolos e indivíduos avaiados não se observa homogeneidade entre eles. A nova faixa de referência para creatinacinase (CK) (repouso) em jogadores de futebol nos norteou na análise dos resultados[1]

Para responder ao objetivo 1, medimos a cinética de aparecimento de marcadores de microlesão muscular e correlacionamos com a intensidade do exercício em recente estudo publicado[2]. Mostramos pela primeira vez a cinética de CK e lactato desidrogenase (LDH) em função da intensidade (r^2=0.99), onde CK teve aumento de 40% e LDH de 300% já nas primeiras 6-8h.

Por supormos que o HIU induzisse a injúria muscular e consequentemente obtvéssemos resultados falso positivo, monitoramos AST, ALT e GGT. Os resultados de GGT nos mostraram que a saída destas enzimas não é hepática e possivelmente muscular. Propomos que a diferença de 260% entre CK e LDH seja pelo seu peso molecular (CK~86kDa e LDH~140kDa).

Acompanhamos o clerance de amônia através da uréia e urato. O urato aumentou cerca de 130-140% nos primeiros 50Km, seguido pela uréia a partir dos 150Km. Mostrando indiretamente a elevada atividade da AMP deaminase com liberação de amônia. Este estado de hiperamoniemia agudo estimula o ciclo da uréia que aumenta sua excreção amônia.

Através da união dos dados do artigo 6[3] desta Tese com Bassini-Cameron (2007)[4] observa-se que a suplementação de cafeína aumenta as microlesões e parece inibir o ciclo da uréia.

[1] Lazarin *et al.,* 2007 The upper values of plasma creatine kinase of professional soccer players during the Brazilian championship. Journal of Science and Medicine in Sport.
2Bessa *et al.*, 2008 High intensity ultraendurance promotes early release of muscle injury markers. British Journal of Sports Medicine.
[3] Bassini-Cameron *et al.* 2008 Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players
[4] Bassini-Cameron *et al.* 2007 Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. British Journal of Sports Medicine.

Para responder ao Objetivo 2:

As mudanças metabólicas causadoras da hiperamônemia geram uma mistura de eventos fisiológicos em humanos descritas por diversos protocolos de exercício. Porém, não há um bom modelo em curto tempo de fácil utilização.

Verificamos que o exercíco intermitente é capaz de gerar aumentos na amonenia de até 200-250% se comparado ao contínuo (800%)[5].

Não observamos diferença nas concentrações de amônia pós-exercício sob suplementação de cafeína/lactose em jogadores de futebol de elite[3].

Para isso, propomos um modelo em que a luta intensa, precedida de dieta cetogênica, é capaz de provocar o aumento da amônemia levando as concentrações de amônia a patamares altíssimos como 600 μM[6].

5 Bassini-Cameron *et al.*, 2008 Glutamine protects against blood ammonia increase in soccer players in an exercise intensity dependent way. British Journal of Sports Medicine.
6Bessa *et al.*, 2008 Arginine supplementation attenuates both lymphocytes and ammonia appearance in blood after high intensity exercise. Scandinavian Journal of Medicine and Science in Sports - Submitted

Para responder ao Objetivo 3:

A neoglicogênese, a ureagênese e o processo anaplerótico são vias ativadas para manutenção do equilíbrio metabólico principalmente em cituações de injúria[7] Dados obtidos no Laboratório de Bioquímica de Proteínas (UNIRIO) demonstraram que a suplementação de CHO e Gln isolados ou combinados têm um efeito semelhante na inibição da elevação da amônemia em corredores[8] e que a suplementação de Ala tem efeito similar ao da Gln em jogadores de futebol[5]. Em exercício de alta intensidade a suplementação de Arg parece auxiliar na regulação do ciclo da uréia e proteger contra a hiperamoniemia[6]

Na tentativa de estimular a gênese de amônia usamos a suplementação de cafeína no exercício intermitente seguido pelo de exaustão e avaliamos as concentrações de aminoácidos plasmáticos[3]. Parece que a cafeína influência na regulação da arginase e da amidinotransferase.

Pudemos observar num estudo de caso que o uso terapêutico de NAC assossiado a metionina (Met) e cisteína (Cys) diminuiu a concentração de GGT séricas dentre outros marcadores de lesão e inflamação[9].

[7] Bachini *et al.* 2006 Nutrition, Metabolism and Exercise in Chronic Obstructive Pulmonary Disease. Brazilian Journal Investigating Pathological Morphological Morphometry

[8] Carvalho-Peixoto *et al.*, 2007 Glutamine and carbohydrate supplements reduce ammonemia increase during endurance field exercise. Applied Physiology, Nutrition, and Metabolism.

[9] Bessa *et al.* 2008 Low Back Pain Followed by Acetaminophen (APAP) Hepatotoxicity: A Case Report. The American Journal of Sports Medicine - Submitted

Os artigos abaixo serão discutidos na tese, onde 3 foram submetidos e 4 publicados:

Paper	Journal	ISSN	Impact Index
1. Lazarin, FL; Antunes-Neto, JMF; SILVA, FOC, Nunes, LAS; Bassini-Cameron; Cameron, LC; Alves, AA; Brenzikofer, R; DV Macedo. The upper values of plasma creatine kinase of professional soccer players during the Brazilian championship. Journal of Science and Medicine in Sport. v.18069060, 2007.	**J SCI MED SPORT**	1440-2440	1.091
2. Bessa, A, Nissembaun, M., Nunes, LAS; Bassini-Cameron, A; Macedo, DV; LC Cameron. Low Back Pain Followed by Acetaminophen (APAP) Hepatotoxicity: A Case Report. The American Journal of Sports Medicine: A Case Report – Submitted	**AM J SPORT MED**	0363-5465	3.397
3. Bachini F, Bassini-Cameron A, Cameron LC. Nutrition, Metabolism and Exercise in Chronic Obstructive Pulmonary Disease. Brazilian Journal Investigating Pathological Morphological Morphometry. 2006. 1(3): 7-14.	**BRAZ J INVESTIG PATHOL MORPHOL MORPHOM**	1519-1192	-
4. Bessa, A, Nissembaun, M., Monteiro, A, Gandra, PG, Nunes, LAS, Bassini-Cameron, A, Werneck-de-Castro, JPS, Macedo, DV, LC Cameron. High intensity ultraendurance promotes early release of muscle injury markers. British Journal of Sports Medicine. v.012008, p.1 - 21, 2008.	**BRIT J SPORT MED**	0306-3674	2.463
5. Bassini-Cameron, A, Monteiro, A, Gomes, ALM, Werneck-de-Castro, JPS, LC Cameron. Glutamine protects against blood ammonia increase in soccer players in an exercise intensity dependent way. British Journal of Sports Medicine. v.42, p.260 - 266, 2008.	**BRIT J SPORT MED**	0306-3674	2.463

6. Bassini-Cameron, A, Sweet, E; Botino, A; Bittar, C; Veiga, C.; LC Cameron, Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players	**AMINO ACIDS**	0939-4451	2.780
7. Bessa, A.; Gonçalves, LCO; Freitas-Dias, R; Bassini-Cameron, A; Werneck-de-Castro JPS; LC Cameron. Arginine modulates both hyperammonemia and lymphocytes appearance in blood after high-intensity exercise - Submitted	**SCAND J MED SCI SPORTS**	0905-7188	2.295

CAPITULO I

Hiperamoniemia transitória associada ao exercício de diferentes intensidades modificada pela dieta e/ou suplementação

CAPÍTULO I

Hiperamoniemia transitória associada ao exercício de diferentes intensidades modificada pela dieta e/ou suplementação

Resumo

A célula pode usar AAs para anabolismo (especialmente síntese de proteínas), transporte de nitrogênio, produção de outras moléculas e transdução de energia. Esta utilização depende da necessidade energética, da disponibilidade deles próprios e/ou outros substratos, e/ou enzimas, do tamanho e complexidade de suas cadeias laterais.

Para manutenção da relação ATP/ADP, acontece interação/integração metabólica, onde principalmente a fosforilação oxidativa e a glicólise são utilizadas na manutenção da capacidade contrátil da musculatura. Esta demanda energética parece ser sinalizadora da troca de AAs entre os órgãos e os tecidos especialmente em modelos de injúria celular. Estas características citadas levam a alteração no tráfico de amônia inter-órgãos, resultando em situações de hiperamoniemia. Propomos que a hiperamoniemia transitória durante o exercício possa ser um agente modificador do metabolismo agindo como sinalizador celular e mobilizador do mecanismo de defesa. Para o maior entendimento destes processos essa tese discutirá alguns dos trabalhos desenvolvidos pelo Laboratório de Bioquímica de Proteínas da Universidade Federal do Estado do Rio de Janeiro.

Palavra-chave: regulação do ciclo da uréia, sondas metabólicas, estresse oxidativo, microlesões, inflamação.

I.I Hiperamoniemia transitória

a) Síntese de amônia

Desde a década de 20, estudos pioneiros demonstraram que durante o trabalho muscular há aumento da concentração da amônia no plasma (Parnas, 1929; Benedict, 1929; Smith, 1929). Embora ainda hoje não esteja completamente estabelecido o número exato de vias, parece haver consenso na literatura sobre a micróbia ser a principal fonte de produção no período pós-prandial (Huizenga, 1994; Manning, 2004).

Lowenstein & Goodman (1978) demonstraram que amônia é um metabólito resultante do catabolismo de AAs e do ciclo das purina nucleotídeos (PNC) em diversos tecidos. Em situação de anabolismo parte da amônia produzida é utilizada para os processos de biossíntese dos compostos nitrogenados, e a outra parte é convertida em uréia no fígado e eliminada pelos rins (Damink, 2002).

A gênese da amônia ocorre durante a síntese de ATP a partir de ADP via miokinase (AMPK) e, simultaneamente há estimulação da creatinacinase (CK) degradando creatinafosfato (CP), ambas para manutenção do balanço energético (Figura 1). As concentrações celulares aumentadas de mono e dinucleotídeos estimulam a AMPK que pode ser inibida pelo aumento de nucleotídeos trifosfatos ou pela CP. Graham & MacLean em 1990 sugeriram que o incremento no trabalho mecânico de contração muscular aumenta a taxa de hidrólise de ATP excedendo sua velocidade de ressíntese, acarretando aumento na concentração intracelular de AMP e amônia.

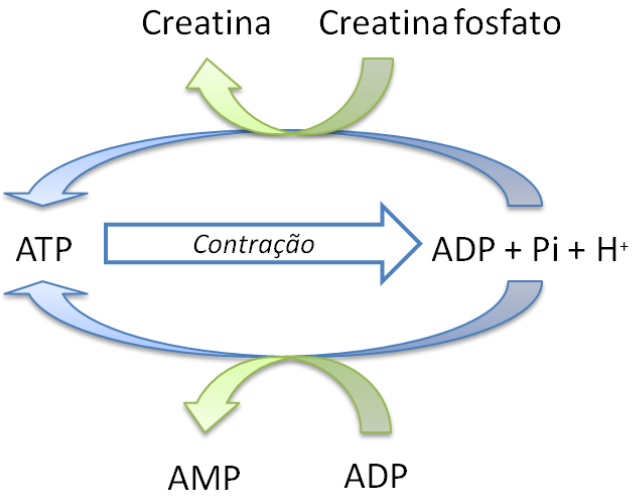


Figura 1: relação ATP/ADP. O aumento das concentrações celulares de ADP estimula a miokinase e a creatinacinase.

O AMP sofre desaminação via mioadenilato desaminase gerando inosina monofosfato (IMP) e amônia, principalmente em situações de estresse celular que é convertido em inosina e hipoxantina. Em condições de baixa demanda energética celular, o IMP é reaminado através da entrada de aspartato (Asp) e a utilização de uma molécula de guanosina trifosfato (GTP) catalisado pela adenilsuccinato sintase formando adenilsuccinato que pode sofrer degradação alimentando conseqüêntemente o ciclo dos ácidos tricarboxilicos (TCA) (Figura 2A) (Schindler, 2005).

Na Figura 2B pode ser observada outra via de escoamento de AMP. Neste caso, o AMP é desfosforilado a adenosina, que é desaminada a inosina, sendo estas reações catalisadas pelas 5'-nucleotidase (5'N) e adenosina deaminase (ADA) respectivamente. O músculo não possui o arsenal enzimático necessário para reverter à síntese de inosina, transformando-a em hipoxantina ou xantina, que deixam o músculo para serem hepaticamente metabolizadas a urato que é excretado pelos rins (Abbracchio & Burnstock, 1998; Tullson *et al.*, 1999)

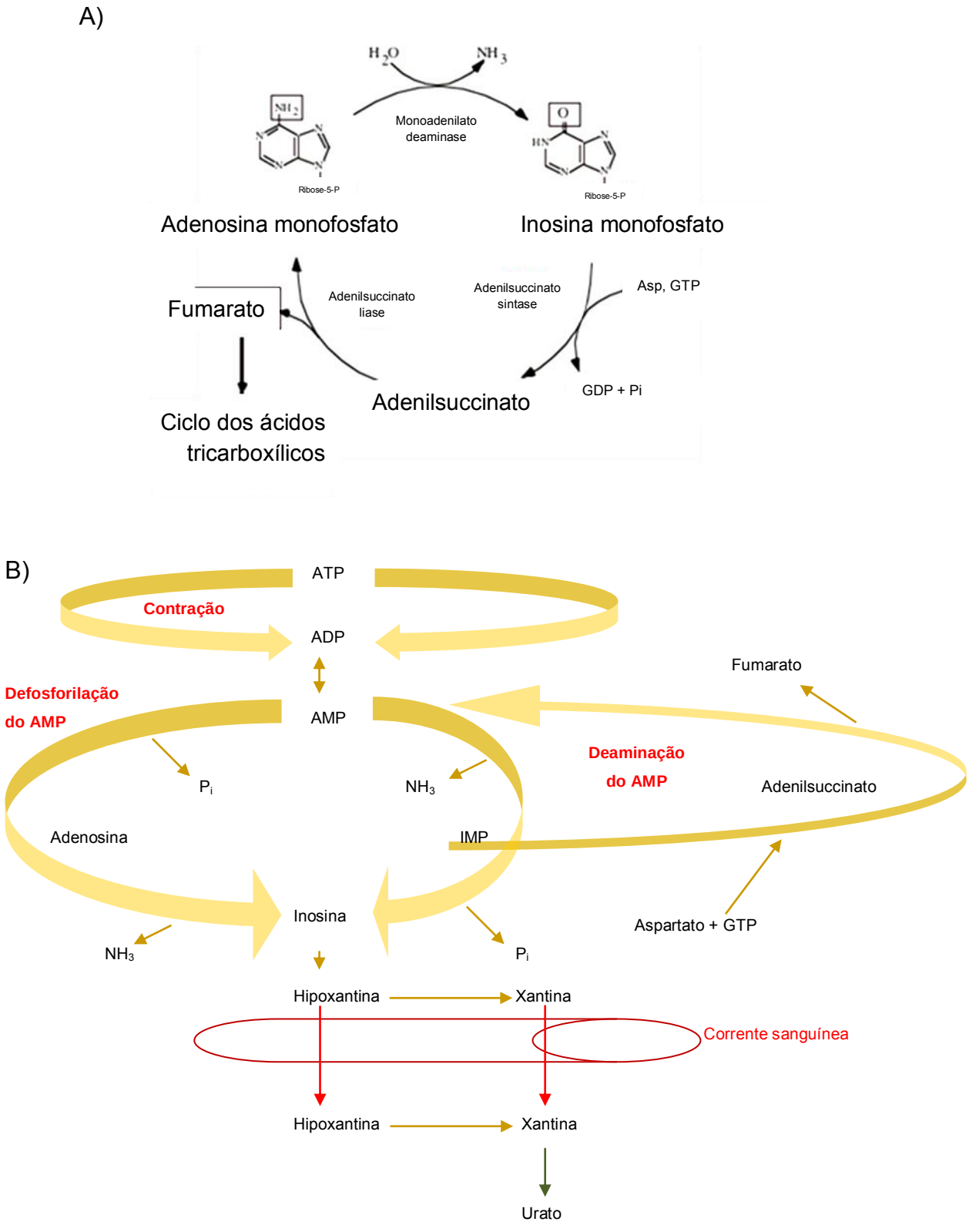


Figura 2: Ciclo das purinas nucleotídeos. (A) Integração do Ciclo Purina-Nucleotídeo com o ciclo de Krebs; (B) Intermediários metabólicos na gênese de amônia e urato. Em alguns casos o processo anaplerótico pode gerar

diretamente amônia que se difunde pela membrana chegando a corrente sangüínea

Além disso, o aumento na concentração intracelular de ADP gera aumento no fluxo de entrada de AAs no TCA, através da desaminação oxidativa, produzindo amônia e cetoácidos que serão utilizados para a produção de ATP e/ou necessários na gliconeogênese (Graham, 1997; Rossi, 1999).

Nesta tese chamaremos de amônia o somatório entre NH_3 + NH_4^+. Dependendo do pH, a amônia esta na forma iônica (NH_4^+) ou gasosa (NH_3); e a 37ºC seu pK é 9,15, ou seja, cerca de 98% encontra-se ionizada nos fluidos fisiológicos (Rose, 2002). Em qualquer dessas formas a amônia (17 kDa) tem trânsito livre através de membranas celulares independende de proteínas transportadoras e pode ser produto ou substrato de diversas reações enzimáticas como ilustrado na Figura 3A (Felipo & Butterworth, 2002a; Butterworth, 2002b).

Recentemente foi descoberto em camundongos a proteína Rhcg no túbulo proximal. A Rhcg funciona como transportador de NH_4^+ do lúmem do túbulo renal para a urina (Figura 3B). Verificou que em ratos *Rhcg-knockout* há diminuição no transporte de amônia de duas a três vezes comparado a camundongos normais com consequente diminuição do pH (Biver et al. 2008; Knepper, 2008). Dessa forma, o metabolismo e o transporte de amônia pelo rim esta intrinsicamente relacionado a regulação do pH sanguíneo (Knepper, 1989; DuBose, et al. 1991). Há diversas proteínas no túbulo renal que transportam amônia, por exemplo: NHE-3, canais de K^+, cotransportador de Na^+-K^+-$2Cl^-$, Na^+-K^+-ATPase, trocador de $K^+/NH_4^+(H^+)$, H^+-K^+-ATPase, aquaporinas, Rh glicoproteínas (RhAG/Rhag, RhBG/Rhbg, RhCG/Rhcg) (Weiner & Hamm, 2007).

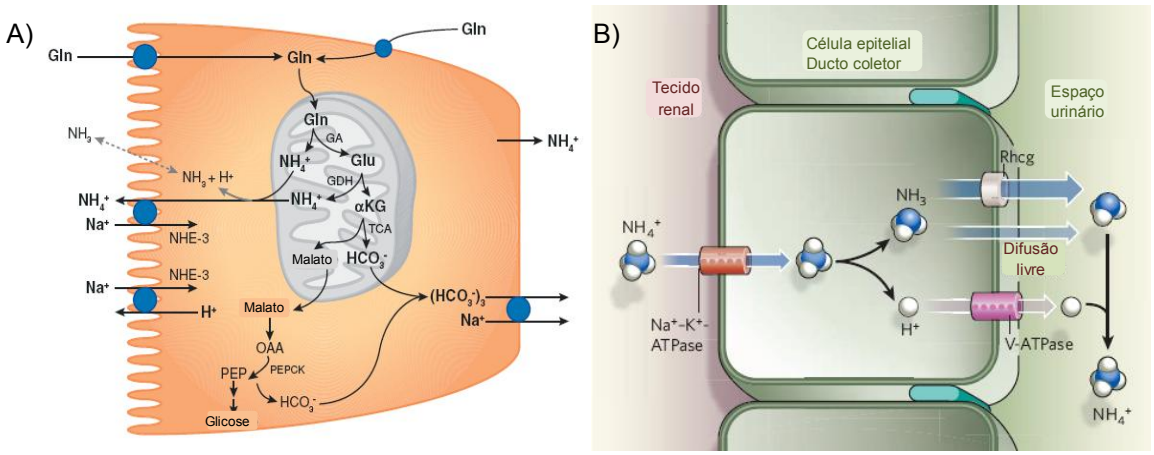


Figura 3: Metabolismo renal de amônia. A) Amoniogênese. A Gln é o principal precursor de amônia, liberando dois íons NH_4^+ e HCO_3^- quando desaminada a αKG via glutaminase e glutamato desidrogenase respectivamente. Os círculos azuis indicam as proteínas tranasportadoras de amônia. A linha cinza pontilhada ilustra o menor transporte de amônia e a contínua o maior transporte. **B) Excreção de amônia.** A amônia pode ser excretada para a urina através de difusão facilitada e pela proteína transportadora Rhcg. A homeostase ácido-básica depende do metabolismo renal de amônia. Cerca de 60 a 70% da produção renal de HCO_3^- é proveniente do metabolismo de glutamina como sistema tampão de amônia.

A) Extraído e adaptado de Weiner & Hamm, 2007.

B) Extraído e adaptado de Knepper, 2008.

Para melhor entendimento das vias metabólicas que serão discutidos nesta tese, faz-se necessário ressaltar os processos de desaminação e transaminação de alguns AAs.

Para que qualquer AA seja quebrado a intermediário do TCA é necessário que o seu grupamento amina seja removido por uma desaminase. Observa-se na reação abaixo a desaminação do Asp a fumarato:

I. Asp + IMP + GTP → GDP + Pi + S-AMP

II. S-AMP → fumarato + AMP

III. AMP → IMP + NH_3

Extraído de Graham *et al.*,1997.

A Figura 4 ilustra os pontos de entrada de diferentes AAs no ciclo de Krebs através da desaminação exceto para o Glu. Cerca de 10 a 15% do consumo total de ATP é derivado da degradação de AAs. Note que cinco destes AAs tem ambas as funções. Os AAs que são degradados a piruvato são potencialmente cetogênicos; sendo a leucina e a lisina exclusivamente cetogênicos. Alguns dos AAs aparecem mais de uma vez podendo ser oxidados a diferentes esqueletos carbonados (Nelson & Cox, 2005).

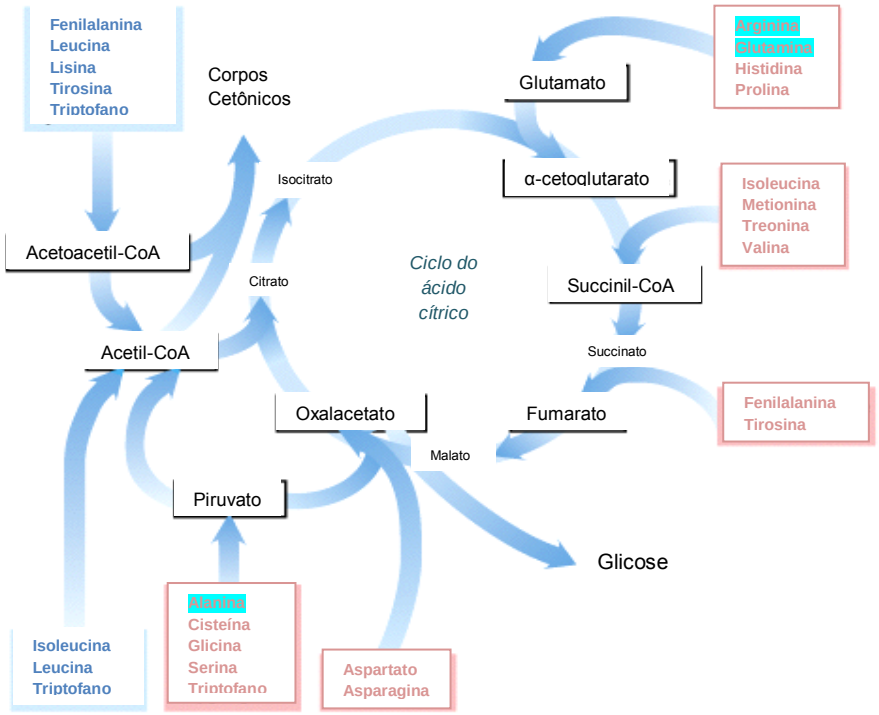


Figura 4: Catabolismo dos AAs. AAs com função gliconeogênica (rosa) e; AAs com função cetogênica (azul). Os AAs estão agrupados no sentido degradação → síntese. os AAs grifados serão discutidos nesta tese. Extraído e adaptado de Nelson & Cox, 2005

Os passos das reações a seguir refletem as principais fontes de amônia envolvendo o Glu e desaminação dos AAs de cadeia ramificada (BCAA):

I. BCAA + KG → Glu + BCKA

II. BCKA + NAD^+ + CoA → Acil CoA derivados, + NADH + CO_2

III. Acil CoA derivados → Acetil CoA, succinil CoA, acetoacetato

IV. Glu → KG + NH_3

V. Glu + NH_3 +ATP → Gln + ADP +Pi

VI. Glu + Pir → KG +Ala

VII. Glu + OA → KG + Asp

NET (1 + 2 + 3 + 4): BCAA + NAD^+ + CoA → NADH + CO_2 +

NH_3 + Acetil CoA, succinil CoA, acetoacetato

Extraído de Graham *et al.* 1997.

No processo de transaminação dos AAs o grupamento amina é reposicionado pela aminotrasferase no seu α-cetoácido utilizando o piridoxal fosfato como co-fator. A reação pode ser verificada no exemplo abaixo (Figura 5):

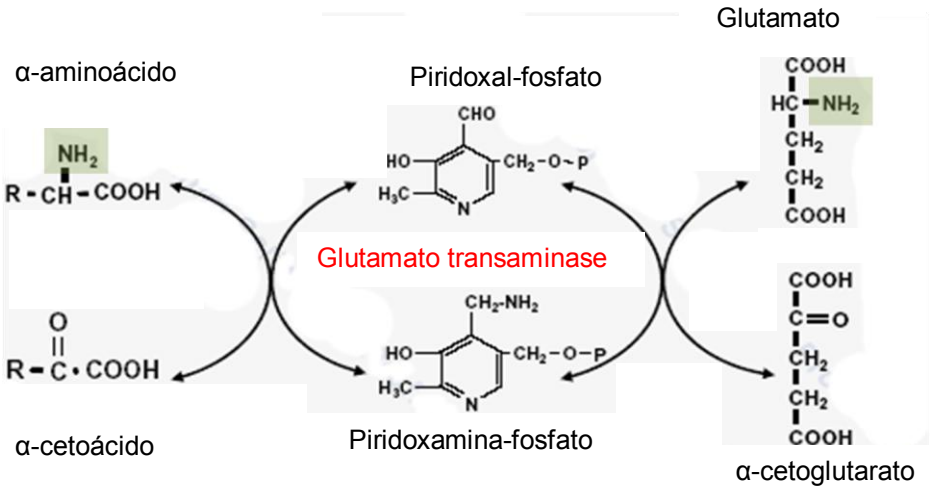


Figura 5: Processo de transaminação envolvido na síntese de Glu. O α-cetoglutarato recebe o grupamento amina oriundo do seu L-aminoácido. O Glu pode ser sintetizado a partir de arginina (Arg), Gln, histidina ou prolina. PLP= piridoxal fosfato.

Outras transaminases merecem foco neste trabalho por possuírem valor clínico no diagnóstico de lesões cardíaca e muscular ou hepática, sendo elas aspartato aminotransferase (AST), alanina aminotransferase (ALT) e gama-glutamil transferase (GGT). Estas enzimas são discutidas nos artigos 2, 4, 5 e 7 desta tese.

b) Metabolismo da amônia

O processo de detoxificação da amônia pelo músculo, foi inicialmente estudado por Bliss (1928). No estudo, o autor postulava que parte da amônia produzida neutralizava os ácidos formados pelo metabolismo e o grupamento amina dos AAs era carreado ligado a proteínas no plasma. No entanto, Krebs descobriu que o fígado responde rapidamente às mudanças sistêmicas nos níveis de amônia

através do ciclo da uréia e do consumo de Glu com liberação de Gln (Buttrose, 1987; Damink, 2002).

A Figura 6 e o anexo (1 A e B) auxiliarão no entendimento do artigo 6 desta tese. Na sessão de Anexo encontra-se a ilustração da suposta entrada de AAs no ciclo da uréia dos grupos suplementados ou não com cafeína.

Ë demonstrado na Figura 6 as diferentes formas do hepatócito de remover a amônia livre. Observem em cinza claro os processos de desaminação e transaminação. O ciclo da uréia didaticamente tem início na síntese de carbamoil fosfato (CTP) pela carbamoil fosfato sintase I (CPS) a partir de NH_3, CO_2, H_2O e ATP dentro da matriz mitocondrial (Makoff & Radford, 1978).

A CPS tem um sítio para hidrólise de Gln e dois de fosforilação, sendo o primeiro para o bicarbonato e o seguinte ao ácido carbâmico. O CTP é precursor intermediário entre as pirimidinas e a biossíntese de Arg, estando presente evolutivamente na arginina desidrolase (Davidson *et al.* 1993; Huang & Graves, 2003). As concentrações mitocondriais de Asp regulam positivamente a atividade da aspartato transcarbamilase (ATCase) estimulando seu escoamento

O ciclo da uréia pode ser assim dividido:

Passo 1: A ortinina recebe a amina do CPT gerando citrulina via ornitina transcarbamilase (OCT) no *mitocondrium;*

Passo 2a e 2b: A hidrólise do ATP em AMP + PPi gera energia para a junção entre a citrulina e aspartato (Asp) via arginosuccinato sintase (AsS);

Passo 3: O arginosuccinato é degrado a Arg e fumarato via Arg succinatoliase (AsL). O aumento das concentrações de fumarato pode estimular a velocidade do TCA auxiliando na produção de ATP e eliminando indiretamente a amônia. Lém disso, a AsL parece ser o passo limitante no direcionamento do arginosuccinato para sua continuidade no ciclo da uréia ou redirecionamento para o ciclo da citrulina-óxido nítrico.

Passo 4: A Arg é hidrolisada em uréia e ornitina via arginase.

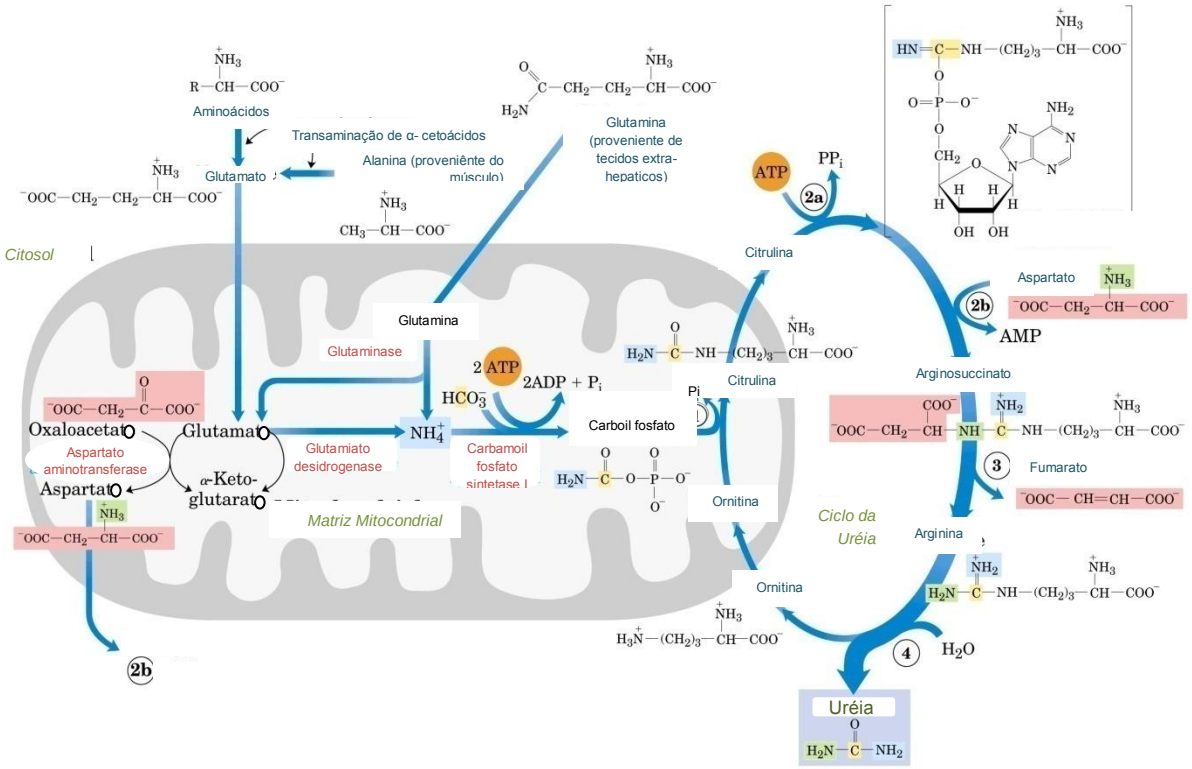


Figura 6-Ciclo da uréia. Extraído de Nelson & Cox, 2005

No fígado encontra-se principalmente a isoforma I da arginse. No entanto a arginase II parece ser principalmente encontrada no rim, cérebro, tecido gastro-intestinal e prostata. Ademais, a arginase II esta envolvida no ciclo da citrulina – oxido nítrico nestes tecidos. Nota-se que a arginase I está mais envolvida na remoção de amônia pelo ciclo da uréia; e a isoforma II parece estar relacionada aos outros caminhos de biossíntese da Arg, por exemplo a síntese de pirimidinas e poliaminas. A arginase II parece ter mais afinidade pelo substrato, pois os seus pI são de 9,3 e 6,8, respectivamente (Cederbaum *et al.*, 2004).

Fatores que corroboram para a sinalização celular durante processos de não homeostasia, tais como: lipopolissacarídeos de membrana (LPS), IL-4, IL-10, IL-13, fator de necrose tumoral (TNF), glicocorticóides, catecolaminas, análogos do cAMP, hipóxia e trauma estimulam a síntese de arginase I e/ou II, que parece inibir as óxido nítrico sintetase (NOSI, NOSII e NOSIII), reduzindo a produção de óxido nítrico (NO) e potencialmente estimulando as defesas celulares. A $V_{máx}$ das arginases em pH fisiológico é mais de 1000 vezes o das NOS (arginases = Km's 2-20 mM e NOS = 2-20 ¹M) diminuendo a entrada de Arg no ciclo da citrulina-óxido

nítrico. Dessa forma, sugere-se que as arginases estejam envolvidas na síntese de colágeno, proliferação celular dentre outros (Morris, 2002).

As enzimas do ciclo da uréia são organizadas no hepatócito, possibilitando eficiente transferência da amônia e seus carreadores. Tem sido evidenciado a interação entre CPS e OTC em *mitocondrium* isolado. O grupo chefiado por Raijman durante toda a década de 80 destacou a co-localização da entrada de ornitina (Orn) no *mitocondrium* associado a OTC. Eles sugerem que a organização destas enzimas é sob a forma de funil, onde a passagem é maior para os intermediários da AsS a AsL afunilando na chegada a arginase (Cohen *et al.*,1982; Cheung *et al.* 1989).

A Figura 7 auxilia na elucidação da integração do TCA com o ciclo da uréia. As enzimas aqui esquematizadas estão expresas em todas células ou tecidos.

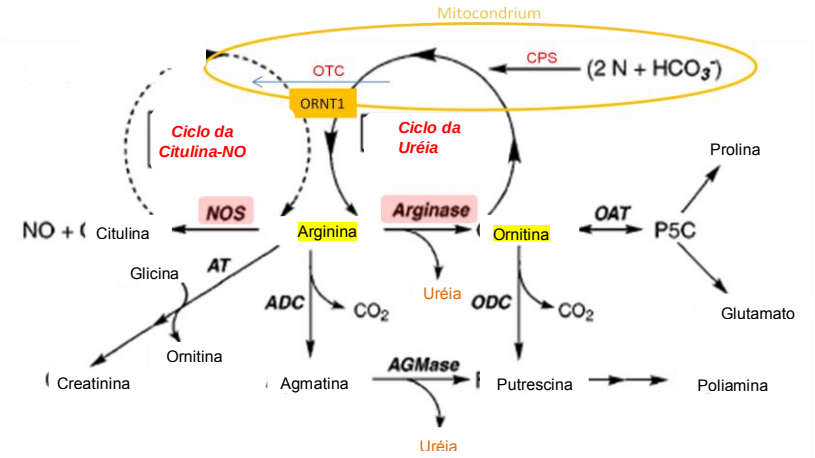


Figura 7 – Localização de arginase e óxido nítrico sintetase. NOS= óxido nítrico sintase; OTC= ornitina transcarbamilase; OAT= ornitina aminotransferase; ODC= ornitina descarboxilase; AT= L-arginina glicina amidinotransferase; ADC=arginina descarboxilase; AGMase= agmatinase; CPS= carbamoil fosfato sintetase. A linha pontilhada demonstra que a eficiência do ciclo citrulina-NO é variável e não quantitativa. Extraído e adaptado de Morris, 2002.

Ao final da década de 70 foi demonstrado em homogenato de fígado e *mitocondrium* isolado de rato o papel inibitório da amônia sobre o TCA resultando na inibição da utilização de substrato diretamente sobre a enzima e indiretamente na respiração celular. A amônia inibe a oxidação de piruvato, citrato/isocitrato via citrato sintase e desidrogenase mais que o 2-oxo-glutarato e succinato via Glu

desidrogenase. Vale ressaltar que nestes estudos não foi observado à influência da amônia sobre o transporte de elétrons. Estudos da época (Katunuma *et al.* 1966; Klahr, *et al.* 1970) sugerem que o primeiro ponto de influência é na redução dos nucleotídeos pirimidínicos intramitocondrial e estimulação da oxidação de nicotinamida adenina dinucleotídeo (NADH+H) e nicotinamida adenina dinucleotídeo fosfato (NADPH+H).

A AST e a ALT contribuem tentando compensar a influência da amônia, aumentando a neoglicogênese e a atividade do ciclo da uréia formando Glu a partir de ornitina. Porém este papel compensatório pode ser inibido pelos BCAAs em particular a ornitina cetoácido transaminase (OKT) (Manning & Gibson,. 2004).

c) Síntese de Gln na remoção da amônia

A Gln é um aminoácido não essencial e não tóxico consistindo de 5 cadeias de carbono e 2 resíduos de nitrogênio, sendo o mais abundante aminoácido extracelular com concentração plasmática de aproximadamente 0,7 μM, correspondente a aproximadamente 50% do *pool* de AAs livres. Sendo importante precursora da síntese de AAs glicogênicos, peptídeos purinas e pirimidinas, ácidos nucléicos e nucleotídeos (Cooper 2001; Newsholme, *et al.*, 2003) (Figuras 8).

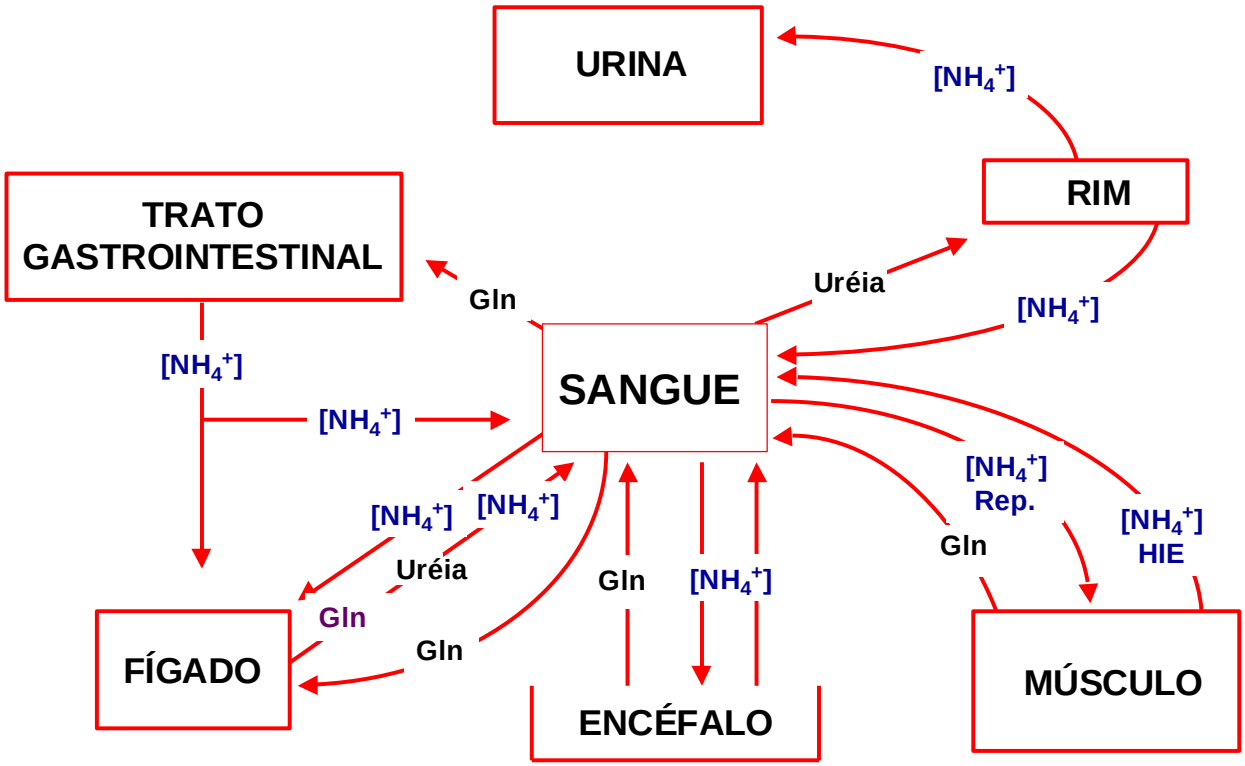


Figura 8- Gênese da amônia. Órgãos e tecidos responsáveis pela formação, utilização e circulação de amônia e de compostos nitrogenados. Extraído e adptado de Banister & Cameron, 1990.

A Gln é metabolizada quase que exclusivamente por somente duas rotas, sendo a hidrólise a Glu pela reação da glutaminase a principal. A glutaminase no fígado é ativada por amônia, portanto o metabolismo alterado de Gln pode contribuir para a elevação da amônia. A adição de uma amônia ao Glu funciona como sistema tampão e agente neoglicogênico (seta dupla amarelo na figura 10) (Greenhaff, 1995; Blomstrand & Saltin, 1999; Fürst, P. 2002; Brustovetsky *et al.* 2001; Machado & Cameron 2002) (Figura 9).

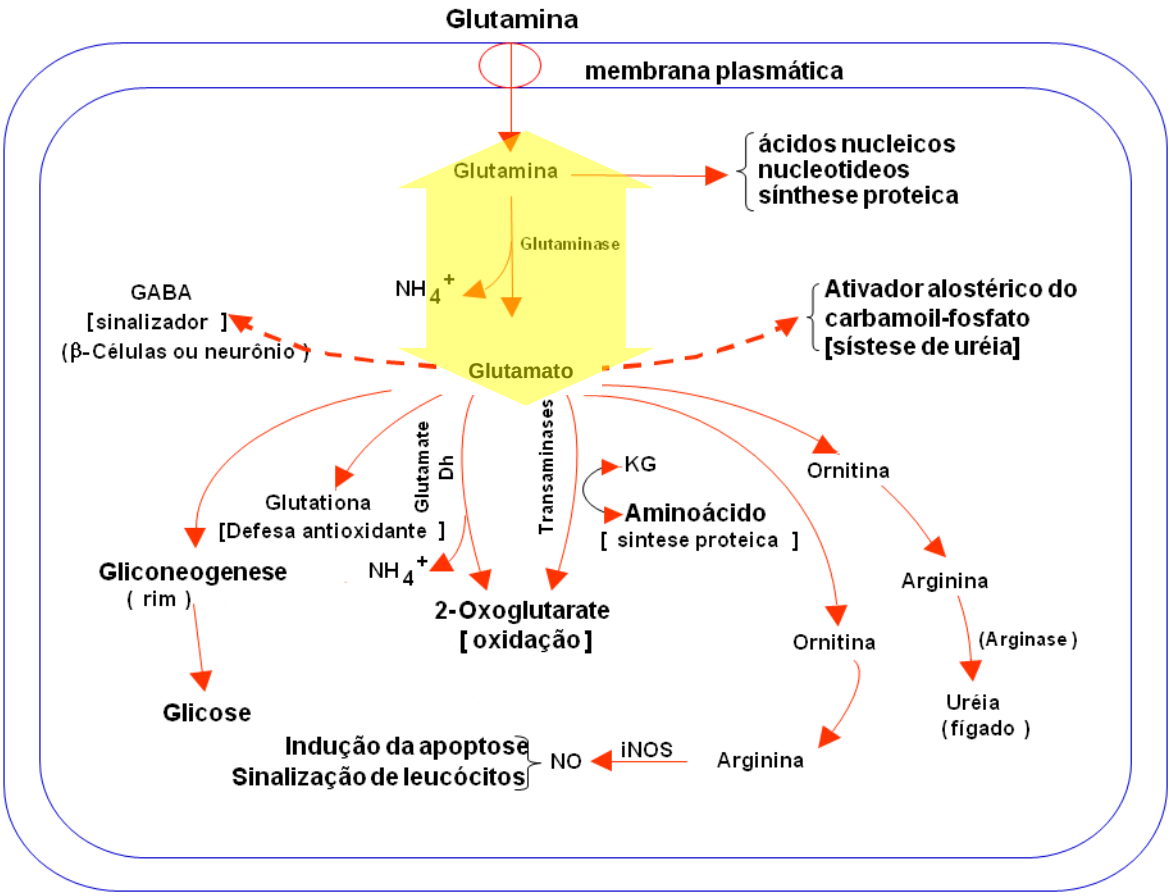


Figura 9- Importância de Gln e Glu nas reações de transaminação e desaminação de AAs. A Glu/Glu são importantes substâncias metabólicas como óxido nítrico, glutationa, uréia e GABA, além também da importância como precursores de glicose através da neoglicogênese. Extraído de Newssholme *et al.*, 2003 a.

O produto metabólico imediato da Gln é o Glu, um dos mais abundantes AAs no fígado, rim, músculo e cérebro. Suas altas concentrações intracelulares em torno de 2 a 5 mmol/L comparados às concentrações extracelulares que é de aproximadamente 0,05 mmol/L, indicam seu importante papel em todos os tecidos (Brosnan, 2000).

O Glu é o centro da carga protéica diária e exerce um papel chave na transaminação e desaminação de AAs, o que inclui a formação de Asp, alanina (Ala) e Gln. O músculo esquelético libera Gln e Ala em grandes quantidades representando 50 a 100 % do efluxo de AAs em jejum e em estado alimentado,

respectivamente. O Glu é o aminoácido predominantemente captado pelo músculo (Grahan et. al., 2000; Newssholme *et al.*, 2003 b).

O Glu também está envolvido com o sistema de lançadeira glutamato/aspartato cuja importância se dá na produção de equivalentes de redução no citoplasma e como substrato anaplerótico para o TCA (Brosnan, 2000; Newsholme, *et al.*, 2003a).

É o principal neurotransmissor excitatório de mamíferos. A ativação excessiva destes receptores pode provocar neurotoxicidade com degeneração neuronal e morte celular (Hermenegildo, et al. 1998; Miñana, *et al.* 1997). Pode ser observada redução das concentrações de Glu do neurônio pré-sináptico na hiperamoniemia o que provoca desequilíbrio no sistema neurotransmissor do Glu e alterações nos sinais de transdução cerebral (Cooper, 2001). Nos astrócitos o aumento da síntese de Gln que excede sua capacidade de liberação, induz o estresse osmótico em regiões críticas do encéfalo (Takahashi *et al.* 1990; Takahashi *et al.* 1991).

Em estado pós-absortivo a Gln é precursora da glicose ao doar seu esqueleto de carbono, aumentando o *pool* de glicose no sangue, via neoglicogênese renal e hepática, o que corresponde a 50 % da produção em situações de jejum prolongado (Stumvoll, *et al.*, 1999; Nissim, 1993; Nissim, 2000).

O músculo, pulmão e tecido adiposo são os maiores contribuidores para o aumento da Gln no plasma enquanto o intestino e o rim os que mais captam. Dependendo das circunstâncias metabólicas o fígado e o músculo estriado esquelético podem alterar sua taxa de captação e liberação, exercendo papel fundamental na regulação da homeostase desse aminoácido (Stumvoll, *et al.*, 1999; Newsholme, *et al.*, 2003b).

d) Metabolismo de carboidratos e amônia

Como já descrito por Cori & Cori (1928) o fígado utiliza ácido lático para manter a glicemia e as reservas hepáticas de glicogênio durante o estresse físico. A utilização de Ala forma glicose liberando amônia permitindo indiretamente que a degradação protéica do músculo continue. No hepatócito, a amônia ativa a hidrólise de Glu, via Glu desidrogenase formando α-cetoglutarato e amônia. Esta

desaminação oxidativa do Glu é de vital importância para a interação das vias metabólicas, pois aumenta a atividade do TCA auxiliando na produção de ATP (Jones, 2002; Monfort, 2002).

O déficit de glicogênio na musculatura, acarreta aumento na concentração plasmática de amônia e lactato durante o incremento de carga, devido à diminuição no fluxo da via glicolítica (Roeykens, 1998), que gera incremento na concentração de IMP (Spencer, 1991a) e de intermediários do TCA (Gibala, 2002). Chicharro (1996) e Ament (1997) observaram que o atleta, especialmente nos eventos de *endurance*, com dieta deficiente em carboidratos (CHO) não tem respostas mecânicas adequadas às exigências da performance alcançando mais rapidamente o ponto de fadiga. Estes achados sugerem que a amônia e o lactato possam ser marcadores bioquímicos do treinamento.

No músculo esquelético em contração intensa, a velocidade da glicólise excede a do TCA, gerando aumentos na concentração de piruvato que é escoado a lactato e a intermediários do TCA, com síntese concomitante de Ala e Gln para manter o fluxo da via glicolítica. Por ser a atividade mitocondrial insuficiente na refosforilação do ADP (Graham & MacLean, 1990; Gibala, 1977).

Aproximadamente 30% do total de amônia produzida pelos rins são liberados para urina e o restante é lançado na veia renal. Em estado subclínico de acidose a situação é reversa, porque o amoniogênese total é elevada e 70% da produção de amônia é eliminada na urina.

Embora questionada por alguns estudos a suplementação de BCAA na atividade física é grandemente difundida e utilizada para aumento da performance (Aparicio, *et al.* 1989; Harris, *et al.*, 1992; Ip, *et al.*, 2003). A utilização metabólica de AAs como precursores neoglicogênicos provoca o aumento da amônia livre no sangue (Walser, *et al.*, 1979; Schloerb, *et al.*, 1996; Fürst, 2002).

A utilização de α-cetoácidos como suplemento poderia ajudar no controle da amonemia, capturando amônia livre para a formação de um novo aminoácido e funcionando como mecanismo para otimização metabólica (Walser, *et al.*, 1987; Schloer, 1996; Snow, 1998; Kato, *et al.* 2003). Pacientes com distúrbios renais crônicos e acidose metabólica obtiveram diminuições significativas nas concentrações séricas de uréia, creatinina e fósforo quando fizeram uso de dieta

associada a suplemento de cetoácidos e AAs essenciais (comparada à dieta convencional com baixa ingestão de proteína).

e) Toxicidade da amônia

A toxicidade da amônia foi descrita no laboratório de Ivan Pavlov (1893) e muitos trabalhos tem sido realizados desde então para se esclarecer os mecanismos moleculares da toxicidade aguda da amônia (Monfort *et al.*, 2002; Shawcross et al., 2005).

Na natureza os danos ao sistema nervoso central (SNC) estão extremamente relacionados ao tempo de exposição e as concentrações de amônia podendo gerar edema cerebral com conseqüente aumento da pressão intracraniana devido ao inchamento do astrócito que acarretará herniação ou Alzheimer tipo II (Kelly & Stanley, 2001). A amônia atravessa a barreira hemato-encefálica por difusão, ou seja, independente de suas concentrações arteriais sob condições fisiológicas normais. O mecanismo de fixação da amônia nos AAs mantém as concentrações em torno de 0,05mM. Estima-se que 25% das concentrações cerebrais de amônia são captadas em situações de pH normal.

São conhecidas pelo menos 16 rotas de produção de amônia no cérebro. Uma das mais importantes é via Glu desidrogenase. A captação do Glu dá-se principalmente pelos astrócitos através de transportadores de *N*-metil-*D*-aspartato (NMDA) sendo desaminado a α-cetoglutarato e redirecionado de acordo com a demanda energética. Da mesma forma que no músculo as enzimas do PNC também são responsáveis pela elevação significativa de amônia no sistema. Além disso, a amônia causa alterações na função mitocondrial e mudanças no metabolismo energético cerebral.

O foco desta tese é discutir algumas das modificações geradas ao SNC que estejam vinculadas ao metabolismo de AAs. Dois distintos mecanismos têm sido propostos para a redução das concentrações de ATP induzidas pela amônia: 1 – inibição do TCA via principalmente α-cetoglutarato desidrogenase (αKGDH), gerando aumento de α-cetoglutarato e piruvato que é escoado a Glu ou GABA e lactato respectivamente; e 2 – mecanismos envolvendo o receptor de NMDA

gerando uma maior depleção nas concentrações de ATP via ativação de Na^+, K^+, ATPase e entrada de Ca^{++} (Felipo & Butterworth 2002a) (Figura 10).

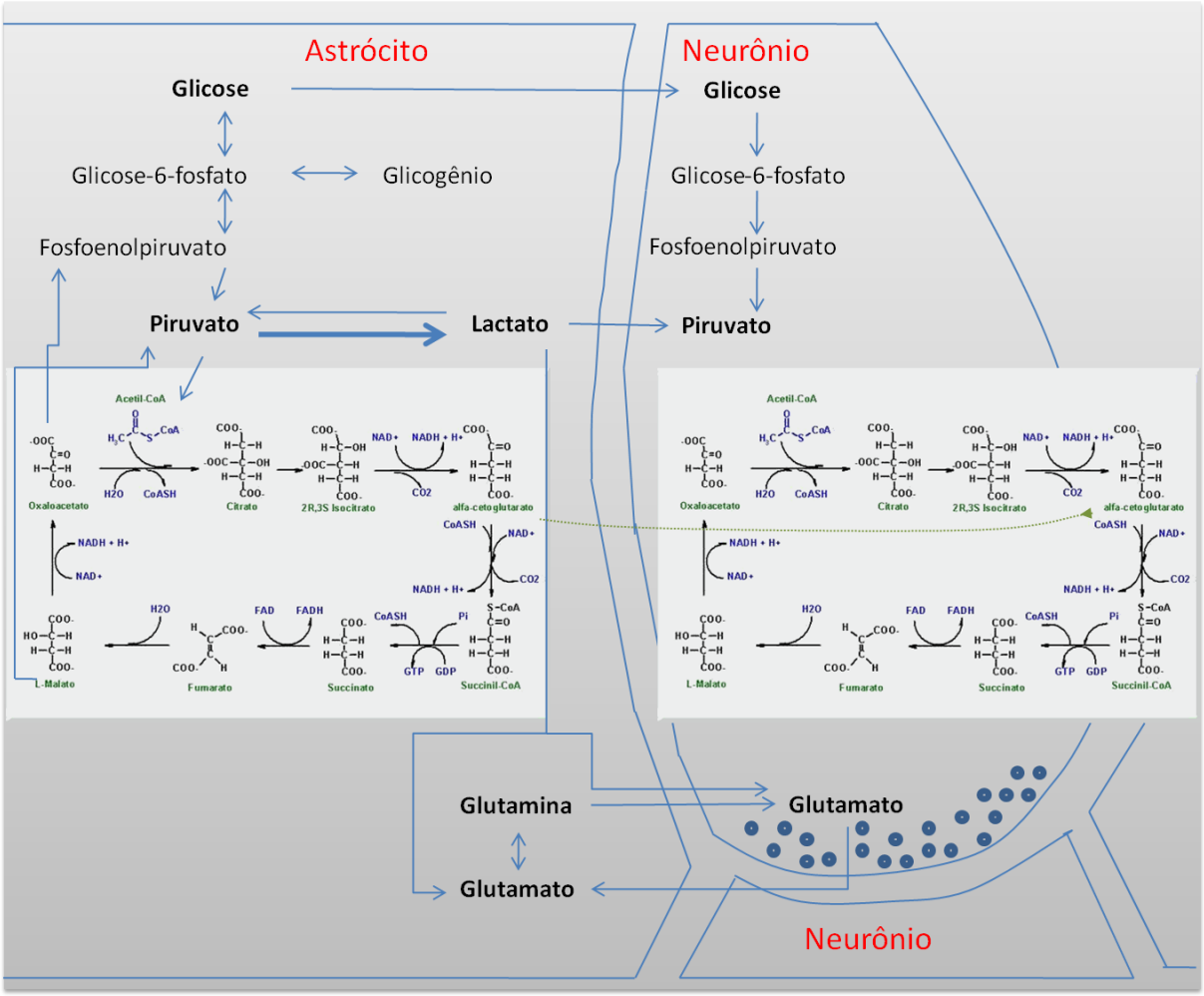


Figura 10- Transito astrócito/neurônio e vice-versa. O Glu é convertido à Gln pela GS. A Gln é exportada para o neurônio e é desaminada a Glu. Extraído e adaptado de Hertz *et al.*, 2000.

A elevação da amônia aguda ativa o NMDA, gerando influxo de Ca++ que liga na calmodulina ativando as três isoformas de NOS que degrada Arg a NO, e estimulando a guanidina ciclase (GC) elevando as concentrações de cGMP resultando em sintomas de desordem neurológica; como pode ser visto na Figura 11B. O Ca^{++} ativa o *calcineurin* que ativa a bomba de Na^+-K^+-ATPase que elimina Na^+ intracelular com consumo de ATP. Este excesso de Ca^{++} é captado em sua maior parte pelas *mitocondria* que se proliferam nesta situação.

A amônia por penetrar no astrócito também aumenta a síntese de radicais superóxidos e diminui a atividade catalítica da glutationa peroxidase, superóxido dismutase, e catalase, e junto com a Gln provocarem o inchamento celular (Figura 11A). Tem sido proposto um mecanismo de neuroproteção pela taurina (Tau) (Figura 11C), que neutraliza as espécies reativas de oxigênio no neurônio pós-sináptico e age como agonista dos receptores do GABA e glicina no neurônio pré-sináptico que diminui a sua liberação de Glu e reduz a ativação do NO/cGMP (Rose & Felipo, 2005).

Outro mecanismo neuroprotetor da toxicidade da amônia pode ser pela síntese e degradação da glutationa. Quando a cultura de astrócito é exposta a concentrações milimolar de amônia há perda da expressão de proteína glial fibrilar ácida (*glial fibrillarly acidic protein* - GFPA), principal constituinte dos filamentos intermediários do citoesqueleto corroborando para perda da morfologia do astrócito (Felipo & Butterworth, 2002b). O aumento excessivo nas concentrações de Ca^{++} no citoplasma ativa proteases, caspases, TNF e citoquinas que induzirão necrose ou apoptose.

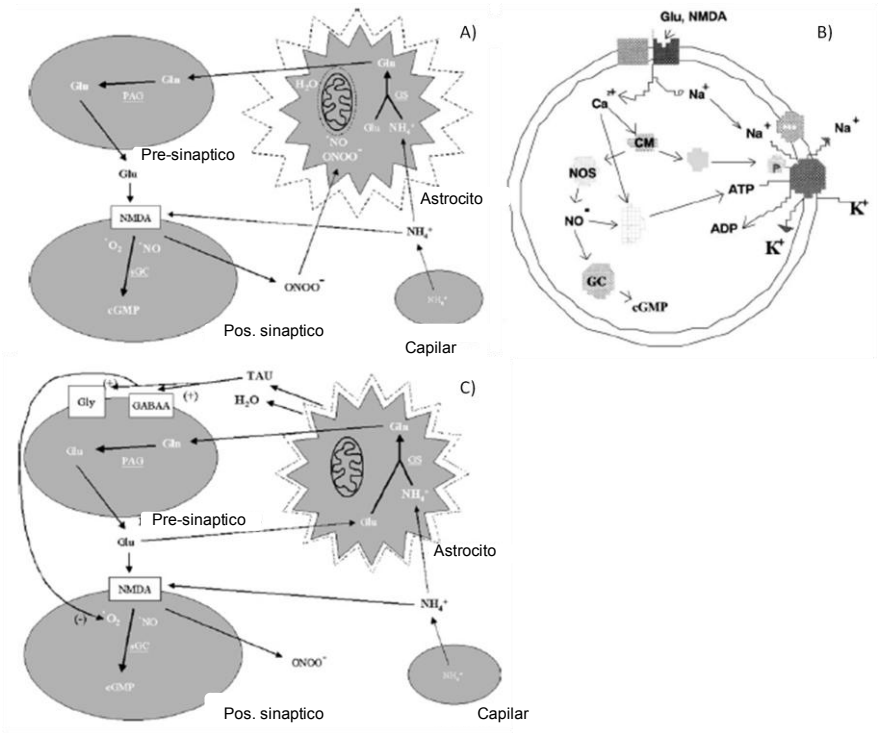


Figura 11- Papéis do receptor de NMDA no inchamento celular causado pela amônia. A) receptor de NMDA/NO/cGMP durante a neurotoxidade; **B)** ação do

receptor de NMDA sob a hiperamoniemia aguda; **C)** mecanismo putativo de neuroproteção por ação da taurina (Tau). As linhas pontilhadas representam o inchamento do astrócito ou do *mitocondrium*. Oxido nítrico sintase= NOS; guanilato ciclase= GC; cGMP; glutamina sintetase= GS. Extraido e adaptado de Felipo & Butterworth, 2002a e Albrecht & Wegrzynowicz, 2005.

O astrócito é o maior responsável pela recaptação de Glu da fenda sináptica através dos transportadores de alta afinidade para Glu (EAAT-1 ou GLAST, e EAAT-2 ou GLT-1). Pequena parte do Glu da fenda é recaptado pelo neurônio pré-sináptico via EAAT-3. O Glu deixa de ter ação excitatória, passando a neurotóxico dependendo do tempo de permanência na fenda (Figura 12). Da mesma forma que o Glu, os íons *ammonium* afetam a transmissão sináptica excitatória e inibitória. No pré-sináptico acontece inibição da liberação de Glu por inibir a síntese de Gln no nervo terminal; e no pós-sináptico tem efeito direto no NMDA (Jones 2004).

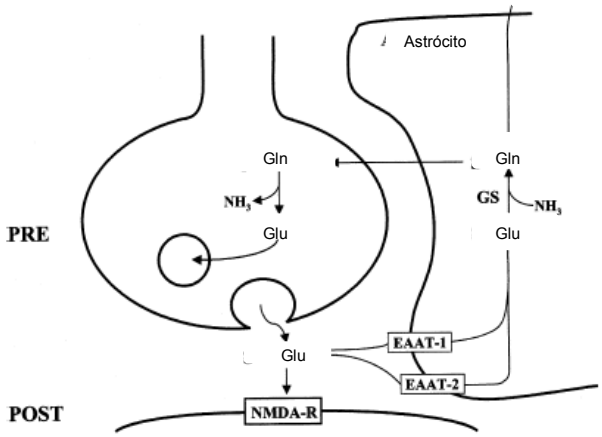


Figura 12- Ciclo do glutamato-glutamina. Recaptação do Glu pelo astrócito. Extraído de Felipe & Butterworth, 2002b

A hiperamoniemia está associada à alteração na regulação de neurotransmissores podendo ocasionar o desequilíbrio nos sistemas glutamatérgico, GABAérgico e serotoninérgico que pode ser suficiente para causar excitotoxidade neural e/ou morte (Sáez, *et al.* 1999; Monfort, *et al.*, 2000; Brustovetsky, *et al.*, 2001; Butterworth, 2001; Butterworth, 2002; Machado, & Cameron, 2002; Monfort, *et al.* 2002 b). Tem sido descrito que a alta concentração

de amônia induzida pelo exercício físico pode gerar distúrbios momentâneos no funcionamento do SNC semelhantes àqueles encontrados em fases iniciais de diversas doenças relacionadas à hiperamoniemia e/ou doenças neuro-degenerativas. (Greenhaff, 1995; Persky & Brazeau, 2001). Durante a atividade física a amonemia pode aumentar em até 400% acima dos valores considerados normais para o indivíduo sadio em repouso (Masud, *et al.*, 1994; Blomstrand & Saltin. 1999).

A maior implicação neurológica das desordens do ciclo da uréia é a hiperamoniemia. Entretanto não é claro se a amônia sozinha é responsável pelas manifestações neurológicas e quando a Gln/Glu é sinergista no processo. A hiperamoniemia leva a mudanças de comportamento cognitivo, de neurotransmissores e falência energética.

A elevação aguda e rápida das concentrações de amônia induz a neurodegeneração via estimulação receptores NMDA, AMPA e Kainato. Sabe-se que a exposição crônica a elevados níveis de amônia afeta o ciclo do óxido nítrico e contribui para deficiência cognitiva. Manifestações neurológicas clássicas do ciclo da uréia são vômitos, anorexia, letargia, ataxia, edema cerebral, hipotermia e coma dentre outros (Gropman et al 2004; Gropman et al 2007).

Tabela 1- Concentração de amônia no sangue arterial, venoso e em doenças hepáticas.

Humanos	**Amônia (μM)**	**Referências**
Sangue e plasma	22-113 (arterial)	Clemmesen *et al.*, 2000.
Sangue e plasma	20-25 (venoso)	Olde Damink, *et al.* 2002.
Indivíduos saudáveis	45 (arterial)	Clemmesen *et al.*, 2000.
Fluido cérebro espinhal (CSF)	20-100	Clemmesen *et al.*, 1999.
Cirrose hepática	60-80	Clemmesen *et al.*, 2000.
Doença aguda hepática	90-120	Olde Damink, *et al.* 2002.
Falência hepática	150-180	Clemmesen *et al.*, 2000.
Falência hepática e grave pressão intracraniana	~340	Clemmesen *et al.*, 1999.

Concentração de amônia cerebro/sangue *Efeito da falência hepática*	$[\textbf{amônia}]_{cerebro}$ / $[\textbf{amônia}]_{sangue}$	
Normal	2	
Falência hepática crônica	3-4	Butterworth, 2002.
Falência hepática aguda	8	
Consumo de amônia pelo cerebro *Efeitos da falência hepática*	Consumo de amônia pelo cerebro (nmol/g/min)	
Normal	-0.1	
Falência hepática crônica	+8.1	Butterworth, 2002.
Falência hepática aguda	+26.7	

Grande parte dos nossos conhecimentos sobre o assunto é derivado de estudos em encefalopatia hepática e passagem (*shunt)* porto – cava. Utilizamos nesta tese a suplementação de Arg, Ala, Gln e cafeína no intuito de verificar proteção no aumento da amonemia durante diferentes situações de estresse celular

É possível que a suplementação adequada possa auxiliar o aumento da performance por causar a diminuição da concentração de amônia durante o exercício. O uso de Asp ou Glu combinado com carboidrato não foi eficaz para a redução da amonemia, em atividades de longa duração e alta intensidade (Tommaso, *et al.*, 1999; Bruce, *et al.*, 2001; Krzywkowski, *et al.*, 2001).

Baseado nestas hipóteses, é possível postular que um exercício intenso e prolongado seja capaz de induzir um estado tóxico de amônemia agudo e subclínico. A amônia pode ser severa a regiões críticas do SNC afetando a continuidade da atividade coordenada.

Dados obtidos em nosso laboratório demonstraram que a suplementação de carboidrato e Gln isolados ou combinados tem um efeito semelhante na inibição da elevação da amônemia em corredores após 60 min. de exercício (Carvalho-Peixoto *et al*, 2007).

I.II O exercício como modelo de estudo

f) No metabolismo de AAs e amônia

O equilíbrio entre as reações anabólicas que conduzem à síntese protéica e às reações catabólicas que controlam a degradação de proteínas é perturbado pelo exercício; a alteração na carga energética celular durante um estresse metabólico gera aumento na lactacidemia e na amonemia (Maclean, 1996). Lowenstein & Goodman (1978) demonstraram que a amônia muscular aumenta em até 400% durante exercício, representando 74% da produção líquida deste metabólito podendo ser produzida a partir da degradação de nucleotídeos com aparecimento concomitante de IMP e urato (Figura 13). Tsintzas (2001) propôs que a gênese de amônia está correlacionada com o tipo de exercício, o tempo de treinamento, aos aspectos nutricionais, ao sexo, a herança genética e ao tipo de fibra recrutada pela atividade.

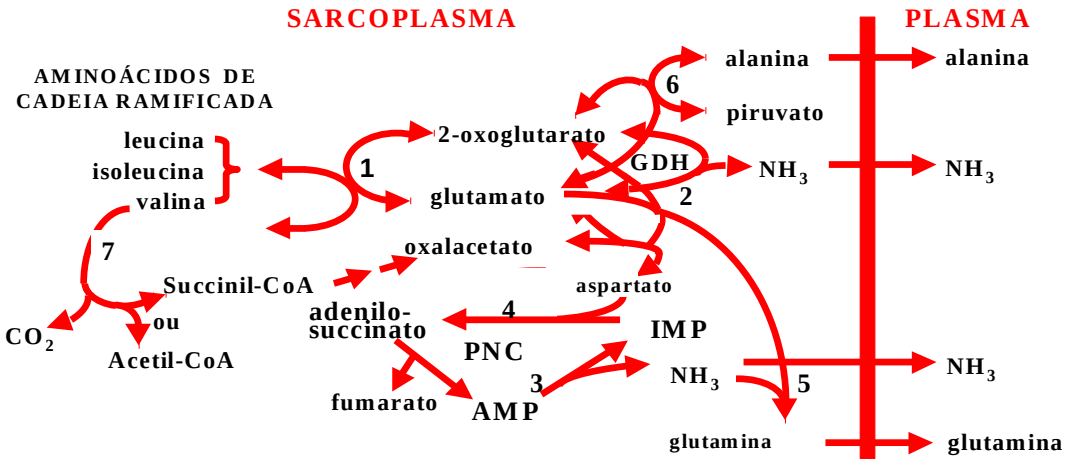


Figura 13- Interação de reações metabólicas envolvendo catabolismo de aminoácidos, ciclo PNC, metabolismo da amônia no músculo e seu efluxo para o plasma. **Enzimas :** 1-aspartato aminotransferase/ 2-glutamato desidrogenase/ 3-AMP desaminase / 4-adenilosuccinato sintetase/ 5-glutamina sintetase/ 6- piruvato carboxilase e alanina aminotransferase. Extraído e adaptado de Terjung R.L. Em: Hragreaves M.; Thompson, M. Exercise Biochemistry X, c.39, p.492, 1999.

Em exercícios de curta duração e alta intensidade a maior quantidade da amônia produzida permanece dentro do músculo, somente uma pequena parte é liberada para o sangue e sua produção total corresponde à degradação de

nucleotídeos a IMP, pelo aumento da atividade do ciclo PNC (Sahlin, 1999). Em concentrações isométricas, há maior produção de amônia pela redução do fluxo sanguíneo no músculo, aumento da taxa de utilização de ATP, fosfofocreatina e desaminação do AMP (Terjung, 1999).

A concentração da amônia aumenta substancialmente em atividades prolongadas entre 70-80% do $VO_{2máx}$, mas os processos que favorecem este aumento ainda não são claros; nestas atividades longas e exaustivas, as possíveis fontes de amônia podem ser a desaminação do AMP e a desaminação oxidativa de AAs como Gln/Glu, Asp e BCAAs (Graham *et al.*, 1990; Graham *et al.*, 1997; Wagenmakers, 1999). O envolvimento desses eventos bioquímicos estabelece uma associação entre metabolismo de AAs, amônia, TCA e PNC em virtude do aumento da demanda energética no músculo esquelético, conforme verificado na Figura 13.

Outro fator que parece estar relacionado à elevação da amônia induzida por atividades exaustivas é a redução do fluxo sanguíneo renal e hepático, que pode diminuir sua captação e excreção aumentando seus níveis na circulação sistêmica (Sahlin, 1999).

O exercício intermitente (por exemplo o futebol) envolve todas as modalidades descritas acima. Sendo assim, de acordo com o fator motivador que estimula o indivíduo a realizar a atividade haverá envolvimento predominantemente mas transitório da contribuição para elevação da amonemia proveniente da desaminação de AAs ou PNC (Bassini-Cameron, 2007).

Os humanos têm sido fascinados pelos diferentes efeitos induzidos pelo exercício prolongado desde a Antiguidade e suas relações com o limite extremo de atividade e seu ponto de exaustão (fadiga). Geralmente o foco destes trabalhos relaciona termorregulação, nutrição, circulação e metabolismo com a intensidade e duração do exercício

As Tabelas 2, 3 e 4 mostram uma linha temporal de diferentes protocolos de teste aplicados ao exercício contínuo, intermitente e/ou de alta intensidade sobre a concentração de amônia sérica.

Investigadores	N	Tipo de Teste	Concentração de amônia	Comentários
Kim *et al.*, 2008	9	Teste em esteira com intensidade e velocidade constante	N redução de 65% na produção de amônia comparada com os animais controles	Os animais suplementados com o extrato da planta P.mume e submetidos ao exercício apresentaram menor concentraçao de amônia comparado com não suplementados.
Marjerczak, *et al.* 2007	19	Exercício com bicicleta ergometrica durante 60 e 120 minutos	S aumento de ~ 400% comparado com os valores de repouso	O aumento é devido a deaminação do AMP no exercícos intenso através da ativação da enzima AMP Deaminase
Meneguello *et al.*, 2003	20	Teste de nataçao com 6% até exaustão	N comparados com os animais controles os animais suplementados com Arg, apresentaram 50% menor na concentraçao da amonia após exercício	Os animais suplementados com Arg, Orn e Cit submetidos ao exercício apresentaram menor concentração de amônia comparado com não suplementados.
Anne ey al., 2001	18	Teste de natação em intensa e baixa intensidade	S aumento de aproximadamente 65% comparado com os animais que realizaram exercicios de baixa intensidade	Os animais que realizaram os exercícios intensos apresentaram aumento na concentração de amônia comparado com os que realizaram exercicios de baixa intensidade
Yamamoto *et al.*, 1997	5	Teste de bicicleta com 65% do VO2 por 25 minutos	S aumento de ~ 60% dos valores pre exercício	Houve um aumento em todos os parâmetros logo após do exercício

Tabela 2- Efeito do exercício contínuo sobre as concentrações séricas de amônia. Note a variedade de protocolos. (M) masculino; (F) feminino; (S) sim e (N) não.

De acordo com o aumento no consumo de oxigênio (aumento do trabalho) há incremento das concentrações sanguíneas de amônia (Gráfico 1A). Indivíduos expostos a depleção de glicogênio, por exemplo pelo teste de Wingate ou dieta pobre em CHO, utilizam maior quantidade de AAs para manter as concentrações

de ATP gerando conseqüentemente maior amônia livre; pela menor quantidade de glicogênio muscular há menor produção de lactato. No entanto, indivíduos treinados em endurance são capazes de consumir maior quantidade de FFA mais rapidamente e possuem maior arsenal enzimático para tolerar situações de estresse celular. Dessa forma, as concentrações de amônia e lactato parecem ter semelhante aparecimento e subida no sangue (Ilustração 1B).

Investigadores	N	Tipo de Teste	Concentração de amônia	Comentários
Liu *et al.*, 2008	10 H	Exercícios em Bicicleta ergométrica com aumento de cargas até exaustão	S aumento de 300% comparados com o grupo suplementado	A elevação está diretamente relacionada com a intensidade do exercício
Burgomaster *et al.*, 2006	16 H	teste de Wingate	N Não houve aumento	Em exercícios máximos boa parte da energia utilizada é defida a utilização do AMP que gera IMP e amônia.

Tabela 3- efeito do exercício intermitente sobre as concentrações séricas de amônia. Note a variedade de protocolos. (M) masculino; (F) feminino; (S) sim e (N) não.

Investigadores	N	Tipo de Teste	Concentração de amônia	Comentários
Seiferti *et al.*, 2007	Revisão	Exercício Intenso	S	O SNC é afetado pela depletação do glicogênio nos astrócitos e pela redução da PaCO2
Nyboa *et al.*, 2004	Revisão	Exercício Intenso	S	Fatores como a hipoglicemia e a hipertermia são possíveis indicadores da Fadiga central.
HARRIS *et al.*, 1997	5	Esteira com Inclinação	S 2.600 % comparado com o valor de repouso	A produçao de amônia está relacionada com diminuição dos estoques de ATP utilizados durante o exercício intenso

Tabela 4- efeito do exercício de alta intensidade sobre as concentrações séricas de amônia. Note a variedade de protocolos. (M) masculino; (S) sim e (N) não.

Fazendo um comparativo ente o gráfico 1A e a ilustração 1B observa-se que para indivíduos normais o ponto de maior síntese/liberação de amônia parece coincidir com o limiar de lactato onde há maior estímulo para descida da via glicolítica aumento as

concentrações de lactato e incremento na velocidade do PNC (Schulz & Heck, 2003).

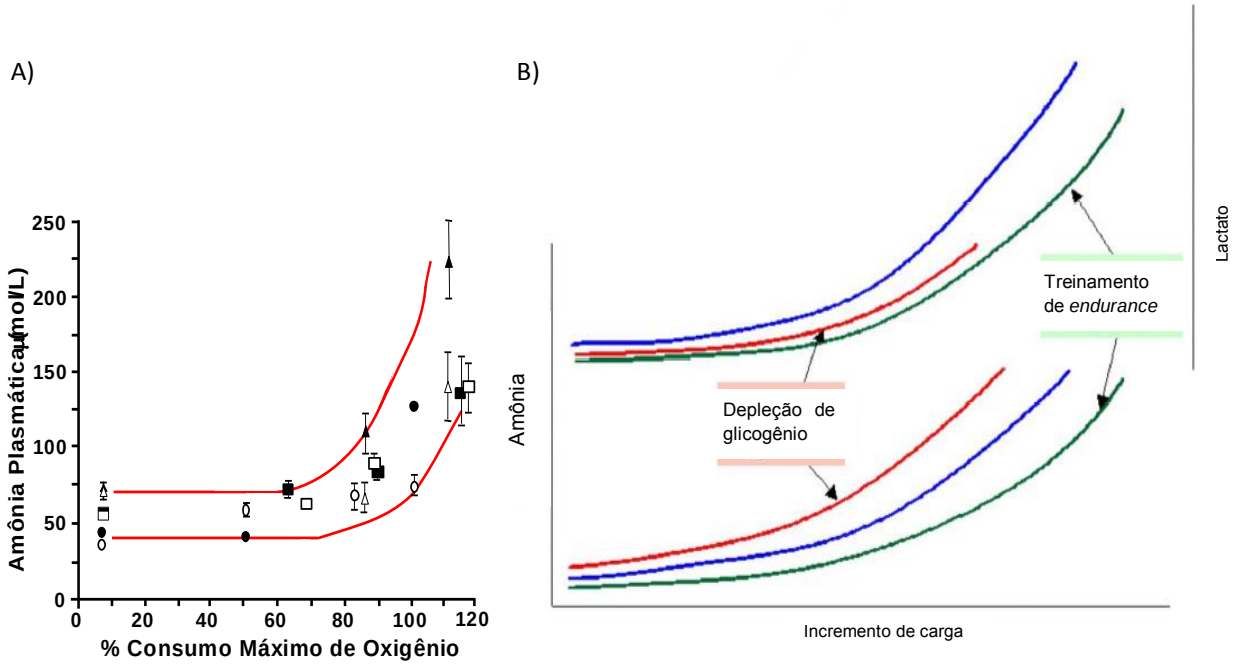


Gráfico 1a- Mudanças na concentração plasmática de amônia em função da intensidade de exercícios de curta duração expresso com % de VO_2 máximo. A área entre as 2 linhas inclui a resposta de indivíduos normais. Adaptado de Terjung, R.L. Ammonia Metabolism in muscle. In: Hargreaves, M.; Thompson, M. Exercise Biochemistry X, c.39, p.489, 1999.

Ilustração 1b- Ilustração esquemática de diferentes efeitos do treinamento de endurance e depleção de glicogênio sobre as concentrações plasmáticas de lactato e amônia durante um exercício progressivo. Extraído de Schulz & Heck (2003)

O exercício tem sido proposto como “vacina” por provocar alterações no sistema humoral pelo aumento de fatores e hormônios pró-inflamatórios. Esta resposta inflamatória desregulada pode estar correlacionada às perturbações induzidas pela atividade física no SNC (Rogers *et al.*, 2008). Acredita-se que a hiperamoniemia influencia na perda de *performance* por estimular/inibir estes dois sistemas podendo ser responsável/contribuinte para a fadiga central (Nybo & Secher, 2004; Nybo et al. 2005; Cooper *et al.*, 2007)

O aumento das concentrações de amônia leva ao desequilíbrio no *pool* de neurotransmissores em resposta a uma elevação da Gln devido à diminuição da capacidade regenerativa do ATP e repolarização da membrana (Guezennec, 1998 Suárez, 2002; Felipo & Butterworth, 2002 a). As alterações na *performance* devido à diminuição do rendimento muscular podem estar associadas ao surgimento da CF. Dois mecanismos de resposta relacionados ao aumento das concentrações de amônia tem sido postulados como responsáveis pelo surgimento da CF: 1) a elevação da síntese de Gln como sistema de desintoxicação, provocando o desequilíbrio do pool de neurotransmissores (Greenhaff, 1995; Snow, *et al.*, 1998) e 2) a alteração do metabolismo energético do conjunto neurônios pré-sináptico/pós-sináptico-astrócito, provocando a diminuição da capacidade regenerativa do ATP (Dechent, *et al.* 1999; Cooper, 2001; Llansola, *et al.*, 2002).

Esta tese não objetiva discutir fadiga central e hiperamoniemia, para melhor entendimento sobre o assunto leia os trabalhos dos seguintes grupos: Febbrario, MA; Nybo, L; Felipo,V.

g) Para desenvolvimento de processos inflamatórios

Desde o início deste milênio, o músculo tem sido proposto como um órgão imunológico e não somente como depósito de substrato. Propõe-se que a célula sadia responda ao exercício estimulando fortemente o sistema neuro-humoral, caracterizado pela mobilização de leucócitos e incremento do seu número na corrente sanguínea. Os leucócitos além dos tecidos adiposo, muscular e cerebral sintetizam a interleucina-6 (IL-6), que é um dos mais potentes mediadores inflamatórios; parece que este fenômeno é transitório e dependente da intensidade e duração da atividade.

Linfócitos, monócitos e natural-killers (NK) aumentam rapidamente em resposta à atividade e diminuem imediatamente ao término da sessão. Os neutrófilos circulantes aumentam mais lentamente e podem permanecer elevado por horas, mesmo que o exercício termine. Sabe-se que mesmo exercícios de 6 min. podem estimular a resposta inata do sistema imune (Cooper et al, 2007; Stewart, et al. 2007; Timmerman *et al.*; 2008).

As citoquinas agem como mediadores do catabolismo de proteína via inibição dos hormônios anabólicos e modulação nas enzimas responsáveis pela síntese e degradação de protéica. Shawcross & Jahan (2005) sugerem que a hiperamoniemia exacerba o processo inflamatório e promove alterações neurofisiológicas. Sabe-se que a hiperamoniemia induz o astrócito a aumentar a expressão de GLUT1, NOS e diminui GLT-1, GFAP e GLY-1; no neurônio há aumento de GLUT-3. Propõe-se que droga anti-inflamatória derivada de não esteróide *(non-steroidal anti-inflammatory drugs* - NSAIDs) sejam eficientes na diminuição da hiperamoniemia. NSAIDs tem sido usado na terapêutica de edema cerebral e *anastomosis* porto - cava.

Na Figura 14 é esquematizado os processos que envolvem inflamação e hiperamoniemia.

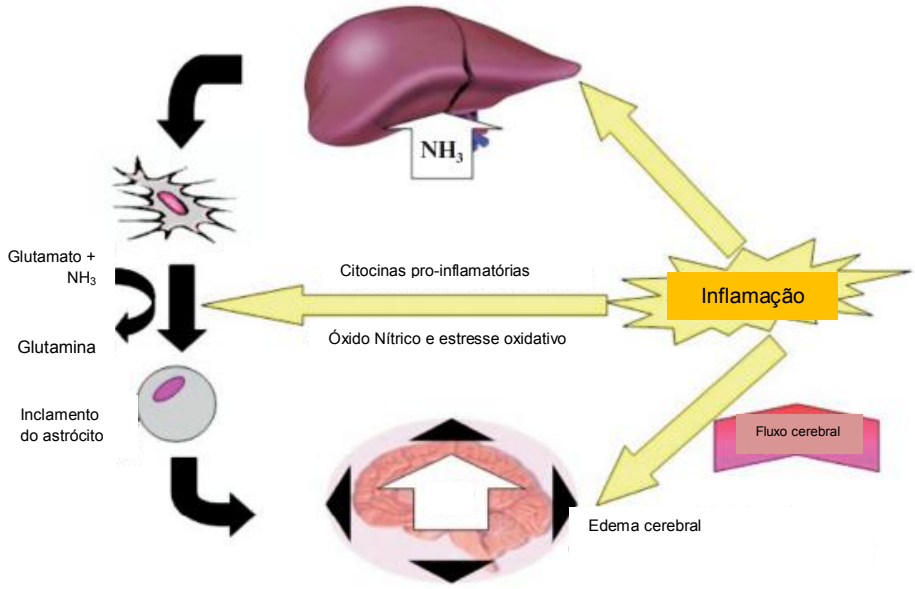


Figura 14- Ilustração da relação entre a inflamação e o fluxo sangüíneo cerebral na encefalopatia hepática. O astrócito detoxifica amônia sintetizando glutamina. Extraído de Shawcross & Jahan (2005).

O músculo expressa e libera miokinas que têm função autócrina e parácrina. Dentre ela a IL-6 age via IL-6Rα/gp130Rβ como homodímero ativando a phosphatidylinositol 3-Kinase e/ou AMPkinase incrementando a glicólise e a

oxidação de ácidos graxos. Durante o exercício há aumento da gliconeogênese e lipólise (Pedersen & Febbraio, 2008).

O exercício intenso induz ao aumento de citoquinas pró-inflamatórias (TNFα and IL-1α) e a uma síntese de IL-6. Esta liberação parece ser o balanço entre a liberação de citiquinas inibitórias (IL-1rα, sTNF-r1 and TNF-r2) e a anti-inflamatória IL-10. No Gráfico 2 observa-se a pequena elevação do TNFα e aumento de IL-6 que parece induzir a síntese de proteína C reativa (PCR). É importante notar que citoquinas anti-inflamatórias, receptores solúveis restringem a magnitude e duração da resposta inflamatória ao exercício (Pedersen, et al. 1999a; Pedersen, et al. 1999b; Ostrowski *et al.*, 1999). Acredita-se que o exercício seja tão eficiente na resposta inflamatória por estimular a expressão gênica de *peripheral blood mononuclear cells* (PBMCs) (Timmerman *et al.*; 2008). Outros efeitos biológicos podem estar associados a estes eventos, tais como: vasodilatação, agregação plaquetária e disfunções renal, hepática, pulmonar e cerebral (Starkie *et al.*, 2000; Starkie *et al.*, 2001a; Starkie *et al.*, 2001b; Pedersen *et al.*, 2007).

Veremos abaixo alguns modelos de injúria hepática para que possamos comparar com os efeitos do exercício e da hiperamoniemia.

Comparando o efeito da septicemia com o exercício observamos que a subida de IL-6 é precedida pela de TNF-α durante processos inflamatórios sistêmicos. Este fato é seguido pelo aparecimento do receptor antagonista de IL-1 (IL-1ra), IL-8, IL-10, *macrophage inflammatory protein α e β* (MIP-1α e MIP-1β) (Pedersen & Febbrario, 2008)

A IL-6 parece ser dependente da intensidade do exercício e seu nível pode aumentar até 100 vezes durante a atividade e retorna ao estado basal assim que o exercício termina. Vale ressaltar que em exercícios extremos, por exemplo numa maratona de 246 Km os níveis de IL-6 aumentaram 8000 vezes (Margeli *et al.*, 2005). Este fato pode explicar parte dos achados de Bessa *et al* (2008) – artigo número 4 desta Tese – onde foi medido elevação de AST, ALT e PCR em 300, 150 e 900% respectivamente, seguido por 300% de aumento da LDH e 30-40% de CK nos primeiros 100-150Km do alta intensidade *ultraendurance (High-Intensity Ultraendurance* - HIU). Em contraponto, Pedersen & Hoffman-Goetz (2000) utilizando citômetro de fluxo não conseguiu observar relação entre a elevação de Il-

6 e o aumento de células do setor branco durante o exercício mesmo com a suplementação de CHO.

Para melhor entendimento dos efeitos da injúria sobre o hepatócito reporte-se ao artigo 3 desta tese.

h) na indução de hepatotoxicidade

A injúria hepática parece estar correlacionada à apnéia do sono pelo aumento das espécies reativas de oxigênio e estresse oxidativo celular (Norman *et al.*, 2008). Savransky *et al.*, 2007 sugerem que uma única alta dose de acetaminofem (APAP) é eficiente para aumentar a lesão, além de ser comum encontrarmos overdose durante seu uso clínico.

O APAP é metabolizado no citocromo P450 no fígado, onde será utilizada pelo sistema glutationa com conseqüente síntese de NO, aumentando sua hepatotoxicidade, e aproximadamente 90% é conjugado com o ácido glicurônico ou sulfato na bile ou no sangue. Supõe-se que a injúria hepática é sinérgica ao APAP causando severo estresse oxidativo mitocondrial, resultando em inflamação.

Se compararmos dois modelos de injúria, os efeitos crônicos da hipóxia intermitente e do uso de APAP em normóxia, notamos aumento 2 vezes maior para ALT no modelo de hipóxia e o uso de APAP gerou aumento na bilirrubinemia, IL-6 e TNF-α e apoptose (Savransky *et al.*, 2009).

O artigo 2 desta tese refere-se a um estudo de caso onde a *N*-acetil-cisteína (NAC) é usada como diminuidor da hepatotoxicidade induzida por APAP. Kelly *et al.* (2008) não viram diferença no tempo de fadiga com suplementação de NAC.

Com base nos dados expostos nas sessões acima se observa a necessidade da avaliação prévia de humanos durante os estudos na tentativa de evitar resultados falso-positivos (Bassini-Cameron *et al.*, 2007a; Bassini-Cameron *et al.*, 2008; Carvalho-Peixoto *et al.*, 2007; Lazarin *et al.*, 2007; artigo número 2 e 7 desta tese).

I.III Suplementação

Underhill (1913 a; c) descreveu que a manipulação dietética, rica em carboidratos, gorduras e pobre em AAs tem uma notável retenção de nitrogênio no organismo de cães, devido à síntese protéica, que teve como conclusão “a presença de carboidratos não é um fator obrigatório na retenção de nitrogênio originário da amônia”.

Diversos estudos (Meneguello, 2003; Rooney, 2003; Spencer, 1991b) têm demonstrado a diminuição da amoniogênese com a utilização de diferentes protocolos de suplementação durante exercícios de duração e intensidades variadas. A suplementação de Arg (Schaefer, 2002), CHO (Snow, 2000), carnitina (Broad, et al. 2008), creatina (Bellinger, 2000), Gln (Bruce, 2001), tiveram como resultado a melhora da performance em atletas. No entanto, a administração de BCAA incrementa produção de amônia, Ala e Glu durante o exercício sub-máximo (MacLean, 1996).

i) suplementação de carboidrato e AAs

Muitos estudos (Tsintzas, 2001; Green, 2003; Nieman, 2003) tem investigado a suplementação de CHO para abastecer energicamente o músculo na tentativa de diminuir a gênese de amônia durante o exercício prolongado de moderada a alta intensidades. Experimentos (Kimber, 2003; Roeykens, 1998; Steensberg, 2002) tem demonstrado o efeito do conteúdo de glicogênio muscular no metabolismo de amônia como indicador da sua produção durante o exercício, ou seja, não é possível mensurar a suplementação de carboidrato sob a produção

e o efluxo de amônia, sem considerar a concentração de glicogênio muscular (que pode ser modificado durante a atividade) (Schulz e Hermann, 2003).

Spencer (1991b) verificou que no grupo suplementado com CHO, há grande utilização de hexose monofosfato a partir da glicose extracelular e diminuição da concentração de IMP ao final do exercício. A ingestão de carboidratos atenua o acúmulo de amônia muscular e plasmática durante os últimos estágios do exercício prolongado submáximo a partir da degradação de AAs (Snow, 2000).

Resultados *in vivo* sugerem que a insulina inibe a degradação protéica e estimula a sua síntese (Jefferson, 1977). Durante a hiperinsulinemia há supressão do catabolismo protéico, reduzindo o suporte de AAs para gerar ATP. Os níveis elevados de glicose e insulina, durante o exercício diminuem a gliconeogênese. Em situações de jejum e/ou hipoglicemia o fígado recebe sinalização do glucagon, que acelera o catabolismo de AAs e a gliconeogênese, havendo aumento da amônia dentro do hepatócito, acelerando o ciclo da uréia (Angus, 2002).

A suplementação de glicose resulta na elevação na concentração de insulina plasmática, durante o exercício prolongado e nas primeiras horas do período de recuperação, utilizando principalmente ácidos graxos livres para manutenção da atividade (Bowtell, 2000).

Snow *et al.* (2000) verificaram que a ingestão de CHO a 8% reduziu a produção de amônia muscular proveniente da degradação de AAs e observaram pequena contribuição do catabolismo de AMP na produção da amônia. Segundo alguns estudos, a associação de CHO com AAs maximiza o efeito de manutenção do *pool* de glicose, ressíntese de glicogênio e síntese protéica durante e após exercícios (Casey, *et al.*, 2000; Casey & Greenhaff; 2000; Burke, *et al.*, 2004; Ivy, *et al.*, 2002). Van Loon, *et al.* (2000) mostraram que 0,8 g de CHO . kg^{-1} associados com uma mistura de AAs resultou em aumento da ressíntese de glicogênio. Dietas pobres em CHO antes e depois de exercícios intensos podem diminuir os estoques de glicogênio de forma aguda e/ou crônica

Burke, *et al.* (2004) mostraram que bebidas a base de carboidratos com acréscimo de proteínas em torno de 20-25 % acelera a recuperação do glicogênio

muscular, principalmente nos primeiros quarenta minutos de esforço, conforme também demonstrou Ivy *et al.* (2003) e Lemmon *et al.*(2003).

Coyle, *et al.* (2001) verificaram que dietas pobres em CHO e gorduras podem modificar a utilização de substratos intramusculares, reduzindo a lipólise e a oxidação de FFA com possível prejuízo para programas de treinamento de endurance. Coyle (2004) verificou que dietas entre 200 a 400 gramas de CHO oferecem menos dificuldade para manutenção de atividades intensas e que acima de 400 gramas podem melhorar o desempenho e reduzir o risco de overtraining.

A toxicidade muscular pode estar relacionada com a queda do rendimento e da performance e manifestar-se com sinais de fraqueza muscular, ataxia, respiração forçada, taquicardia, apatia e incordenação motora. A toxicidade cerebral pode induzir redução da atenção e controle motor, manifestando sinais de letargia, alterações de comportamento e do pensamento coerente, irritabilidade, perda de lucidez e memória (Banister & Cameron, 1990; Monfort, *et al.*, 2002).

O Gráfico 4 sumariza parte dos resultados descritos acima, onde a depleção de glicogênio e a suplementação com BCAAs incrementam a produção de amônia em comparação a indivíduos normais sob a mesma intensidade de exercício.

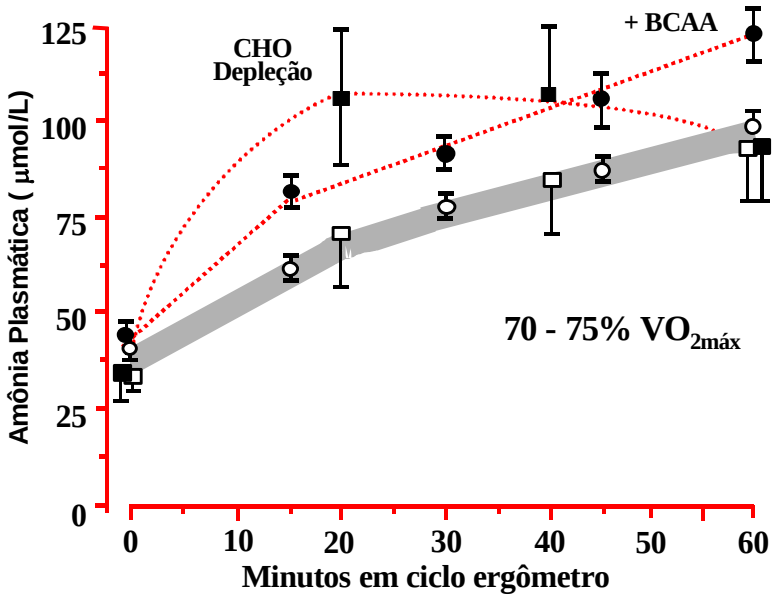


Gráfico 2- Depleção de CHO e a variação da amonemia em relação à suplementação com BCAAs. Mudanças na concentração plasmática de amônia em relação ao tempo de performance em cicloergomêtro a 70 -75 % do $VO_{2máx}$. A área escura é característica de resposta normal. O gráfico mostra o aumento da amônia plasmática causada pela depleção de CHO e a variação da amonemia em relação à suplementação com BCAAs. Adaptado de MacLean & Graham, 1993.

Estudos do Laboratório de Bioquímica de Proteínas tem demonstrado que a suplementação de CHO e Glu protegem da hiperamoniemia a partir de 60 min. (Carvalho-Peixoto, *et al.,* 2007 e Bassini-Cameron, *et al.*, 2008). Nesta tese praticantes de Jiu-Jitsu foram submetidos à dieta cetogênica e suplementação de Arg, que parece proteger da elevação da amonemia (artigo 7)

j) Suplementação de cafeína

Muitos estudos tem analisado o efeito ergogênico da cafeína sobre a *performance*, força e *endurance* em diversos protocolos empregados a atletas. Diferenças substanciais na dose e técnicas de investigação tem sido utilizada nestes estudos (Graham & Spriet, 1995, Graham, 1998; Kovacs *et al.*, 1998; Pasman, 1995).

Os efeitos ergogênicos da cafeína em doses de 3 a 5 mg . kg^{-1}, foram principalmente observados nos exercícios de endurance (Bell *et al.*, 1998; Bell & McLellan, 2003; Conway *et al.*, 2003; Cox *et al.*, 2002). Estas ações se embasam na liberação de catecolaminas, na lipólise e na glicogenólise levando ao menor consumo de glicogênio muscular (James *et al.*, 2004; James & Gregg, 2004a; James & Gregg, 2004b; Kalmar *et al.*, 2004). Já que é um inibidor PHD, a cafeína pontecializa a sinalização do AMPc, com conseqüente ativação das fosforilases e da lipase hormônio sensível (Thong *et al.*, 2002). Além disso, aquela xantina aumenta a mobilização do cálcio do retículo sarcoplasmático estimulando a contração muscular (Herrmann-Frank *et al.*, 1999; Warren *et al.*, 2001).

Devido ao antagonismo dos ARs, a cafeína aumenta a concentração e o estado de alerta diminuindo a percepção a fadiga (Denadai & Denadai, 1998; Dager *et al.*, 1999). O aumento do cortisol e das β–endorfinas atenua a percepção do sofrimento e promove euforia, capacitando o atleta a realizar longos períodos de atividade, evitando a exaustão e o desconforto (Laurent *et al.*, 2000; Motl *et al.*, 2003, Motl & Dishman, 2004).

O SNC é a via de ligação do sistema neuroendócrino com o sistema imune (Karlund *et al.*, 2000). Durante o exercício agudo, a cafeína promove aumento das concentrações plasmáticas de adrenalina, noradrenalina, insulina, GH, cortisol e β–endorfinas, melhorando a sinalização aos ARs que estão expressos tanto na membrana celular dos neutrófilos, linfócitos e plaquetas quanto no endotélio vascular, onde ambos estão implicados em processos inflamatórios. A ativação destes receptores regula a produção de mediadores inflamatórios ativados pelos receptores A_1 e inibidos pelos receptores A_{2A} e A_3 de adenosina (Holffman *et al.* 2002; Ikarugi *et al.*, 2003).

A Figura 15 demonstra o efeito do exercício extenuante e sugere a ação da adrenalina, GH e cortisol sobre as concentrações relativas de linfócitos e neutrófilos além do efeito “janela aberta”. Em condições de aumento de adenosina nos pulmões há uma super expressão dos receptores A_{2B} e A_3 de adenosina nos mastócitos pelo aumento histamina (Burns *et al.*, 1997; Pedersen & Toft, 2000;).

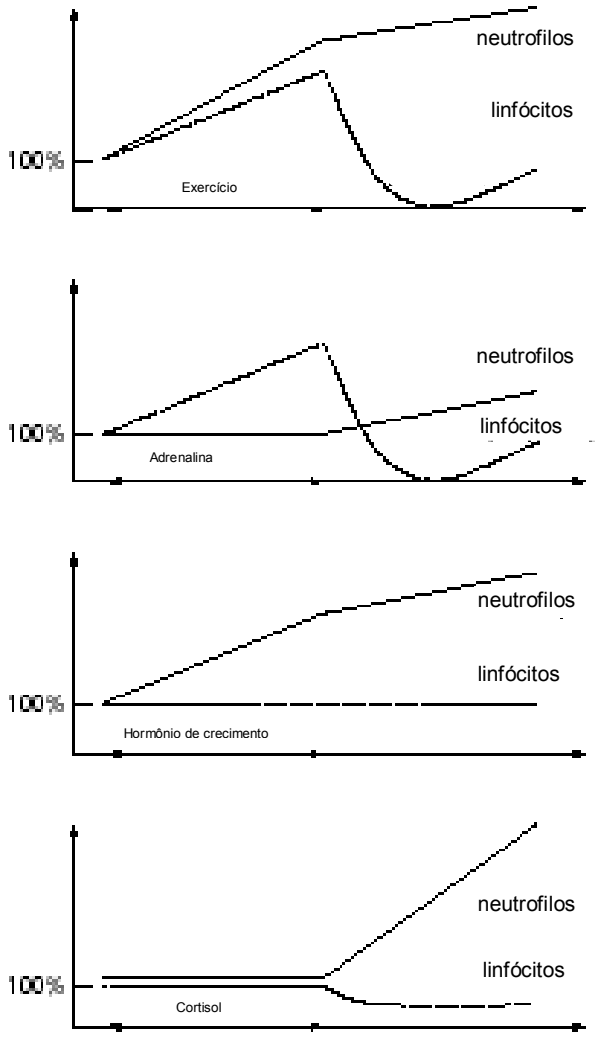


Figura 15- Modelo proposto do mecanismo neuroendócrino sobre o sistema imune. Extraído de Pedersen & Toft (2000).

No exercício prolongado a elevação dos hormônios supracitados afeta positivamente as respostas metabólicas durante o *endurance*, tais como: aumento no FFAs, AMPc, glicerol, glicose e lactato, diminuindo a espoliação de glicogênio (Graham *et al.*, 1991 b, 1995; Greer *et al.*, 2000; Thong *et al.*, 2002; Shearer *et al.*, 2003; Battran *et al.*, 2004).

Poucos estudos têm investigado os efeitos ergogênicos da cafeína sobre o desempenho físico em exercícios de alta intensidade e curta duração (força, velocidade e potência) nos primeiros 10 min. (Pasman, 1995). Além disso, os

resultados encontrados até o momento têm sido bastante controversos, impossibilitando conclusões mais definitivas a esse respeito. As maiores dificuldades para interpretação dos resultados produzidos por esses estudos concentram-se nos diferentes delineamentos utilizados, nas diferentes doses de cafeína administradas, nas diferenças entre os protocolos experimentais e na falta de uma maior rigidez metodológica. Parte destes estudos têm investigado a influência das catecolaminas sobre as respostas metabólicas durante este tipo de atividade (Graham *et al.*, 1991b; Jackman *et al.*, 1996; Greer *et al.*, 1998; Van Soeren *et al.*, 1998; Ikarugi *et al.*, 2003).

Costill *et al.* (1978) foi um dos o primeiros a demonstrar que a ingestão de cafeína (330 mg) uma hora antes do exercício exaustivo aumenta significativamente a performance em ciclistas. Outros estudos comprovaram este achado e demonstraram que a cafeína aumenta a contratilidade muscular, o tempo de aparecimento da exaustão e a performance durante o exercício prolongado de moderada a alta intensidade. Poucas investigações não observaram efeito ergogênico da cafeína (Lehmann *et al.*, 1992; Ferrauti *et al.*, 1997; Bell *et al.*, 1998).

A suplementação da cafeína em humanos foi estudada em doses entre 3 a 9 mg . kg^{-1} com resultados que mostraram o incremento de 20 - 50% do tempo de exercício e o retardo da fadiga (Spriet *et al.*, 1995; Grandjean *et al.*, 2000; McLean *et al.*, 2002; Davis *et al.*, 2003). Graham & Spriet (1995) mostraram que doses entre 3 e 6 mg . kg^{-1} de cafeína exercem os mesmos efeitos ergogênicos, indicando que não existe relação entre as dosagens de cafeína e o aumento na performance outro estudo postulou que o efeito ergogênico máximo da cafeína ocorre em doses próximas a 3 mg . kg^{-1} (Conway *et al.*, 2003). Em diversas investigações foram detectados benefícios ergogênicos na dose de 5 mg . kg^{-1}, comparando sujeitos habituados ou não a cafeína e diferentes protocolos de utilização (Bell *et al.*, 2002; Cox *et al.*, 2002; Graham *et al.*, 1998).

Thong *et al.* (2002) observaram um aumento significativo na mobilização de FFAs e glicerol como substrato energético após suplementação com cafeína (5 mg . Kg^{-1}), onde a sinergia da cafeína e o exercício reduziram a atividade da glicogênio sintetase (GS) antes e durante a infusão de insulina, concluindo que a cafeína

melhora a resposta insulínica (Shearer *et al.*, 2003; Battran *et al.*, 2004; Petrie *et al.*, 2004). A administração de cafeína isoladamente ou associada à efedrina, levou a um aumento significante nos ácidos graxos livres, glicerol e lactato. A melhora no tempo de desempenho até a exaustão somente foi observada na forma associada (Bell *et al.*, 1998) De acordo com os achados parece que a cafeína altera as funções metabólicas, porém estas alterações não são suficientes para prolongar o tempo de aparecimento da exaustão durante o exercício.

REFERÊNCIA BIBLIOBRÁFICA

- Abbracchio, M.P. & Burnstock, G. Purinergic signalling: pathophysiological roles. Jpn J Pharmacol. 1998; 78:113-45.
- Albrecht, J. & Wegrzynowicz, M. Endogenous neuro-protectants in ammonia toxicity in the central nervous system: facts and hypotheses. Metab Brain Dis. 2005; 20:253-63.
- Ament, W.; Huizenga, J.R.; Mook, G.A.; Gips, C.H.; Verkerke,G.J. Lactate and ammonia concentration in blood and sweat during incremental cycle ergometer exercise. Int J Sports Med.1997; 18:35-9.
- Angus, D.J.; Febbraio, M.A.; Hargreaves, M. Plasma glucose kinetics during prolonged exercise in trained humans when fed carbohydrate. Am J Physiol Endocrinol Metab. 2002; 283:E573-7.
- Aparicio, M.; Gin, H.; Potaux, L.; Bouchet, J.L.; Morel, D.; Aubertin, Effect of a ketoacid diet on glucose tolerance and tissue insulin sensitivity. J. Kidney Intern. 1989; 36:231-235.
- Baldwin, J.; Snow, R.J.; Gibala, M.J.; Granham, A.; Howarth, K.; Febbraio, M.A. Glycogen availability does not affect the TCA cycle or TAN pools during prolonged, fatiguing exercise. J Appl Physiol. 2003; 94:2181-7.
- Banister, E.W. & Cameron, B.J., Exercise-induced hyperammonemia: Peripheral and central effects. Int J Sports Med. 1990; 11:S129-142.
- Bassini-Cameron, A.; Monteiro, A.; Gomes, A.; Werneck-de-Castro, J.P.; Cameron, L.C. Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way. Br J Sports Med. 2008; 42:260-6.
- Bassini-Cameron, A.; Sweet, E.; Bottino, A.; Bittar, C.; Veiga, C.; Cameron, L.C. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions.Br J Sports Med. 2007; 41:523-30.
- Battram, D.S.; Shearer, J.; Robinson, D.; Graham, T.E. Caffeine ingestion does not imped the resyntesis of proglicogen and macroglicogen after prolonged exercise and carboydrate supplementation in humans. J Appl Physiol. 2004; 96:943-50.

- Bavransky, V.; Bevans, S.; Nanayakkara, A.; Li, J.; Smith, P.L.; Torbenson, M.S.; Polotsky, V.Y. Chronic intermittent hypoxia causes hepatitis in a mouse model of diet-induced fatty liver. Am J Physiol Gastrointest Liver Physiol. 2007; 293:G871-7.
- Bell, D.G.; Jacobs, I.; Zamecnik, J. Effects of caffeine, ephedrine and their combination on time to exhaustion during high-intensity exercise. Eur J Appl Physiol Occup Physiol. 1998; 77:427-33.
- Bell, D.G.; McLellan, T.M. Effect of repeated caffeine ingestion on repeated exhaustive exercise endurance. Med Sci Sports Exerc. 2003; 35:1348-54.
- Bellinger, B.M.; Bold, A.; Wilson, G.R.; Noakes, T.D.; Myburgh, K.H. Oral creatine supplementation decreases plasma markers of adenine nucleotide degradation during a 1-h cycle test. Acta Physiol Scand. 2000; 170:217-24.
- Benedict, S.R. & Nash, T.P. Jr. On the question of the origin of urinary ammonia. J Biol Chem. 1929; 82:673-8.
- Bessa, A.; Nissenbaum, M.; Monteiro, A.; Gandra, P.G.; Nunes, L.S.; Bassini-Cameron, A.; Werneck-de-Castro JP, de Macedo, D.V.; Cameron, L.C. High-intensity ultraendurance promotes early release of muscle injury markers. Br J Sports Med. 2008; 42:589-93.
- Biver, S.; Belge, H.; Bourgeois, S.; Van Vooren, P.; Nowik, M.; Scohy, S.; Houillier, P.; Szpirer, J.; Szpirer, C.; Wagner, C.A.; Devuyst, O.; Marini, A.M. A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility. Nature. 2008; 456:339-43.
- Bliss, S. The amide nitrogen of blood. Science. 1928; 67:515-6.
- Blomstrand, E. & Saltin, B. Effect of muscle glycogen on glucose, lactate and amino acid metabolism during exercise and recovery in human subjects. J Physiol. 1999; 514:293-302.
- Bowtell, J.L.; Leese, G.P.; Smith, K.; Watt, P.W.; Nevill, A.; Rooyackers, O.; Wagnmakers, A.J.M.; Rennie, M.J. Effect of oral glucose on leucine turnover in human subjects at rest and during exercise at two levels of dietary protein. J Physiol. 2000; 525:271-81.
- Broad, E.M.; Maughan, R.J.; Galloway, S.D. Carbohydrate, protein, and fat metabolism during exercise after oral carnitine supplementation in humans. Int J Sport Nutr Exerc Metab. 2008; 18:567-84.

- Brooks, G.A., Mercier, J. Balance of carbohydrate and lipid utilization during exercise: the “crossover” concept. J Appl Physiol. 1994; 76:2253-61.
- Brosnan, J.T. Glutamate, at the interface between amino acid and carbohydrate metabolism. J Nutr. 2000; 130:988S-90S.
- Bruce, M.; Constantin-Teodosiu, D.; Greenhaff, P.L.; Boobis, L.H.; Williams, C.; Bowtell, J.L. Glutamine supplementation promotes anaplerosis but not oxidative energy delivery in humans skeletal muscle. Am J Physiol Endocrinol Metabol. 2001; 280:E669-E675.
- Brustovetsky, N.; Brustovetsky, T.; Dubinsky, J.M. On the mechanisms of neuroprotection by creatine and phosphocreatine. J Neurochem. 2001; 76:425-34.
- Burke, L.M.; Kiens, B.; Ivy, J.L. Carbohydrates and fat for training and recovery. J Sports Sci. 2004; 22:15-30.
- Burns, A.M.; Keogan, M.; Donaldson, M.; Brown, D.L.; Park, G.R. Effects of inotropes on human leucocytes numbers, neutrophil funcion and lymphocyte subtypes. Br J l Anaesthesia 1997; 78:530-5.
- Butterworth, R.F. Glutamate transporter and receptor function in disorders of ammonia metabolism. Ment Retard Dev Disabil Res Rev. 2001; 7:276-9.
- Butterworth, R.F. Mental Retardation and Developmental Disabilities. Res Rev. 2001; 7:276-9.
- Butterworth, R.F. Pathophysiology of hepatic encephalopathy: a new look at ammonia. Metab Brain Dis. 2002; 17:221-7.
- Buttrose, M.D.; McKellar, D.; Welbourne, T.C. Gut-liver interaction in glutamine homeostasis: portal ammonia role in uptake and metabolism. Am J Physiol. 1987; 252:E746-50.
- Cairns, S.P. Lactic acid and exercise performance: culprit or friend? Sports Med. 2006; 36:279-91.
- Campbell, B.; Roberts, M.; Kerksick, C.; Wilborn, C.; Marcello, B.; Taylor, L.; Nassar, E.; Leutholtz, B.; Bowden, R. Pharmacokinetics, safety, and effects on exercise performance of l-arginine alpha-ketoglutarate in trained adult men. Nutrition. 2006; 22:872–81.

- Carvalho-Peixoto, J.; Alves, R.; Cameron, L.C. Glutamine and carbohydrate supplements reduce ammonemia increase during endurance field exercise. Appl Physiol Nutr Metab. 2007; 32:1186-90.
- Casey, A.; Greenhaff, P.L. Does dietary creatine supplementation play a role in skeletal muscle metabolism and performance? Am J Clin Nutr. 2000; 72:607S-17S.
- Casey, A.; Mann, R.; Banister, K.; Fox, J.; Morris, P.G.; Macdonald, I.A.; Greenhaff, P.L. Effect of carbohydrate ingestion on glycogen resynthesis in human liver and skeletal muscle, measured by (13)C MRS. Am J Physiol Endocrinol Metab. 2000; 278:E65-75.
- Cederbaum, S.D.; Yu, H.; Grody, W.W.; Kern, R.M.; Yoo, P.; Iyer, R.K.; Arginases I and II: do their functions overlap? Mol Genet Metab. 2004; 81:S38-44.
- Cheung, C.W.; Cohen, N.S.; Raijman, L. Channeling of urea cycle intermediates in situ in permeabilized hepatocytes. J Biol Chem. 1989; 264:4038-44.
- Chicharro, J.L.; Vaquero, A.F.; Tello, R.; Pérez, M.; Lucía, A. Relationship between lactate and ammonia thresholds in heart transplant patients. Chest. 1996; 110:693-7.
- Cohen, N.S.; Cheung, C.W.; Kyan, F.S., Jones E.E.; Raijman, L. Mitochondrial carbamyl phosphate and citrulline synthesis at high matrix acetylglutamate. J Biol Chem. 1982; 257:6898-907.
- Cohen, N.S.; Cheung, C.W.; Raijman, L. Channeling of extramitochondrial ornithine to matrix ornithine transcarbamylase. J Biol Chem. 1987; 262:203-8.
- Colombatto, S.; Fasulo, L.; Mondardini, A.; Malabaila, A.; Grillo, M.A. Effect of caffeine on ornithine metabolism in rat brain, liver and kidney. J Biochem. 1989; 38:75-82.
- Conway, K.J.; Orr, R.; Stannard, S.R. Effect of a divided caffeine dose on endurance cycling performance, postexercise urinary caffeine concentration, and plasma paraxanthine. J Appl Physiol. 2003; 94:1557-62
- Cooper, A.F. Whose illness is it anyway? Why patient perceptions matter. Int J Clin Pract. 1998; 52:551-6.
- Cooper, A.J. Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. Ment Retard Dev Disabil Res Rev. 2001; 7:280-6.

- Cori, C.F. & Cori, G.T. Glicogen formation in the liver from d- and l-lactic acid. J Biol Chem. 1928; 79:389-403.
- Costill, D.L.; Dalsky, G.P.; Fink, W.J. Effects of caffeine ingestion on metabolism and exercise performance. Med Sci Sports. 1978; 10:155-8.
- Cox, G.R.; Desbrow, B.; Montgomery, P.G.; Anderson, M.E.; Bruce, C.R.; Macrides, T.A.; Martin, D.T.; Moquin, A.; Roberts, A.; Hawley, J.A.; Burke, L.M. Effect of different protocols of caffeine intake on metabolism and endurance performance. J Appl Physiol. 2002; 93:990-9.
- Coyle, E.F.; Jeukendrup, A.E.; Oseto, M.C.; Hodgkinson, B.J.; Zderic, T.W. Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise. Am J Physiol Endocrinol Metab. 2001; 280:E391-8.
- Dager S.R.; Layton, M.E.; Strauss, W.; Richards, T.; Heide, A.; Friedman, S.; Artru, A.; Hayes, C.E.; Posse, S. Human brain metabolic response to caffeine and the effects of tolerance. Am J Psychiatry. 1999; 156:229-37.
- Damink, O.S.W.; Deutz, N.E.; Dejong, C.H.; Soeters, P.B.; Jalan, R. Interorgan ammonia metabolism in liver failure. Neurochem Int. 2002; 41:177-88.
- Davidson, J.N.; Chen, K.C.; Jamison, R.S.; Musmanno, L.A.; Kern, C.B. The evolutionary history of the first three enzymes in pyrimidine biosynthesis. Bioessays. 1993; 15:157-64.
- Davis, J.; Zhao, M.Z.; Stock, H.S.; Mehl, K.A.; Buggy, J.; Hand, G.A. Central nervous system effects of caffeine and adenosine on fatigue. Am J Physiol Regul Integr Comp Physiol. 2003; 284:R399-R404.
- Dechent, P.; Pouwels, P.J.W.; Wilken, B.; Hanefeld, F.; Frahm, J. Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. Am J Physiol. 1999; 277:R698–R704.
- Denadai B.S. & Denadai, L.D.R. Effects of caffeine on time to exhaustion in exercise performed below and above the anaerobic threshold. Braz J Med Biol Res. 1998; 31:581-5.
- Derave, W.; Bouckaert, J.; Pannier, J.L. Gender differences in blood ammonia response during exercise. Arch Physiol Biochem. 1997; 105:203-9.
- DuBose, T.D. Jr.; Good, D.W.; Hamm, L.L.; Wall, S.M. Ammonium transport in the kidney: new physiological concepts and their clinical implications. J Am Soc Nephrol. 1991; 1:1193-203.

- Febbraio, M.A. & Dancey, J. Skeletal muscle energy metabolism during prolonged, fatiguing exercise. J Appl Physiol. 1999; 87:2341-7.
- Febbraio, M.A.; Flanagan, T.R.; Snow, R.J.; Zhao, S.; Carey, M.F. Effect of creatine supplementation on intramuscular TCr, metabolism and performance during intermittent, supramaximal exercise in humans. Acta Physiol Scand. 1995; 155:387-95.
- Felipo, V. & Butterworth, R.F. Neurobiology of ammonia. Prog Neurobiol. 2002; 67:259-79 a.
- Felipo, V. Chronic hyperammonemia impairs the glutamate-nitric oxide-cyclic GMP pathway in cerebellar neurons in culture and in the rat in vivo. Eur J Neurosci. 1998, 10:3201-9.
- Felipo, V. Modulation of glutamine synthesis in cultured astrocytes by nitric oxide. Cell Mol Neurobiol. 1997; 17:433-45.
- Felipo, V. & Butterworth, R.F. Mitochondrial dysfunction in acute hyperammonemia. Neurochem Int. 2002; 40:487-91a.
- Felipo, V. & Butterworth, R.F. Neurobiology of ammonia. Prog Neurobiol. 2002; 67:259-79b.
- Ferrauti, A.; Weber, K.; Strüder, H.K. Metabolic and ergogenic effects of carbohydrate and caffeine beverages in tennis. J Sports Med Phys Fitness. 1997; 37:258–66.
- Fürst, P. Amino acid metabolism in uremia. J Am Coll Nutr. 1989; 8:310-23.
- Furst, A. Can nutrition affect chemical toxicity? Int J Toxicol. 2002; 21:419-24.
- Garg, S.K.; Banerjee, R.; Kipnis, J. Neuroprotective immunity: T cell-derived glutamate endows astrocytes with a neuroprotective phenotype. J Immunol. 2008; 180:3866-73.
- Gibala, M.J.; MacLean, D.A.; Graham, T.E.; Saltin, B. Anaplerotic processes in human skeletal muscle e during brief dynamic exercise. J Physiol. 1997; 502:703-13.
- Gibala, M.J.; MacLean, D.A.; Graham, T.E.; Saltin, B. Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. Am J Physiol. 1998; 275:E235-42.

- Gibala, M.J.; Peirce, N.; Constantin-Teodosiu, D.; Greenhaff, P.L. Exercise with low muscle glycogen augments TCA cycle anaplerosis but impairs oxidative energy provision in humans. J Physiol. 2002; 540:1079-86.
- Giordano, G.; Sanchez-Perez, A.M.; Burgal, M.; Montoliu, C.; Costa, L.G.; Felipo, V. Chronic exposure to ammonia induces isoform-selective alterations in the intracellular distribution and NMDA receptor-mediated translocation of protein kinase C in cerebellar neurons in culture. J Neurochem. 2005; 92:143-57.
- Godel, H.; Graser, T.; Földi, P.; Pfaender, P.; Fürst, P. Measurement of free amino acids in human biological fluids by high-performance liquid chromatography. J Chromatogr. 1984; 297:49-61.
- Graham, T.E.; Hibbert, E.; Sathasivam, P. Metabolic and exercise endurance effects of coffee and caffeine ingestion. J Appl Physiol. 1998; 85:883-9.
- Graham, T.E.; Sgro, V.; Friars, D.; Gibala, M.J. Glutamate ingestion: the plasma and muscle free amino acid pools of resting humans. Am J Physiol Endocrinol Metab. 2000; 278:E83-9.
- Graham, T.E. & Spriet, L.L. Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. J Appl Physiol. 1995; 78:867-74.
- Graham, T.E. & MacLean, D.A. Ammonia and amino acid metabolism in human skeletal muscle during exercise. Am J Physiol Pharmacol. 1990; 70:132-41.
- Graham, T.E.; Bangsbo, J.; Gollnick, P.D.; Juel, C.; Saltin, B. Ammonia metabolism during intense dynamic exercise and recovery in humans. Am J Physiol. 1990; 259:E170-6.
- Graham, T.E.; Turcotte, L.P.; Kiens, B.; Richter, E.A. Effect of endurance training on ammonia and amino acid metabolism in humans. Med Sci Sports Exerc. 1997; 29: 646-53.
- Grandjean, A.C.; Bannick, K.E.; Haven, M.C. The effect of caffeinated, non-caffeinated, caloric and non-caloric beverages on hydration. Am Coll Nutr. 2000; 9: 591-600.
- Granham, A.; Howarth, K.; Febbraio, M.A. Glycogen availability does not affect the TCA cycle or TAN pools during prolonged, fatiguing exercise. J Appl Physiol. 2003; 94:2181–7.

- Green, J.K.; Croaker, S.J.; Rowbottom, D.G. Carbohydrate supplementation and exercise-induced changes in T-lymphocyte function. J Appl Physiol. 2003; 95:1216-23.
- Greenhaff, P.L. Creatine and its application as an ergogenic aid. Int J Sport Nutr. 1995; 5:S100-10.
- Greer, F., McLean, C.; Graham, T.E. Caffeine, performance and metabolism during repeated Wingate exercise tests. J Appl Physiol. 1998; 85:1502-8.
- Greer, F.; Friars, D.; Graham, T.E. Comparison of caffeine and treophylline ingestion: exercise metabolism and endurance. J Appl Physiol. 2000; 89:1837-44.
- Gropman, A.L. & Batshaw, M.L. Cognitive outcome in urea cycle disorders. Mol Genet Metab. 2004; 81:S58-62.
- Gropman, A.L.; Summar, M.; Leonard, J.V. Neurological implications of urea cycle disorders. J Inherit Metab Dis. 2007; 30:865-79.
- Guezennec, C.Y.; Abdelmalki, A.; Merino, D.; Bigard, X.; Berthelot, B.M.; Pierard, C.; Peres, C.P. Effects of prolonged exercise on brain ammonia and acids. Int J Sports Med. 1998; 19:323-27.
- Hahn, M.; Massen, O.; Nencki, M.; Pavlov, I. Die Eck'sche fistel zwischen der unteren hohlvene und der pfortader und ihre folgen fur den organismus. Arch Exp Pathol Pharm. 1893; 32:161–210.
- Harris, R.C.; Söderlund, K.; Hultman, E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. Clin Sci (Lond). 1992; 83:367-74.
- Hellsten, Y.; Richter, E.A.; Kiens, B.; Bangsbo, J. AMP deamination and purine exchange in human skeletal muscle during and after intense exercise. J Physiol. 1999; 520:909-20.
- Hermenegildo, C.; Montoliu, C.; Llansola, M.; Muñoz, M.D.; Gaztelu, J.M.; Miñana, M.D.; Hertz, L.; Yu, A.C.; Kala, G.; Schousboe, A. Neuronal-astrocytic and cytosolic-mitochondrial metabolite trafficking during brain activation, hyperammonemia and energy deprivation. Neurochem Int. 2000; 37:83-102.
- Herrmann-Frank, A.; Luttgau, H.C.; Stephenson, D.G. Caffeine and excitation-contraction coupling in skeletal muscle: a stimulating story. J Muscle Res and Cell Motility. 1999; 20:223-37.

- Hoffman, J.R.; Maresh, C.M.; Newton, R.U.; Rubin, M.R.; French, D.N.; Volek, J.S.; Sutherland, J.; Robertson, M.; Gomes, A.L.; Ratamess, N.A.; Kang, J.; kraemer, W. Performance, biochemical, and endocrine changes during a competitive football game. Med Sci Sports Exerc. 2002; 34: 1845-53.
- Huang, M. & Graves, L.M. De novo synthesis of pyrimidine nucleotides; emerging interfaces with signal transduction pathways. Cell Mol Life Sci. 2003; 60:321-36.
- Huizenga, J.R.; Tangerman, A.; Gips, C.H. Determination of ammonia in biological fluids. Ann Clin Biochem. 1994; 31:529-43.
- Ikarugi, H., Shibata, M.; Shibata, S.; Ishii, H.; Taka, T.; Yamamoto, J. High intensity exercise enhances platelet reactivity to shear stresss and coagulation during and after exercise. Pathophysiol. Haemost. Thromb. 2003; 33:127-33.
- Ip, Y.K.; Tam, W.L.; Wong, W.P.; Loong, A.M.; Hiong, K.C.; Ballantyne, J.S.; Chew, S.F. A comparison of the effects of environmental ammonia exposure on the Asian freshwater stingray Himantura signifer and the Amazonian freshwater stingray Potamotrygon motoro. J Exp Biol. 2003; 206:3625-33.
- Ivy, J.L.; Goforth, H.W. Jr; Damon, B.M.; McCauley, T.R.; Parsons, E.C.; Price T.B. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. J Appl Physiol. 2002; 93:1337-44.
- Ivy, J.L.; Res, P.T.; Sprague, R.C.; Widzer, M.O. Effect of a carbohydrate-protein supplement on endurance performance during exercise of varying intensity. Int J Sport Nutr Exerc Metab. 2003; 13:382-95.
- Jackman, M.; Wendling, P.; Graham, T.E. Metabolic, catecholamine, and endurance responses to caffeine during intense exercise. J Appl Physiol. 1996: 81: 1658-63.
- James, J.E. Critical review of dietary caffeine and blood pressure: a relationship that should be taken more seriously. Psychosom Med. 2004; 66:63-71 b.
- James, R.S.; Wilson, R.S.; Askew, G.N. Effects of caffeine on mouse skeletal muscle power output during recovery from fatigue. J Appl Physiol. 2004; 96:545-52.
- James, J.E. & Gregg, M.E. Hemodinamic effects of dietery caffeine sleep restriction, and laboratory stress. Psychophysiology. 2004; 41:914-23 a.

- Jefferson, L.S. & Rannels, S.R. Regulation by insulin of amino acid release and protein turnover in the perfused rat hemicorpus. J Biolog Chemistry. 1977; 252:1476-83.
- Jones, E.A. Fatigue complicating chronic liver disease. Metab Brain Dis. 2004; 19:421-9.
- Jones, E.A. Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy. Metab Brain Dis. 2002; 17:275-81.
- Kato, T.; Matsumura, Y.; Tsukanaka, A.; Harada, T.; Kosaka, M.; Matsui, N. Effect of low oxygen inhalation on changes in blood pH, lactate, and ammonia due to exercise. Eur J Appl Physiol. 2004; 91:296-302.
- Katunuma, N.; Okada, M.; Nishii, Y. Regulation of the urea cycle and TCA cycle by ammonia. Adv Enzyme Regul. 1966; 4:317-36.
- Kelly, A. & Stanley, C.A. Disorders of glutamate metabolism. Ment Retard Dev Disabil Res Rev. 2001; 7:287-95.
- Kelly, M.K.; Wicker, R.J.; Barstow, T.J.; Harms, C.A. Effects of N-acetylcysteine on respiratory muscle fatigue during heavy exercise. Respir Physiol Neurobiol. 2009; 165:67-72.
- Kimber, N.E.; Heigenhauser, G.J.F.; Spriet, L.L.; Dyck, J.D. Skeletal muscle fat and carbohydrate metabolism during recovery from glycogen-depleting exercise in humans. J Physiol. 2003; 548:919-27.
- Kipnis, J.; Derecki, N.C.; Yang, C.; Scrable, H. Immunity and cognition: what do age-related dementia, HIV-dementia and 'chemo-brain' have in common? Trends Immunol. 2008; 29:455-63.
- Klahr, S.; Tripathy, K.; Lotero, H. Renal regulation of acid-base balance in malnourished man. Am J Med. 1970; 48:325-31.
- Knepper, M.A.; Packer, R.; Good, D.W. Ammonium transport in the kidney. Physiol Rev. 1989; 69:179-249.
- Knepper, M.A. Physiology: Courier service for ammonia. Nature. 2008; 456:336-7.
- Korf, J. Intracerebral trafficking of lactate in vivo during stress, exercise, electroconvulsive shock and ischemia as studied with microdialysis. Dev Neurosci. 1996; 18:405-14.

- Kovacs, E.M.; Stegen, J.H.C.H.; Brouns, F. Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. J Appl Physiol. 1998; 85:709-15.
- Krzywkowski, K.; Petersen, E.W.; Ostrowski, K.; Link-Amster, H; Boza, J.; Halkjaer-Kristensen, J.; Pedersen, B.K. Effect of glutamine and protein supplementation on exercise-induced decreases in salivary IgA. J Appl Physiol. 2001; 91:832-8.
- Laurent, D., Schneider, K.E.; Prusaczyk, W.K.; Franklin, C.; Vogel, S.M.; Krssak, M.; Petersen, K.F.; Goforth, H.W.; Shulman, G.I. Effects of caffeine on muscle glycogen utilization and the neuroendocrine axis during exercise. J Cli Endocrino & Metabolism. 2000; 85:2170-5.
- Lazarim, F.L.; Antunes-Neto, J.M.; da Silva, F.O.; Nunes, L.A.; Bassini-Cameron, A.; Cameron, L.C.; Alves, A.A.; Brenzikofer, R.; de Macedo, D.V. The upper values of plasma creatine kinase of professional soccer players during the Brazilian National Championship. J Sci Med Sport. 2009; 12:85-90.
- Lehmann, M.; Dickhuth, H.H.; Gendrisch, G.; Lazar, W.; Thum, M.; Kaminsky, R.; Aramendi, E.P.; Wieland, W.; Keul, J. Training-Overtraing. A Prospective, Experimental Study with Experimental Middle-and long-distance runners. Int J Sports Med. 1991; 12:444-52.
- Lehmann, M.; Baumgartl, P.; Wiesenack, A.; Seidel, H.; Baumann, S.; Fischer, S.; Spori, U.; Gendrisch, G.; Kaminsky, R.; Keul, J. Training-Overtraing. Influence of a defined increase in training volume vs training intensity on performance, catecholamines and some metabolic parameters in experienced middle and long distance runners. Eur J Appl Physiol. 1992; 64:169-77.
- Lemmon, S.K.; Sens, D.A.; Buse, M.G. Insulin stimulation of glucose transport and metabolism in a human Wilms' tumor-derived myoblast-like cell line: modulation of hormone effects by glucose deprivation. J Cell Physiol. 1985; 125:456-64.
- Llansola, M.; Erceg, S.; Hernández-Viadel, M.; Felipo, V. Prevention of ammonia and glutamate neurotoxicity by carnitine: molecular mechanisms. Metab Brain Dis. 2002; 17:389-97.
- Lowenstein, J.M.; Goodman, M.N. The purine nucleotide cycle in skeletal muscle. Fed Proc. 1978; 37:2308-12.

- Lowenstein, J.M. The purine nucleotide cycle revisited. Int J Sports Med. 1990; 11:411.
- Machado, M. & Cameron, L.C. Suplementação aguda de creatina em praticantes de musculação: efeito na composição corporal. F & P J. 2002; 1:41-5.
- MacLean, D.A.; Graham, T.E.; Saltin, B. Stimulation of muscle ammonia production during exercise following branched-chain amino acid supplementation in humans. J Physiol. 1996; 493:909-22.
- MacLean, D.A.; Graham, T.E. Branched-chain amino acid supplementation augments plasma ammonia responses during exercise in humans. J Appl Physiol. 1993; 74:2711-7.
- MacLean, D.A.; Graham, T.E.; Saltin, B. Stimulation of muscle ammonia production during exercise following branched-chain amino acid supplementation in humans. J Physiol. 1996; 493:909-22.
- Makoff, A.J.; Radford, A.; Genetics and biochemistry of carbamoyl phosphate biosynthesis and its utilization in the pyrimidine biosynthetic pathway. Microbiol Rev. 1978; 42: 307-28.
- Manning, T.S.; Gibson, G.R. Microbial-gut interactions in health and disease. Prebiotics. Best Pract Res Clin Gastroenterol. 2004; 18:287-98.
- Margeli, A.; Skenderi, K.; Tsironi, M.; Hantzi, E.; Matalas, A.L.; Vrettou, C.; Kanavakis, E.; Chrousos, G.; Papassotiriou, I. Dramatic elevations of interleukin-6 and acute-phase reactants in athletes participating in the ultradistance foot race spartathlon: severe systemic inflammation and lipid and lipoprotein changes in protracted exercise. J Clin Endocrinol Metab. 2005; 90:3914-8.
- Masud, T.; Young, V.R.; Chapman, T.; Maroni, B.J. Adaptive responses to very low protein diets: the first comparison of ketoacids to essential amino acids. Kidney Int. 1994; 45:182-92.
- McCullough, H. The determination of ammonia in whole blood by a direct colorimetric method. Clin Chim Acta. 1967; 17:297-304.
- McLean, C. & Graham, T.E. Effects of exercise and thermal stress on caffeine pharmacokinetics in men and eumenorrheic women. J Appl Physiol. 2002; 93:1471-1478.

- Meneguello, M.O.; Mendonça, J.R.; Lancha, A.H.; Rosa, L.B.P.C. Effect of arginine, ornithine and citrulline suplementation upon performance and metabolism of trained rats. Cell Biochem Funct. 2003; 21:85-91.
- Mitch, W.E.; Abras, E.; Walser, M. Long-term effects of a new ketoacid-amino acid supplement in patients with chronic renal failure. Kidney Int. 1982; 22:48-53.
- Mitch, W.E.; Walser, M.; Steinman, T.I.; Hill, S.; Zeger, S.; Tungsanga, K. The effect of a keto acid-amino acid supplement to a restricted diet on the progression of chronic renal failure. N Engl J Med. 1984; 311:623-9.
- Monfort, P.; Montoliu, C.; Hermenegildo, C.; Muñoz, M.; Felipo, V. Differential effects of acute and chronic hyperammonemia on signal transduction pathways associated to NMDA receptors. Neurochem Int. 2000; 37:249-53.
- Monfort, P.; Kosenko, E.; Erceg, S.; Canales, J.J.; Felipo, V. Molecular mechanism of acute ammonia toxicity: role of NMDA receptors. Neurochem Int. 2002; 41:95-102 a.
- Monfort, P.; Muñoz, M.D.; Elayadi, A.; Kosenko; Felipo, V. Effects of hyperamonemia and liver failure on glutamatergic Neurotransmission. Metabolic Brain Disease. 2002; 17:237-50 b.
- Monteiro, A.; Bassini, A.; Cameron, L.C. Perfil hematológico das reservas de macronutrients em jogadores de futebol em fase de pré-temporada. F & P J. 2006; 5:129-33.
- Morris, S.M. Jr. Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr. 2002; 22:87-105.
- Motl, R.W; O'Connor, P.J.; Dishman, R.K. Effect of caffeine on perceptions of leg muscle pain during moderate intensity cycling exercise. J Pain. 2003; 4:316-21.
- Motl, R.W. & Dishman, R.K. Effects of acute exercise on the soleus H-reflex and self-reported anxiety after caffeine ingestion. Physiol Behav. 2004; 80: 577-85.
- Mujika, I. & Padilla, S. Creatine supplementation as an ergogenic aid for sports performance in highly trained athletes: a critical review. Int J Sports Med. 1997; 18:491-6.
- Nelson, D.L.; Cox, M.M. Lehninger Principles of Biochemistry, Fourth Edition, 2005.

- Newsholme, P.; Lima, M.M.; Procopio, J.; Pithon-Curi, T.C.; Doi, S.Q.; Bazotte, R.B.; Curi, R. Glutamine and glutamate as vital metabolites. Braz J Med Biol Res. 2003; 36:153-63 b.
- Newsholme, P.; Procopio, J.; Lima, M.M.; Pithon-Curi, T.C.; Curi, R. Glutamine and glutamate--their central role in cell metabolism and function. Cell Biochem Funct. 2003; 21:1-9 a.
- Nielsen, S. & Pedersen, B.K. Skeletal muscle as an immunogenic organ. Curr Opin Pharmacol. 2008; 8:346-51.
- Nieman, D.C.; Davis, J.M.; Brown, V.A.; Henson, D.A.; Dumke, C.L.; Utter, A.C.; Vinci, D.M.; Downs, M.F.; Smith, J.C.; Carson, J.; Brown, A.; McAnulty, S.R.; McAnulty, L.S. Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. J Appl Physiol. 2004; 96:1292-8.
- Nieman, D.C.; Davis, J.M.; Henson, D.A.; Rankin, J.W.; Shute, M.; Dumke, C.L.; Utter, A.C.; Vinci, D.M.; Carson, J.A.; Brown, A.; Lee, W.J.; McAnulty, S.R.; McAnulty, L.S. Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. J Appl Physiol. 2003; 94:1917-25.
- Nikolic, J.; Bjelakovic, G.; Stojanovic, I. Effect of caffeine on metabolism of L-arginine in the brain. Mol Cell Biochem. 2003; 244:125-8.
- Nissim, I.; Cattano, C.; Lin, Z.; Nissim, I. Acid-base regulation of hepatic glutamine metabolism and ureagenesis: study with 15N. J Am Soc Nephrol. 1993; 3:1416-27.
- Nissim, I. Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. Am J Physiol. 1999; 277:F493-7.
- Norman, D.; Bardwell, W.A.; Arosemena, F.; Nelesen, R.; Mills, P.J.; Loredo, J.S.; Lavine, J.E.; Dimsdale, J.E. Serum aminotransferase levels are associated with markers of hypoxia in patients with obstructive sleep apnea. Sleep. 2008; 31:121-6.
- Nybo, L.; Dalsgaard, M.K.; Steensberg, A.; Møller, K.; Secher, N.H. Cerebral ammonia uptake and accumulation during prolonged exercise in humans. J Physiol. 2005; 563:285-90.
- Nybo, L.; Secher, N.H. Cerebral perturbations provoked by prolonged exercise. Prog Neurobiol. 2004; 72:223-61.

- Ofluoglu, E.; Pasaoglu, H.; Pasaoglu, A. The Effects of Caffeine on L: -Arginine Metabolism in the Brain of Rats. Neurochem Res. 2009; 34:395-9.
- Ostrowski, K.; Rohde, T.; Asp, S.; Schjerling, P.; Pedersen, B.K. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol. 1999; 515:287-91.
- Parnas, J.K. Ammonia formation in muscle and its source. Am J Physiol. 1929; 90:467.
- Pasman, W.J.; van Baak, M.A.; Jeukendrup, A.E.; de Haan, A. The effect of different dosages of caffeine on endurance performance time. Int J Sports Med. 1995; 16:225-30.
- Pedersen, B.K.; Akerström, T.C.; Nielsen, A.R.; Fischer, C.P. Role of myokines in exercise and metabolism. J Appl Physiol. 2007; 103:1093-8.
- Pedersen, B.K.; Bruunsgaard, H.; Jensen, M.; Krzywkowski, K.; Ostrowski, K. Exercise and immune function: effect of ageing and nutrition. Proc Nutr Soc. 1999; 58:733-42 a.
- Pedersen, B.K.; Bruunsgaard, H.; Jensen, M.; Toft, A.D.; Hansen, H.; Ostrowski, K. Exercise and the immune system--influence of nutrition and ageing. J Sci Med Sport. 1999; 2:234-52 b.
- Pedersen, B.K. & Febbraio, M.A. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev. 2008; 88:1379-406.
- Pedersen, B.K. & Hoffman-Goetz, L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev. 2000; 80:1055-81.
- Pedersen, B. K. & Toft, A.D. Effects of lymphocytes and cytokines. Br J Sports Med. 2000; 34:246-51.
- Pedersen, B.K. & Hoffman-Goetz, L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev. 2000; 80:1055-81.
- Persky, A.M. & Brazeau, G.A. Clinical pharmacology of the dietary supplement creatine monohydrate. Pharmacol Rev. 2001; 53:161-76.
- Petrie, H.J; Chown, S.E.; Belfie, L.M.; Duncan, A.M.; McLaren, D.H.; Conquer, J.A.; Graham, T.E. Caffeine ingestion increases the insulin response to an oral-glucose-tolerance test in obese men before and after weight loss. Am J Clinical Nutrition. 2004; 80:22–8.

- Roeykens, J.; Magnus, R.R.; Meeusen, K.; Meirleir, D. Bood ammonia – Heart rate relationship during graded exercise is not influenced by glycogen depletion. Int J Sports Med. 1998; 19:26-31.
- Rogers, K.S. Variable sulfhydryl activity toward silver nitrate by reduced glutathione and alcohol, glutamate and lactate dehydrogenases. Biochim Biophys Acta. 1972; 263:309-14.
- Rooney, K.B.; Bryson, J.M.; Digney, A.L.; Rae, C.D.; Thompson, C.H. Creatine supplementation affects glucose homeostasis but not insulin secretion in humans. Ann Nutr Metab. 2003; 47:11-5.
- Rose, C. Increased extracellular brain glutamate in acute liver failure: decreased uptake or increased release? Metab Brain Dis. 2002; 17:251-61.
- Rose, C. & Felipo, V. Limited capacity for ammonia removal by brain in chronic liver failure: potential role of nitric oxide. Metab Brain Dis. 2005; 20:275-83.
- Rossi, L. & Tirapegui, J. Aspectos atuais sobre exercício físico, fadiga e nutrição. Ver Paul Educ Fís. 1999; 13:67-82.
- Rouach, N.; Koulakoff, A.; Abudara, V.; Willecke, K.C.G. Astroglial Metabolic Networks Sustain Hippocampal Synaptic Transmission. Science. 2008; 322:1551-55.
- Sáez, R.; Llansola, M.; Felipo, V. Chronic exposure to ammonia alters pathways modulating phosphorylation of microtubule-associated protein 2 in cerebellar neurons in culture. J Neurochem. 1999; 76:2555-62.
- Sahlin, K.; Tonkonogi, M.; Söderlund, K. Plasma hypoxanthine and ammonia in humans during prolonged exercise. Eur J Appl Physiol Occup Physiol. 1999; 80:417-22.
- Savransky, V.; Nanayakkara, A.; Vivero, A.; Li, J.; Bevans, S.; Smith, P.L.; Torbenson, M.S.; Polotsky, V.Y. Chronic intermittent hypoxia predisposes to liver injury. Hepatology. 2007; 45:1007-13.
- Savransky, V.; Reinke, C.; Jun, J.; Bevans-Fonti, S.; Nanayakkara, A.; Li, J.; Myers, A.C.; Torbenson, M.S.; Polotsky, V.Y. Chronic intermittent hypoxia and acetaminophen induce synergistic liver injury in mice. Exp Physiol. 2009; 94:228-39.

- Savransky, V.; Reinke, C.; Jun, J.; Bevans-Fonti, S.; Nanayakkara, A.; Li, J.; Myers, A.C.; Torbenson, M.S.; Polotsky, V.Y. Chronic Intermittent Hypoxia and Acetaminophen Induce Synergistic Liver Injury. Exp Physiol. 2009; 94:228-39.
- Schaefer, A.; Piquard, F.; Geny, B.; Doutreleau, S.; Lampert, E.; Mettauer, B.; Lonsdorfer, J. L-arginine reduces exercise-induced increase in plasma lactate and ammonia. Int J Sports Med. 2002; 23:403-7.
- Schaefer, A.; Piquard, F.; Geny, B.; Doutreleau, S.; Lampert, E.; Mettauer, B.; Lonsdorfer, J. L-arginine reduces exercise-induced increase in plasma lactate and ammonia. Int J Sports Med. 2002; 23:403-7.
- Schindler, C.W.; Karcz-Kubicha, M.; Thorndike, E.B.; Müller, C.E.; Tella, S.R.; Ferré, S.; Goldberg, S.R. Role of central and peripheral adenosine receptors in the cardiovascular responses to intraperitoneal injections of adenosine A1 and A2A subtype receptor agonists. Br J Pharmacol. 2005; 144:642-50.
- Schloerb, P.R. Essential L-amino acid administration in uremia. Am J Med Sci. 1966; 252:650-9.
- Schulz, H. & Heck, H. Glycogen depletion as indication for ammonia determination in exercise testing. Eur J Sport Sci. 2003; 3:1-9.
- Seiler, N. Ammonia and Alzheimer`s disease. Neurochem Int. 2002, 41:189-207.
- Shawcross, D.L.; Olde Damink, S.W.; Butterworth, R.F.; Jalan, R. Ammonia and hepatic encephalopathy: the more things change, the more they remain the same. Metab Brain Dis. 2005; 20:169-79.
- Shawcross, D. & Jalan, R. The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation. Cell Mol Life Sci. 2005; 62:2295-304.
- Shearer, J., A. Farah, T. Paulis, D. P. Bracy. Quinides of roasted coffe enhance insulin action in rats. Am Soc Nut Sci. 2003; 3529-33.
- Sidney, B. The amide nitrogen of bood.III – Muscular exercise: The role of ammonia in the neutralization of lactic acid. 1928; 137-58.
- Smith, W.H. The excretion of ammonia and urea by the gills of fish. J Biol Chem. 1929; 81:727-42.

- Snow, R.J.; Carey, M.F.; Stathis, C.G. Febbraio, M.A.; Hargreaves, M. Effect of carbohydrate ingestion on ammonia metabolism during exercise in humans. J Appl Physiol. 2000; 88:1576-80.
- Snow, R.J.; McKenna, M.J.; Selig, S.E.; Kemp, J.; Stathis, C.G.; Zhao, S. Effect of creatine supplementation on sprint exercise performance and muscle metabolism. J Appl Physiol. 1998; 84:1667-73.
- Spencer, M.K. & Katz, A. Role of glycogen in control of glycolisis and IMP formation in human muscle during exercise. Am J Physiol. 1991; 260:E859-64 a.
- Spencer, M.K.; Yan, Z.; Katz, A. Carbohydrate supplementation attenuates IMP accumulation in human muscle during prolonged exercise. Am J Physiol. 1991; 261:C71-6 b.
- Spriet, L.L. Caffeine and performance. Int J Sports Nutr. 1995; 5:84-99.
- Starkie, R.L.; Angus, D.J.; Rolland, J.; Hargreaves, M.; Febbraio, M.A. Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. J Physiol. 2000; 528:647-55.
- Starkie, R.L.; Rolland, J.; Angus, D.J.; Anderson, M.J.; Febbraio, M.A. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. Am J Physiol Cell Physiol. 2001; 280:C769-74 a.
- Starkie, R.L.; Rolland, J.; Febbraio, M.A. Effect of adrenergic blockade on lymphocyte cytokine production at rest and during exercise. Am J Physiol Cell Physiol. 2001; 281:C1233-40 b.
- Starkie, R.L.; Angus, D.J.; Rolland, J.; Hargreaves, M.; Febbraio, M.A. Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. J Physiol. 2000; 528:647-55.
- Steensberg. A.; van Hall, G.; Keller C, Osada T, Schjerling P, Pedersen BK, Saltin B, Febbraio MA. Muscle glycogen content and glucose uptake during exercise in humans: influence of prior exercise and dietary manipulation. J Physiol. 2002; 541:273-81.
- Steensberg, A.; Hall, G.V.; Keller, C.; Osada, T.; Schjerling, P.; Pedersen, B.K.; Saltin, B.; Febbraio, M.A. Muscle glycogen content and glucose uptake during exercise in humans: influence of prior exercise and dietary manipulation. J Physiol. 2002; 541:273-81.

- Stewart, L.K.; Flynn, M.G.; Campbell, W.W.; Craig, B.A.; Robinson, J.P.; Timmerman, K.L.; McFarlin, B.K.; Coen, P.M.; Talbert, E. The influence of exercise training on inflammatory cytokines and C-reactive protein. Med Sci Sports Exerc. 2007; 39:1714-9.
- Stumvoll, M.; Meyer, C.; Mitrakou, A.; Gerich, J.E. Important role of the kidney in human carbohydrate metabolism. Med Hypotheses. 1999; 52:363-6.
- Suárez, I.; Bodega, G.; Fernández, B. Glutamine synthetase in brain: effect of ammonia. Neurochem Int. 2002; 41:123-42.
- Takahashi, H.; Koehler, R.C.; Brusilow, S.W.; Traystman, R.J. Glutamine synthetase inhibition prevents cerebral edema during hyperammonemia. Acta Neurochir Suppl. 1990; 51:346-7.
- Terjung, R.L. Ammonia Metabolism in muscle. Em: Hargreaves, M.; Thompson, M. Exercise Biochemistry X, c.39, p.489, 1999.
- Thompson, W.J. & Appleman, M.M. Characterization of cyclic nucleotide phosphodiesterases of rat tissues. J Biol Chem. 1971; 246:3145-50.
- Thong F.S.L., Derave, W.; Kiens, B.; Graham, T.E.; Urso, B.; Wojtaszewski, J.F.P.; Hansen, B.F.; Richter, E.A. Caffeine-induced impairment of insulin action but not insulin signaling in human skeletal muscle is reduced by exercise. Diabetes. 2002; 51:583–90.
- Timmerman, K.L.; Flynn, M.G.; Coen, P.M.; Markofski, M.M.; Pence, B.D. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? J Leukoc Biol. 2008; 84:1271-8.
- Tipton, C.M. Current issues in exercise metabolism: the crossover concept. Clin Exp Pharmacol Physiol. 1997; 24:887-8.
- Tommaso, A.; Caterina, P.; Di Cocco, M.E.; Arduini, A., Ricciolini, R.; Scalibastri, Filippo, C.M. Entry of [(1,2-13C2)acetyl]-L-carnitine in liver tricarboxylic acid cycle and lipogenesis: a study by 13C NMR spectroscopy in conscious, freely moving rats. Eur J Biochem. 1999; 263:287-93.
- Tsintzas, K.; Williams, C.; Constantin-Teodosiu, D.; Hultman, E.; Boobis, L.; Clarys, P.; Greenhaff, P. Phosphocreatine degradation in type I and type II muscle fibres during submaximal exercise in man: effect of carbohydrate ingestion. J Physiol. 2001; 537:305-11.

- Tullson PC, Terjung RL. IMP degradative capacity in rat skeletal muscle fiber types. Mol Cell Biochem. 1999; 199:111-7.
- Underhill, F.P. Ammonium salt. J. Biol. Chsvrt. 1913; 15: 321-35.
- Underhill, F.P. Studies on the metabolism of ammoniun salts. I. The elimination of ingested ammonium salts in the dog upon an adequate mixed diet. J Biol Chem. 1913; XV:327-40 a.
- van Loon LJ, van Rooijen JJ, Niesen B, Verhagen H, Saris WH, Wagenmakers AJ. Effects of acute (-)-hydroxycitrate supplementation on substrate metabolism at rest and during exercise in humans. Am J Clin Nutr. 2000; 72:1445-50.
- Van Soeren, M.H. & Graham, T.E. Effect of caffeine on metabolism, exercise, and catecholamine responses after withdrawal. J Appl Physiol. 1998; 85:1493-1501.
- Van Soeren, M.H.; Sathasivam, P.; Spriet, L.L.; Graham, T.E. Caffeine metabolism and epinephrine responses during exercise in users and nonusers. J Appl Physiol. 1993; 75:805.
- Wagenmakers, A.J. Tracers to investigate protein and amino acid metabolism in human subjects. Proc Nutr Soc. 1999; 58:987-1000.
- Walser, M.; Mitch, W.E.; Collier, V.U. The effect of nutritional therapy on the course of chronic renal failure. Clin Nephrol. 1979; 11:66-70.
- Walser, M.; LaFrance, N.D.; Ward, L.; VanDuyn, M.A. Progression of chronic renal failure in patients given ketoacids following amino acids. Kidney Int. 1987; 32:123-8.
- Warren, G.L.; Ingalls, C.P.; Lowe, D.A.; Armstrong, R.B. Excitation-contraction uncoupling: Major role in contraction-induced muscle injury. Exerc. Sports Sci. Rev. 2001; 29:82-7.
- Weiner, I.D.; Hamm, L.L. Molecular mechanisms of renal ammonia transport. Annu Rev Physiol. 2007; 69:317-40.

ANEXO

Anexo 1.

Enzima	Comparte-mento	Atividade	M_r	pH opt	K_m, mM	Constante de equilíbrio	Distribuição tecidual
N-acetil glutamato sintetase EC 2.3.11	Matrix mitocondrial	0.30-1.49	200,000	8.5	Glu,3.0 Ac.CoA 0.7 Arg,0.01	Irreversível	Fígado, intestine, rim, baço
Carbamil fosfato sintetase	Matrix mitocondrial	279[+]	310,000 dimero	6.8-7.6	NH_4, 0.8 HCO_3, 6.7 MgATP,1.1 NAG, 0.1	Irreversível	Fígado, intestine, rim
Ornitina transcarbamilase EC 6.3.4.16	Matrix mitocondrial	6600	108,00 tretramero	7.7	CP, 0.16 Orn,0.40	$\frac{(cit)(p)}{(Orn)(CP)} = 10^{5}$	Fígado, intestine, rim
Arginino succinato sintetase EC 6.3.4.5	Citosol	90	185,000 tetramero	8.7	Asp, .03 Cit, .03	$\frac{(ASA)(AMP)(MgPP)(2H)}{(Cit)(Asp)(MgATP)} = 0.89^{++}$	Fígado, rim, fibroblastos, cérebro
Arginino succinase liase EC4.3.2.1	Citosol	220	173,200 tetramero	7.5	Asp,0.017 Cit, 0.016 ATP, 0.041	$\frac{(Arg)(fumarato)}{(ASA)} = 11.4x10^{-3}$	Fígado, rim, cérebro, fibroblastos
Arginase EC 3.5.3.1	Citosol	86,600	107,000 tetramero	9.5	Arg 10.5	Irreversível	Fígado, eritrócitos, rim, cerebro

Anexo 1.A- As enzimas do ciclo da uréia. *a atividade da enzima esta expressa em micromol por grama de peso molhado. Extraído de *inherited disease - Urea cycle enzymes chapter* 85, pag 1912.

Enzime	Comparte-mento	Pesso Molecular Massa kDA	Estrutura	Cofactor	Parametros cinéticos	Distribuição tecidual
Ornitina aminotransferase EC2.6.1.13	Matrix mitocondrial	49 → 45+	Homohexamero ou homotetramero	Piridoxal fosfato	$K_{m\ orn\ =}$ 1.8mM K_{mKG} = 2.7mM K_{mPLP} = 0.7 µM	General
Ornitina transcarbamilase EC2.1.3.3	Matrix mitocondrial	40 → 36+	Homotrimero	-	$K_{m\ orn}$ = 0.47mM $K_{m\ CAP}$ = 0.7mM	Principalmente no fígado; e baixa concentração no intestino
Ornitina decarboxilase (EC4.1.1.17)	Citoplasma	51	Monomero	Piridoxal fosfato	$K_{m\ orn}$ = 0.1mM	Generamente em tecidos com grande capacidade de divisão
Arginase (EC3.5.3.1)	Citoplasma	30	Homotetramero	Mn^{2+}	$K_{m\ arg}$ = 10mM	Fígado e eritrocito principalmente, rim
Glicina transamidinase EC2.1.4.1	⇕	45	Homodimero	-	$K_{m\ arg}$ = 2.5mM $K_{m\ gly}$ = 2.5mM	Pancreas, rim, fígado
Óxido nítrico sintetase EC1.14.23	Ligada a membrana	144	Homodimero (?)	BH_4 , NADPH, FAD, FMN	$K_{m\ arg}$ ≈ 3µM	Célula endotelial

Anexo 1.B- Enzimas do metabolismo da ornitina.

CAPITULO II

Lazarin, FL; Antunes-Neto, JMF; SILVA, FOC, Nunes, LAS; Bassini-Cameron; Cameron, LC; Alves, AA; Brenzikofer, R; DV Macedo. The upper values of plasma creatine kinase of professional soccer players during the Brazilian championship.Journal of Science and Medicine in Sport. v.18069060, 2007.

Journal of Science and Medicine in Sport (2009) 12, 85—90

ELSEVIER

Journal of Science and Medicine in Sport

www.elsevier.com/locate/jsams

ORIGINAL PAPER

The upper values of plasma creatine kinase of professional soccer players during the Brazilian National Championship

Fernanda L. Lazarim[a], Joaquim M.F. Antunes-Neto[b], Fernando O.C. da Silva[a], Lázaro A.S. Nunes[a], Adriana Bassini-Cameron[c,d], Luiz-Cláudio Cameron[c,d,e], Armindo A. Alves[f], René Brenzikofer[g], Denise Vaz de Macedo[a,*]

[a] *Instituto de Biologia, Unicamp, 13083-970 Campinas, Sao Paulo, Brazil*
[b] *Faculdades Integradas Metropolitanas de Campinas (Metrocamp), Campinas, Brazil*
[c] *Universidade Federal do Estado do Rio de Janeiro (UniRio), Rio de Janeiro, Brazil*
[d] *Universidade Federal de Uberlândia, Uberlândia, Brazil*
[e] *Universidade Castelo Branco, Rio de Janeiro, Brazil*
[f] *Centro Universitário Hermínio Ometto, Uniararas — Rua Maximiliano Baruto, 500 Araras, Brazil*
[g] *Laboratório de Instrumentação para Biomecânica (LIB), Faculdade de Educação Física, Unicamp, Campinas, Brazil*

Received 18 April 2007; received in revised form 19 September 2007; accepted 18 October 2007

KEYWORDS
Soccer;
Muscular damage;
Overload;
Creatine kinase

Summary The current schedule of the Brazilian Soccer Championship may not give players enough recovery time between games. This could increase the chances of muscle damage and impaired performance. We hypothesized that plasma creatine kinase (CK) activity could be a reliable indirect marker of muscle overload in soccer players, so we sought to identify the reference values for upper limits of CK activity during a real-life elite competition. This study analyzed changes in plasma CK activity in 128 professional soccer players at different times during the Brazilian Championship. The upper limits of the 97.5th and 90th percentiles determined for CK activity were 1.338 U/L and 975 U/L, respectively, markedly higher than values previously reported in the literature. We also evaluated a team monthly throughout the Championship. The upper limit of the 90th percentile, 975 U/L, was taken as the decision limit. Six players showing plasma CK values higher than this were asked to decrease their training for 1 week. These players presented lower CK values afterwards. Only one player with a CK value higher than the decision limit

* Corresponding author.
E-mail address: denisevm@unicamp.br (D.V. de Macedo).

1440-2440/$ — see front matter
doi:10.1016/j.jsams.2007.10.004

(1800 U/L 1 day before a game) played on the field and was unfortunately injured during the game. The CK activity in all the other players showed a significant decrease over the course of the Championship, and the values became more homogeneous at the end. The results presented here suggest that plasma CK upper limit values can be used as a practical alternative for early detection of muscle overload in competing soccer players.

Introduction

The games of the National Brazilian Soccer Championship generally occur every Saturday/Sunday and Wednesday/Thursday, which means that training and matches overlap. As a consequence, the players, especially the infield ones, may not have enough time to recover between games. This may result in muscular overload, leading to varying degrees of microtrauma in muscle, connective tissue and/or bones and joints. This stress can progress from the initial benign microtrauma stage to a sub-clinical injury that will impair the athlete's performance.[1,2]

In the event of muscle damage, cell membranes rupture and some proteins leak out reaching the bloodstream.[3] Some of the enzymes and proteins commonly analyzed after exercise-induced muscle damage include creatine kinase (CK), lactate dehydrogenase, aspartate transaminase and myoglobin.[3,4] Of all of these analytes, plasma CK activity seems to be the best indicator of exercise severity and its effect on tissues.[4] However, the idea of using plasma CK analysis as an indirect marker of sub-clinical muscular injury in competing athletes has not been explored in the literature.

Doubts about the application of CK analysis are derived from studies suggesting that this measurement is an unreliable marker for histological muscle lesions.[5] In addition, CK values measured in individuals exercising to a similar degree showed high variability and a non-Gaussian distribution.[6] However, the majority of these studies involved subjects performing specific exercises for defined short periods.[7] While these studies make important contributions to the field of exercise physiology, they do not provide enough information on CK levels in actively competing athletes. Such a study could help advance sports science, so we carried out the present investigation with soccer athletes competing under normal conditions.

We hypothesized that plasma CK activity could be a reliable indirect marker of muscle overload for soccer players, so we began by identifying the reference interval for this parameter during a real-life elite competition. We hypothesized further that plasma CK values below the upper limit of the reference interval could indicate an adaptive muscular response. Some studies have suggested that plasma CK activity can arise in the absence of histological lesions, as a consequence of changes in muscle membrane permeability.[8–10] The same muscle membrane alterations that may increase plasma CK activity also affect the release of growth factors by muscle cells,[11] which could be one way in which changes in plasma CK activity reflect muscular adaptation. According this point of view the individual plasma CK activity above the upper reference limit may indicate the transition from adaptive microtrauma to a sub-clinical muscular injury, increasing the potential for damage.

Reference intervals refer to the range of values for a laboratory test observed in a specific population, typically described by upper and lower reference limits and containing the central 95% of results.[12] In many cases, such as the commonly measured enzymes, low values are unimportant and only the upper limit of the reference interval is used in medical decision-making. In these cases, the one-sided reference interval can be calculated. The 95% reference region would then consist of a single value, i.e., the 97.5th percentile, representing the upper limit cutoff.[13]

The purpose of the present study was to analyze the distribution of plasma CK activity to determine the upper limits for professional soccer players who were participating in the 2001 Brazilian National Championship. We also tracked CK activity in one team during this Championship to investigate whether it could be used as a marker for muscle overload.

Materials and methods

The analyzed population included active professional soccer players from five clubs belonging to the Brazilian Soccer First Division. To avoid interference with the experiment, athletes who were taking medications or who were injured and not training were excluded from the study. The athletes ($n = 134$) were 24 ± 4 years old and weighed 74.7 ± 8.0 kg (means $\pm$ S.D.). This study was performed with the approval of the Ethical Committee

on Human Research (Proc. 019/2004). All subjects provided written consent after the purpose, content and possible risks of the study had been explained to them.

Experimental protocol

Plasma CK activity was measured in the soccer players at different times in the Brazilian Championship in order to cover a large range of competitive stress conditions. One team included in the group ($n = 29$) was evaluated monthly throughout the 5 months of the Championship. The acute hemodiluting effects of exercise were not considered in the present study as blood sampling was always performed after 2 days of reduced sporting activity and before the next game.[14,15] After six athletes were excluded, a total of 128 analyses were used to establish the upper percentiles.[16]

Blood sampling and analyses

Blood sampling took place under standardized conditions in a supine position in the morning after fasting. For the analyses, 4 mL of blood was collected in heparin using the Vacutainer® BD system and centrifuged at 2000 × *g* for 15 min under refrigeration (4 °C) to separate the plasma. Plasma CK activity was analyzed with Wiener lab kits run in an automatic analyzer (Autolab Boehringer). The enzymatic plasma CK activity was measured at 37 °C. The internal quality control for the analyte was performed in parallel with the tests by measuring the levels of the commercial serum control, Wiener lab Standatrol SE 2.

Statistical analysis

To determine the reference interval for plasma CK activity, we followed the criteria established by the International Federation of Clinical Chemistry (IFCC). Under these criteria, the establishment of a reference interval depends on the size of the data set (at least 120 samples) and the method of evaluation.[13] The first recommendation is to detect outliers before any estimate of the reference interval.[13] Outliers were detected and removed by Horn's test.[17] Table 1 shows the number of analyses, the number of outliers and their values, the total number of analyses used after outlier removal, and their mean and median values.

The RefVal program,[18] including practical approaches and formulas recommended by IFCC, was used to calculate the upper reference limits for the non-parametric 97.5th and 90th percentiles, together with their 90% confidence intervals (CI), by Bootstrap methodology.[13,17,19] Boxplots were made to show the behavior of plasma CK activity in players throughout the 5 months of the championship. The analyses of the results and statistics were carried out with the aid of Matlab® 7.0 software. One-way analysis of variance (ANOVA) was used with Tukey's post-test. Values of $p < 0.05$ were considered significant.

Table 1 Number of athletes analyzed for plasma CK activity, the number and values of outliers, and the median and mean of the retained measurements

Total number of analyses	134
Number of detected outliers	6
Values of outliers	63, 82, 90, 2178, 2833, 3047
Number of used analyses	128
CK (U/L) mean ± S.D.	493 ± 315
CK (U/L) median	371

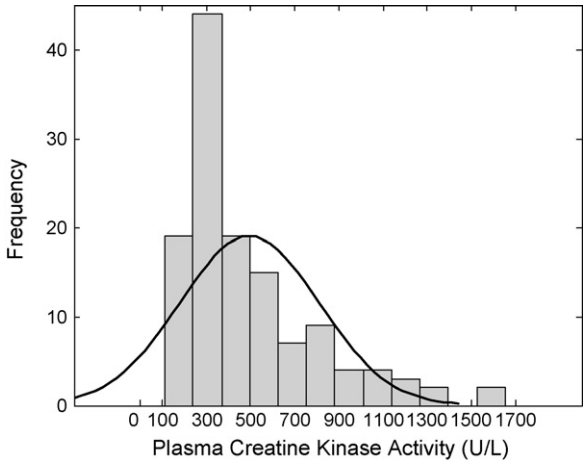


Figure 1 Frequency of plasma creatine kinase activity in 128 professional soccer players in the range of 100—1700 U/L.

Results

Determination of the upper reference limit

Fig. 1 shows the histogram of plasma CK activity. We found a non-Gaussian, left-skewed data distribution even after the exclusion of the outliers. The majority of the data were concentrated between 150—1000 U/L, with a high variability (200—1600 U/L).

The upper limit values determined for plasma CK activity are shown in Table 2. Different per-

Table 2 Upper limit values with their 90% confidence intervals for CK activity measurements

	CK values (U/L)
Percentile 97.5	1338
CI[a]	1191—1639
Percentile 90	975
CI[a]	810—1090

[a] CI is the 90% confidence interval for the upper limit of the indicated percentiles.

centiles 97.5th and 90th were calculated due to higher values inherent in the soccer player population compared to the general population. The plasma CK upper limits of the 97.5th and 90th percentiles were 1.338 U/L (CI = 1191—1639 U/L) and 975 U/L (CI = 810—1090 U/L), respectively.

Plasma CK activity throughout the Championship

Fig. 2 shows a box plot of the behavior of plasma CK activity of one team throughout 5 months of the Brazilian Championship. We adopted the upper limit of the 90th percentile as the decision limit to detect muscle overload, and this value was exceeded by two players in July (pre-competition) and four players in August (beginning of the championship). These players were asked to decrease their sport activity level, and their CK activity was reanalyzed 1 week later. Between them, five players presented lower CK activity values after this rest period. The data are shown in Table 3.

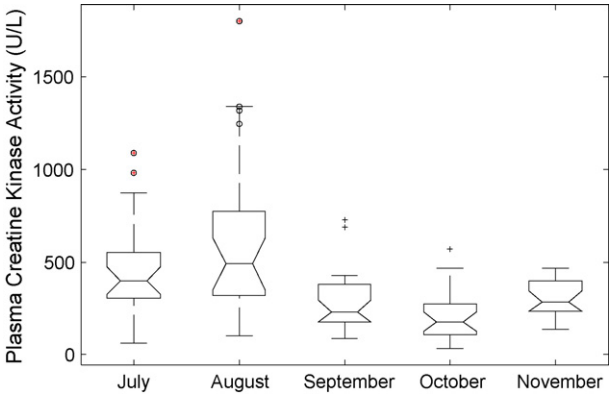


Figure 2 Box plot of plasma creatine kinase activity of soccer players during 5 months of the competitive season. (◯) represents athletes with CK activity above the defined ''upper limit.'' According to the ANOVA test, July is significantly different from October ($p < 0.01$) and August is significantly different from September, October, and November ($p < 0.001$).

Table 3 Plasma CK activity from athletes whose resting time was increased

Athletes	CK values	
	1[a]	2[b]
A	1316	729
B	1090	482
C	983	513
D	1245	507
E	1340	687
F	1800	Not done

[a] The first analysis 48 h after a game or training session.
[b] The analysis after 1 week of reduced sporting activity.

It is important to point out that one player was found to have CK activity of 1800 U/L on the eve of a match. He subsequently participated in the game and unfortunately was injured. He was excluded from the analysis. The other players did not participate in the next game after their plasma CK levels were found to be higher than the 90th percentile upper limit. In the subsequent months, plasma CK values of all players significantly decreased and showed a more homogeneous distribution (Fig. 2).

Discussion

In this study we used the IFCC-recommended methodology to analyze the distribution of plasma CK activity in professional soccer players from five clubs belonging to the Brazilian Soccer First Division. The values varied greatly between individuals, and one reason may be physical differences between the athletes. We used these values to determine an upper limit that might be applied when managing athletes' performance. Our procedure allowed us to derive an upper limit from soccer players during a championship, and this value may be more appropriate for this population than other values reported previously in the literature.

The upper limits of the 97.5th and 90th percentiles for plasma CK activity were 1.338 U/L and 975 U/L, respectively, which are markedly higher than the level of 398 U/L recently reported for non-athletes by Stromme et al. [19] These marked discrepancies are most likely related to the bouts of intense exercise experienced by the soccer players daily, which causes a significant amount of

skeletal muscle stress and may lead to the elevated plasma CK values. In agreement with this interpretation, Lev et al. [20] reported that the mean plasma CK measurements of 428 male soldiers from the Israeli Army at different moments were around 544 U/L. In addition, Thompson et al. [21] showed that individuals doing uninterrupted exercise (90 min hard effort interspersed with walking) had peaks of plasma CK activity with a mean value of 774 U/L.

As the upper limit of the 97.5th percentile is higher than the majority of the values found in soccer players (200—1000 U/L), we used the upper limit of the 90th percentile (975 U/L) as the threshold value for muscular overload. However, the CI of the 90th percentile's upper limit (810—1090 U/L) should be taken into account, because it may indicate an individual muscular adaptive response as proposed by McNeil and Khakee [11]. The data shown in Fig. 2 reinforce this proposition, as the median values of plasma CK were lower than the 90th percentile's upper limit. Further evidence for muscular adaptation may be the observation that players had their most intense moment at the beginning of the Championship in August, and then in the months afterwards, the group's median CK activity decreased and the distribution of the data became more homogeneous. This response was more pronounced at the end of the championship, suggesting a positive muscular adaptive response in all players during the main competitive period. These data may reflect the positive results of the team: a 6th place finish at the 2001 Brazilian Championship.

Our results suggest the possibility of using plasma CK activity analysis as a marker for the early detection of fatigue or muscle overload in soccer players. Determining an upper limit for CK activity and measuring players against that value may contribute not only to protecting them physically but also to optimizing their training schedule. Players with CK values beneath the upper limit probably exhibit adaptive muscular responses specific to this sport, which may allow them to play with less chance of overload or injury. In our study, only the athletes with plasma CK values higher than the 90th percentile upper limit were asked to reduce their sporting activity. After 1 week, the players showed a decrease in CK activity and they returned to their previous routine (Table 3). The one player in our study who was injured due to muscular overstress during a game showed a CK value (1800 U/L) above the upper limit 1 day before, which is consistent with our hypothesis that plasma CK activity can reflect muscle overload and damage potential.

Practical implications

- The availability of portable apparatus for this measurement would allow assessment of muscle stress in real-time during a championship.
- This may allow for the development of optimal physical conditioning plans that take into account the stress limits for individual players and thereby ensure muscle protection.
- It is important to point out that the specific reference intervals for the portable kits must be determined before application.
- In addition, the reference values for athletes in other sports are likely to be different from those for soccer players, so they should be determined in each case.

Acknowledgements

The authors thank the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) Brazilian agencies for financial support (Pronex-03/09923-2 and CNPq Proc. 523383-96-7). FL Lazarim is the recipient of a Ph.D. grant from Capes. FOC da Silva is the recipient of a Ph.D. grant from FAPESP (Proc. 04/07203-5). The authors also thank the biologist Ana Maria Marçal Porto for technical support, the physiologist Prof. Helio Moura, and all of the athletes who accepted the invitation to participate in this study.

References

1. Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc* 2000;**32**:317—31.
2. Fry RW, Norton AR, Keast D. Overtraining in athletes: an update. *Sports Med* 1991;**12**:32—65.
3. Sayers SP, Clarkson PM. Short-term immobilization after eccentric exercise. Part II. Creatine kinase and myoglobin. *Med Sci Sports Exerc* 2003;**35**(5):762—8.
4. Chevion S, Moran DS, Heled Y, Shani Y, Regev G, Abbou B, et al. Plasma antioxidant status and cell injury after severe physical exercise. *PNAS* 2003;**100**(9):5119—23.
5. Malm C. Exercise-induced muscle damage and inflammation: fact or fiction? *Acta Physiol Scand* 2001;**171**(3): 233—9.
6. Clarkson PM, Ebbeling C. Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clin Sci* 1988;**75**(3):257—61.
7. Stone MH, Sands WA, Stone ME. The downfall of sports science in the United States. *Strength Conditioning J* 2004;**26**(2):72—5.
8. Manfredi TG, Fielding RA, O'Reilly KP, Meredith CN, Lee HY, Evans WJ. Plasma creatine kinase activity and exercise-

induced muscle damage in older men. *Med Sci Sports Exerc* 1991;**23**(9):1028—34.
9. Volfinger L, Lassourd V, Michaux JM, Braun JP, Toutain PL. Kinetic evaluation of muscle damage during exercise by calculation of amount of creatine kinase released. *Am J Physiol* 1994;**266**:R434—41.
10. Van der Meulen JH, Kuipers H, Drukker J. Relationship between exercise-induced muscle damage and enzyme release in rats. *J Appl Physiol* 1991;**71**(3): 999—1004.
11. McNeil PL, Khakee R. Disruptions of muscle fiber plasma membranes. Role in exercise-induced damage. *Am J Pathol* 1992;**140**(5):1097—109.
12. Dufour DR, Lott JA, Nolte FS, et al. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. *Clin Chem* 2000;**46**(12):2050—68.
13. Horn SP, Pesce AJ. Reference intervals: an update. *Clin Chim Acta* 2003;**334**:5—23.
14. Fellmann N. Hormonal and plasma volume alterations following endurance exercise: a brief review. *Sports Med* 1992;**13**:37—49.
15. Schumacher YO, Schmid A, Grathwohl D, Bultermann D, Berg A. Hematological indices and iron status in athletes of various sports and performances. *Med Sci Sports Exerc* 2002;**34**(5):869—75.
16. PetitClerc C, Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. *Clin Chim Acta* 1987;**170**:S3—12.
17. Solberg HE, Lahti A. Detection of outliers in reference distributions: performance of Horn's Algorithm. *Clin Chem* 2005;**51**(12):2326—32.
18. Solberg HE. The IFCC recommendation on estimation of reference intervals. The RefVal program. *Clin Chem Lab Med* 2004;**42**:710—4.
19. Stromme JH, Rustad P, Steensland H, Theodorsen L, Urdal P. Reference Intervals for eight enzymes in blood of adult females and males measured in accordance with the International Federation of Clinical Chemistry reference system at 37°C: part of the Nordic Reference Interval Project Scand. *J Clin Lab Invest* 2004;**24**:371—84.
20. Lev EI, Tur-Kaspa I, Ashkenazy I, Reiner A, Faraggi D, Shemer J. Distribution of serum creatine kinase activity in young healthy persons. *Clin Chim Acta* 1999;**279**(1-2):107—15.
21. Thompson D, Nicholas CW, Willian C. Muscle soreness and damage parameters after prolonged intermittent shuttle-running. *J Sport Sci* 1999;**17**(5):387—95.

ANEXO

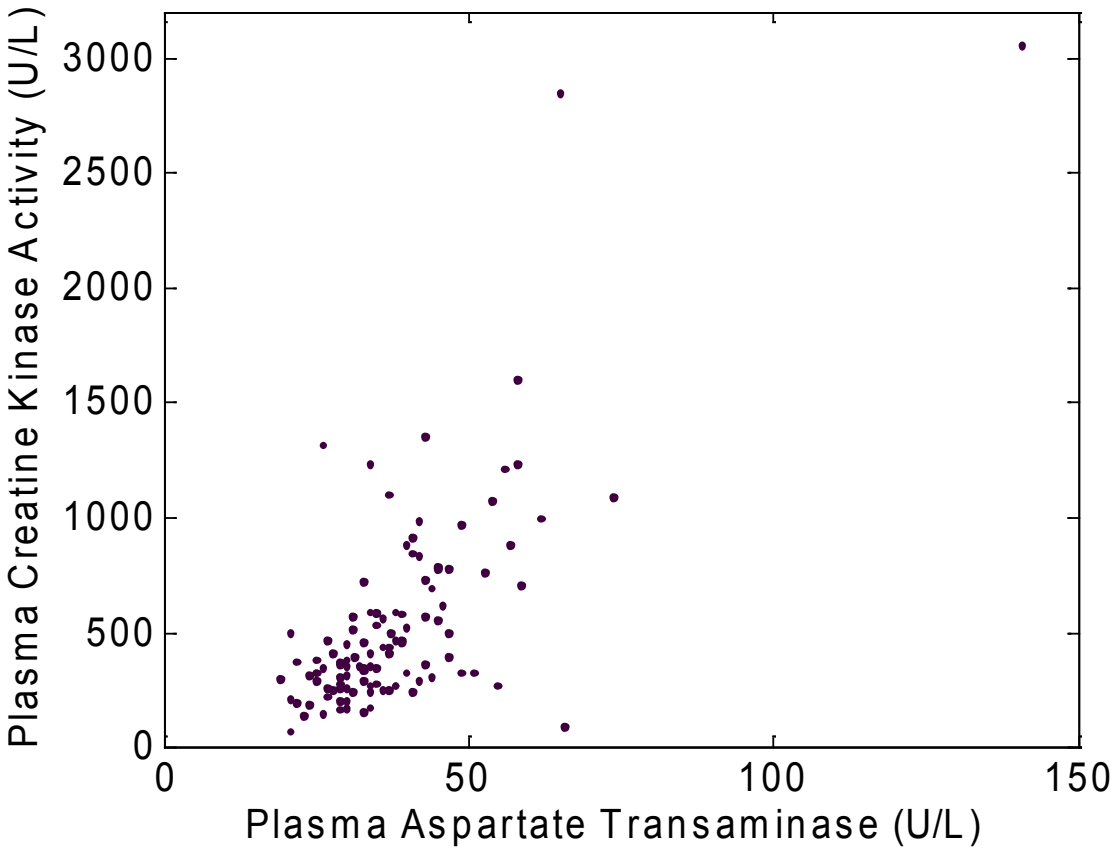
.

Enzyme	Number of Analyses	Number of outliers detected	Values of outliers	Total number of analyses	Mean ± SD	Median
CK	134	6	63, 82, 90, 2178, 2833, 3047	128	493,5 ± 315,6	371,5
AST	113	2	19, 141	111	37 ± 10,4	35

Table I: Number of analyses, outliers detected and their values, and the median and mean of the values analyzed. CK – creatine kinase; AST – aspartate transaminase.

Enzyme	95% reference interval			90% reference interval			85% reference interval			80% reference interval		
CK	Percentiles		90% CI*	Percentiles		90% CI*	Percentiles		90% CI*	Percentiles		90% CI*
	2.5	*97.5*		*5*	*95*		*7.5*	*92.5*		*10*	*90*	
Simple non-param.	132	1338	1208-1656	156	1215	983-1347	174	1069	875-1226	191	975	803-1208
BootStrap	135	1338	1191-1639	156	1215	983-1328	177	1062	884-1222	191	975	810-1090

Table IIa: CK reference limits for different reference intervals and methods. CK - creatine kinase. *CI – 90% confidence interval of the upper percentiles.



Grafic 1: AST vs CK activity

CAPITULO III

Bessa, A, Nissembaun, M., Nunes, LAS; Bassini-Cameron, A; Macedo, DV; LC Cameron. Low Back Pain Followed by Acetaminophen (APAP) Hepatotoxicity: A Case Report. The American Journal of Sports Medicine: A Case Report

Low Back Pain Followed by Acetaminophen (APAP) Hepatotoxicity: A Case Report

Running head: Exercise and acetaminophen induced hepatotoxicity

Key Terms: acetylcysteine; liver injury; exercise biochemistry; muscle soreness

Introduction

Back pain is a common problem seen in sports medicine. Not only can this be difficult to manage but it is also a frustrating experience for the athlete, unable to compete or train effectively[4]. Although modern management of acute low back pain emphasizes self care[5], and acetaminophen's effectiveness in relieving pain is widely accepted[10], self-medication still represents a risk to athletes due to the possibility of acetaminophen hepatotoxicity exacerbated by vigorous exercise[17].

Here we present a case of silent liver injury caused by self-medication with large doses of acetaminophen (APAP) taken to relieve pain after lower back injury caused by a cycling accident.

Case Report

A team of triathletes (n=4) came to us requesting metabolic evaluation and assessment. We evaluated the VO_2 of the athletes, as well as their hematologic, biochemical, and enzymatic profiles. They had been following the same training and nutritional program, and their performance was very similar based on ergospirometry (Fig. 1).

During the initial analysis, one athlete showed unexpected levels of muscle and liver injury markers. The athlete's value for creatine kinase (CK) level was almost 275% higher, the lactate dehydrogenase (LDH) level was approximately 25% greater, and C-reactive protein (CRP) was 140% greater than the average value of three teammates. We also noticed a higher level (36%) of aspartate aminotransferase (AST), a 184%

increase in γ-glutamyltransferase (γGT), and a 350% increase in total bilirubin level, when compared with his teammates. In contrast, the subject of interest had levels of liver injury markers alkaline phosphatase (ALP) and alanine aminotransferase (ALT) similar to the group (Table 1).

After a careful history, we found that the subject had been experiencing back pain for 96 h before testing. He attributed this pain to a previous training session in which a bike drift occurred, triggering severe muscle pain. As the athlete was training for a major competition, he decided to keep that information from his medical staff, fearing to be withdrawn from the team, and started taking over-the-counter painkillers without the knowledge of his trainers. The athlete indicated that he used up to 9 g of acetaminophen (APAP) per day over four days to relieve muscle soreness. After biochemically ruling out diseases such as hepatitis and cirrhosis, we hypothesized that the athlete had suffered a muscle injury, after which his use of APAP resulted in liver injury.

Based on his recent history and biochemical profile, we suggested that the athlete decrease both his training intensity and duration to 30-40% of his previous effort and increase rest and sleeping time. We did not ask the athlete to completely refrain from training, since he was scheduled to compete in two weeks. We also suspended the use of APAP and advised dietary changes. Our dietary plan called for high carbohydrates (≥ 6 g of carbohydrates/kg/day) and high protein (≥2 g of proteins/kg/day). We also recommended a high intake of dietary methionine (Met) and cysteine (Cys), and a daily dose of 3600-4200 mg of N-acetylcysteine (NAC).

After two weeks following our training and dietary recommendations, the athlete reported that the pain had been relieved. This was corroborated by biochemical analysis, which showed that the amounts of CK and γGT in the blood were less than 20% and 70% of pre-treatment levels, respectively. We did not, however, detect a change in the level of total bilirubin in the blood; the athlete's level following treatment was still higher than the average value of his teammates. Nevertheless, we did detect an improvement in the bilirubin conjugation measured by the way of both direct and indirect bilirubin (Table 1).

Discussion

Endurance exercise is very stressful for the body. In fact, it has been proposed that endurance training can cause chronic liver injury [16]. Given the high CK values of this athlete, we initially thought that he might be suffering from overtraining syndrome[12]. However, the athlete's VO_{2max} test analysis showed him to have similar performance to his teammates. Since the VO_{2max} test is widely accepted as the gold standard for OTS diagnosis, we discarded this possibility.

The athlete reported having back pain after a bike drift. Due to the role of this muscle group in torso stabilization, we suspected that muscle injury was the principal cause of the high CK values. Consistent with this hypothesis, the athlete had higher levels of the classical injury markers CK and LDH than in his teammates. In fact, the CK level was greater by almost 275% and the LDH by 25%, and these increases are consistent with our recent report of the kinetics of CK and LDH appearance in the blood [3]. Our

explanation of muscle injury is further supported by the elevated CRP value, which indicates acute phase inflammatory response.

In the present case study, the athlete had a γGT level nearly twice as high as teammates following the same training schedule, and his AST level showed a slightly smaller increase. We previously showed that it is possible to separate muscle and liver injuries using alkaline phosphatase and γGT as hepatocyte integrity markers [1, 2]. These findings support the hypothesis of liver injury.

APAP is a widely used over-the-counter painkiller in Brazil. Following the bike drift, the athlete had begun taking a large daily dose of APAP to reduce back pain. Excessive doses of APAP have been linked to hepatocyte injury [11, 13]. It has also been proposed that APAP may abolish normal increase in post-exercise protein synthesis by blocking the production of $PGF_{2\alpha}$ via the cyclooxygenase enzyme, leading to impairment in muscle regeneration[14, 15].

Although we cannot ignore the reported correlation of endurance training to chronic liver injury [16], we believe that the hepatotoxic effect of APAP is to blame in this case. The high dose alone could lead to liver damage[11] and in addition it is known that acetaminophen consumers perceive lower exertional levels than do their counterparts not taking it,[6] and that APAP toxicity is enhanced by exercise[17]. It is also known that APAP does not influence protein catabolism [15], therefore we concluded that the athlete was suffering from acute back pain caused by muscle injury due to mechanical stress, and that subsequent APAP overdose led to liver injury. We recommended that he use N-acetylcysteine, an APAP antidote widely used in clinical treatment[9]. We further

recommended that he increase his dietary intake of naturally occurring Met and Cys, which have also been proposed to protect against APAP hepatotoxicity[8].

A second evaluation was conducted 15 days after the first, the CK and CRP levels in the athlete had fallen within the range observed in his teammates, during this evaluation, we were also able to discard inherited and chronic metabolic diseases. The level of γGT had decreased by 38% of the level measured at the first evaluation, but it was still higher than in his teammates. We observed an increase in hepatic function based on measurement of the liver's ability to conjugate bilirubin. Later evaluations showed no persistent liver damage or dysfunctions in the athlete.

Taking into consideration that the intake of APAP to relieve pain among athletes is almost 1000% higher than the non athlete population[7] , monitoring the consumption of APAP and monitoring hepatic indices is highly relevant to the sports medicine community. Our results suggest that our diagnosis was correct, and that the interventions, specially the merge of oral N-acetylcysteine with dietetic sulfured amino acids and rest is effective for treating mild APAP intoxication in athletes.

Rererences

1. Bassini-Cameron A, Monteiro A, Gomes A, Werneck-de-Castro JP, Cameron L. Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way. *Br J Sports Med.* Apr 2008;42(4):260-266.

2. Bassini-Cameron A, Sweet E, Bottino A, Bittar C, Veiga C, Cameron LC. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. *Br J Sports Med.* Aug 2007;41(8):523-530; discussion 530.

3. Bessa A, Nissembaum M, Monteiro AN, et al. High Intensity Ultraendurance Promotes Early Release Of Muscle Injury Markers. *Br J Sports Med.* Jan 18 2008.

4. Clark A, Stanish WD. An unusual cause of back pain in a young athlete. A case report. *Am J Sports Med.* Jan-Feb 1985;13(1):51-54.

5. Frank A. Low back pain. *Bmj.* Apr 3 1993;306(6882):901-909.

6. Garcin M, Mille-Hamard L, Billat V, Humbert L, Lhermitte M. Influence of acetaminophen consumption on perceived exertion at the lactate concentration threshold. *Percept Mot Skills.* Dec 2005;101(3):675-683.

7. Garcin M, Mille-Hamard L, Billat V, Imbenotte M, Humbert L, Lhermitte Z. Use of acetaminophen in young subelite athletes. *J Sports Med Phys Fitness.* Dec 2005;45(4):604-607.

8. Glazenburg EJ, Jekel-Halsema IM, Scholtens E, Baars AJ, Mulder GJ. Effects of variation in the dietary supply of cysteine and methionine on liver concentration of glutathione and "active sulfate" (PAPS) and serum levels of sulfate, cystine, methionine and taurine: relation to the metabolism of acetaminophen. *J Nutr.* Jul 1983;113(7):1363-1373.

9. Heard KJ. Acetylcysteine for acetaminophen poisoning. *N Engl J Med.* Jul 17 2008;359(3):285-292.

10. Kinkade S. Evaluation and treatment of acute low back pain. *Am Fam Physician.* Apr 15 2007;75(8):1181-1188.

11. Larson AM. Acetaminophen hepatotoxicity. *Clin Liver Dis.* Aug 2007;11(3):525-548, vi.

12. Margonis K, Fatouros IG, Jamurtas AZ, et al. Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis. *Free Radic Biol Med.* Sep 15 2007;43(6):901-910.

13. Terneus MV, Kiningham KK, Carpenter AB, Sullivan SB, Valentovic MA. Comparison of S-Adenosyl-L-methionine and N-acetylcysteine protective effects on acetaminophen hepatic toxicity. *J Pharmacol Exp Ther.* Jan 2007;320(1):99-107.

14. Trappe TA, Fluckey JD, White F, Lambert CP, Evans WJ. Skeletal muscle PGF(2)(alpha) and PGE(2) in response to eccentric resistance exercise: influence of ibuprofen acetaminophen. *J Clin Endocrinol Metab.* Oct 2001;86(10):5067-5070.

15. Trappe TA, White F, Lambert CP, Cesar D, Hellerstein M, Evans WJ. Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. *Am J Physiol Endocrinol Metab.* Mar 2002;282(3):E551-556.

16. Wu HJ, Chen KT, Shee BW, Chang HC, Huang YJ, Yang RS. Effects of 24 h ultra-marathon on biochemical and hematological parameters. *World J Gastroenterol.* Sep 15 2004;10(18):2711-2714.

17. Yoon MY, Kim SN, Kim YC. Potentiation of acetaminophen hepatotoxicity by acute physical exercise in rats. *Res Commun Mol Pathol Pharmacol.* Apr 1997;96(1):35-44.

Table 1

Parameter	Injured athlete (1st analysis)	Health athletes (1st analysis)	Injured athlete (2nd analysis)	Health athletes (2nd analysis)
CK (UI/L)	868	182.7 ± 19.8	147	190.3 ± 20.8
LDH (UI/L)	383	308 ± 50.6	408	296.3 ± 53.0
AST (UI/L)	57.3	42.0 ± 3.1	55.6	46.9 ±11.4
ALT (UI/L)	27.7	25.3 ± 3.1	19.8	35.2 ±14.4
γGT (UI/L)	47.1	16.6 ±2.7	33.9	15.3 ± 2.3
ALP (UI/L)	54	75.3 ± 11.3	44	81.3 ± 16.8
CRP (nmol/L)	62.53	25.9 ± 10.8	3.4	12.7 ± 5.1
Total bilirubin (µmol/L)	44.3	9.7 ± 1.6	40.0	18.1 ± 2.3
Direct bilirubin (µmol/L)	9.6	4.4 ± 0.7	18.8	13.7 ± 4.8
Indirect bilirubin (µmol/L)	34.7	5.3 ± 1.6	21.0	4.3 ± 2.5

Table title: Muscle and liver injury markers measured before and after treatment.

Column heads:

Parameter

Injured athlete (1st analysis)

Health athletes (1st analysis)

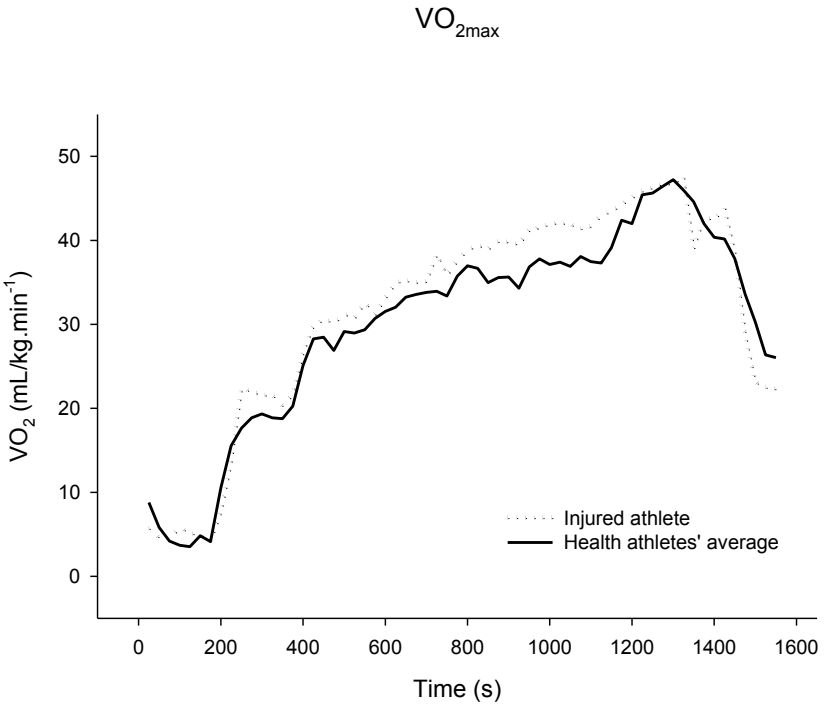
Injured athlete (2nd analysis)

Health athletes (2nd analysis)

Explanatory legends: Liver and muscle injury parameters measured before and after team counseling and athlete treatment. Data for health athletes are average ± SE.

FIGURE CAPTION

Figure 1. No difference was found in injured athlete's VO_{2max} when compared with his team average. Dashed line, injured athlete; straight line, health athletes' average.



CAPITULO IV

Bachini F, Bassini-Cameron A, Cameron LC. Nutrition, Metabolism and Exercise in Chronic Obstructive Pulmonary Disease. Brazilian Journal Investigating Pathological Morphological Morphometry. 2006. 1(3): 7-14.

Nutrição, Metabolismo e o Exercício na Doença Pulmonar Obstrutiva Crônica

Nutrition, Metabolism and Exercise in Chronic Obstructive Pulmonary Disease

Bachini, F. [1]; Bassini-Cameron, A.[1,2]; and Cameron, L. C.[1,2,3].

[1]Laboratório de Bioquímica de Proteínas. Universidade Federal do Estado do Rio de Janeiro
[2] Instituto de Genética e Bioquímica - Universidade Federal de Uberlândia.
[3] Programa de Pós-Graduação Stricto Sensu em Ciência da Motricidade Humana. Universidade Castelo Branco.

✉ Av Pasteur, 296 – Térreo
Urca - Rio de Janeiro – Brazil.
CEP: 22290-240
Fax: 55 21 25425204
Phone: 55 21 25425280
Email: cameron@unirio.br

Resumo.

Durante a evolução da Doença Pulmonar Obstrutiva Crônica (DPOC) há desequilíbrio no metabolismo com maior consumo de aminoácidos e ácidos graxos como substrato energético. O processo catabólico nos pacientes com DPOC é regulado iminentemente pela liberação de mediadores inflamatórios e hôrmonios liberados em resposta ao stress. Como conseqüência do baixo nível energético celular ocorre sinalização para a neoglicogênese e anaplerose levando a maior degradação de aminoácidos musculares. Ademais o uso crônico de corticosteróides amplia o desvio metabolico, podendo levar à redução da força respiratória e periférica, fadiga e morte. Indivíduos em catabolismo aumentado necessitam de suplementação nutricional e exercício para estimular o aumento da massa livre de gordura (MLG), de peso e melhora da capacidade física. Na presente revisão, discutimos os mediadores inflamatórios responsáveis pelas mudanças metabólicas ocorridas na DPOC.

Palavras - chave: Inflamação, corticosteróides, catabolismo, suplementação de aminoácidos, *turnover* protéico.

Abstract

During the evolution of Chronic Obstructive Pulmonary Disease (COPD) there is an imbalance in the metabolism increasing the use of both amino acid and fatty acid as energetic substrates. The catabolic process in these subjects is mainly regulated by the release of inflammatory mediators and stress hormones. Signalization for neoglucogenesis and anaplerosis takes place as a consequence of the low energetic status of the cell, increasing the muscular amino acid breakdown. Furthermore, the chronic administration of corticosteroids increases the metabolic deviation, leading to a decrease in both respiratory and peripheral strength, fatigue and death. Subjects with enhanced catabolism require nutritional supplementation and physical activity to develop free fat mass (FFM), in order to gain weight and to improve physical fitness. In this review, we discuss the inflammatory mediators involved in the metabolic changes of COPD.

Keywords: inflammation, corticosteroids, catabolism, amino acids supplementation, protein turnover

Introdução

A Doença Pulmonar Obstrutiva Crônica (DPOC) cursa com diversas alterações metabólicas que levam ao balanço nitrogenado negativo e ao comprometimento do estado nutricional (1-7). Este processo catabólico é disparado por mediadores inflamatórios e uso de grandes doses de corticosteróides que aumentam o fluxo de proteínas e geram uma diminuição de força dos músculos respiratórios e periféricos e um aumento do consumo de oxigênio (O_2) (1, 8-19). O resultado é a perda de massa muscular, a redução da capacidade de produzir força e a diminuição da tolerância aos esforços (2, 15, 19, 20). Estes eventos estão associados à redução dos níveis circulantes de insulin-like growth factor-1 (IGF-1) e leptina com conseqüente diminuição da síntese de proteínas e redução da ingestão de alimentos (7, 11, 17, 18, 21, 22).

Na tenativa de manter o nível energético, o aumento de mediadores inflamatórios e proteínas de fase aguda (PFA) levam a redução da massa muscular esquelética (1). Como conseqüência do baixo nível energético celular ocorre sinalização para a neoglicogênese e anaplerose levando a maior degradação de aminoácidos musculares (23). A gênese aumentada de metabólitos nitrogenados e ureagênese resultam em perda de massa muscular e disfunção muscular (16). Portanto, indivíduos em catabolismo aumentado necessitam de suplementação nutricional e exercício para estimular o aumento da massa livre de gordura (MLG), de peso e melhora da capacidade física (14, 20, 24-29).

A DPOC cursa com diversas alterações metabólicas que levam ao balanço nitrogenado negativo e ao comprometimento do estado nutricional (1-7). Este processo catabólico é relacionado ao aumento do consumo de oxigênio (O_2), a inflamação sistêmica, ao elevado *turnover* protéico, a ineficiência mecânica dos músculos respiratórios e ao uso terapêutico de corticosteróides (1, 8-19). A interação destes processos leva a perda de massa muscular, a redução da capacidade de produzir força, a diminuição da tolerância aos esforços.

Resultando em aumento do consumo de O_2 e do gasto energético basal (GEB) além da perda de massa magra e reinicia todo o processo (2, 15, 19, 20).

O aumento da concentração de citoquinas e PFAs exacerbam a redução da massa muscular esquelética para manutenção do nível energético celular (1). Em adição, a corticoterapia agrava o aumento no GEB e o efluxo de nitrogênio muscular contribuindo para instalação do processo catabólico. Estes eventos estão associados à redução dos níveis circulantes de insulin-like growth factor-1 (IGF-1) e leptina, tendo redução da síntese protéica e hipertrofia (7, 11, 17, 18, 21, 22).

A neoglicogênese, a ureagênese e o processo anaplerótico são vias ativadas para manutenção do equilíbrio metabólico (23) resultando em disfunção muscular (16). Assim, indivíduos catabólitos necessitam de suplementação nutricional e exercício para estimular a síntese de proteína e a MLG (14).

A reabiltação pulmonar associada a suplementos/complementos nutricionais aumentam a oferta de substrato para os processos anabólicos culminando em aumento de peso corporal, melhora da capacidade física e sobrevida; além de diminuir o tempo de internação (20, 24-29).

Discussion.

2. GASTO ENERGÉTICO

Na DPOC o desequilíbrio entre o aumento do consumo e redução do suprimento energético resulta na perda de peso corporal e diminuição da capacidade laboral pelo incremento REE. Causado pela ineficiência mecânica e metabólica dos músculos respiratórios; inflamação sistêmica; elevado metabolismo protéico e uso de fármacos (9, 13, 19). Assim, em indivíduos hipermetabólicos portadores de DPOC há dificuldade na ressíntese de proteínas que contribui para a redução da força muscular periférica e diafragmática, intolerância ao exercício, perda de peso corporal e maior consumo de O_2 (2, 8, 9, 11, 12, 13, 15, 18, 20).

2.1 METABOLISMO PROTÉICO

A diminuição do metabolismo protéico é observada em DPOCs com hipóxia pela diminuição do IGF-1 (14). Engelen et al (2000a) avaliaram o fluxo de proteínas em repouso e demonstraram que a degradação protéica é maior que a síntese de proteínas em DPOCs estáveis da mesma faixa etária. Ademais, a gravidade do enfisema pode sofrer influência do REE e do metabolismo de proteínas. O metabolismo protéico medido pela síntese de uréia pós–exercício nos pacientes com enfisema é similar a de sujeitos saudáveis em exercício de baixa intensidade, o que não exacerba o catabolismo de pacientes com enfisema (14).

Se separarmos os pacientes com enfisema em 2 subgrupos: macroscópico (E+) e microscópico (E-) de acordo com a tomografia de alta-resolução, estes apresentam respostas diferentes a suplementação de aminoácidos. Assim, a suplementação em E+ não alcança a mesma resposta comparado a E-, devido a diminuição da síntese de proteínas e a supressão do metabolismo de proteína pós-exercício (14).

As mudanças no metabolismo de proteínas mediadas pela inflamação ocorrem pela ativação de citoquinas que diminuem a concentração dos aminoácidos totais no plasma [alanina (Ala), glutamina (Gln), glutamato (Glu), asparagina (Asn)] e aumentam o metabolismo protéico em resposta à fase aguda de pacientes clinicamente estáveis portadores de DPOC e sem exacerbação dos sintomas (Figura 1) (13, 16). Esta resposta metabólica aumenta a perda de FFM no DPOC (1, 13). Como conseqüência da perda prolongada de massa magra haverá diminuição

da força muscular respiratória e periférica, redução da capacidade de exercício e risco de morte (2, 3, 17, 20).

2.2 INFLAMAÇÃO SISTÊMICA

Citoquinas são proteínas sinalizadoras extracelulares, normalmente menores do que 80 kDa de massa e produzidas por diferentes tipos de células envolvidas na interação célula-célula, funcionando de forma autócrina, endócrina e parácrina. As citoquinas podem induzir a expressão de receptores que mudam a responsividade da célula, além de terem ação sinergista ou antagônica, dependendo da afinidade dos receptores celulares, que são reguladas pelo trabalho celular (30). O processo inflamatório crônico com elevação das citoquinas séricas e APPs ocorre no indivíduo com DPOC estável (1, 10, 11, 12, 16). As citoquinas envolvidas neste processo são, principalmente, o fator de necrose tumoral-α (TNF-α), a interleucina-1 (IL-1) e a interleucina-6 (IL-6) (12, 31). O TNF-α e a IL-1β são citoquinas pró-inflamatórias que provocam a perda de massa muscular e podem levar a resistência ao IGF-1 em mioblastos dificultando a regeneração muscular (22). As citoquinas são classificadas de acordo com a Tabela 1 (31).

A inflamação crônica é a maior causa de aumento do REE no DPOC. Mesmo em DPOC com índice de massa corpórea (BMI) normal. Pode-se medir o aumento dos níveis séricos de proteína C reativa (CRP), IL-6 e TNF-α (1, 11, 12, 13, 31). Este fato, tem sido correlacionado a perda de massa muscular esquelética via sinalização por leptina relacionada ao status pró-inflamatório, diminuição da ingestão dietética e aumento do catabolismo (1, 11, 12, 13, 31, 32).

É sabido que em animais anoréxicos, o lipopolissacarídeo (LPS), o TNF-α e a IL-1 induzem a produção de leptina que reduz a ingestão de alimento (33). A diminuição do aporte energético contribui para perda de peso em pacientes estáveis com enfisema podendo ser considerado uma conseqüência dos efeitos metabólicos do TNF-α p55 e leptina (9, 32). Embora, Takabatake et al (1999) postulem não haver relação entre o sistema TNF-α e leptina, pois a regulação fisiológica da leptina seria mantida apesar da perda de peso. Por outro lado, a redução do consumo dietético e o aumento do TNF-α poderiam levar à anorexia e funcionar como indutores da perda de peso, o que explicaria o observado clinicamente (11).

O aumento das citoquinas pró-inflamatórias vem sendo associadas a depressão em idosos sendo relacionados à redução da função cognitiva e distúrbios do sono. Estas mudanças são

induzidas pelo stress psicológico e ativação do eixo hipotálamo-pituitário-adrenal (HPA) com aumento do nível de cortisol e redução do dehidroepiandrosterona (DHEA) (34, 35).

Este fato já foi observado em portadores de DPOC, indicando ativação do eixo HPA e dificuldade de hipertrofia (31). E pode ser explicado pela imunesenescência ligada a endocrisenescência que induz ao aumento da produção na proporção IL-6/TNF no qual eleva a razão cortisol/sulfato de dehidroepiandrosterona (DHEAS) (34, 35). Em particular, a redução da concentração de DHEAS aumenta a produção de IL-6 no envelhecimento ou na doença inflamatória (34). O conhecimento destas relações inflamatórias é importante para estabelecer alvos de combate durante o processo inflamatório da doença (35).

2.2.1 Fator de Necrose Tumoral

O TNF-α é liberado por macrófagos, células T, mastócitos e células epiteliais das vias aéreas desempenhando um importante papel no mecanismo de defesa (30). São conhecido pelo menos dois receptores de superfície celular do TNF-α (p55 e p75) que podem ser liberados como moléculas solúveis e agir como intermediários da atividade desta citoquina. O p55 parece exacerbar o mecanismo de resposta inflamatória por aumentar a migração dos neutrófilos para o pulmão, e o p75 parece ser um modulador da ação inflamatória, pois atenua o efeito tóxico do TNF-α. Assim, a sinalização do TNF-α não é dependente somente da sua concentração, mas também da ação dos receptores solúveis, p55 e p75 (36) (Figura 2):

Na DPOC, o TNF-α pode ser ativado por 2 sistemas: pela hipoxemia, que é apontada como a ativadora do sistema TNF-α e pela exposição a fumaça de cigarro, que aumenta os níveis de TNF-α (37, 38).

No portador de DPOC estável, principalmente em enfisematosos, o aumento da concentração plasmática de TNF-p55 parece estar envolvido na perda de massa muscular (1, 12, 19, 32). Embora, o aumento da secreção sorológica do TNF-α parece não envolvido na perda de peso em pacientes desnutridos com DPOC (39). Para explicar tal fato, tem sido proposto que citoquinas, de forma análoga as endotoxinas, promovam a expressão de leptina que reduz a ingestão de alimentos e contribui para a perda de massa muscular (1, 11, 12, 32, 33).

2.2.2 Interleucina–1

A IL-1é sintetizada nas células β, endoteliais, das vias aéreas, fibroblastos e em diversas células brancas (macrófagos, monócitos e neutrófilos) (30). São conhecidos dois tipos principais de IL-1 a α e β que se ligam a dois receptores: 1) IL-1RI encontrados nos fibroblastos, células T, endoteliais e da musculatura lisa; e 2) IL-1RII formado nas células β, monócitos e linfócitos polimorfonucleares (40).

O paciente com DPOC estável apresenta o mesmo nível sérico de sIL-RII que sujeitos saudáveis (12). O sIL-RII é um anti-inflamatório que se liga a IL-1β e reduz a disponibilidade desta citoquina e a sinalização inflamatória (40). Um dos efeitos do tratamento com corticosteróides é o aumento dos níveis de sIL-RII, que pode contribuir para a melhora clínica desses pacientes (12). Embora a IL-1β e a IL-6 possam estimular em hepatócitos a síntese do receptor antagonista da IL-1 (IL-1RA) que inibe os efeitos da IL-1 (41). Parece que com ausência de mudanças nos níveis de receptor solúvel de interleucina II (sIL-1RII) vem associado o aumento dos níveis de mediadores pró-inflamatórios, tais como: APP, sTNF–Rs e o número de leucócitos, estes achados sugerem um desequilíbrio entre os mediadores pró e anti-inflamatórios na circulação de indivíduos com DPOC estável (12). O aumento de mediadores pró-inflamatórios e IL-1 estimulam a produção de leptina levando a redução do apetite, aumento do REE e do metabolismo de proteínas (13, 14, 16, 33). Acrescentando a esta situação de caquexia, o TNF-α e a IL-1β induzem a resistência de IGF-1 em mioblastos levando a redução da miogênese (22).

2.2.3 Interleucina-6

A IL-6 é produzida pelas células T e pelas mesmas células que produzem IL-1 (30). A IL-6 liberada pelos macrófagos pode induzir a síntese de novo de proteína ligada a lipoproteína (LBP) pelo redirecionamento dos aminoácidos (Ala, Gln, Glu e Asn) do músculo para o fígado e promover aumento da resposta inflamatória induzindo a síntese hepática de LBP e CRP, auxiliando o desequilíbrio protéico e perda de massa muscular magra (Figura 1) (3, 42).

A IL-6 também pode ativar o eixo HPA e, conseqüentemente, as interações hormonais de fatores catabólicos (IL-6 e cortisol) e anabólicos [testosterona livre (Tbio) DHEAS e IGF-1], contribuindo para relações sinalizadoras do catabolismo (Tabela 2) (31).

2.2.4 Proteínas de Fase Aguda

Diversas proteínas podem ser usadas como marcadores inflamatórios agudos e de lesão teciduais. Clinicamente APPs são classificadas em dois grupos. As APPs 1 (CRP, substância amilóide A e α_1-glicoproteína ácida) são estimuladas por TNF-α, IL-1β, IL-6, dexametasona (DEX) e LBP. E as APPs 2 (fibrinogênio, haptoglobulina, ceruloplasmina, antiproteases) são reguladas pela IL-6 e a DEX (42, 43, 44).

A LBP é uma proteína plasmática que age na fase aguda da resposta de defesa das bactérias Gram–negativas que liberam LPS. Sabe-se que o LPS forma um complexo de alta afinidade com o CD14 de monócitos, provocando a liberação de TNF, IL-1 e IL-6 que contribuem para o aumento do REE e do metabolismo de proteínas (13, 14, 16, 42). Portadores de DOPC hipermetabólicos apresentam elevados níveis de CRP e LBP que sinalizam o redirecionamento de aminoácidos musculares para o fígado com objetivo de manter concentrações normais de proteínas no sangue e no músculo (11, 12, 16). Por isso, o aumento do fibrinogênio plasmático e da IL-6 são considerados indicativos do risco de hospitalização de pacientes com DPOC. Estes marcadores têm sido associados ao decréscimo da função pulmonar e aumento da inflamação das vias aéreas (43, 44).

Quando esta situação não é equilibrada pela ressíntese de proteína, há exacerbação do estado catabólico que pode levar à morte (3, 17, 18).

2.3 FÁRMACOS E METABOLISMO NO DPOC

Corticosteróides

Foi demonstrado em diferentes modelos animais que a triancinolona pode provocar hipotrofismo no fígado e nas fibras tipo IIb do diafragma e gastrocnêmios. Este efeito acontece devido a diminuição da síntese de IGF-1 ocasionando redução da força e massa muscular (21). A metilpredinisolona também provoca redução nos níveis séricos de IGF-1 (1, 7, 11, 21). E em

doses elevadas (66mg/dia) pode induzir ao hipermetabolismo e levar ao maior requerimento protéico, exacerbando o processo catabólico em DPOC hospitalizados e risco de morte (17). No paciente entubado, altas doses de metilpredinisolona (240 mg nas primeiras 48 horas) pode desenvolver miopatia quadriplégica aguda levando ao aumento da duração da ventilação mecânica e o tempo de internação (45). Contudo, em doses terapêuticas é capaz de melhorar a reação inflamatória das vias aéreas e o espasmo durante as exacerbações em pacientes internados (46).

Há evidências que predinisolona administrada em altas doses possa aumentar a concentração de leptina culminando em redução da ingestão alimentar, perda de peso e diminuição da força muscular periférica e respiratória (2, 9, 11, 18). E em baixas doses a predinisolona não prejudica a força muscular e respiratória, não altera as concentrações de enzimas glicolíticas e oxidativas e não piora a ressíntese de ATP, demonstrando que administrado em doses baixas pode ser seguro em pacientes estáveis (5, 47).

O tratamento com corticosteróides pode induzir à miopatia. Quando ocorre tal fato pode-se mudar o esquema terapêutico reduzindo as doses altas ou mudando por exemplo de triancinolona para predinisolona que é considerado mais seguro (48). E ainda, pode-se descontinuar a terapia em pacientes estáveis dependentes do corticosteróide, pois não causa um aumento de exacerbações e proteja o paciente da perda de peso (49).

3. ALTERAÇÕES MUSCULARES

O portador de DPOC apresenta hipotrofia e/ou atrofia das fibras musculares ocasionando dificuldade na síntese e metabolização de substratos devido à redução da atividade de enzimas oxidativas e glicolítica e diminuição da capacidade de realizar exercícios de endurance (20, 50). A redução da atividade da citrato sintase e 3-hidroxiacil CoA desidrogenase promovem uma diminuição na velocidade da β-oxidação e metabolização da acetil-CoA acompanhada de diminuição do consumo de O_2 máximo e da função pulmonar (4). Além disso, a redução da atividade de citocromo oxidase e succinato desidrogenase em pacientes hipoxêmicos pode ocasionar dificuldade de ressíntese de ATP e redução da reação ATP/ADP (23, 50). Em adição a este quadro enzimático, a ineficiência respiratória em repouso que ocorre pelo aumento da resistência das vias aéreas devido a produção de muco e fibrose pode estar vinculada a maior

hiperinsuflação dinâmica que exige aumento na resposta ventilatória. Assim, indivíduos com ineficiência mecânica (DPOC malnutridos e hipermetabólicos) e com redução da atividade de enzimas oxidativas e glicolíticas têm maior consumo de O_2 durante o exercício submáximo, que associado a inflamação sistêmica e a redução dietética podem levar a perda de peso, a redução da força muscular respiratória e redução da tolerância aos esforços (2, 8, 9, 15, 20, 50).

Os corticosteróides levam ao aumento do REE, redução da massa muscular e aceleração do catabolismo, elevando o risco de morte em pacientes com DPOC (17, 18).

4. EXERCÍCIO NO PORTADOR DE DPOC

Diversos fatores contribuem para a redução de oferta de O_2 para os tecidos, redução da capacidade do exercício e piora dos sintomas no portador de DPOC (20, 51). A redução da capacidade de exercício acontece em parte devido ao aumento da atividade glicolítica e redução da oxidação mitocondrial, associadas à redução da razão ATP/ADP e fosfocreatina/creatina (PCr/Cr) que resultam em acúmulo de piruvato (4, 23, 50, 52, 53).

A produção excessiva de citoquinas (IL-6, TNF-α e interferon-γ) inibe a produção e o efeito da eritropoetina nas células eritróides causando resistência ao hormônio diminuindo a maturação da hemácia (51). A limitação ao exercício no paciente com DPOC também pode acontecer via processos anêmicos causados pela inflamação.

O hipermetabolismo reduz a capacidade de realizar exercício devido ao aumento da necessidade de O_2 múscular (2, 8, 9, 11, 12, 13, 15, 18, 20). A perda de massa muscular e a disfunção muscular estão relacionados à hipoxemia em DPOCs aumentando o catabolismo do Glu intracelular que pode levar ao aumento da concentração de piruvato via alanina aminotransferase (ALT). Em seguida, o piruvato pode ser transformado em lactato com a finalidade de manter o potencial redox da célula (50, 53). A amônia produzida durante esta via é exportada do músculo ligada a Ala e Gln (carreadores não tóxicos), que são usados na gliconeogênese ou ureagênese (16, 54) (Figura 3).

Um programa de exercício de endurance é capaz de diminuir a freqüência respiratória de repouso pela redução da ventilação minuto e do espaço morto fisiológico ou aumento da taxa de eliminação do CO_2 (55). A redução da produção de catecolaminas e o aumento da extração de O_2 pode levar a angiogênese e incremento da capacidade oxidativa mitocondrial que culmina na

redução de lactato sérico ou geração de CO_2 do bicarbonato (29). Este aumento da capacidade oxidativa mitocondrial é verificado pela dimuição na relação fosfato inorgânico/fosfocreatina ([Pi]/[PCr]) e ligeiro aumento do pH intracelular após treinamento (28).

O sistema de regeneração muscular do DPOC parece ser preservado uma vez que pós-internação hospitalar há cerca de 8% de aumento na força de quadríceps após 90 dias somente com atividade de vida diária (7).

6. SUPLEMENTAÇÃO

Alguns pacientes com DPOC apresentam uma resposta inadequada a dieta devido a: anemia, anorexia, envelhecimento, inflamação sistêmica, e treinamento. Resultando em um equilíbrio energético negativo que afeta o trato gastrointestinal levando a diminuição da absorção (10, 14). A intervenção de dieta terapêutica precisa ser considerada com objetivo anabólico durante o treinamento, sinalizando equilíbrio energético positivo (14, 54, 56).

Assim, a suplementação nutricional durante o treinamento em pacientes depletados de FFM é necessária porque o exercício sozinho não é capaz de aumentar a síntese de proteínas e nos pacientes não-depletados é opcional, pois estes pacientes podem aumentar a força e a resistência muscular sem intervenções ergogênicas (3, 56).

Existem várias propostas para ergogênese na DPOC, por exemplo: as dietas ricas em carboidratos, ácidos graxos polinsaturados (PUFA), creatina, O_2, hormônio de crescimento (GH) e esteróides anabólicos que visão ganho de massa muscular e recuperação funcional (24, 25, 26, 27, 56, 57, 58, 59).

O primeiro estudo a investigar os benefícios de uma suplementação rica em carboidrato combinado a um programa de reabilitação verificou aumento da capacidade física e ganho de peso (56). A suplementação de PUFA também aumenta a capacidade física, mas sem elevar a FFM (24). Não foi observado melhora no desempenho físico durante duas semanas de suplementação com creatina antes do exercício. Por outro lado, a creatina parece ter efeito ergogênico positivo na manutenção do treinamento, já que foram observados: aumento da performance muscular; elevação na FFM e aumento nos níveis basais e de ressíntese da PCr (27).

Em pacientes desnutridos o uso de GH associado a suplementação de O_2 gera aumento da FFM, do desempenho físico e diminuição da dispnéia (25). Contudo, somente a suplementação de O_2 durante o exercício possibilita a melhora da performance, devido a inibição da vasoconstrição hipóxica e redução na pressão de oclusão da artéria pulmonar leva a uma redução do airtrapping (26).

O uso de testosterona (dose de ataque) e estanazolol em desnutridos culmina em aumento da FFM, mas sem melhorar a endurance (58). No entanto, a melhora da capacidade física e a redução da velocidade de hipotrofia do diafragama podem ser obtidos pelo uso de nandrolona devido a uma taxa mais elevada na eritropoese e na oferta de O_2 (57, 60). E a oxandrolona aumenta a massa corporal e não melhora a função pulmonar (59). Todos esses agentes anabólicos demonstram sinalizar o aumento do peso corporal, contudo há diferenças quanto ao desenvolvimento da performance física.

Conclusão.

Os distúrbios metabólicos nos pacientes com DPOC resultam em redução da tolerância aos esforços e risco de morte. A liberação de mediadores inflamatórios, o aumento do metabolismo de proteína, a liberação de APP e o uso crônico de corticosteróide iniciam o processo de debilidade muscular que levam a alteração da mecânica respiratória, resultando em disfunção muscular e fadiga precoce.

Os mediadores inflamatórios funcionam como sinalizadores do sistema hormonal reduzindo a ingestão alimentar e favorecendo a elevação dos hormônios catabólicos. Esta exacerbação do desequilíbrio metabólico diminui o metabolismo de proteína e dificulta o anabolismo. Assim, instala-se o estado de inanição na tentativa de manter as concentrações protéicas músculares e sanguíneas. Os mediadores inflamatórios sinalizam o efluxo de aminoácidos musculares para o fígado na tentativa de manter o suporte de substrato para os órgãos e tecidos, sintetizando principalmente, glicose, uréia e APP. Entretanto, durante este maior funcionamento hepático há exacerbação e retroalimentação do estado catabólico e morte. Que pode ser agravado pelo uso prolongado de corticosteróides pela redução do nível sérico de IGF-1.

Todos esses acontecimentos metabólicos juntos associados a hipóxia, ao envelhecimento, a hiperinsuflação pulmonar e resistência das vias aéreas culminam em aumento do consumo do O_2 e catabolismo.

O catabolismo leva a diminuição da capacidade de realizar exercício pela redução da atividade de enzimas oxidativas e glicolítica dificultando a ressíntese de ATP.

Exercícios de longa duração são capazes de aumentar o perfil oxidativo mesmo em pacientes com peso reduzido (20), junto à suplementação com O_2 e orientação nutricional feita por profissional competente. Desta forma, a fisioterapia surge como uma ferramenta importante para se conseguir melhorar a função muscular, o desempeno físico e tolerância às atividades do dia a dia.

References

1. Eid AA, Ionescu AA, Nixon LS, Lewis-Jenkins V, Matthes SB, Griffiths TL, Shale DJ. Inflammatory response and body composition in chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2001; 164: 1414-8.
2. Engelen MPKJ, Schols AMWJ, Baken WC, Wesseling GJ, Wouters EFM. Nutritional depletion in relation to respiratory and peripheral skeletal muscle fuction in out-patients with COPD. **Eur Respir J** 1994; 7: 1793-7.
3. Engelen MPKJ, Schols AMWJ, Does JD, Wouters EFM. Skeletal muscle weakness is associated with wasting of extremity fat-free mass but not with airflow obstruction in patients with chronic obstructive pulmonary disease. **Am J Clin Nutr** 2000c; 71: 733-8.
4. Maltais F, LeBlanc P, Whittom F, Simard C, Marquis K, Bélanger M, Breton M-J, Jobin J. Oxidative enzyme acticvities of the vastus lateralis muscle and the functional status in patients with COPD. **Thorax** 2000; 55: 848-53.
5. Pouw EM, Koerts-de Lang E, Gosker HR, Freling G, van der Vusse, EFM Wouters, Schols AMWJ. Muscle metabolic status in patients with severe COPD with and without long-term predinisolone. **Eur Respir J** 2000; 16: 247-52.
6. Salueda J, García-Palmer F, Wiesner RJ, Tarraga S, Harting I, Tomás P, Gómez C, Saus C, Palou A, Agustí AGN. Cytochrome oxidase activity and mitochondrial gene expression in skeletal muscle of patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1998; 1557: 1413-7.
7. Spruit MA, Gosselink R, Trooster T, Kasran A, Gayan-Ramirez G, Bogaerts P, Boullon R, Decramer M. Muscle force during an acute exacerbation in hospitalised patients with copd and its relationship with CXCL8 and IGF-1. **Thorax** 2003; 58: 752-6.
8. Baarends EM, Schols AMWJ, Akkermans MA, Wouters EFM. Decreased mechanical efficiency in clinically stable patients with COPD. **Thorax** 1997a; 52: 981-6.

9. Cohen RI, Marzouk K, Berkoski P, O`Donnel CP, Polotsky VY, Scharf SM. Body composition and resting energy expenditure in clinically stable, non-weight-losing patients with severe emphysema. **Chest** 2003; 124: 1365-72.
10. Creutzberg EC, Schols AMW, Weling-Scheepers CAPM, Buurman WA, Wouters EFM. Characterization of nonresponse to high caloric oral nutrition therapy in depleted patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2000a; 161: 745-52.
11. Creutzberg EC, Wouters EFM, Vanderhoven-Augustin IML, Dentener MA, Schols AMW. Disturbances in leptin metabolism are related to energy imbalance during acute exacerbations of chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2000b; 162: 1239-45.
12. Dentener MA, Creuztberg EC, Schols AMWJ, Mantovani A, van´t Veer C, Buurman WA, Wouters EFM. Systemic anti–inflammatory mediators in COPD: increase in soluble interleukin 1 receptor II during treatment of exacerbations. **Thorax** 2001; 56: 721-6.
13. Engelen MPKJ, Deutz NEP, Wouters EFM, Schols AMWJ. Enhaced levels of whole-body protein turnover in patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2000a; 162: 1488-92.
14. Engelen MPKJ, Deutz NEP, Mostert R, Wouters EFM , Schols AMWJ. Response of whole-body protein and urea turnover to exercise differs between patients with chronic pulmonary obstructive disease with and without emphysema. **Am J Clin Nut** 2003; 77: 868-74.
15. Mannix ET, Manfredi F, Farher MO. Elevated O_2 cost of ventilation contributes to tissue wasting in COPD. **Chest** 1999; 115; 708-13.
16. Pouw EM, Schols AMWJ, Deutz NEP, Wouters EFM. Plasma and muscle amino acid levels in relation to resting energy expenditure and inflammation in stable chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1998a; 158: 797-801.
17. Saudny-Unterberger H, Martin JG, Gray-Donald K. Impact of nutritional support on functional status during an acute exacerbation of chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1997; 156: 794-9.

18. Schols AMWJ, Wesseling G, Kester ADM, de Vries G, Mostert R, Slangen J, Wouters EFM. Dose dependent increased mortality risk in COPD patients treated with oral gkucocorticoids. **Eur Respir J** 2001; 17: 337-42.
19. Baarends EM, Schols AMWJ, Westerterp KR, Wouters EFM. Total daily energy expenditure relative to energy expenditure in clinically stable patients with COPD. **Thorax** 1997b; 52: 780-5.
20. Palange P, Forte S, Onorati P, Paravati V, Manfredi F, Serra P, Carlone S. Effect of reduced body weight on muscle aerobic capacity in patients with COPD. **Chest** 1998; 114: 12-8.
21. Gayan-Ramirez G, Vanderhoydonc F, Verhoeven G, Decramer M. Acute treatment with corticosteroids decreases IGF-1 and IGF-2 expression in the rat diaphragm and gatrocnemius. **Am J Respir Crit Care Med** 1999; 159: 283-9.
22. Strle K, Broussard SR, McCusker RH, Shen W-H, Johnson RW, Freund GG, Dantzer R, Kelley KW. Proinflammatory cytokine impairment of insulin-like growth factor I-induced protein synthesis in skeletal muscle myoblasts requires ceramides. **Endocrinology** 2004; 145(10): 4592-602.
23. Pouw EM, Schols AMWJ, van der Vusse GJ, Wouters EFM. Elevated inosine monophosphate levels in resting muscle of patients with stable chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1998b; 157: 453-7.
24. Broekhuizen R, Wouters EFM, Creutzberg EC, Weling-Scheepers CAPM, Schols AMWJ. Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. **Thorax** 2005; 60: 376-82.
25. Burdet L, de Muralt R, Schutz Y, Richard C, Fitting JW. Administration of growth hormone to underweight patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1997; 156: 1800-6.
26. Fujimoto K, Matsuzawa Y, Yamaguchi S, Koizumi T, Kubo K. Benefits of oxygen on exercise performance and pulmonary hemodynamics in patients with COPD with mild hypoxemia. **Chest** 2002; 122: 457-63.
27. Fuld JP, Kilduf LP, Neder JA, Pitsiladis Y, Lean MEJ, Ward SA, Cotton MM. Creatine supplementation during pulmonary rehabilitation in chronic obstructive pulmonary disease. **Thorax** 2005; 60; 531-7.

28. Sala E, Roca J, Marrades RM, Alonso J, Gonzalez de Suso JM, Moreno A, Barberà JA, Nadal J, Lluis de Jover, Rodriguez-Roisin R, Wagner PD. Effects of endurance training on skeletal muscle bioenergetics in chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1999; 159: 1726-34.
29. Vogiatzis I, Williamsom AF, Miles J, Taylor IA. Physiological response to moderate exercise workloads in a pulmonary rehabilitation program in patients with varying degrees of airflow obstruction. **Chest** 1999; 116: 1200-7.
30. Chung KF. Cytokines in chronic obstructive pulmonary disease. **Eur Respir J** 2001; 18(34): 50-9.
31. Debigaré R, Marquis K, Côté CH, Tremblay RR, Michaud A, LeBlanc P, Maltais F. Catabolic/anabolic balance and muscle wasting in patients with COPD. **Chest** 2003; 124: 83-9.
32. Schols AMW, Creutzberg EC, Buurman WA, Campfield LA, Saris WHM, Wouters, EFM. Plasma leptin is related to proinflammtory status and dietary intake in patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1999; 160(4); 1220-6.
33. Grunfeld C, Zhao C, Fuller J, Pollock A, Moser A, Friedman J, Feingold KR. Endotoxin and cytokines induce expression of leptin, the *ob* gene product, in hamster. **J Clin Invest** 1996; 97(9): 2152-7.
34. Straub RH, Konecna L, Hrach S, Rothe G, Kreutz M, Schölmerich J, Falk W, Lang B. Serum dehydroepiandrosterona (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man *in vitro*: possible link between endocrinosenescence and immunosenescence. **J Clin Endocrinol** *Metab* 1998; 83; 2012-7.
35. Straub RH, Schuld A, Mullington J, Haack M, Schölmerich J, T Pollmächer. The endotoxin-induced increase of cytokines is followed by an increase of cortisol relative to dehydroepiandrosterone (DHEA) in healthy male subjects. **Journal of Endocrinology** 2002; 175: 467-74.
36. Peschon JJ, Torrance DS, Stocking KL, Glaccum MB, Otten C, Willis CR, Charrier K, Morrissey PJ, Ware CB, Mohler KM. TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. **The Journal of Immunology** 1998; 160: 943-52.

37. Churg A, Dai J, Xie C, Wright JL. Tumor Necrosis Factor-α is central to acute cigarette smoke-induced inflammation and connective tissue breakdown. **Am J Respir Crit Care Med** 2002; 166: 849-54.
38. Takabatake N, Nakamura H, Abe S, Inque S, Hino T, Saito H, Yuki H, Kato S, Tomoike H. The relationship between chronic hypoxemia and activation of the tumor necrosis factor–α system in patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2000; 161: 1179-84.
39. Takabatake N, Nakamura H, Abe S, Hino T, Saito H, Yuki , Kato S, Tomoike H. Circulating leptin in patient with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 1999; 159: 1215-19.
40. Bourke M, Casseti A, Villa A, Fadlon E, Coletta F, Mantovani A. IL-1 scavenging by type II IL-1 decoy receptor in human neutrophils. **The Journal of Immunology** 2003; 170: 5999-6005.
41. Gabay C, Smith MF, Eidlen D, Arend WP. Interleukin receptor antagonist (IL-1Ra) is an acute - phase protein. **J Clin Invest** 1997; 99: 2930-40.
42. Grube BJ, Cochane CG, Ye RD, Green CE, McPhail ME, Ulevitch RJ, Tobias PS. Lipopolysaccharide binding protein expression in primary human hepatocytes and HepG2 hepatoma cells. **The Journal of Biological Chemistry** 1994; 269: 8477-82 .
43. Dahl M, Tybjaerg-hansen, Vestbo J, Lange P, Nordestgaard BG. Elevated plasma fibrinogen associated with reduced pulmonary function and increased risk of chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2001; 164(6): 1008-11.
44. Donaldson GC, Seemungal TAR, Patel IS, Bhowmik A, Wilkinson TMA, Hurst JR, MacCallum PK, Wedzicha JA. Airway and systemic inflammation and decline in lung function in patients with COPD. **Chest** 2005; 128: 1995-2004.
45. Amaya-Villar R, Garnacho-Montero J, García-Garmendía JL, Madrazo-Osuna J, Garnacho-Montero MC, Luque R, Ortiz-Leyba C. Steroid-induced myopathy in patients intubated due to exacerbation of chronic obstructive pulmonary disease. **Intensive Care Med** 2005; 31(1): 157-161.
46. Li H, He G, Chu H, *et al*. A step-wise application of methylprednisolone versus dexamethasone in the treatment of acute exacerbations of COPD. **Respirology** 2003; 8: 199-204.

47. Hopkinson NS, Man WD-C, Dayer MJ, Ross ET, Nickol AH, Hart N, Moxham J, Polkey MI. Acute effect of oral steroids on muscle function in chronic obstructive pulmonary disease. **Eur Resp J** 2004; 24: 137-42.
48. Dekhuijzen PNR, van Balkom RHH. Steroid-induced change in the respiratory muscles: its relevance in patients with obstructive airways disease. **Respiratory Medicine** 1994; 88: 335-41.
49. Rice KL, Rubins JB, Lebahn F, Parenti CM, Duane PG, Kuskowski M, Joseph AM, Niewoehner DE. Withdrawal of chronic systemic corticosteroids in patients with COPD. **Am J Respir Crit Care Med** 2000; 162: 174-8.
50. Gosker HR, van Mameren H, van Dijik PJ, Engelen MPKJ, van der Vusse GJ, Wouters EFM, Scchols AMWJ. Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. **Eur Respir J** 2002; 19: 617-25.
51. John M, Hoerning S, Doehner W, Okonko DD, Wit C, Anker SD. Anemia and inflammation in COPD. **Chest** 2005; 127: 825-9.
52. Engelen MPKJ, Schols AMWJ, Does JD, Deutz NEP, Wouters EFM. Alterated glutamate metabolism is associated with reduced muscle glutathione levels in patients with emphysema. **Am J Respir Crit Care Med** 2000b; 161: 98-103.
53. Engelen MPKJ, Schols AMWJ, Does JD, Gosker HR, Deutz NEP, Wouters EFM. Exercise – induce lactate increase in relation to muscle substrates in patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2000d; 162: 1697-704.
54. Engelen MPKJ, Wouters EFM, Deutz NEP, Does JD, Schols AMWJ. Effects of exercise on amino acid metabolism in patients with chronic obstructive pulmonary disease. **Am J Respir Crit Care Med** 2001; 163; 859-64.
55. O´Donnel DE, McGuire M, Samis L, Webb KA. General exercise training improves ventilatory and peripheral muscle strength and endurance in chronic airflow limitation. **Am J Respir Crit Care Med** 1998; 157: 1489-97.
56. Steiner MC, Barton RL, Singh SJ, Morgan MDL. Nutritional enhacement of exercise performance in chronic obstructive pulmonary disease; a randomised controlled trial. **Thorax** 2003; 58: 745-51.

57. Creutzberg EC, Wouters EFM, Mostert R, Pluymers BJ, Schols AMWJ. A role for anabolic steroids in the rehabilitation of patient with COPD? **Chest** 2003; 124: 1733-42.
58. Ferreira IM, Verreschi IT, Nery LF, Goldstein RS, Zamel N, Brooks D, Jardim JR. The influence of 6 months of oral anabolic steroids on body mass and respiratory muscles in undernourished COPD patients. **Chest** 1998; 114: 19-28.
59. Yeh S, Deguzman B, Kramer T. Reversal of COPD-asssociated weight loss using the anabolic agent oxandrolone. **Chest** 2002; 122: 421-8.
60. Van Balkom RHH, Dekuijzen PNR, van der Heijden HFM, Folgering HTM, Fransen JAM, van Herwaarden CLA. Effects of anabolic steroids on diaphragm impairment induced by methylprednisolone in emphysematous hamsters. **Eur Respir J** 1999; 13(5): 1062-9.

Tables
Table 1

Categoria	Citoquinas
Linfoquinas	IL-4, IL-5, IL-6, IL-10,IL-13
Fatores quimiotáticos para	
Neutrófilos	CXC quimioquinas (IL-8,GRO-α,ENA-78),IL-1,TNF,IL-17
Eosinófilos	CC quimioquinas (eotaxina, RANTES, MCP-4), GM-CSF
Monócitos/macrófagos	MCP-1, MIP-1α, RANTES
Células T	IL-16 (CD4+), MIP-1α, (CD8+), STCP-1(Th2), RANTES (memória), MCP-1
Pró – inflamatório	IL-1β, TNF-α, IL-6
Anti – inflamatória	IL-10, IL-1RA, IFN-γ
Fatores de crescimento	TGF-β, PDGF, EGF, IGF

Tabela 1. Citoquinas na doença pulmonar obstrutiva crônica. A tabela demonstra a classificação das citoquinas na DPOC: linfoquinas, fatores quimiotáticos (para neutrófilos, eosinófilos, monócitos/macrófagos, células T), pró-inflamatórios, anti-inflamatórios e fatores de crescimento (Tabela extraída de Chung, K.F., 2001). EGF: fator de crescimento da epiderme; ENA-78: neutrófilo ativador 78 derivados do epitélio; GM-CSF: Fator estimulante da colônia macrófago-granulócito; GRO-α: oncogene-α relacionada ao crescimento; 2; IFN-γ: interferon gama; IGF: fator de crescimento da insulina; IL: interleucina; IL-1RA antagonista do receptor IL-1; MCP: proteína quimiotática de monócitos; MIP-1α: 1α proteína inflamatória de macrófago; PDGF: plaquetas derivadas do fator de crescimento; RANTES: reguladas na ativação, células T normais expressadas e secretadas; STCP-1:1 proteína de células T estimuladas; TGF-β: fator de crescimento transformante-β; TNF: fator de necrose tumoral; Th2: célula helper tipo .

Table 2

Fatores anabólicos e catabólicos	MTCSA < 70cm^2	MTCSA > ou =70cm^2
DHEAS	↓	
IL-6	↑	↑
Cortisol		
Cortisol/DHEAS		↑
IL-6/DHEAS	↑	
IL-6/Tbio	↑	
IL-6/IGF-1	↑	

Tabela 2. Fatores anabólicos e catabólicos e sua relação com a área transversa do quadríceps de pacientes com DPOC. Os níveis de DHEAS estão reduzidos, enquanto os níveis IL-6 e suas relações com hormônios anabólicos se encontravam aumentados em pacientes com MTCSA < 70 cm^2 . O cortisol está elevado em pacientes com MTCSA < 70 cm^2 , como indicado pela proporção cortisol/DHEAS em pacientes com MTCSA > ou = 70 cm^2 estar aumentada. Em ambos os grupos, a IL-6 estava aumentada. MTCSA= área de secção transversa do músculo; ↑= aumenta; ↓= diminui (Adaptado de Debigaré *et al*, 2003).

Figures

Figure 1

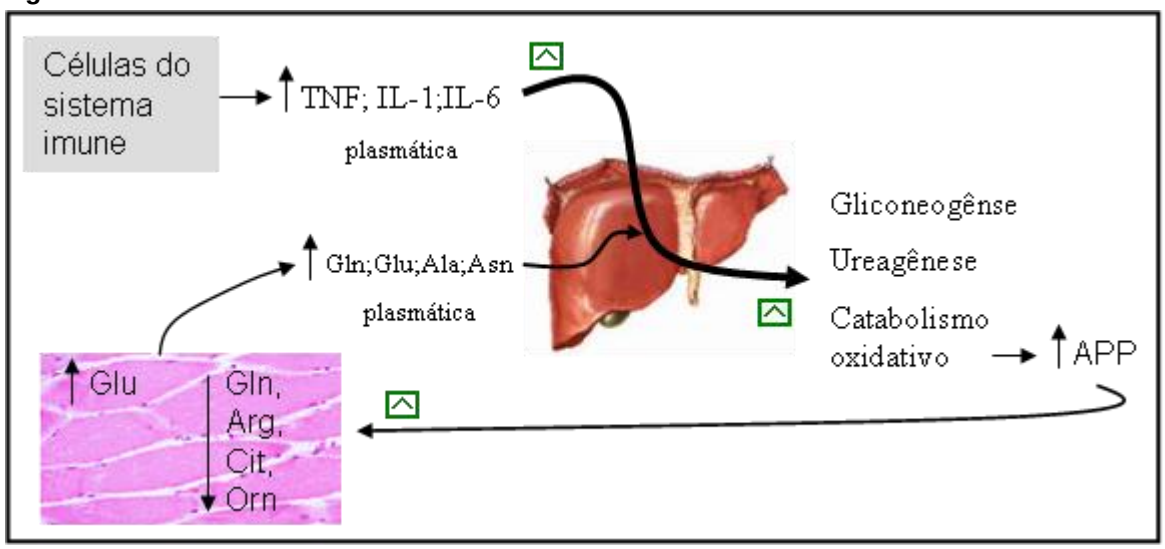


Figura 1. Regulação metabólica através da sinalização inflamatória. A ativação pelas citoquinas e redução da concentração plasmática de aminoácidos totais no plasma produzem o aumento do turnover protéico em resposta à fase aguda (Ala: alanina; APP: proteínas de fase aguda; Arg: arginina; Asn: asparagina; Cit: citrulina; IL-1: interleucina-1; IL-6: interleucna-6; Gln: glutamina; Glu: glutamato; Orn: ornitina; TNF: fator de necrose tumoral).

Figure 2

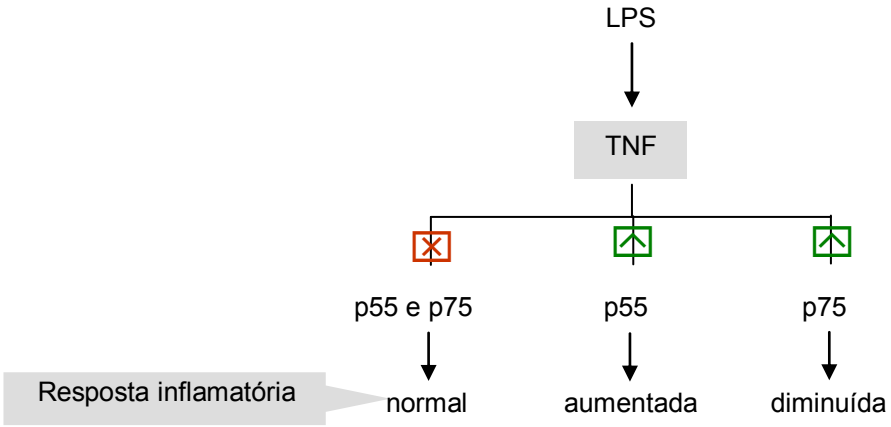


Figura 2. Resposta inflamatória induzida do TNF. A ausência dos receptores induz à resposta inflamatória normal. O TNF-α com o receptor p55 aumenta a resposta inflamatória. A presença do p75 modula a resposta inflamatória por atenuar os efeitos tóxicos do TNF-α (norm: normal).

Figure 3

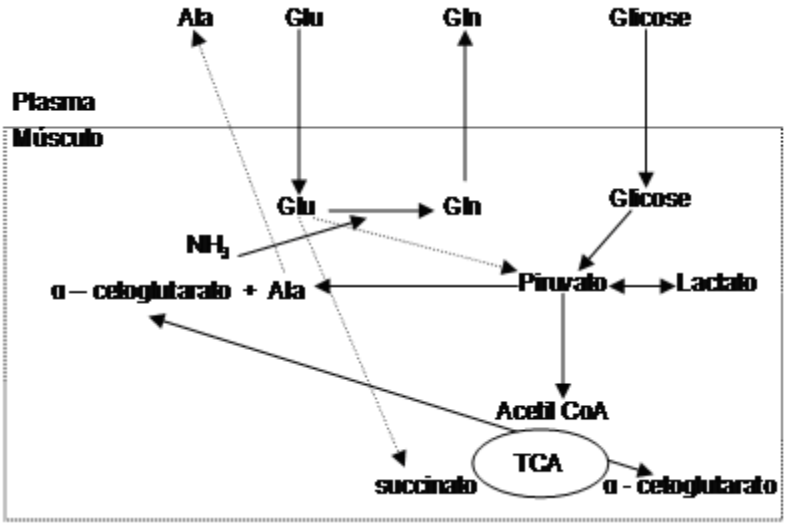


Figura 3. Metabolismo de aminoácidos durante o exercício. Observe a reação do Glu e piruvato na reação da alanina aminotransferase (ALT) para recompor a concentração de Ala e α-cetoglutarato. E a captação de amônia pelo Glu formando Gln. Extraído de Engelen *et al*, 2001 (Ala: alanina; Glu: glutamato; Gln: glutamina: NH_3: amônia; TCA: ciclo do ácido cítrico)

CAPITULO V

Bessa, A, Nissembaun, M., Monteiro, A, Gandra, PG, Nunes, LAS, Bassini-Cameron, A, Werneck-de-Castro, JPS, Macedo, DV, LC Cameron. High intensity ultraendurance promotes early release of muscle injury markers. British Journal of Sports Medicine. v.012008, p.1 - 21, 2008.

High Intensity Ultraendurance Promotes Early Release Of Muscle Injury Markers

Artur Bessa, Marcelo Nissembaum, André Nascimento Monteiro, Paulo Guimarães Gandra, Lázaro Alessandro Soares Nunes, Adriana Bassini-Cameron, João Pedro Saar Werneck-de-Castro, Denise Vaz de Macedo and Luiz-Claudio Cameron

Br. J. Sports Med. published online 18 Jan 2008;
doi:10.1136/bjsm.2007.043786

BJSM Online First, published on January 18, 2008 as 10.1136/bjsm.2007.043786

HIGH INTENSITY ULTRAENDURANCE PROMOTES EARLY RELEASE OF MUSCLE INJURY MARKERS

Artur Bessa[1#]; Marcelo Nissenbaum[1#]; André Monteiro[1]; Paulo Guimarães Gandra[4]; Lázaro Soares Nunes[4]; Adriana Bassini-Cameron[1,3]; João Pedro Saar Werneck-de-Castro[1,5,6]; Denise Vaz de Macedo[4] and Luiz-Claudio Cameron[1,2,3].

[1]Laboratory of Protein Biochemistry – Federal University of State of Rio de Janeiro

[2]Graduate Program in Human Movement Science – University Castelo Branco

[3]Institute of Genetics and Biochemistry – Federal University of Uberlândia

[4] Laboratory of Exercise Biochemistry – State University of Campinas

[5] Department of Bioscience of Physical Activity – EEFD - Federal University of Rio de Janeiro

[6] Laboratory of Cellular and Molecular Biology – IBCCF – Federal University of Rio de Janeiro

[#]Have made equal contribution for the paper.

Key words: muscle metabolism, cycling, exercise, inflammatory response, liver metabolism

Running title: WBC, Muscle Injury and Ultraendurance

Word count: 4339

ABSTRACT

Objective: To evaluate the impact of high intensity ultraendurance (HIU) cycling, using it as a possible way to understand muscle injury kinetics and blood immune cells' release during high intensity prolonged exercises.
Design: Male amateur triathletes enrolled during a cycling race of the International Bike Championship 800 km cycling relay (~23 h). Each athlete alternately cycled 20-25 minutes until exhaustion and performed a total of approximately 200 km.
Results: Creatine kinase levels in blood reached a 300% rise in a sigmoidal pattern, while lactate dehydrogenase levels increased 30-40% following a hyperbolic pattern. Aspartate aminotransferase and alanine aminotransferase levels increased up to 250% and 140%, respectively. Liver injury markers such as alkaline phosphatase and γ-glutamyltransferase remained stable. Platelets increased 20-30% from pre-exercise, and there was no change in hematocrit during the race. White blood cells rose nearly 200%. Leucocytes rose 210% during the race, with a major component coming from neutrophils, which increased more than 300%. Triacylglycerol levels were decreased at the finish and total cholesterol levels remained unchanged. Urate increased (up to 35%) during the first half of the race, and urea levels increased with a different pattern, increasing 45% in the second half.

Conclusions: Here we showed the blood appearance kinetics of muscle injury markers and some metabolites. We suggest that the increase in these enzymes came primarily from muscle damage instead of liver and that white blood cells are selectively mobilized independently of hemoconcentration. We also had shown the early appearance of muscle injury markers in this kind of exercise.

INTRODUCTION

Endurance and ultraendurance exercise are an extreme challenge to human metabolism. High intensity ultraendurance (HIU) exercise has been defined as repeated bouts of high-intensity exercise (>75% VO_{2max}) during an ultraendurance race with limited recovery[1]. Several studies have described metabolic changes during and after endurance exercise[2], high intensity exercise[3], and ultraendurance exercise[4], but very little is known about metabolism in HIU. This kind of activity exposes athletes to exercise intensities higher than in regular endurance events[1, 5, 6] and is poorly studied, specially in field protocols that conserves the psychological and environmental stress. The high level physical demand during these events induces acute changes in metabolism, including muscle injury[5]. Some of these changes can be accessed by hematological and biochemical analysis of blood, powerful tools in understanding exercise intensity and metabolism in physical stress[7-9].

Among all of the changes imposed on the metabolism by exercise, ammonia and its metabolites has been focus of several recent studies, for review see[10, 11]. Blood ammonia concentration increases during endurance exercise mainly due to

myokinase activity and deamination by muscle[4], and has been proposed as a cause for both peripheral and central fatigue[10-12]. Also, metabolites such as urate and urea increase during high intensity exercise in response to the IMP and ammonia clearance demand[2].

The strong muscle contractions during exercise may cause micro-tears in both muscle and the vascular endothelium, which increases the migration of white blood cells into the muscle, inducing acute-phase inflammatory reactions. We have shown muscle injury markers appear in an early window with leukocytosis during intermittent exercise[13]. Since HIU exercise is high intensity and intermittent, we decided to evaluate its impact on classical muscle injury markers, white blood inflammatory cells and muscle and liver metabolism[14-19].

Here, we use HIU exercise to evaluate extreme physical stress on biochemical and hematological parameters. Since athletes were subjected to high intensities for a long period of exercise, we also hypothesized that HIU exercise is a good model for understanding part of nitrogen metabolism and the relationship between muscle injury markers kinetics and the white blood cell response. There are a couple of unique features of our study. First, this research took place on a highway without regular traffic interruption (night and day, ~22 h), which adds a considerable amount of environment stress. In addition, we also measured several biochemical and hematological parameters, making this a more accurate investigation on this subject[1, 5]. And more we here show the appearance of muscle injury markers as short as six hours of exercise.

MATERIALS AND METHODS

Subjects

An entire cycling team, consisting of four healthy male amateur triathletes (37 ± 2.7 years old, 82.5 ± 3.5 kg body mass, height = 180.2 ± 2.2 cm; VO_{2max} = 47.6 ± 1.8 ml of $O_2.Kg^{-1}.min^{-1}$; HR_{max} = 170 ± 5 bpm) enrolled voluntarily in this study. The subjects were initially submitted to an anthropometric and laboratorial analysis and denied using ergogenic resources or drugs. Written informed consent was obtained from all athletes. This study was approved by the ethics committee for human research of the Universidade Castelo Branco and conformed to the requirements for carrying out research in human subjects (Health National Council, Brazil, 1996).

The race and blood sampling

We collected blood samples during one cycling race of the International Cycling Championship. Briefly, the race consisted of 800 km relay cycling (~23 h); each athlete alternately cycled 20-25 minutes until exhaustion and performed approximately a total of 200 km (1/4 of the race). The athletes cycled on a regular highway without transit interruption and with a safety car to protect them. After each cycle, the athletes were picked up by a support team car and were able to rest until the next ride set. We used two more vehicles: a mobile laboratory that had to be developed to collect and to pre-treat blood samples in order to avoid the loss of volatile compounds and protein degradation and another car to transport the blood collection team.

Blood samples were collected after the exercise set whenever the subject completed 50 km. In other words, we collected blood right after the maximum effort of each athlete at 50, 100, 150 and 200 km. We weighed the athletes immediately after each cycle for drink counseling regarding weight loss. While resting between the exercises sets (60-75 min), athletes were allowed to eat *ad libitum* and drink under our counseling. Water, sports drinks, carbohydrate gels, fruits and pasta were available in the support car.

Hematologic and biochemical analysis

Venipuncture made by a certified phlebotomist was performed before and during the race. After each running cycle, the athlete stopped and in a 30 sec window had his blood collected. Samples for biochemical assay were collected into tubes with coagulation enhancer and splitting gel (*Vacuette, Greiner Bio-One)* and immediately centrifuged (3,000 x g; 10 min). Blood serum or plasma was aliquoted and stored in liquid nitrogen for later analysis. Samples were analyzed for muscle injury markers and biochemical variables. Biochemical analysis were done in an automatic device (*Autolab 18 - Boehringer Mannheim)* for creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γGT), lactate dehydrogenase (LDH), alkaline phosphatase

(ALP), glucose, urea, creatinine, urate, cholesterol, triacylglycerols (TG), total proteins, albumin, serum iron, bilirubin, C-reactive protein (CRP) and acid α-1-glycoprotein.

Hematological analysis was performed by automated analysis (KX-21N Sysmex) from blood collected into tubes containing EDTA and stored at 4 ºC. Total and differential white cell count were performed, red blood cells and platelets were also measured.

Statistics

All data were normalized to pre-race values and are expressed as means ± SE, this is so that the changes between pre and post race values are more clear. Statistical significance was calculated by analysis of variance (*One-way ANOVA*) and the level of significance was set at $P < 0.05$. Linear regression was done by Pearson's correlation.

Nonlinear regression was done to determine LDH and CK kinetics.

The increase in LDH activity curve was fitted according to data following a two parameters hyperbolic equation:

$$y = \frac{ax}{b + x}$$

The calculated parameters for the data were: a = 49.8 ± 1.8 and b = 30.1 ± 4.8.

The sigmoidal increase in CK activity curve was fitted according to data following a three parameter equation:

$$y = \frac{a}{1 + e^{-\left(\frac{x - x0}{b}\right)}}$$

The calculated parameters for the data were: a = 351.3 ± 23.8; b = 36.5 ± 5.0 and x_0 = 117.0 + 7.6.

RESULTS

Injury Markers

We observed different blood markers changes during the race. Creatine kinase (EC 2.7.3.2; CK) blood levels rose in the race, reaching a 300% increase in a sigmoidal pattern. Conversely, lactate dehydrogenase (EC 1.1.1.27; LDH) levels increased with a hyperbolic behavior to 30-40%, reaching most of its raise in the first 50 km (fig 1). Aspartate aminotransferase (EC 2.6.1.1; AST) and alanine aminotransferase (EC 2.6.1.2; ALT) had a clear increase up to 250% (fig 2A) and 140% (fig 2B) from pre-exercise values, respectively, at 50 km. ALT remained greater than before exercise at the end of the race.

Typical liver injury markers such as alkaline phosphatase (EC 3.1.3.1; ALP) and γ-glutamyltransferase (EC 2.3.2.2; γGT) remained stable during the race 22.4 ± 9.6 U/L and 75.5 ± 26.7 U/L respectively, due to personal differences.

Blood cells

To understand the effect of high intensity ultraendurance exercise on blood cells, we measured the amount of platelets and both red and white blood cells. There was no change in hematocrit, hemoglobin, MCV, MCH or MCHC during the race. Platelets increased 20-30% from pre-exercise at 100 km, returning to pre-exercise levels (table 1).

	Pre (absolute value)	50Km (% of pre)	100Km (% of pre)	150Km (% of pre)	200Km (% of pre)
Hematocrit	44.6 ± 0.9 (%)	99.2 ± 1.6	98.2 ± 2.2	99.0 ± 2.1	99.5 ± 2.0
Haemoglobin	2.32 ± 0.06 (mmol/L)	100 ± 2.08	99 ± 2.1	99.2 ± 1.7	100.3 ± 1.6
MCV	88.7 ± 2.7 (fL)	99.5 ± 0.3	99 ± 0.4	97.2 ± 1.4	98.7 ± 0.2
MCH	29.7 ± 0.97 (pg)	100 ± 0.4	100 ± 0.4	98.2 ± 2.1	100.3 ± 0.4
MCHc	32,2 ± 1,3 (g/dL)	105.3 ± 4.6	106 ± 4.7	105.5 ± 4.6	106.3 ± 4.9
Platelets	251 ± 31 ($x10^9$/L)	115.0 ± 8.4	122.3 ± 2.1*	127 ± 7.1*	121.0 ± 2.0

Table 1. Hematological parameters measured during high intensity ultraendurance cycling. Data are mean ± SE. Statistical analyses were done using normalized values. * $p<0.05$ compared to pre-exercise. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHc, mean corpuscular hemoglobin concentration

Total white blood cells count (WBC) rose to nearly 200% with hyperbolic kinetics from pre-exercise over the whole race, without differences between the points. To assess the extent of exercise influence on WBC, we also did a differential WBC count, measuring the leukocyte sub-populations. Lymphocytes increased up to 210% during the race (fig 3A). The major contribution to the leukocyte increase came from neutrophils, which increased more than 300% from the first measurement (50 km). Even with an increase from 300-740%, CRP was higher than pre-exercise only at 150 km (fig 3B). We plotted the erythrocyte counts together with leukocyte sub-populations to control for blood volume variations. The red blood cells remained at the same level during the competition.

Glucose, Fat Metabolism, Urate and Urea

Glucose levels fluctuated during the contest, as expected, due to CHO intake (Fig 4A). Triacylglycerol levels decreased at the end of the race to 87% compared to

those at 50 km (Fig 4B), and total cholesterol levels remained unchanged (data not shown). Urate increased (up to 35%) during the first half of the race, and urea levels increased with a different pattern, reaching a 45% growth at the second half (Fig 5A and 5B, respectively).

DISCUSSION

Distinctly of usual ultraendurance competitions, athletes have time for partial metabolic recover after each exercise bout. We believe that the interpretation of data in this study leads to the understanding of some metabolic responses in HIU and also give some directives about the metabolic response during this kind of exercise.

Due to the high difficulty of data collection during the race we evaluated an apparently small number of athletes. It was possible due to previous biochemical and hematological analysis showing group similarity. During an 800 km relay cycling race we measured markers of inflammation, muscle injury and nitrogen metabolism. To our knowledge, this is the first study to examine hematological and biochemical responses in a non-staged field protocol of a HIU cycling race. It is important to figure out that we used an in-field experimental design allowing us to keep all the race stress.

Injury Markers

It is clear that exercise is a powerful inducer of muscle injury. In our study, LDH (~140 kDa) had a burst with a hyperbolic increase, tending to saturation at 100 km. As expected, the smaller protein CK (~86 kDa) had a bigger increase, reaching a 300% increase with a Hill number of ~1.8. This represents a cooperative behavior to its appearance in blood, probably because most of CK is bound to the sarcomere M-line. Our data are in agreement with the changes in injury biomarkers being more pronounced during the second half of a 200 km ultramarathon race[20].

An interesting finding in this study is that we were able to show an increase in the muscle injury markers CK and LDH much earlier than classically described[21, 22], a result also recently depicted by another group[23]. This could be due to the race design, where the athletes rested and were able to keep at high intensity exercise, or simply because these premature time frames were not exhaustively investigated previously.

Since injury markers such as LDH, ALT and AST increase following both liver and muscle injury; it is unclear whether hepatic damage occurs in ultraendurance exercise[24]. Furthermore, it has been proposed that basal values of these enzymes in ultraendurance athletes are high due to chronic liver damage following long-term strenuous exercise[9]. In the present study, we observed increased amount of blood CK, LDH, AST and ALT. These raises could be due to muscle and/or liver damage. Our laboratory recently proposed that muscle damage can be dissociated from liver harm by using more specific hepatic injury markers as ALP and γGT[13]. Taking these data together with maintenance of in either ALP or γGT amount in blood found here, we suggest that the increases of these enzymes came primarily from muscle damage instead of liver.

Blood Cells

Athletes' body weights were constant during the race (data not shown). During the experiment, there were no differences in hematocrit, hemoglobin, MCV, MCH and

MCHC, which represents a lack in volemic variation. On the other hand, there is a previously described inverse correlation between body weight and hematocrit after a marathon[8]. In addition, some studies reported increases in hematocrit, hemoglobin, MCV, and MCH, indicating hemoconcentration at the end of a 36 h continuous marathon[25, 26]. Since the athletes' were allowed to rest and to drink (water and isotonic beverages) during our study, this maintenance of volemia can be explained by the relay race dynamics and drink counseling by our group. In accordance with our results, previous studies did not find differences in hematological parameters during a 24 h ultraendurance marathon in relay runners or non-stop runners[5, 9].

White blood cell count increases after both endurance and ultraendurance exercise have been extensively reported[8, 9, 25]. We measured an important increase in total WBC, especially neutrophils and lymphocytes. This 280% increment in the total WBC population, was already observed six hours after the beginning of the race. More importantly, erythrocyte counts did not change at all over the race. Taken together, these results suggest that WBC mobilization is not due to a non specific exercise induced spleen release, but rather from a specific signal. In addition, platelets also seem to be affected by exercise in a spleen-independent manner, since we observed thrombocytosis without any change in erythrocyte counts. These data suggest that leukocytosis and thrombocytosis could be induced by muscle injury as proposed previously[9, 20].

Muscle lesions stimulate immune cell mobilization to the blood stream and migration to muscle tissue, which is consistent with WBC mobilization due to muscle injury[13]. We show here that there was an increase in skeletal muscle injury markers in smaller time frames than classically reported. We were also able to measure the increase in WBC together with classical muscle injury markers, suggesting a possible signaling role from muscle injury to promote this early WBCs' migration. Hence, it is possible to propose that we can relate the WBC increase measured in this study with precocious muscle damage.

Metabolism

It is easily predictable that HIU exercise could promote high metabolic stress. To access this, we evaluated the impact of HIU exercise on indicators of exercise metabolism intensity[3]. Since the athletes had accessibility to food, it is difficult to perform a metabolic interpretation of glucose levels. It is interesting to note that the levels of triacylglycerols decreased without changes in cholesterol. Since the athletes were not feed with significant amount of lipids, these findings may reflect the use of fatty acids as fuels, as predicted in long term exercise[27] in addition to a smaller synthesis of triacylglycerols.

In high intensity exercise part of ammonia production comes from amino acid deamination which contributes less than IMP production. Even having a huge amount of CHO supply during the race both urate and urea increase near of 40-50 %. Urate was significantly increased above baseline values in the first half of the race, and decreased after that. On the other hand, urea increased in the second

half, with a linear progression (r^2 = 0.98). Some attempts were tried before predict the behavior of metabolic pathways in whole organisms which is difficult even taking apart compartmentalization[28-30]. We suppose that the blood urea increase in the second race half followed a slowest ammonia metabolism than IMP degradation, probably due to the need of mitochondrial contribution. These findings lead us to believe that in the first part of the race, the athletes had an energetic demand with great IMP production, which was partially equilibrated by the use of less entropic energetic substrates in the latter half of the race. Taking all these data together our results lead us to suggest that the exercise demand of the first half of HIU is metabolic compensated after that probably due the consumption of fat acids.

WHAT IS ALREADY KNOWN

HIU exercise is an enormous challenge to the body. Muscle damage is known to affect many athletes and its measurement become an important tool to avoid injuries and performance decrease[1, 5]. The analysis of muscle enzymes such as CK and LDH released to the blood stream have been used to quantify muscle injury extension which an blood appearance window of 24-72h after exercise [1, 21, 24].

WHAT THIS ARTICLE ADDS

The increase in WBC population after endurance, ultra-endurance and HIU exercise have been reported but never had their kinetics evaluated. Furthermore, we show an early appearance of several injury markers as CK, LDH, AST and ALT; blood cells and metabolic indicators during the HIU event. We also show that during HIU there is a bigger metabolic demand in the first half of the race followed by a metabolic adaptation.

REFERENCES

1 Laursen PB, Ahern SM, Herzig PJ, Shing CM, Jenkins DG. Physiological responses to repeated bouts of high-intensity ultraendurance cycling--a field study case report. *J Sci Med Sport* 2003;**6**:176-86.
2 Boyum A, Ronsen O, Tennfjord VA, Tollefsen S, Haugen AH, Opstad PK, et al. Chemiluminescence response of granulocytes from elite athletes during recovery from one or two intense bouts of exercise. *Eur J Appl Physiol* 2002;**1**:20-8.
3 Bassini-Cameron A, Monteiro AN, Gomes A, Cameron LC. Glutamine protects against blood ammonia increase in soccer players in a exercise dependent way.
4 Banister EW, Cameron BJ. Exercise-induced hyperammonemia: peripheral and central effects. *Interl J Sports Med* 1990;**2**:129-42.
5 Nagel D, Seiler D, Franz H. Biochemical, hematological and endocrinological parameters during repeated intense short-term running in comparison to ultra-long-distance running. *J Sci Med Sport* 1992;**13**:337-43.
6 Neumayr G, Pfister R, Mitterbauer G, Gaenzer H, Sturm W, Eibl G, *et al*. Exercise intensity of cycle-touring events. *Int Sci Med Sport* 2002;**7**:505-9.

7 Page AJ, Reid AS, Speedy DB, Mulligan GP, Thompson J. Exercise-associated hyponatremia, renal function, and nonsteroidal antiinflammatory drug use in an ultraendurance mountain run. *Clin J Sport Med* 2007;**1**:43-8.
8 Reid SA, Speedy DB, Thompson JM, Noakes TD, Mulligan G, Page T, *et al*. Study of hematological and biochemical parameters in runners completing a standard marathon. *Clin J Sport Med* 2004;**6**:344-53.
9 Wu HJ, Chen KT, Shee BW, Chang HC, Huang YJ, Yang RS. Effects of 24 h ultra-marathon on biochemical and hematological parameters. *World J Gastroenterol* 2004;**18**:2711-4.
10 Nybo L, Dalsgaard MK, Steensberg A, Moller K, Secher NH. Cerebral ammonia uptake and accumulation during prolonged exercise in humans. *J Physiol* 2005;**1**:285-90.
11 Nybo L, Secher NH. Cerebral perturbations provoked by prolonged exercise. *Prog Neurobiol* 2004;**4**:223-61.
12 Carvalho-Peixoto J, Alves RC, Cameron LC. Glutamine and carbohydrate supplements reduce ammonemia increase during endurance field exercise. *Appl Physiol Nutr Metab* 2007;*in press*.
13 Bassini-Cameron A, Sweet E, Bottino A, Bittar C, Veiga C, Cameron LC. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. *Br J Sports Med* 2007;**8**:523-30.
14 Horrigan LA, Kelly JP, Connor TJ. Immunomodulatory effects of caffeine: friend or foe? *Pharmacol Ther* 2006;**3**:877-92.
15 Howatson G, Gaze D, van Someren KA. The efficacy of ice massage in the treatment of exercise-induced muscle damage. *Scand J Med Sci Sports* 2005;**6**:416-22.
16 Ikarugi H, Shibata M, Shibata S, Ishii H, Taka T, Yamamoto J. High intensity exercise enhances platelet reactivity to shear stress and coagulation during and after exercise. *Pathophysiol Haemost Thromb* 2003;**3**:127-33.
17 Li Y, Koike K, Che Q, Yamaguchi M, Takahashi S. Changes in lactate dehydrogenase and 3-hydroxyacetyl-CoA dehydrogenase activities in rat skeletal muscle by the administration of Eucommia ulmoides OLIVER leaf with spontaneous running-training. *Pharm Bull* 1999;**9**:941-6.
18 Paulsen G, Benestad HB, Strom-Gundersen I, Morkrid L, Lappegard KT, Raastad T. Delayed leukocytosis and cytokine response to high-force eccentric exercise. *Med Sci Sports Exerc* 2005;**11**:1877-83.
19 Pedersen BK. Special feature for the Olympics: effects of exercise on the immune system: exercise and cytokines. *Immunol Cell Biol* 2000;**5**:532-5.
20 Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur J Appl Physiol* 2007;**4**:443-7.
21 Chen TC, Hsieh SS. Effects of a 7-day eccentric training period on muscle damage and inflammation. *Med Sci Sports Exerc* 2001;**10**:1732-8.
22 Milias GA, Nomikos T, Fragopoulou E, Athanasopoulos S, Antonopoulou S. Effects of eccentric exercise-induced muscle injury on blood levels of platelet

activating factor (PAF) and other inflammatory markers. *Eur J Appl Physiol* 2005;**6**:504-13.
23 Siegel AJ, Verbalis JG, Clement S, Mendelson JH, Mello NK, Adner M, et al. Hyponatremia in marathon runners due to inappropriate arginine vasopressin secretion. *Am J Med* 2007;**5**:461-511.
24 Fallon KE, Sivyer G, Sivyer K, Dare A. The biochemistry of runners in a 1600 km ultramarathon. *Br J Sports Med* 1999;**4**:264-9.
25 Skenderi KP, Kavouras SA, Anastasiou CA, Yiannakouris N, Matalas AL. Exertional Rhabdomyolysis during a 246-km continuous running race. *Med Sci Sports Exerc* 2006;**6**:1054-7.
26 Spiropoulos K, Trakada G. Hematologic and Biochemical Laboratory Parameters Before and After a Marathon Race. *Lung* 2003;**2**:89-95.
27 de Lange P, Moreno M, Silvestri E, Lombardi A, Goglia F, Lanni A. Fuel economy in food-deprived skeletal muscle: signaling pathways and regulatory mechanisms. *Faseb J*. 2007;*in press*.
28 Curto R, Voit EO, Sorribas A, Cascante M. Mathematical models of purine metabolism in man. *Math Biosci* 1998;**1**:1-49.
29 Heinmets F. Supercomputer analysis of purine and pyrimidine metabolism leading to DNA synthesis. *Cell Biophys* 1989;**3**:283-323.
30 Franco R, Canela EI. Computer simulation of purine metabolism. *Eur J Biochem* 1984;**2**:305-15.

FIGURE CAPTIONS

Figure 1. Muscle injury markers increase during high intensity ultraendurance cycling. Data are plotted as blood creatine kinase (■, CK) and lactate dehydrogenase (●, LDH) against distance cycled. Lines show non linear fitting curves using the parameters described under material and methods. Data are shown as average ± SE of the increase in normalized activity from pre-exercise (0%). Absolute pre-exercise values are shown within the graphs. * $p<0.05$ compared to pre-exercise. # $p<0.05$ compared to 50 km.

Figure 2. Blood presence of aminotransferases during HIU. A) Aspartate aminotransferase (AST) and B) Alanine aminotransferase (ALT) measured in blood. Data are normalized (average ± SE) to pre-exercise values (100%). Absolute pre-exercise values are shown within the graphs (U/L). * $p<0.05$ compared to pre-exercise.

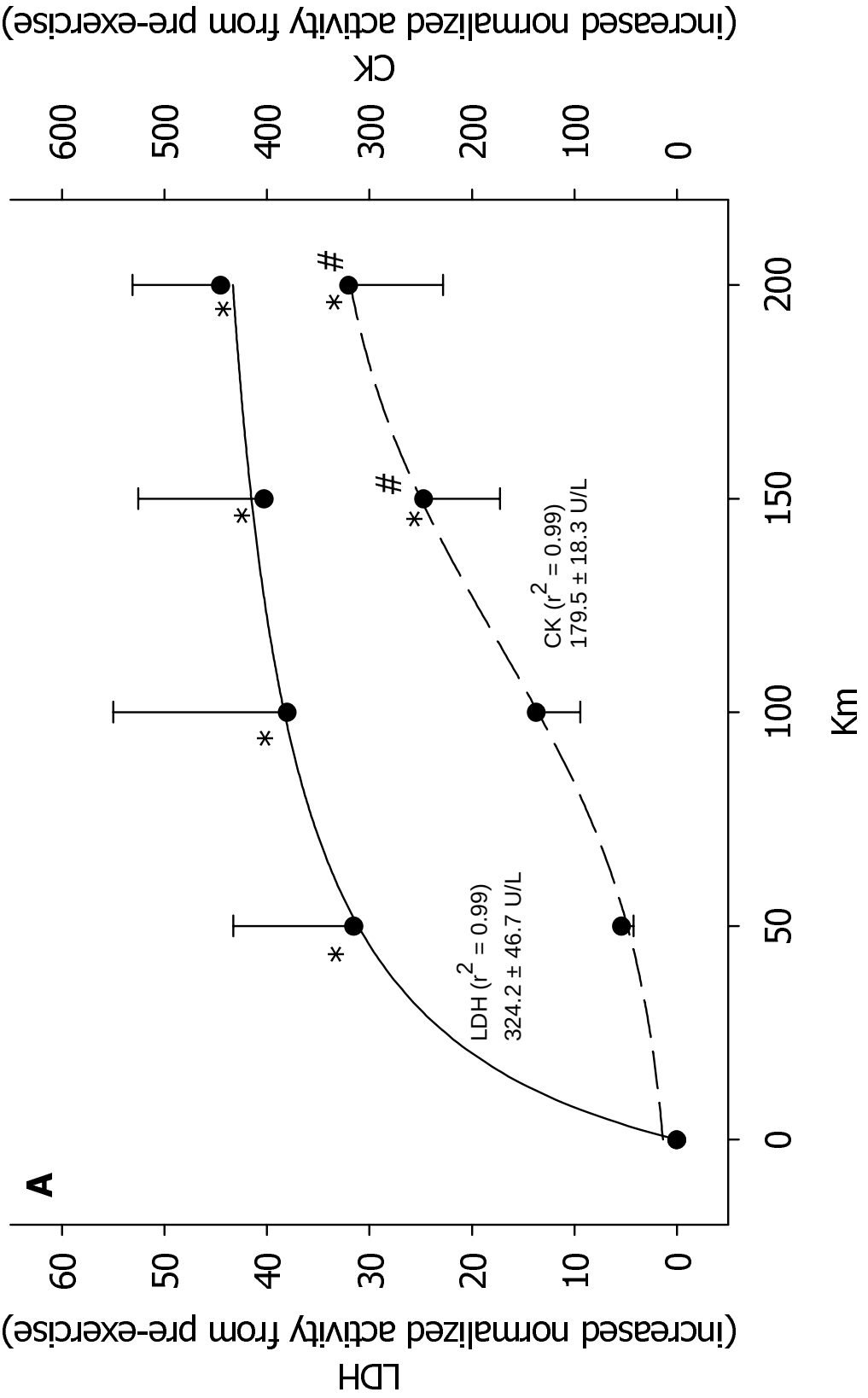
Figure 3 HIU effect on blood cells and CRP. (A) Both white blood cells and erythrocytes presence are plotted in response to exercise; (B) C-reactive protein (CRP) is also showed during HIU cycling. Data are normalized to pre-exercise values (100%), and absolute pre-exercise values are shown within the graphs (U/L). Data are showed as average ± SE. In erythrocyte counts SE bars are inside the symbols. * $p<0.05$ compared to pre-exercise.

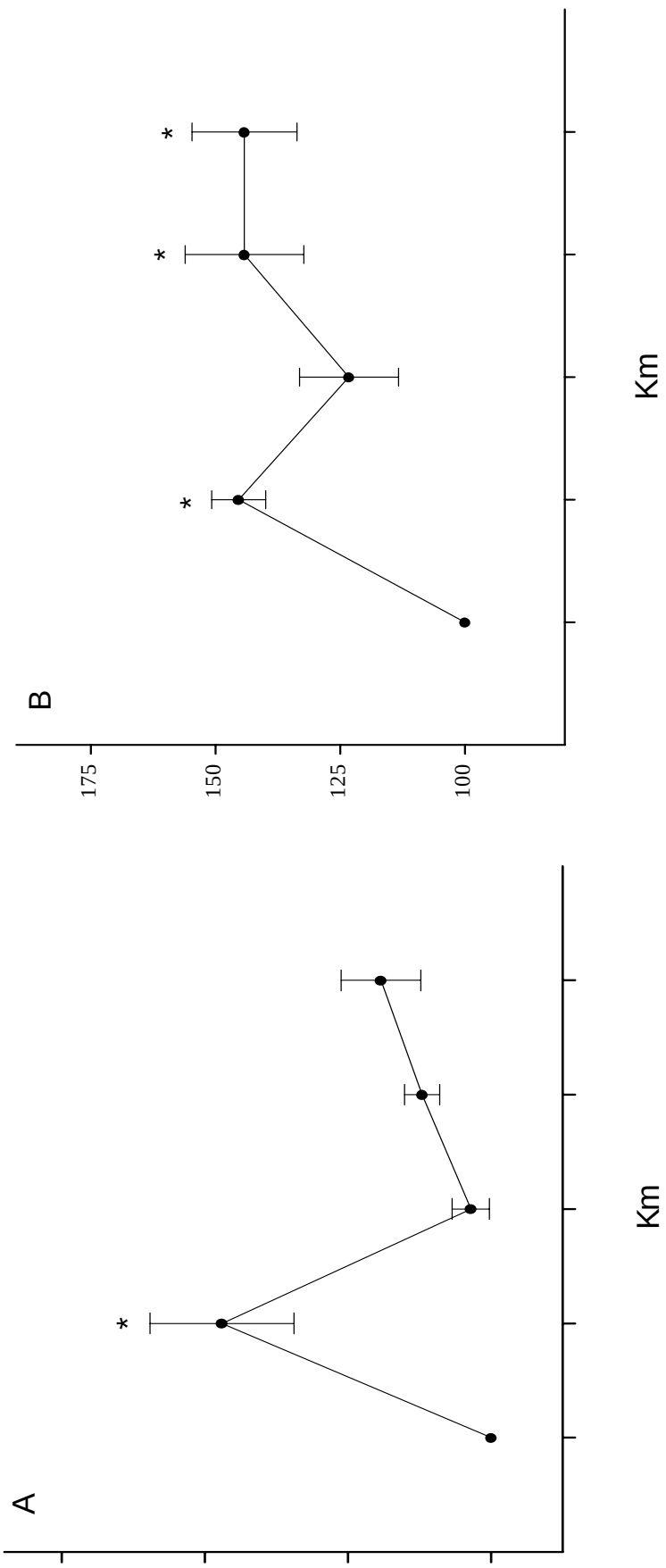
Figure 4. Glicemia (A) and tryacylgliceridemia (B) during HIU exercise. Data are normalized (average ± SE) to pre-exercise values (100%), and absolute pre-exercise values are shown within the graphs (U/L). * $p<0.05$ compared to pre-exercise. # $p<0.05$ compared to 50 km.

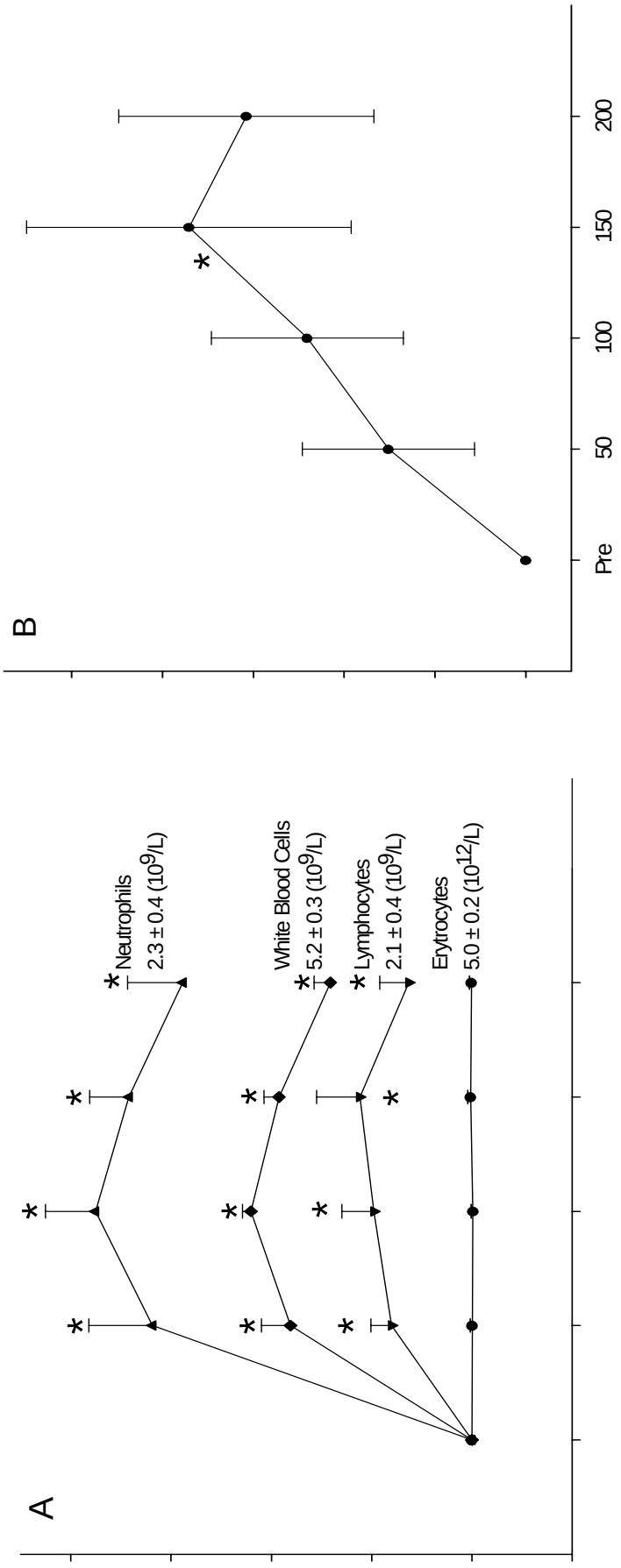
Figure 5. Blood Urate (A) and Urea (B) during race. Data are normalized (average ± SE) to pre-exercise values (100%) and absolute pre-exercise values are shown within the graphs (U/L). * $p<0.05$ compared to pre-exercise.

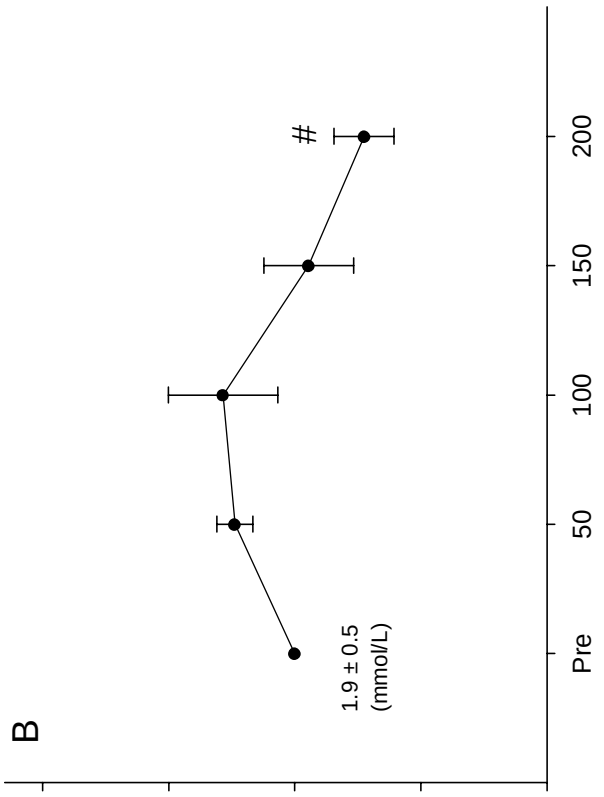
FINANCIAL DISCLOSURE: Each author certifies that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

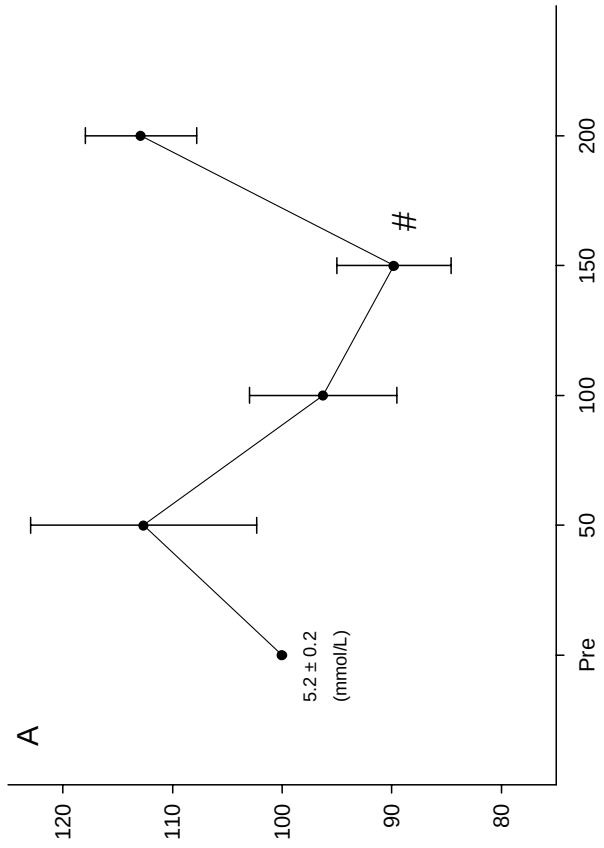
The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its licencees, to permit this article (if accepted) to be published in BJSM and any other BMJ Group products and to exploit all subsidiary rights, as set out in our licence (http://bjsm.bmjjournals.com//ifora/licence.pdf)"

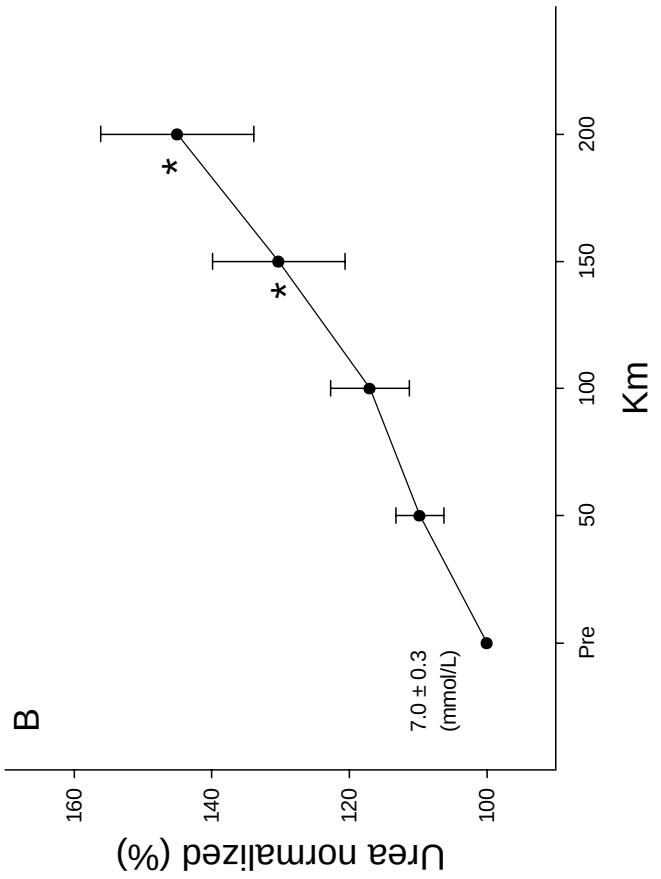


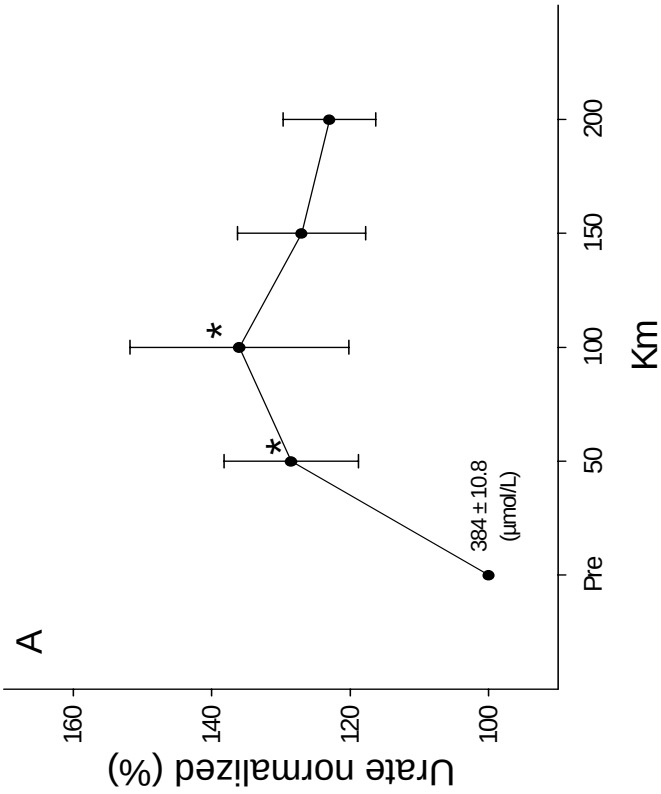












CAPITULO VI

Bassini-Cameron, A, Monteiro, A, Gomes, ALM, Werneck-de-Castro, JPS, LC Cameron. Glutamine protects against blood ammonia increase in soccer players in an exercise intensity dependent way. British Journal of Sports Medicine. v.42, p.260 - 266, 2008.

Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way

A Bassini-Cameron, A Monteiro, A Gomes, J P S Werneck-de-Castro and L Cameron

Br. J. Sports Med. 2008;42;260-266; originally published online 5 Nov 2007; doi:10.1136/bjsm.2007.040378

Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way

A Bassini-Cameron,[1,2] A Monteiro,[1,3,4] A Gomes,[1,5] J P S Werneck-de-Castro,[1,6,7] L Cameron[1,2,3]

[1] Laboratório de Bioquímica de Proteínas, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; [2] Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Uberlândia, Brazil; [3] Programa de Pós Graduação em Ciência da Motricidade Humana-Universidade Castelo Branco, Rio de Janeiro, Brazil; [4] Universidade Iguçu, Rio de Janeiro, Brazil; [5] Universidade Estácio de Sá, Rio de Janeiro, Brazil; [6] Departamento de Biociências da Atividade Física, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [7] Insituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Correspondence to:
L C Cameron, Av Pasteur, 296-térreo. Urca - Rio de Janeiro, RJ, CEP 22290-240, Brazil;
cameron@unirio.br

ABC and AM contributed equally to this paper

Accepted 4 September 2007
Published Online First
5 November 2007

ABSTRACT

Objective: High-intensity and prolonged exercise significantly enhances the levels of plasma ammonia, a metabolite with toxic effects on the central nervous system. The main purpose of the present study was to evaluate the metabolic response of athletes to glutamine (Gln) and alanine (Ala) supplementation, since these amino acids have a significant influence on both anaplerosis and gluconeogenesis.

Methods: Professional football players were assigned to groups receiving either Gln or Ala supplementation (100 mg kg^{-1} body weight); this supplementation was either short-term or long-term and was given immediately before exercise. The players were evaluated using two exercise protocols, one with intervals (n = 18) and the other with continuous intensity (n = 12).

Results: Both types of exercises increased ammonia, urate, urea and creatinine in blood. Chronic Gln supplementation partially protected against hyperammonemia after a football match (intermittent exercise: Gln −140 (SEM 13)% vs Ala −240 (SEM 37)%) and after continuous exercise at 80% of the maximum heart rate (Gln −481 (SEM 44)% vs placebo −778 (SEM 99)%). Urate increased by 10−20% in all groups, independently of supplementation. Glutamine once a day supplementation induced a greater elevation in urate as compared to alanine at the end of the game; however, long-term supplementation provoked a lesser increment in urate. Exercise induced similar increases in creatinine as compared to their respective controls in either acute or chronic glutamine administration.

Conclusions: Taken together, the results suggest that chronically supplemented Gln protects against exercise-induced hyperammonemia depending on exercise intensity and supplementation duration.

Athletic performance in football is affected by the subject's energy-transducing capacity.[1] The average work intensity in football is close to the lactate threshold (80–90% of maximum heart rate (HR_{max})).[2] However, football matches are characterised by intermittent short periods of high-intensity activity where accumulation of lactate takes place followed by periods of low-intensity activity during which lactate is removed from the working muscles.[3] High-intensity endurance training is important to improve the physical performance of the athletes[4] and requires amino acids as an energy substrate. Because high amounts of energy are required during training, amino acid supplementation has been used to increase the availability of this substrate and so decrease the depletion of muscle protein and the effect of over training.[5]

Ammonia (NH_3+NH_4^+) has been used as an indicator of metabolic activity during exercise because it is associated with the requirement and production of ATP.[6] Several studies have shown that high-intensity and prolonged exercises are correlated to the appearance of ammonia in blood. Furthermore, it has been suggested that high ammonia levels can be toxic to both muscles and the central nervous system (CNS), and induce peripheral and central fatigue.[7–10]

In order to decrease the toxic effect caused by increased levels of ammonia, the CNS enhances glutamine (Gln) synthesis to buffer free ammonia.[11] In humans, Gln is the most abundant circulating amino acid. It is synthesized in large amounts by the muscles, takes part in carbon transport and is a non-toxic ammonia carrier. The concentrations of plasma Gln decrease as a function of exercise intensity because of the increase in gluconeogenesis and urea synthesis.[12] It has been widely shown that oral Gln supplementation increases its plasma concentration and its efficiency as an energy substrate during rest or metabolic stress.[13–15]

The use of amino acids as energy substrates may increase the pool of ammonia from amino acid deamination.[16] The intramuscular concentration of alanine (Ala) is decreased during exercise because this amino acid effluxes from the muscle and is used as gluconeogenic substrate. The supplementation of Ala alone or in association with creatine was recently proposed as both a fatigue delayer and a potential endurance performance enhancer.[17 18] Even though Ala is a gluconeogenic substrate, it is not commonly studied as an ergogenic supplement during exercise.

In the present study we investigated the production of nitrogenous metabolites in football players performing at various exercise intensities and under different regimes of Ala or Gln supplementation. We used the gluconeogenic amino acid Ala as a control for Gln supplementation. We hypothesized that Ala supplementation may have a weaker effect on ammonia production than Gln because it releases a single ammonia molecule during anaplerosis.

METHODS

Participants

Professional football players (n = 18, mean (SEM) age 22.6 (0.6) years, weight 70.8 (1.4) kg) volunteered to

take part in this double-blind, randomized study. Users of pharmaceutical drugs or nutritional ergogenic aids were excluded from the study. Biochemical and haematological characteristics of the participants were controlled through initial diagnosis. We informed the participants about the nature and procedures of the study and they gave their written informed consent as required by the Research Ethics Committee of the Universidade Castelo Branco, Rio de Janeiro.

Exercise protocols and amino acid supplementation

The participants received 100 mg kg^{-1} body weight of either L-glutamine (Gln) or L-alanine (Ala) dissolved in a non-nutritional beverage. The supplements were weighed, packed in opaque containers and had indistinguishable organoleptic characteristics. Both amino acids were either administrated 1 hour before (short-term) or over 5 consecutive days (long-term), including the exercise day, before the exercise protocol.

We followed the players during their pre-season training and submitted them to two different exercise protocols with a 60-day interval (fig 1A and 1B). The first exercise protocol involved playing two matches 1 week apart; the matches started at 10:00 hours. Because football matches consist of two halves of 45 min each with a 15 min interval, we classified this as infield intermittent exercise.

All the athletes received acute supplementation for the first match (D1: soccer (football) glutamine short-term supplementation (SQS); soccer (football) alanine short-term supplementation (SAS)) and chronic supplementation for the second game (D8: soccer (football) glutamine long-term supplementation (SQL); soccer (football) alanine long-term supplementation (SAL)) (fig 1A).

The second exercise protocol involved running continuously for 60 min at 80% of the HR_{max} determined by a modified Bruce protocol.[19] Twelve players from the first group performed three treadmill runs in a controlled indoor environment at 1-week intervals under different supplementation regimens (fig 1B). Athletes were supplemented with placebo (IP) in the first treadmill run (D75), with acute Gln (indoor glutamine short-term supplementation (IQS)) in the second run (D82) and chronic Gln (indoor glutamine long-term supplementation (IQL)) in the last run (D89).

Blood sampling and analysis

All the blood sampling procedures, centrifugation and blood storage were carried out immediately after collection to avoid the loss of volatile compounds. The separated sample was frozen in liquid nitrogen and kept at −70°C. Laboratório Pedra Verde Ltda. (Campos, Brazil) carried out blood collection and analysis.

Before pre-season training, the athletes underwent anthropometric, haematological, hormonal and nutritional studies to ensure homogeneity among the participants. We evaluated haematocrit, cholesterol, triacylglycerol, serum Fe, Na^+, K^+, thyroid hormones, total and free testosterone, cortisol, aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyltranspeptidase (γGT), serum bilirubin, urea, urate, and creatinine. Also, we analysed coagulation and bleeding time, capillary fragility, clot retraction, platelet count, prothrombin time and activity.

During the intermittent exercise protocol, venepuncture was done in the antecubital vein to collect blood before, at the time

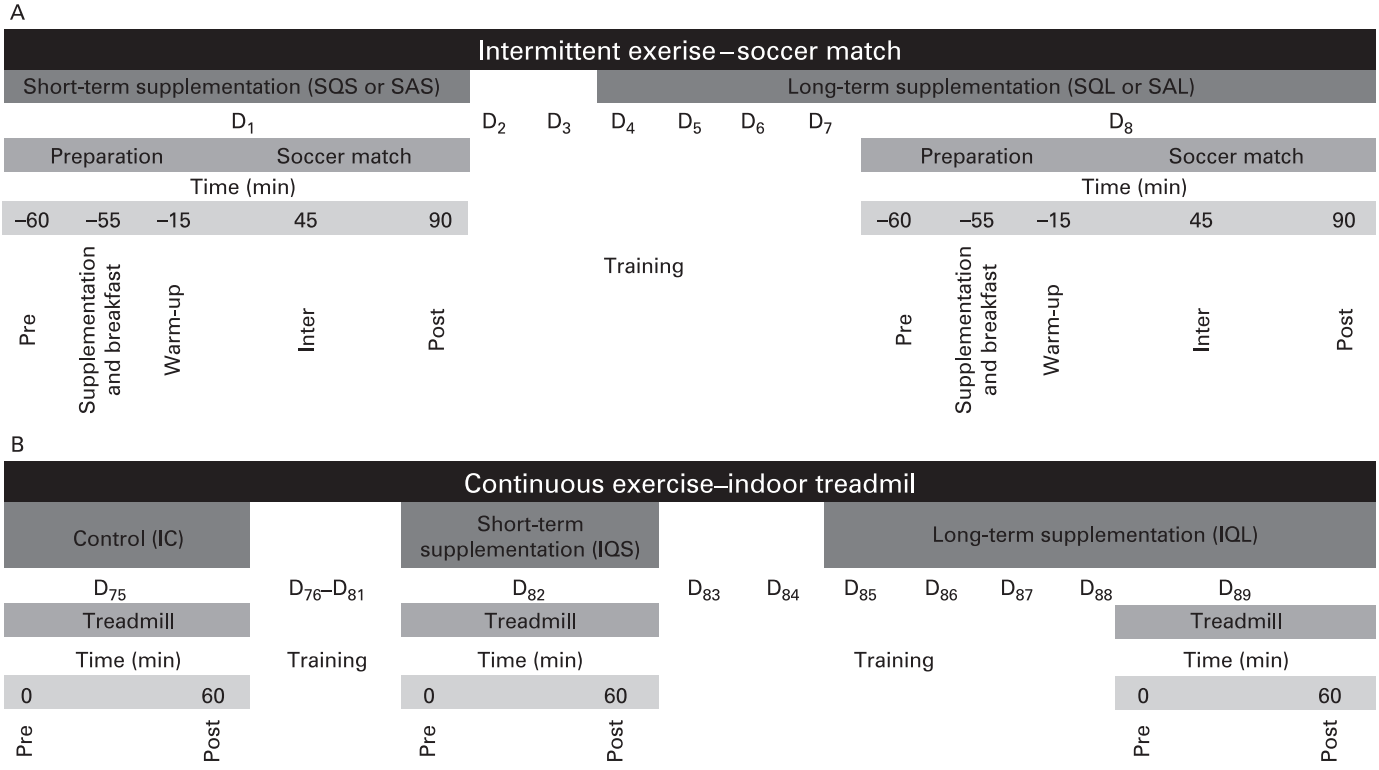


Figure 1 Experimental design. (A) In the intermittent exercise protocol (football) the athletes were supplemented with glutamine (SQS; n = 8) or alanine (SAS; n = 10) 1 h before the matches (short-term supplementation). Later, the same participants were given supplements (SQL and SAL) for 5 consecutive days (long-term supplementation). Blood samples were collected before supplementation, at the middle and at the end of the game (0, 60 and 120 min). (B) In the indoor continuous experiment, some of the athletes (n = 12) received placebo (IP) and glutamine supplementation, either short-term (IQS) or long-term (IQL). Blood was collected before and after the exercise (0 and 60 min). The time scale is in days (D) and the protocol details (min) are shown.

out period and after exercise. Blood samples were collected before and after continuous exercise. Glucose, ammonia, urate and urea were determined by an enzymatic method and creatinine by the alkaline picrate method.

Statistics

Data are shown as mean (SEM). Vertical bars show pre-exercise absolute values and line scatter graphs demonstrate data normalized by pre-exercise values. Gosset's t test was performed on the intermittent exercise data for comparisons among groups and ANOVA was done to the continuous exercise protocol data. In addition, we tested basal glucose values for a linear trend in the continuous exercise data. Significant differences were considered at $p<0.05$.

RESULTS

To guarantee homogeneity among the participants enrolled in the present study, we underwent anthropometric, haematological and biochemical evaluation before the study and randomly divided the football players into two groups. There was no difference in the anthropometric, biochemical, haematological, hormonal and nutritional parameters measured before the protocols (data not shown). To date, participants from both groups presented higher hematocrit values than the standard population, as expected for people who engage in endurance activities (data not shown). Two athletes previously considered healthy were excluded from the study. One presented with a left skewed distribution of the white blood cell count, diagnosed later as a silent urinary infection; the other presented with glucose intolerance.

Intermittent exercise

Glycemia increased around 20% and 30% in all groups at 60 min (15 min of warm-up plus 45 min of the first half) (fig 2A and 2B) and returned to basal levels at 120 min (15 min of time out plus 45 min of the second half). The glucose increment during intermittent exercise was not different between the alanine and glutamine supplementation groups either in the short-term or the long-term supplementation groups. The basal glucose level was higher (20%) in both long-term supplementation protocols as compared to SQS and SAS.

To evaluate the effect of Gln and Ala on ammonia production caused by energy expenditure during exercise, ammonia concentration was measured during the football matches. By the end of the game (120 min), plasma ammonia concentration increased twofold in the athletes under acute supplementation (SQS and SAS) as compared to the beginning of the match (fig 3A), with no difference between the groups. On the other hand, long-term glutamine supplementation reduced the rise in plasma ammonia by nearly 65% when compared to the control chronic alanine ingestion (fig 3B). During SQL and SAL protocols, the athletes developed a higher basal ammonia level (100–130%) than in SQS and SAS.

To elucidate ammonia genesis during exercise we evaluated urate as an indicator of inosine monophosphate (IMP) metabolism. During the match (60 min), urate levels increased by 10−20% in all groups, independently of type of amino acid or supplementation regimen (fig 4A,B). Acute glutamine supplementation (SQS) induced a greater elevation in urate as compared to alanine at the end of the game (fig 4A). However, long-term glutamine supplementation provoked a smaller increase in urate at 120 min (fig 4B).

The concentrations of blood urea and creatinine were evaluated as indicators of both hepatic and renal metabolic responses. Long-term supplementation increased basal urea in relation to short-term supplementation. At the end of the game, urea levels increased 10–20% in short-term supplementation (fig 5A) with no significant differences between groups ($p = 0.07$). In the long-term protocol, alanine and glutamine increased plasma urea by 10% at 60 min (fig 5B). Exercise induced similar increases in creatinine as compared to their respective controls (SAS and SAL) in either acute or chronic glutamine administration (fig 6A,B).

Continuous exercise

To investigate whether nitrogen metabolism was masked by exercise intensity variation we submitted 12 players to a continuous indoor exercise protocol by running on a treadmill at 80% of their maximum heart rate. Participants received both acute and long-term glutamine supplementation and were compared with a placebo situation (fig 1B).

Glucose basal levels were higher in long-term glutamine supplementation as compared to the placebo (fig 2C). Similar to the long-term amino acid supplementation in the first protocol, there was an increasing trend from IP toward IQL ($p<0.05$). After 60 min of exercise glycemia was diminished by about 10% in all groups.

Ammonia levels increased more than 600% in the IP and IQS groups; on the other hand, the IQL group was partially protected from increases in blood ammonia levels (fig 3C). Urate and urea increased after continuous exercise whatever the experimental situation (figs 4C and 5C). However, when chronically supplemented with glutamine, athletes exhibited less urate increases than with IP and IQS. The continuous exercise led to a creatinine increase of 38% in the IQL group and 50% in the IP and IQS groups, with no significant difference between them.

DISCUSSION

The intensity and duration of exercise may affect biochemical and haematological parameters in athletes, interfering directly with their performance.[20–22] To achieve high methodological accuracy and eliminate any variables that could mask the results during our study, the participants underwent haematological and biochemical tests before the experiments. These indicated that the metabolic profiles of the participants were adequate and homogeneous. Moreover, this analysis of the athletes allowed us to group them first into two groups and then into three groups in the second protocol, reaching functional homogeneity of the groups during the distinct experiments.

The prevalent energy source during physical activity is determined by the intensity and duration of the exercise.[1] Prolonged exercise may deplete glycogen stores and increase the use of amino acids as an energy substrate[12] thereby increasing ammonia production.[23 24] Hence, it is relevant to the investigation that the experimental protocol matches the sport as it is performed in a real situation. Therefore, we used a field protocol simulating the situation imposed on the athletes during a competition, i.e., high psychological stress and intermittent energy demand.

Both Gln and Ala are anaplerotic and gluconeogenic substrates, and contribute to the synthesis of ATP or glucose. The ergogenic action promoted by Gln supplementation has been extensively studied (for a review see[25 26]). On the other hand, the use of Ala as a supplement has not been reported, nor have its effects on metabolism during exercise. With the use of equimolar amounts of amino acids for ATP net synthesis, Gln

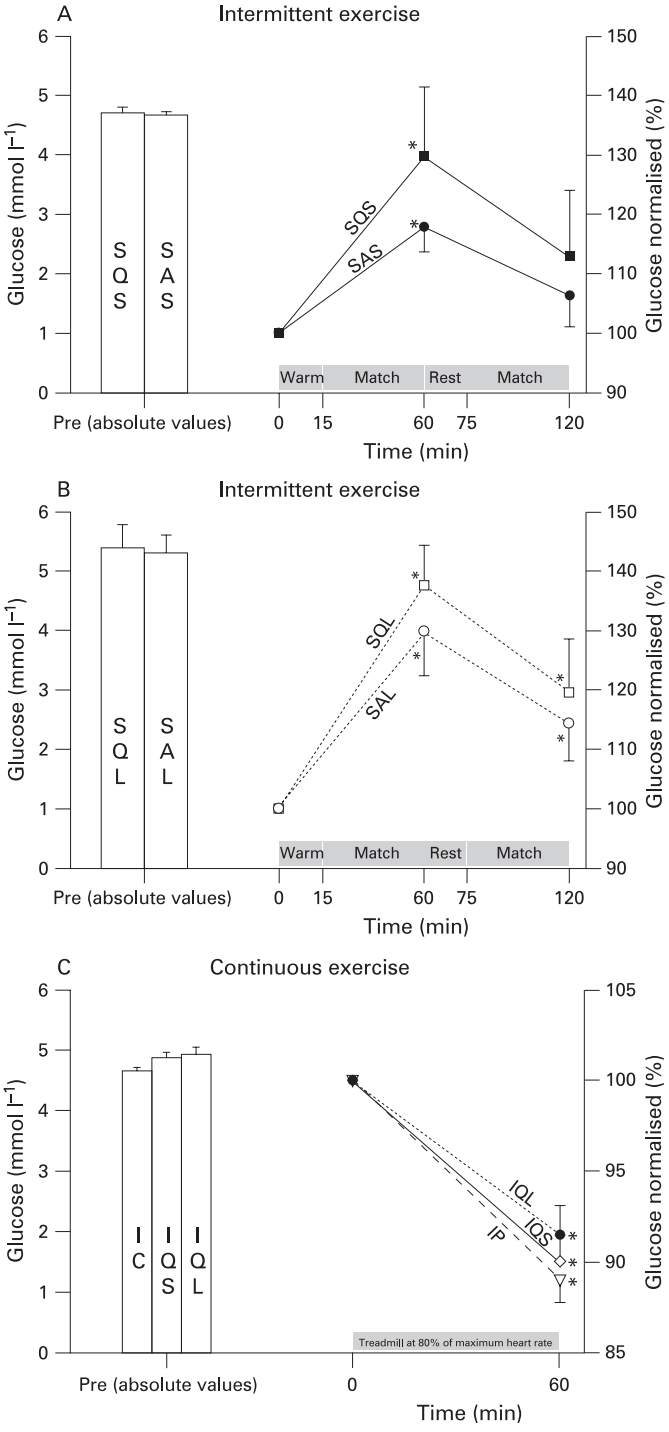


Figure 2 Glycemia during intermittent (A,B) and continuous exercise protocols (C). (A) Amino acid short-term supplementation. (B) Amino acid long-term supplementation. Absolute pre-exercise values are shown in bars. The line scatter shows data normalized to pre-exercise values. IP, indoor placebo; IQL, indoor glutamine long-term supplementation; IQS, indoor glutamine short-term supplementation; SAL, soccer (football) alanine long-term supplementation; SAS, soccer (football) alanine short-term supplementation; SQL, soccer (football) glutamine long-term supplementation; SQS, soccer (football) glutamine short-term supplementation; *p<0.05 compared with pre-exercise.

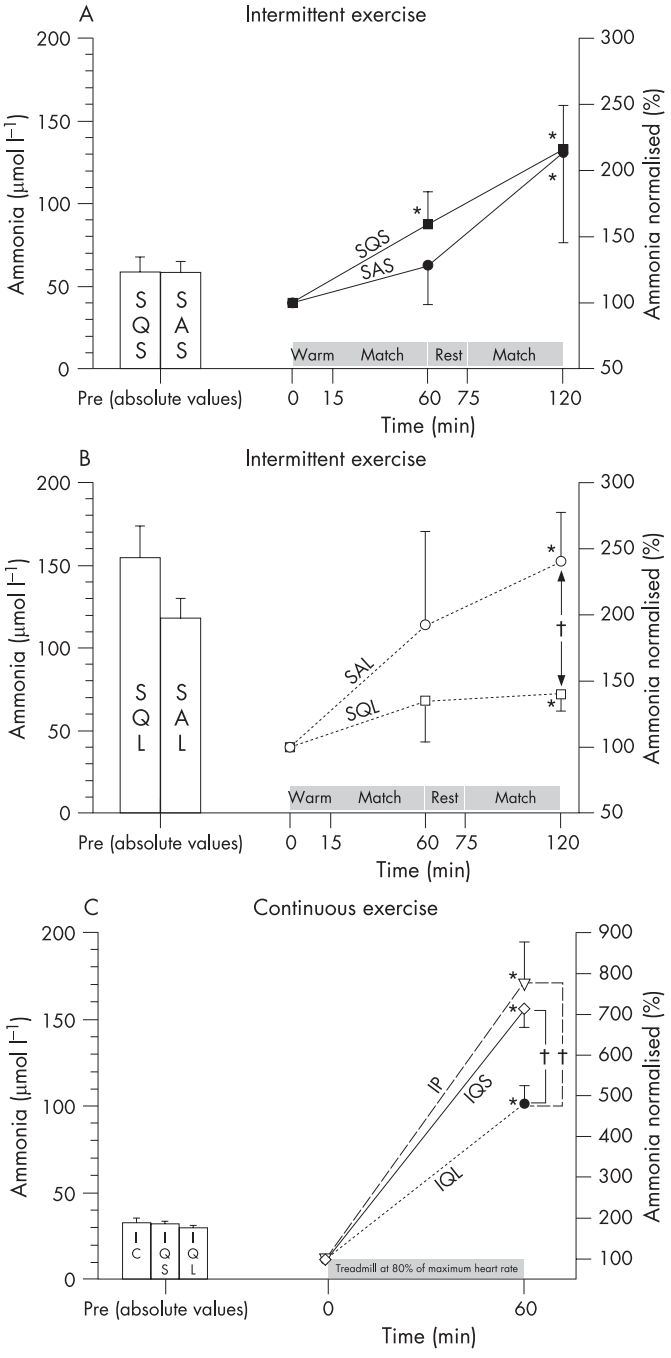


Figure 3 Ammonemia during intermittent (A,B) and continuous exercise protocols (C). (A) Amino acid short-term supplementation. (B) Amino acid long-term supplementation. Absolute pre-exercise values are shown in bars. The line scatter shows data normalized to pre-exercise values. IP, indoor placebo; IQL, indoor glutamine long-term supplementation; IQS, indoor glutamine short-term supplementation; SAL, soccer (football) alanine long-term supplementation; SAS, soccer (football) alanine short-term supplementation; SQL, soccer (football) glutamine long-term supplementation; SQS, soccer (football) glutamine short-term supplementation; *p<0.05 compared with pre-exercise; †p<0.05.

will release more ammonia than Ala.[27] Thus, we compared these amino acids under different supplementation regimes using Ala as a control in the intermittent protocol.

During the intermittent protocol, at no point did glycemia fall below the pre-exercise measurement.[28] In fact, a rise in glycemia was recorded during the interval due to athletes' feeding and supplementation. The use of long-term supplementation kept glycemia above basal levels throughout the protocol,

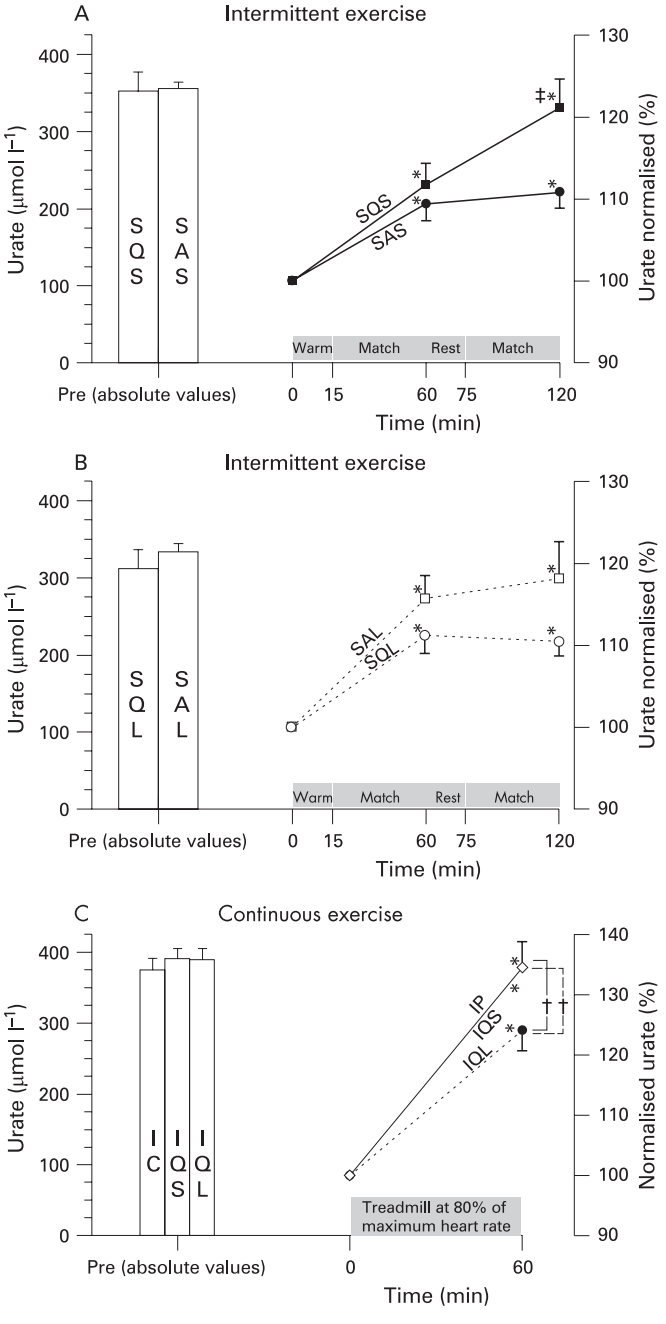


Figure 4 Urate levels during both intermittent (A,B) and continuous exercise protocols (C). (A) Amino acid short-term supplementation. (B) Amino acid long-term supplementation. Absolute pre-exercise values are shown in bars. The line scatter shows data normalized to pre-exercise values. IP, indoor placebo; IQL, indoor glutamine long-term supplementation; IQS, indoor glutamine short-term supplementation; SAL, soccer (football) alanine long-term supplementation; SAS, soccer (football) alanine short-term supplementation; SQL, soccer (football) glutamine long-term supplementation; SQS, soccer (football) glutamine short-term supplementation; *p<0.05 compared with pre-exercise; †p<0.05; ‡p<0.05 compared with SAS.

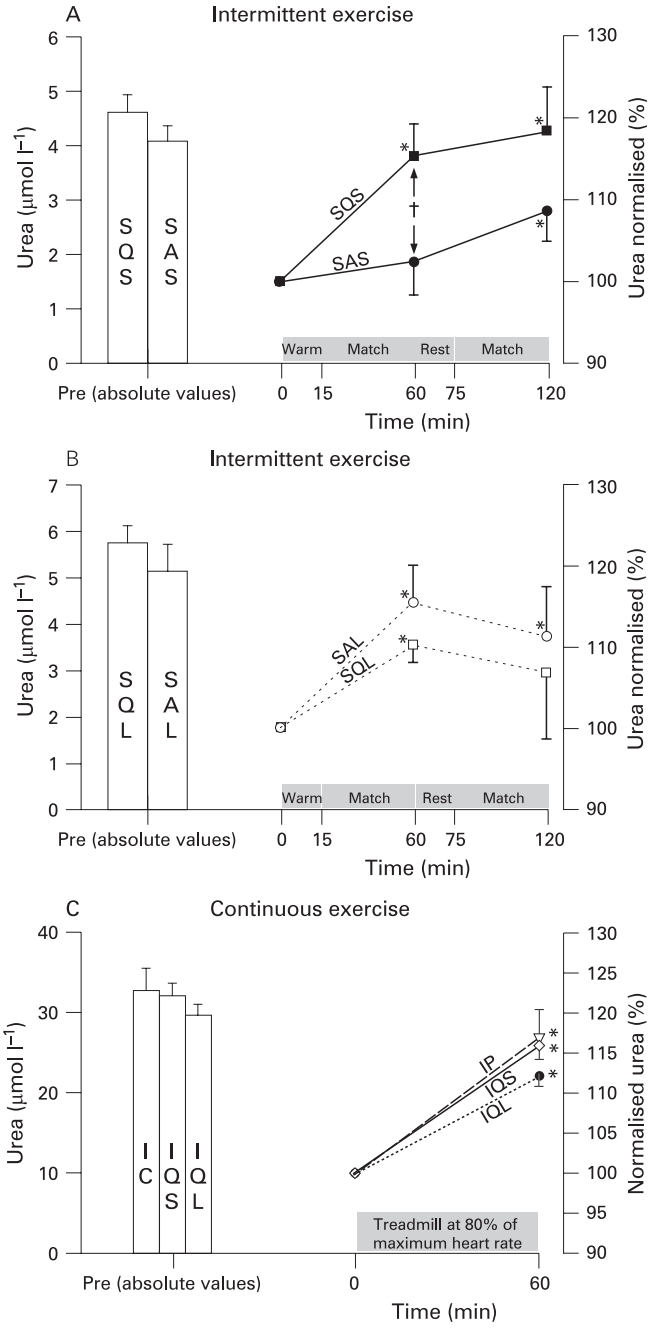


Figure 5 Urea levels during intermittent (A,B) and continuous exercise protocols (C). (A) Amino acid short-term supplementation. (B) Amino acid long-term supplementation. Absolute pre-exercise values are shown in bars. The line scatter shows data normalized to pre-exercise values. IP, indoor placebo; IQL, indoor glutamine long-term supplementation; IQS, indoor glutamine short-term supplementation; SAL, soccer (football) alanine long-term supplementation; SAS, soccer (football) alanine short-term supplementation; SQL, soccer (football) glutamine long-term supplementation; SQS, soccer (football) glutamine short-term supplementation; *p<0.05 compared with pre-exercise; †p<0.05.

while short-term supplementation promoted a rise in glycemia during the first 60 min before dropping back to basal levels. This feature is probably explained by a bigger glycogen stock following long-term supplementation.[29 30] On the other hand, in the continuous protocol, glucose levels decreased by 10% in all athletes in an exercise-intensity-correlated manner.

At rest, arterial ammonia levels are maintained at 50–100 µM.[31] Athletes engaged in our study exhibited normal basal venous ammonia levels (40–60 µM) as in our previous studies with football players[22] and marathon runners.[32] Ammonia has been proposed as a physiological marker of prolonged intense exercise and its appearance in blood is positively correlated with exercise intensity.[33–36] Moreover, ammonia can be toxic to both

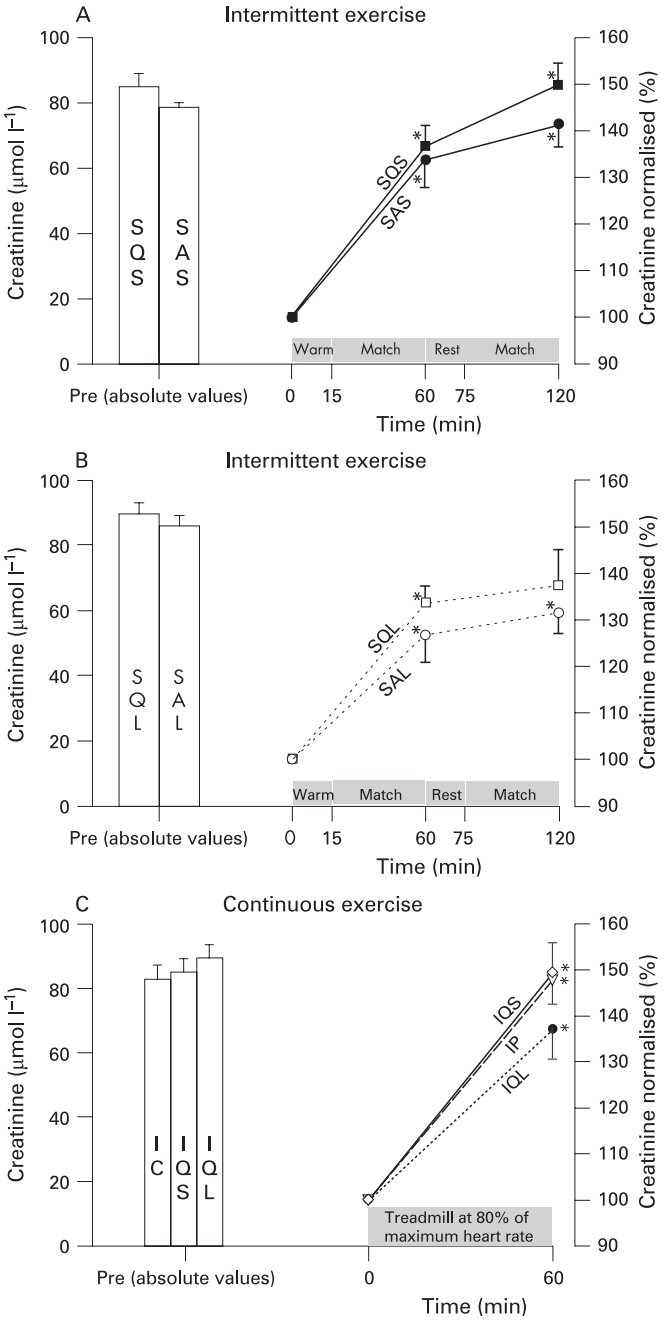


Figure 6 Creatinine levels during both intermittent (A,B) and continuous exercise protocols (C). (A) Amino acid short-term supplementation. (B) Amino acid long-term supplementation. Absolute pre-exercise values are shown in bars. The line scatter shows data normalized to pre-exercise values. IP, indoor placebo; IQL, indoor glutamine long-term supplementation; IQS, indoor glutamine short-term supplementation; SAL, soccer (football) alanine long-term supplementation; SAS, soccer (football) alanine short-term supplementation; SQL, soccer (football) glutamine long-term supplementation; SQS, soccer (football) glutamine short-term supplementation; *$p<0.05$ compared with pre-exercise.

muscles and the central nervous system (CNS), and can induce central and peripheral fatigue.[7–10] In the worst case scenario, ammonia (300–500 μM) induces severe encephalopathy[37] and could lead to coma. Therefore, any strategies to protect against hyperammonemia could enhance physical performance or prevent CNS injuries. During our study, ammonia levels rose significantly at the end of the experiment in both protocols regardless of the amino acid used. Placebo administration in the indoor experiment resulted in high levels of ammonia (272 μM on average). However, we found that long-term Gln supplementation partially protected against increased ammonia levels in both continuous (indoor), as previously described,[32] and intermittent exercise protocols compared to Ala supplementation. The increase in ammonia levels was higher in the treadmill activity because high-intensity continuous exercise impaired athletes' ATP regeneration capacity. Although of longer duration than our continuous protocol, intermittent exercise allows ATP re-synthesis, reducing the production of ammonia from AMP deamination.

In the intermittent protocol, resting ammonia levels were higher in participants receiving chronic supplementation. This suggests that constant Ala and Gln supplementation increases ammonia production. Ammonia levels released by Gln during the anaplerotic processes were twofold: when comparing them to those of ammonia released by Ala, we found a bigger basal ammonemia in the SQL group when compared to SAL. In the indoor protocol, the basal ammonia levels of the three groups were the same, probably owing to the better physical conditioning obtained during the training between the two protocols.[24] In the more intense exercise (treadmill), long-term glutamine supplementation provided less protection (40%) than in intermittent exercise (70%). These findings support the idea that part of the protection from Gln occurs in an AMP deamination-independent way. These findings and interpretations are reinforced by the complementary urea data obtained in both continuous and intermittent protocols.

The serum urate level increased along with ammonia during the exercise, which suggests that the higher ammonia production during the study was probably caused by AMP deamination to maintain the ATP/ADP ratio.[6 8 37] In the intermittent protocol, the ammonia level curve for SQL and SAL athletes had a higher base level than that of SQS and SAS. The urate level was the opposite during the pre-exercise. Ammonia production was already high in the pre-exercise metabolism, since it increased significantly with the different supplementations even when no exercise was performed. Taking these data together, we suggest that this difference in basal levels of ammonia has a higher contribution from the amino acid deamination process than from AMP deamination. These findings suggest a pattern of increased ammonia production prior to physical effort as a response to the use of amino acids for gluconeogenesis, glycogenesis and lipid production.

It is relevant to point out that we compared our data with two different controls: Ala, a monoamine amino acid used to control the different supplementation protocols, and an internal control using continuous high-intensity exercise, a model already used by our laboratory.[32] These present findings indicated that both exercise intensity and supplementation are limiting factors in the protective effect of Gln. Also, the present study suggests that the protective effect of Gln in ammonemia rise during exercise should be primarily due to a reduction in muscle AMP deamination, due to an increase of carbon skeleton availability or to a contribution of the amino acid to glycogen stocks as previously described.[38–40] Our current results clearly show that 5 days of Gln supplementation prior to exercise partially prevents the hyperammonemia observed after both intermittent and continuous exercise. Furthermore, since a football game is characterized by intermittent exercise, these findings can be applied to other types of exercise with similar metabolic characteristics.

Competing interests: None.

REFERENCES

1. **Madsen K,** MacLean B, Christensen D. Effects of glucose, glucose plus branched chain amino acids, or placebo on bike performance over 100 km. *J Appl Physiol* 1996;**81**:2644–50.
2. **Helgerud JL,** Fngein C, Wisloff U, *et al*. Aerobic endurance training improves soccer performance. *Med Sci Sports Exerc* 2001;**33**:1925–31.
3. **Stolen T,** Chamari K, Castagna C, *et al*. Physiology of soccer: an update. *Sports Med* 2005;**35**:501–36.
4. **Chamari K,** Hachana Y, Kaouech F, *et al*. Endurance training and testing with the ball in young elite soccer players. *Br J Sports Med* 2005;**39**:24–8.
5. **Hiscock N,** Mackinnon L. A comparison of plasma glutamine concentration in athletes from different sports. *Med Sci Sports Exerc* 1998;**30**:1693–4.
6. **Eriksson LS,** Broberg S, Björkman O, *et al*. Ammonia metabolism in man. *Clin Physiol* 1985;**5**:325–36.
7. **Sahlin K,** Katz A, Broberg S. Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. *Am J Physiol* 1990;**259**:C834–41.
8. **Banister E,** Cameron J. Exercise-induced hyperammonemia: peripheral and central effects. *Int J Sports Med* 1990;**11**:129–42.
9. **Castell L,** Newsholme E. The effects of oral glutamine supplementation on athletes after prolonged, exhaustive exercise. *Nutrition* 1997;**13**:7–8.
10. **Graham T,** Turcotte L, Kiens B, *et al*. Effect of endurance training on ammonia and amino acid metabolism in humans. *Med Sci Sports Exerc* 1997;**29**:646–53.
11. **Butterworth RF.** Glutamate transporter and receptor function in disorders of ammonia metabolism. *Ment Retard Dev Disabil Res Rev* 2001;**7**:276–9.
12. **Rohde T,** MacLean D, Pedersen B. Effect of glutamine supplementation on changes in the immune system induced by repeated exercise. *Med Sci Sports Exerc* 1998;**30**:856–62.
13. **Walsh N,** Blannin A, Clark A, *et al*. The effects of high-intensity intermittent exercise on the plasma concentrations of glutamine and organic acids. *Eur J Appl Physiol* 1998;**77**:434–8.
14. **Nissim I.** Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am J Physiol* 1999;**277**:F493–7.
15. **Newsholme P,** Castell LM, Poortmans JR, *et al*. Some aspects of the acute phase response after a marathon race, and the effects of the glutamine supplementation. *Eur J Appl Physiol* 1997;**75**:47–53.
16. **Pitkanen H,** Nykanen T, Knuutinen J, *et al*. Free amino acid pool and muscle protein balance after resistance exercise. *Med Sci Sports Exerc* 2003;**35**:784–92.
17. **Zoeller RF,** Stout JR, O'Kroy JA, *et al*. Effects of 28 days of beta-alanine and creatine monohydrate supplementation on aerobic power, ventilatory and lactate thresholds, and time to exhaustion. *Amino Acids*. Published Online First: 5 September 2006. doi:10.1007/s00726-006-0399-6.
18. **Stout JR,** Cramer JT, Zoeller RF, *et al*. Effects of beta-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. *Amino Acids* 2007;**32**:381–6.
19. **Bruce RA,** Hossack KF, Belanger L, *et al*. A computer terminal program to evaluate cardiovascular functional limits and estimate coronary event risks. *West J Med* 1981;**135**:342–50.
20. **Lehmann M,** Baumgartl P, Wiesenack A, *et al*. Training-overtraining. Influence of a defined increase in training volume vs training intensity on performance, catecholamines and some metabolic parameters in experienced middle and long distance runners. *Eur J Appl Physiol* 1992;**64**:169–77.
21. **Selby G,** Eichner E. Hematocrit and perfomance: The effect of endurance training on blood volume. *Sem Hemat* 1994;**31**:122–7.
22. **Bassini-Cameron M,** Sweet E, Bottino A, *et al*. Effect of caffeine supplementation on hematological and biochemical parameters in elite soccer players under physical stress conditions. *Br J Sports Med* 2007;**47**:1–8.
23. **Roeykens J,** Magnus L, Rogers R, *et al*. Blood ammonia – heart rate relationship during graded exercise is not influenced by glycogen depletion. *Int J Sports Med* 1998;**19**:26–31.
24. **Schulz H,** Hermann H. Glycogen depletion as indication for ammonia determination in exercise testing. *Eur J Sports Sci* 2003;**3**:1–9.
25. **Castell M,** Poortmans J, Lieclery R, *et al*. Some aspects of the acute phase response after a marathon race, and effects of glutamine supplementation. *Eur J Appl Physiol* 1997;**75**:47–53.
26. **Bowtell J,** Gelly K, Jackman M, *et al*. Effect of oral glutamine on whole body carbohydrate storage during recovery from exhaustive exercise. *J Appl Physiol* 1999;**86**:1770–7.
27. **Newsholme P,** Lima R, Procopio J, *et al*. Glutamine and glutamate as vital metabolites. *Brazil J Med Biol Res* 2003;**36**:153–63.
28. **Mourtzakis M,** Graham TE. Glutamate ingestion and its effects at rest and during exercise in humans. *J Appl Physiol* 2002;**93**:1251–9.
29. **Rogero MM,** Tirapegui J, Pedrosa RG, *et al*. Effect of alanyl-glutamine supplementation on plasma and tissue glutamine concentrations in rats submitted to exhaustive exercise. *Nutrition* 2006;**22**:564–71.
30. **Iwashita S,** Williams P, Jabbour K, *et al*. Impact of glutamine supplementation on glucose homeostasis during and after exercise. *J Appl Physiol* 2005;**99**:1858–65.
31. **Felipo V,** Butterworth RF. Neurobiology of ammonia. *Prog Neurobiol* 2002;**67**:259–79.
32. **Carvalho-Peixoto J,** Alves RC, Cameron LC. Glutamine and carbohydrate supplements reduce ammonemia increase during endurance field exercise. *Appl Physiol Nutr Metab*. In press.
33. **Ament W,** Huizenga J, Mook G, *et al*. Lactate and ammonia concentration in blood and sweat during incremental cycle ergometer exercise. *Int J Sports Med* 1997;**18**:35–9.
34. **Gibala M,** MacLean DA, Graham TE, *et al*. Anaplerotic processes in human skeletal muscle during brief dynamic exercise. *Am J Physiol* 1997;**502**:703–13.
35. **Rodney J,** Carey M, Stathis C, *et al*. Effect of carbohydrate ingestion on ammonia metabolism during exercise in humans. *J Appl Physiol* 2000;**88**:1576–80.
36. **Hellsten Y,** Richter EA, Kiens B, *et al*. AMP deamination and purine exchange in human skeletal muscle during and after intense exercise. *J Physiol* 1999;**520**:909–20.
37. **Clemmensen P,** Holmvang L, Grande P, *et al*. "Add-on" research in clinical trials: are we asking the right questions? *J Electrocardiol* 1999;**32**:108–10.
38. **Linnewalker J,** Heigenhauser E, Spriet L. Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. *J Appl Physiol* 2000;**88**:2151–8.
39. **Rennie M,** Bowtell J, Bruce M, *et al*. Interaction between glutamine availability and metabolism of glycogen, tricarboxylic acid cycle intermediates and glutathione. *J Nutr* 2001;**131**:2488–90S.
40. **Steenberg A,** Van Haal G, Keller C, *et al*. Muscle glycogen content and glucose uptake during exercise in humans: influence of prior exercise dietary manipulation. *J Physiol* 2002;**541**:273–81.

CAPITULO VII

Bassini-Cameron,A; Sweet, E; Bottino, A. Bittar, C; Veiga, C; Tozzi, MB; Bloom, M; LC Cameron.Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players

CAPÍTULO VII

Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players

Adriana Bassini-Cameron[1,2,3]; Eric Sweet[4]; Altamiro Bottino[5]; Christina Bittar[6]; Carlos Veiga[7], Marta Brandão Tozzi[8]; Megan Bloom[9] and Luiz-Claudio Cameron[1,2,3].

[1]Laboratory of Protein Biochemistry – Federal University of State of Rio de Janeiro, Brazil

[2]Graduate Program in Human Movement Science – University Castelo Branco, Rio de Janeiro, Brazil

[3]Institute of Genetics and Biochemistry – Federal University of Uberlândia, Brazil

[4]Department of Anatomy - Federal University of Rio de Janeiro, Brazil

[5]Botafogo de Futebol e Regatas - Rio de Janeiro, Brazil

[6]Bittar Laboratory Ltda – Niterói, Brazil

[7]National Institute of Traumatic Orthopedic - Rio de Janeiro, Brazil

[8]Joquei Clube Brasileiro - Rio de Janeiro, Brazil

[9]University of Virginia

Corresponding author:

L. C. Cameron, Ph. D.

Laboratório de Bioquímica de Proteínas

Universidade Federal do Estado do Rio de Janeiro

Av. Pasteur, 296

Urca - Rio de Janeiro - RJ

Brazil

CEP 22290-240

Phone +55 21 2542 5204

Fax +55 21 2542 5280

www.unirio.br/lbp

cameron@unirio.br

METHODS

Subjects

This double-blind randomized study was approved by the Ethics Committee for Human Research from University Castelo Branco and met the requirements for carrying out research in human subjects (Health National Council, Brazil, 1996). Written informed consent was obtained from the subjects, who were instructed as to the nature and procedures of the study. This experiment was conducted was described in a previous study (Bassini-Cameron *et al.*, 2007), briefly:

Professional soccer players (n=19) from a first-division team affiliated in the Confederação Brasileira de Futebol (CBF, Brazilian Soccer Confederation) participated in this study voluntarily. To ensure the control of many experimental variables that could affect the results, we conducted the experiment during a contention for the Brazilian Soccer Championship. This procedure guaranteed that all the athletes had similar diets, training regime, resting and sleep conditions.

The subjects had no medical history of health problems and were not using ergogenic substances or any other drugs. In addition clinical examinations, anthropometric measurements and laboratory tests were performed on the subjects to assure integrity and homogeneity among the groups. The initial laboratory evaluation tests included hematobiochemical analyses, which allowed the diagnosis of metabolism distortions that could affect results or impair any interpretation.

Experimental Protocol

No caffeine, xanthine or other substance that could mask the results was ingested by the athletes for 72h before blood collection.

On the experiment day blood was collected from fasting soccer players, i.e., before breakfast (PRE). Players were randomly divided into two groups and received a specific breakfast diet and a supplement caffeine or lactose.

After receiving breakfast and the supplements, the subjects were driven to the test place, which took 15 min. After 20 min of warming up (articular mobilization and

elongation exercises), the subjects performed the test protocol under cardiac monitoring, simulating a soccer match (Figure 1).

Specific Procedures

Caffeine supplementation

The different supplements were in indistinguishable capsules so that the subjects were not aware of the substance they were ingesting. Caffeine (Purifarma, China) was given at 5mg·kg^{-1} in two 500mg-capsules that were then completed with lactose (Via Farma, Brazil) to groups CEx and C. The control group, LEx, received 2 capsules with 500mg lactose each.

Test protocol

The variable distance run protocol (VDR) was used to simulate a soccer match and was performed for 45 min in a 50 x 50 m court with 5 x 5 m marks. The athletes received drink containing electrolytes and glucose (Gatorade®) *ad libitum* throughout training. After the VDR, Yo-Yo intermittent recovery test (Yo-Yo IRT) (ref) was performed to drive athletes to exhaustion. This test finished at a different time for each athlete, and immediately after, blood was collected for laboratory analyses (POST).

Data Collection

Blood sampling

Venous blood samples were collected from athletes' forearms in heparinized tubes. Immediately after the collection, the blood samples were centrifuged for plasma separation, which were quickly frozen and stored at -70 ºC. The samples were analyzed by Laboratório Bittar Ltda (Niterói, Brazil).

A range of analyses was carried out to detect any variable that could affect the results: Urinary myoglobin and troponin I were also determined for evaluation of muscle integrity (data not show).

Statistical Analyses

The data were analyzed using an ANOVA with condition and time as repeated measures variables, followed by post hoc t-tests where necessary. Statistical significance was set at $\alpha < 0.05$. Data are expressed as mean $\pm$ standard error.

RESULTS

To guarantee homogeneity among the participants enrolled in the present study, we underwent anthropometric, hormonal, hematological and biochemical evaluation before the study and randomly divided the soccer players into two groups. (Bassini-Cameron *et al.*, 2007) in addition, we evaluated urinary amino acids (Ile, Leu, Met, Phe, Tyr and Val) corrected by the creatinine clearance rate. All the subjects exhibited normal metabolic functions and there were no differences between the subjects (data not shown).

Caffeine absorption and group division

To evaluate blood caffeine concentration during the experiment we measured plasmatic caffeine. Caffeine clearly increased following two patterns so we decided to split the caffeine supplemented group in two: CEx (~ 900% increase, n = 5) and SCEx (~ 10,000% increase, n = 6). There was no difference between blood caffeine in all groups before experiment and in non supplemented group either before or after the test (LEx, n = 8) (Figure 2).

Caffeine effect on metabolism

Exercise increased the blood concentrations of glucose (26-53%), lactate (>600%) and insulin (53-84%). Caffeine supplementation did not affect their response to exercise (Table 1). On the other hand, there was a dose dependent effect on uremia. Subjects with the biggest caffeine (SCEx) concentrations exhibit a lack of uremia increase in response to exercise. No effect was measured regarding blood concentrations of urate, ammonia or creatinine (table 2).

Caffeine effect on plasmatic amino acids

Caffeine and exercise have had different effects in blood amino acid concentrations. Gly, Ser and His blood concentration raise were prevented by large amounts of caffeine. Pro raise in blood was inhibited even in the CEx group. Blood concentration of Lys, Thr, Tau, Asn, Asp, and Met were not affected by both exercise and caffeine in our study (Table 3).

Exercise increased the BCAA concentration on blood by 28%. This increase was suppressed by caffeine in both groups. Analysis of Val presence in blood showed that it was the amino acid more affected by caffeine. Control group had an exercise induced Val increase of 29%, prevented by the use of the xanthine. Leu and Ile were less affected and no significant difference was found between groups. The blood concentration of Trp was neither affected by exercise nor caffeine. Tyr equally rises in the three groups and Phe had its exercise induced raise decreased by large concentrations of caffeine in blood (Table 3).

Gln and Ala are the major amino acid sources for neoglucogenesis. The rise of Gln in response to exercise was abolished by higher caffeinemia, without effect in both Ala and Glu blood concentration enhance caused by exercise (Table 3).

Caffeine is described as an urea cycle inhibitor. In our study caffeine promoted an effect in the blood concentrations of the three urea cycle intermediates Arg; Cit and Orn. The SCEx group had a significantly deprivation of Cit and Orn blood rise due to exercise. Blood Arg concentration was also affected (Table 3).

Analyses of amino acid blood exercise induced increase leads to the separation of caffeine effect in two. Ile, Trp, Val and BCAA blood concentration was affected in both groups. Meanwhile, Orn, Gln, Gly, His, Phe, Ser and Tyr in blood is less affected by the smallest caffeine concentration (Figure 3).

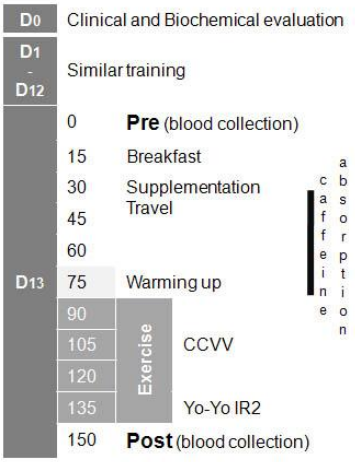
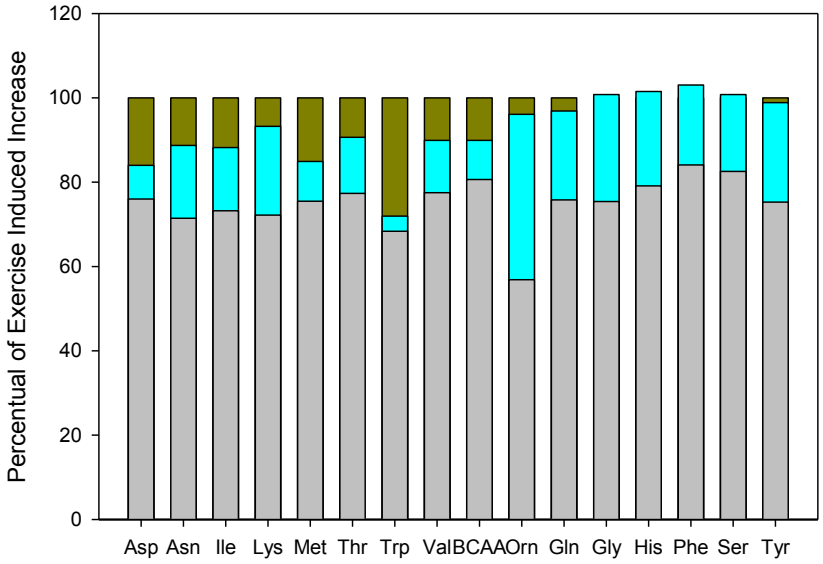
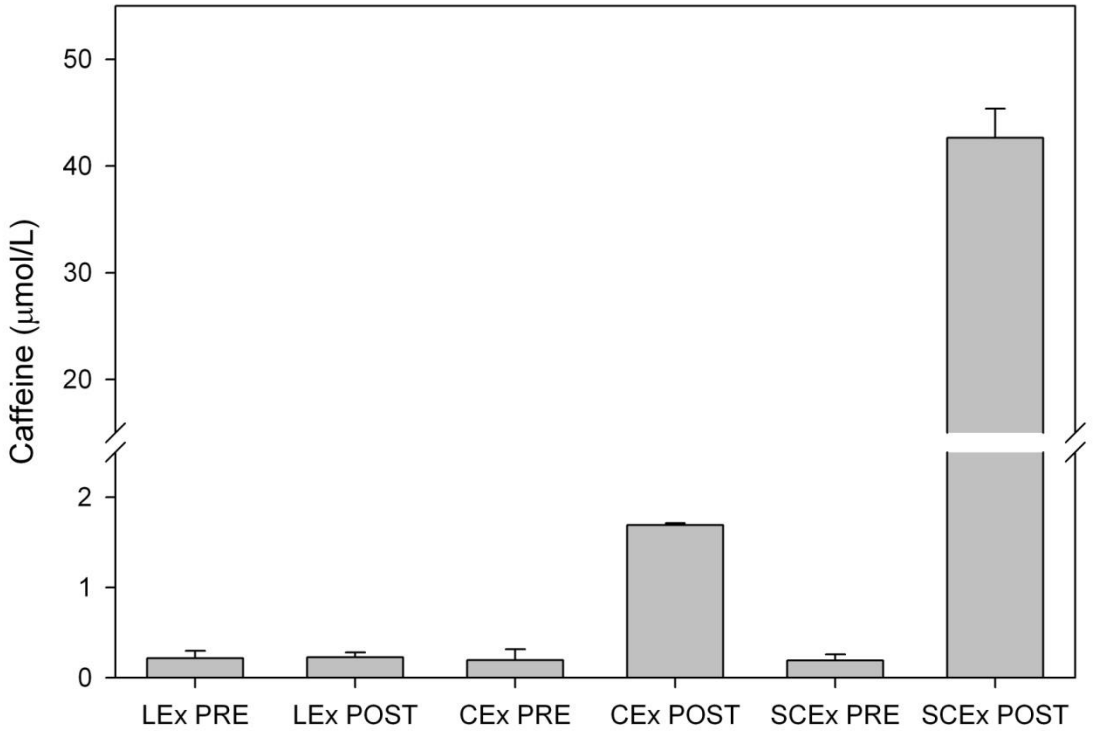


Figure 1 - Experimental design. Professional soccer players (n=19) from a first-division team affiliated in the Confederação Brasileira de Futebol (CBF, Brazilian Soccer Confederation) participated in this study. On the experiment day blood was collected from fasting soccer players, i.e., before breakfast (PRE). Players were randomly divided into two groups and received a specific breakfast diet and a supplement caffeine or lactose. After that the subjects were driven to the test place and warmed up. The subjects performed a variable distance run protocol (VDR), simulating a soccer match After the VDR, Yo-Yo intermittent recovery test (Yo-Yo IRT) was performed to drive athletes to exhaustion. This test finished at a different time for each athlete, and immediately after, blood was collected for laboratory analyses (POST). The athletes received drink containing electrolytes and glucose *ad libitum*. Described caffeine time is showed for comparison.



Grafic 1 - Summary of the post exercise plasma amino acids caffeine group normalized against LEx.



Grafic 2 – Caffeine plasma concentration variation in response to exercise. The exercise induced a 20 times increase in SCEx post on plasma caffeine.

	SCEx (n=5)		**CEx** (n=6)		**LEx** (n=8)	
	PRE	POST	PRE	PRE	POST	PRE
Glucose (mmol/L) *	4.5 ± 0.1	6.9 ± 0.3	4.6 ± 0.1	6.6 ± 0.4	4.6 ± 0.1	5.8 ± 0.4
Insulin (µmolU/L)*	5.1 ± 0.2	9.4 ± 0.3	5.3 ± 0.1	9.1 ± 0.7	5.2 ± 0.2	8.0 ± 0.7
Lactate (mmol/L) *	2.1 ± 0.4	15.5 ± 3.6	1.9 ± 0.2	23.2 ± 3.2	1.9 ± 0.2	17.0 ± 1.6

Table 1 – Plasma glucose, insulin and lactate in response to caffeine supplementation. Values are means ± SE. *(PRE x POST, $p < 0.05$)

	SCEx (n=5)		**CEx** (n=6)		**LEx** (n=8)	
	PRE	POST	PRE	PRE	POST	PRE
Ammonia (μmol/L) *	14.3 ± 4.5	88.8 ± 30.7	10.6 ± 2.3	54.6 ± 16.0	10.7 ± 1.6	70.6 ± 24.8
Creatinine (μmol/L) *	94.3 ± 6.0	116.4 ± 9.0	93.7 ± 2.2	140.7 ± 6.8	103.9 ± 4.3	118.2 ± 5.0
Urate (μmol/L)	434.3 ± 35.9	440.0 ± 44.7	416.9 ± 21.1	489.6 ± 45.1	426.9 ± 27.0	378.0 ± 23.7
Urea (mmol/L)	**6.5 ± 0.5**	**6.7 ± 0.5** [a, b]	**8.5 ± 0.9**	**9.5 ± 1.0** [a]	**7.7 ± 0.5** [b]	**8.4 ± 0.6**

Table 2 – Nitrogen metabolites changes in response to caffeine supplementation. Values are means ± SE. * means difference after exercise (PRE x POST). There are difference by condition for urea in bold; Difference by condition and time for creatinine; and difference by time for all metabolites. Significant difference from SCEx versus: [a] CEx; and [b] LEx. Repeated measures ANOVA: There are differences by time points in all of these variables Main effect of condition; Multivariate ANOVA – Metabolism by condition and Time Interception is different for all metabolites.

	SCEx (n=5)		**CEx** (n=6)		**LEx** (n=8)	
	PRE	POST	PRE	PRE	POST	PRE
Asp	21.6 ± 2.0	19.8 ± 1.2	21.3 ± 1.8	22.4 ± 1.3	18.9 ± 0.4	23.6 ± 2.3
Glu	63.6 ± 5.2	97.0 ± 7.9 *	58.8 ± 5.5	84.3 ± 10.2 *	64.5 ± 4.2	103.5 ± 6.8 *
Ala	308.8 ± 13.1	651.4 ± 47.5 *	262.5 ± 15.3	680.7 ± 74.0 *	287.5 ± 29.8	626.5 ± 43.3 *
Arg	**54.5 ± 3.7**	**63.1 ± 5.2** [a]	**65.6 ± 6.6**	**70.2 ± 5.5** [a, c]	**49.8 ± 3.1**	**62.6 ± 3.9** [c]
Asn	68.0 ± 2.6	64.6 ± 3.9	61.3 ± 6.2	72.2 ± 7.8	56.3 ± 3.2	75.0 ± 5.1
Cit	**20.8 ± 2.0**	**24.1 ± 2.9** [b]	**20.6 ± 1.9**	**31.0 ± 3.7 ***	**24.5 ± 1.9**	**33.3 ± 2.6 *** [b]
Gln	522.2 ± 22.0	508.8 ± 31.4	486.1 ± 30.9	602.3 ± 47.2 *	482.6 ± 25.0	616.7 ± 26.3 *
Gly	217.4 ± 11.3	207.8 ± 7.6	188.5 ± 12.0	240.1 ± 22.3 *	194.0 ± 11.5	244.0 ± 9.9 *
His	*70.5 ± 2.4*	*74.7 ± 3.9*	*64.7 ± 2.3*	*88.3 ± 7.5* *	*62.3 ± 3.5*	*83.6 ± 3.1* *
Ile	95.7 ± 3.6	88.9 ± 10.6	82.5 ± 6.9	92.7 ± 9.4	75.3 ± 4.6	95.5 ± 6.3
Leu	148.8 ± 7.8	178.2 ± 13.7	153.3 ± 8.2	181.3 ± 20.3	137.5 ± 8.4	177.0 ± 8.6
Lys	222.8 ± 15.3	214.1 ± 19.1	192.3 ± 26.1	238.4 ± 16.3	161.3 ± 12.9	214.6 ± 12.8
Met	30.5 ± 1.7	36.6 ± 2.1	30.5 ± 2.4	41.2 ± 5.3	27.0 ± 1.7	42.9 ± 2.7
Orn	**43.6 ± 6.6**	**38.1 ± 4.1** [b]	**34.8 ± 5.9**	**51.1 ± 7.8** [c]	**44.7 ± 4.5**	**68.6 ± 5.8** [b, c]
Phe	65.9 ± 0.9	73.5 ± 5.9	59.9 ± 5.1	81.5 ± 6.8 *	61.5 ± 5.3	81.0 ± 4.6 *
Pro	156.6 ± 13.0	197.2 ± 26.2	155.0 ± 8.1	196.0 ± 24.6	172.9 ± 15.3	239.7 ± 19.3 *
Ser	74.1 ± 2.9	76.8 ± 4.2	71.9 ± 4.9	91.5 ± 7.9 *	66.5 ± 3.7	83.9 ± 3.4 *
Tau	82.3 ± 4.0	89.8 ± 10.0	69.9 ± 8.0	97.3 ± 15.9	63.3 ± 9.2	82.4 ± 9.5
Thr	115.6 ± 5.2	114.9 ± 4.1	118.0 ± 10.8	136.9 ± 13.8	110.7 ± 9.8	142.0 ± 10.4
Trp	49.8 ± 3.0	47.3 ± 4.1	41.2 ± 3.1	41.4 ± 10.2	36.8 ± 2.8	51.2 ± 3.4
Tyr	62.2 ± 1.8	83.7 ± 6.2 *	53.5 ± 3.5	94.2 ± 8.1 *	50.1 ± 2.8	89.0 ± 6.4 *
Val	289.9 ± 10.8	290.1 ± 29.4	258.9 ± 15.3	302.0 ± 25.2	238.8 ± 15.7	307.4 ± 14.8 *
BCAA	534.3 ± 22.3	557.2 ± 53.7	494.7 ± 30.4	576.0 ± 54.9	451.7 ± 28.6	579.9 ± 29.7 *
TAA	2785.3 ± 141.0	3240.4 ± 251.2	2551.2 ± 191.0	3537.1 ± 351.1 *	2486.8 ± 179.2	3544.1 ± 211.2

Table 3 – Sumary of the plasma amino acids pre and post exercise. Multivariate ANOVA by condition and time folling by Post Hoc tests. * Means difference by time; Arg, Cit and Orn in bold means difference by condition; *His* italic means interaction by time and condition. Significant difference from SCEx versus: [a] CEx; and [b] LEx. [C] means difference from CEx vs. Lex.

ANEXO

Artigo número 06: Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players

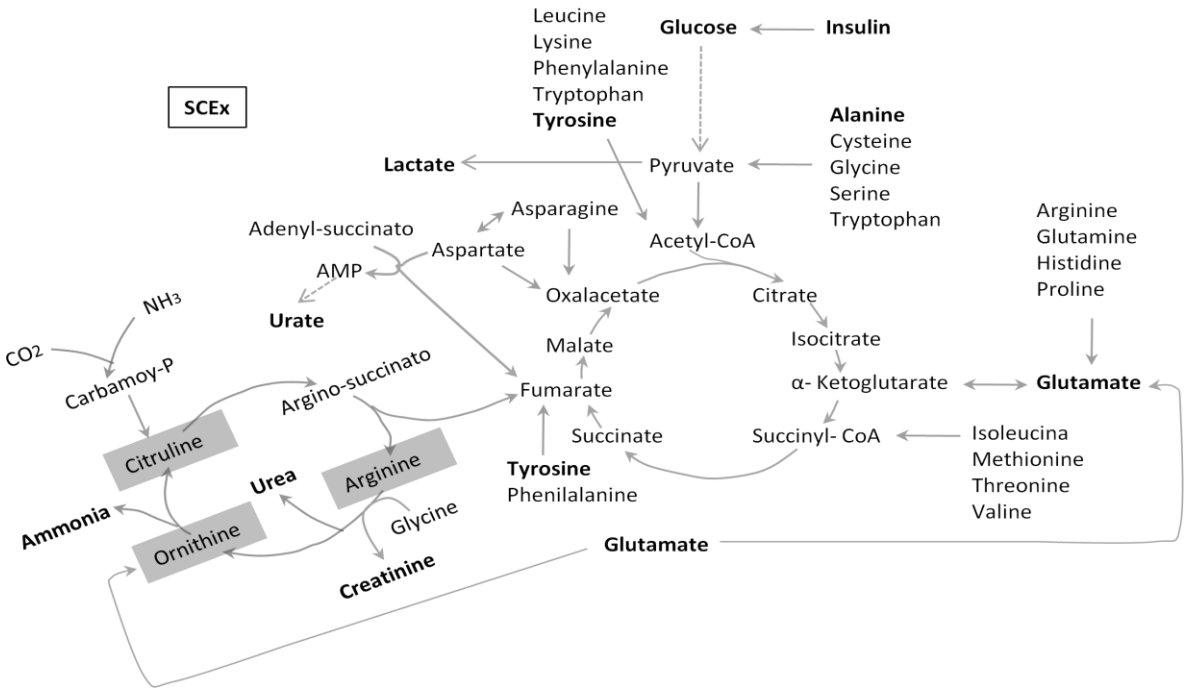


Ilustração da interação do ciclo da uréia com o dos ácidos tricarboxílicos no hepatócito do grupo suplementado com cafeína (SCEX) com concentrações cerca de 9000 vezes mais elevadas em comparação aos outros grupos. Os aminoácidos em negrito tiveram sua concentração aumenta na corrente sanguine. A linha continua cinza demonstra uma das possíveis vias de entrada dos aminoácidos. A linha pontilhada cinza significa que vários passos da via não foram esquematizados.

Artigo número 06: Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players

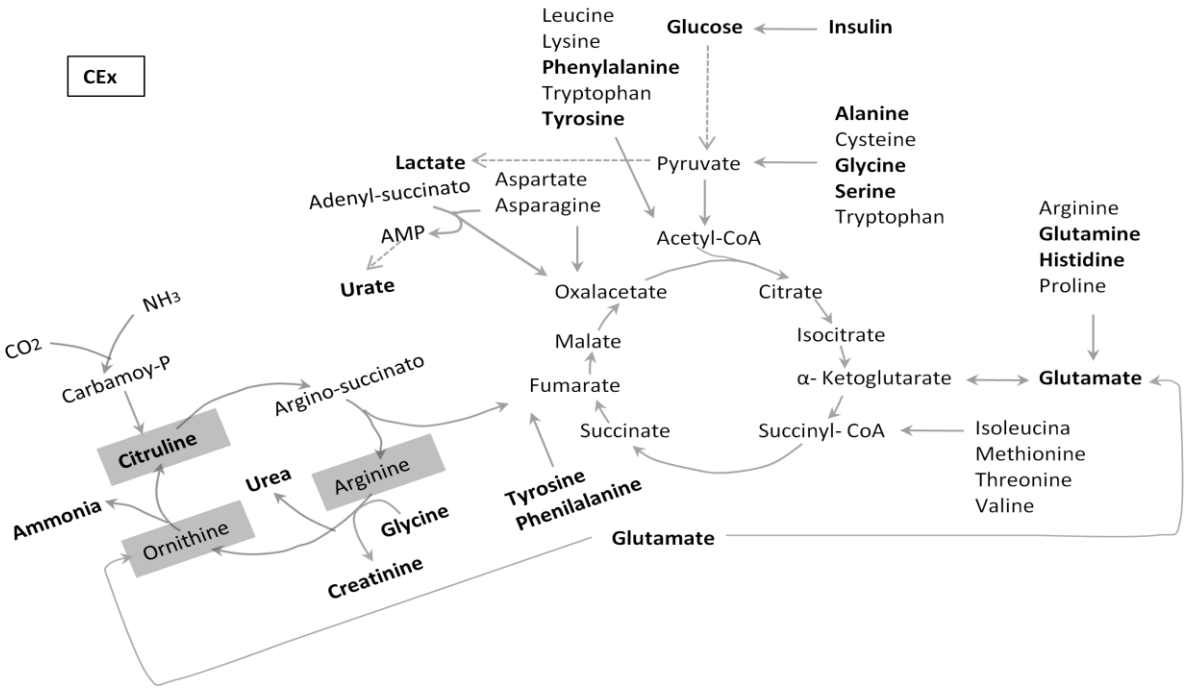


Ilustração da interação do ciclo da uréia com o dos ácidos tricarboxílicos no hepatócito do grupo suplementado com cafeína (CEX). Os aminoácidos em negrito tiveram sua concentração aumenta na corrente sanguine. A linha continua cinza demonstra uma das possíveis vias de entrada dos aminoácidos. A linha pontilhada cinza significa que vários passos da via não foram esquematizados.

Artigo número 06: Caffeine supplementation effects on plasma aminoacids and metabolism in elite soccer players

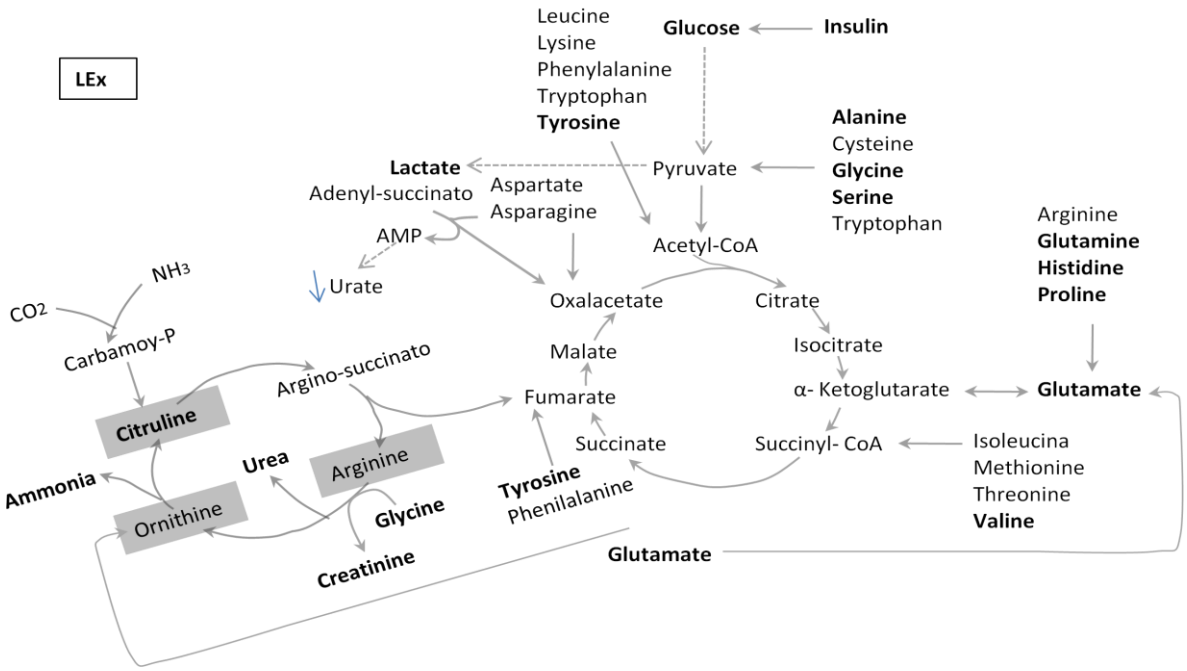


Ilustração da interação do ciclo da uréia com o dos ácidos tricarboxílicos no hepatócito do grupo não suplementado que realizou somente exercício (LEX). Os aminoácidos em negrito tiveram sua concentração aumenta na corrente sanguine. A linha continua cinza demonstra uma das possíveis vias de entrada dos aminoácidos. A linha pontilhada cinza significa que vários passos da via não foram esquematizados.

CAPITULO VIII

Bessa, A.; Gonçalves, LCO; Freitas-Dias, R; Bassini-Cameron, A; Werneck-de-Castro JPS; LC Cameron. Arginine modulates both hyperammonemia and lymphocytes appearance in blood after high-intensity exercise

SCANDINAVIAN JOURNAL OF MEDICINE & SCIENCE IN SPORTS

Arginine modulates both hyperammonemia and lymphocyte appearance in blood after high-intensity exercise.

Journal:	*Scandinavian Journal of Medicine and Science in Sports*
Manuscript ID:	draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Bessa, Artur; UniRio, LBP Gonçalves, Luis; UniRio, LBP Freitas-Dias, Ricardo; UniRio, LBP Bassini-Cameron, Adriana; UniRio, LBP Werneck-de-Castro, João; UniRio, LBP Cameron, Luiz-Claudio; UniRio, Laboratório de Bioquímica de Proteínas
Keywords:	White blood cells, Granulocytes, Monocytes, Amino Acids, Immune Response

Arginine modulates both hyperammonemia and lymphocyte appearance in blood after high-intensity exercise.

Artur Bessa[1,3], Luis C. O. Gonçalves[1,2] , Ricardo Freitas-Dias[1,2]; Adriana Bassini-Cameron[1,3], João P. S. Werneck-de-Castro[1,4,5] and Luiz-Claudio Cameron[1,2,3].

[1]Laboratório de Bioquímica de Proteínas, Universidade Federal do Estado do Rio de Janeiro, Brazil

[2]Universidade Castelo Branco, Rio de Janeiro, Brazil

[3]Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Brazil

[4]Laboratório de Biologia Muscular e do Exercício, Departamento de Biociências e Atividade Física, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

[5]Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Corresponding author:

L. C. Cameron

Laboratório de Bioquímica de Proteínas

Universidade Federal do Estado do Rio de Janeiro

Av. Pasteur 296 - Urca

CEP 22290-240

Rio de Janeiro – Brazil

Phone: 55 21 2542 5204

Fax: 55 21 2542 5280

cameron@unirio.br

Key words: White blood cells; Granulocytes; Monocytes; Amino Acids; Immune Response

Running head: Arginine effects on hyperammonemia and WBCs

ABSTRACT

Exercise is a good model to study hyperammonemia in humans without requiring external ammonia exposure. Here we used a new fight-based model to better understand arginine modulation of both hyperammonemia and the appearance of leukocytes in the blood. Brazilian Jiu-Jitsu practitioners (men, n = 39) volunteered to this study. The subjects followed a ketogenic diet for four days before the trials and received either arginine supplementation (100 mg/kg of body mass/day) or a placebo. We used an experimental model consisting of a six-minute fight with athletes wearing full gear. The rate of ammonemia increase during the fight in the control group was almost twice that of the arginine group (25 μmol/L.min^{-1} and 13 μmol/L.min^{-1}, respectively). The exercise induced an increase in leukocytes of almost 75%. An even greater difference was observed in the lymphocyte counts, which rose 2.2–fold in the control group; this increase was partially prevented by arginine. The ammonemia curve shape suggests that arginine is helping to prevent ammonia increase. In this study, we showed a high correlation between increases in both lymphocytes and ammonia, prevented by arginine supplementation. We also propose that an increase in lymphocytes could be a metabolic mechanism to protect from hyperammonemia.

INTRODUCTION

Hyperammonemia can occur due to urea cycle enzyme diseases or liver failure. Ammonia ($NH_3 + NH_4^+$) is highly toxic to humans, and the liver metabolism maintains its blood concentration in the 20–100 μM range. The metabolite can cross the blood–brain barrier and reach levels greater than 800 μM inside the central nervous system (CNS), which can lead to a decrease in cerebral function, neuropsychiatric disorders and death(Felipo e Butterworth, 2002). Ammonia-mediated excitotoxicity was proposed as a way to spread damage in CNS(Munoz, Monfort *et al.*, 2000). Data obtained from exercise have been used to help to explain hyperammonemia effects, since several studies have demonstrated that a rise in ammonemia occurs after different types of exercise(Guezennec, Abdelmalki *et al.*, 1998; Felipo e Butterworth, 2002; Degoutte, Jouanel *et al.*, 2003; Nybo e Secher, 2004; Nybo, Dalsgaard *et al.*, 2005; Bassini-Cameron, Sweet *et al.*, 2007; Bassini-Cameron, Monteiro *et al.*, 2008). We have previously proposed that exercise can be used as a tool to study the interactions between metabolic stress and the immune system(Bassini-Cameron, Sweet *et al.*, 2007). It is widely known that exercise impacts the immune response (reviewed in (Gleeson, 2007), and these effects depend on the intensity, duration and chronicity of the exercise. Changes in the number of white blood cells in circulation were previously described in various exercise models, including both high and low intensity exercise(Degoutte, Jouanel *et al.*, 2003; Natale, Brenner *et al.*, 2003; Ramel, Wagner *et al.*, 2003). Immunological effects due to exercise have been associated with the mechanical release of leukocytes from the vessel walls due to blood flow increase or catecholamine release, a mechanism that can be partially explained by cell adhesion signaling(Van Eeden, Granton *et al.*, 1999; Simonson e

Jackson, 2004). It has already been proposed that exercise can be employed as a model of the temporary immunosuppression that occurs after severe physical stress(Pedersen e Nieman, 1998; Pedersen e Hoffman-Goetz, 2000).

It has been shown that changes in ammonia in response to exercise can be managed by using amino acids or carbohydrates to interfere with metabolism(Graham, Turcotte *et al.*, 1997; Carvalho-Peixoto, Alves *et al.*, 2007; Bassini-Cameron, Monteiro *et al.*, 2008). Arginine (Arg) is a versatile amino acid in animal cells. It can be used as a precursor not only for protein synthesis but also for the synthesis of nitric oxide, urea, and other amino acids such as glutamate(Morris, 2006). Exercise studies showed that mammals that received Arg supplementation had greater concentrations of urea cycle intermediates in the serum, less lactatemia and better ammonia buffering than controls(Schaefer, Piquard *et al.*, 2002; Meneguello, Mendonca *et al.*, 2003). Arginine supplementation has also been described as an immune system stimulator, mainly in the production of T-cells(Field, Johnson *et al.*, 2000; Sureda, Tauler *et al.*, 2006). Arginine vasodilatory properties, via NO and its effect on growth hormone secretion, are well documented(Wu e Morris, 1998; Schaefer, Piquard *et al.*, 2002), but it remains unclear whether its use may confer an ergogenic effect in healthy individuals exposed to physical exercise stress(Paddon-Jones, Borsheim *et al.*, 2004).

In this study, we used for the first time a fight-based short duration high-intensity exercise combined with dietary manipulation as a model to produce an increase in blood ammonia to better understand arginine modulation of both hyperammonemia and leukocyte appearance in the blood. We developed this new exercise model to understand the impact of exercise metabolism in acute rises of ammonemia.

MATERIALS AND METHODS

Subjects

World class Brazilian Jiu-Jitsu male competitors (n = 39) volunteered to take part in this double blind, randomized study. The enrolled athletes had a minimum of three years of Jiu-Jitsu practice. Users of pharmaceutical drugs or nutritional ergogenic aids were excluded from the study. The included athletes had not sustained any injuries in the previous six months.

The subjects were randomly divided into two groups. The arginine supplemented group (RG, n = 16) ingested 100 mg/kg of body mass/day, and the control group (PG, n = 23) took 100 mg/kg of body mass/day of lactose, with supplement doses as previously described(Colombani, Bitzi *et al.*, 1999; Schaefer, Piquard *et al.*, 2002; Paddon-Jones, Borsheim *et al.*, 2004; Ohtani, Sugita *et al.*, 2006; Ruel, Beanlands *et al.*, 2008; Shao e Hathcock, 2008). Each athlete received packs of indistinguishable pills containing his daily doses and used them for four days, including the experimental day.

The athletes were briefed about the aim and the protocol of the study. Informed written consent was obtained from all the subjects, and the experiments were carried out in accordance with guidelines from the ethics committee for human research of the Universidade Castelo Branco, as well as the requirements for carrying out research in human subjects (Health National Council, Brazil, 1996).

Diet

Athletes of both groups followed a ketogenic diet (caffeine, ethanol and smoke-free) for three days before the trials, ingesting no more than 50 g of carbohydrates/day and at least 2 g of proteins/kg of body mass/day; ketogenic diet adherence was

verified by diet evaluation before the experiment. The experiment was conducted after 12 h of fasting. The last supplemental doses were given 90 min before the fight.

The Experiment

We used a model consisting of a six-minute Brazilian Jiu-Jitsu fight, with athletes wearing full gear. The fights were performed at similar temperatures and humidity and began with the athletes kneeling to avoid injuries from falling. The subjects were instructed to keep high mobility and avoid finishing the fight. The opponent fighter was not submitted to a ketogenic diet and was exchanged for a rested athlete after three minutes of elapsed fight time to keep the intensity as high as possible. Fights occurred between subjects in the same weight category.

The exercise intensity was evaluated during a pilot experiment, and athletes displayed a range from 85% to 90% of the maximum heart rate; we also observed that the fight promoted a similar kinetic ammonia serum rise for all the athletes (data not shown).

Blood sampling

Blood samples were collected following venipuncture at rest before and after the fight (1; 3; 5; 7 and 10 min). The pre-fight sample was collected before the last dose of arginine/lactose and the post-fight sample was collected immediately after the fight ended.

Blood was collected in tubes containing EDTA and kept refrigerated until hematological analysis (up to 2 h). Total and differential white cell counts were performed at least from three different samples. Red blood cells and platelets were also measured.

Samples for the biochemical assay were collected into tubes with coagulation enhancer and splitting gel (*Vacuette, Greiner Bio-One)* and immediately centrifuged

(3,000 x g, 10 min). Blood serum was aliquoted and stored in liquid nitrogen for later analysis. Sera were analyzed using clinical kits for the following muscle injury markers and biochemical variables: ammonia, creatine kinase (CK), creatine kinase-MB (CK-MB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γGT), lactate dehydrogenase (LDH), alkaline phosphatase - (ALP), glucose, urea, creatinine, urate, total protein, albumin, bilirubin, globulin, and serum hemoglobin. No changes in plasma volume were detected during the experiment.

Calculations and Statistics

The area under the curve (AUC) for the blood ammonia data for each individual in each treatment was determined using the equation AUC = {Ai(Ti+1 - Ti) + (1/2)(Ai+1 - Ai)(Ti+1 - Ti)}, where A denotes ammonia concentration (μmol/L) and T denotes time (min).

Data are shown as mean and standard error. Data were normalized to pre-exercise values. Intergroup statistical significance was calculated by analysis of variance (one-way ANOVA), and intragroup significance was established by Student's t-test. Data correlations were calculated using Pearson's test. Significant differences were assumed at $P < 0.05$.

RESULTS

Ammonia and its metabolites

In order to evaluate the effect of blood ammonia concentration from a high intensity exercise, we used a Brazilian Jiu-Jitsu fight as an exercise stress inducer (Figure 1). The rate of ammonemia increase during the fight in the control group was almost twice that of the Arg-supplemented group (25 μmol/L.min^{-1} and 13 μmol/L.min^{-1}, respectively). Statistical analysis of the area under the curve (AUC) showed that the Arg-supplemented group maintained lower ammonemia (~30%) compared to the control (Figure 2). Additionally, normalized data analysis showed that the fight induced a four- to sixfold increase in blood ammonia levels after exercise with a distinct curve shape for Arg-supplemented subjects (data not shown).

Ammonia production during high intensity exercise is mainly due to AMP deamination. To evaluate the previous increase in urea due to the higher ammonia production due to supplementation, we measured the urea and urate concentrations before and after exercise. Neither urea nor urate changed in response to the fight (Figures 3A and 3B). On the other hand, the fight led to a 30% increase in glycemia that remained high until the last measurement was done ten minutes after the fight ended (Figure 3C).

Proteins and Injury Markers

To ensure that the athletes were at similar training levels and also had similar liver integrity, we measured classical muscle and liver injury markers. The athletes of both groups had similar anthropometric conditions (Table 1).

Despite high levels of classical muscle injury markers, such as creatine kinase (CK; EC 2.7.3.2) and lactate dehydrogenase (LDH; EC 1.1.1.27), the concentration of

these enzymes in the blood did not change after the fight. Liver injury markers, such as alkaline phosphatase (ALP; EC 3.1.3.1) and γ-glutamyltransferase (γGT; EC 2.3.2.2), also remained stable in both groups. The same stability was seen with the less specific markers, aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) (Table 2). The amount of globulins in the blood increased in both groups after exercise. In the arginine-supplemented group, there was an 11% increase, while PG globulins rose 15% (Table 2).

Blood Cells and Platelets

The six minutes of exercise induced an increase in leukocytes of nearly 75%. This heightened level did not decrease in the ten minutes following the experiment, and we were not able to find a difference between the groups (Figure 4A). To avoid misinterpretations due to volemic variations, we evaluated red blood cells as well. Packed cell volume was not altered by exercise (Figure 4B). Platelet count was not affected by either exercise stress or Arg supplementation (data not shown). We did not measure differences in red blood cell counts, volume or hemoglobin content in response to fighting or supplementation.

Differential white blood cell analyses showed a distinct response to both exercise and Arg supplementation. Basophil counts rose twofold in the control group without changes in the supplemented one (Figure 5A). Analysis of eosinophil counts revealed a significant difference between the groups after the end of exercise (Figure 5B). On the other hand, neutrophils seemed to not significantly respond to either exercise or Arg (Figure 5C).

An even greater difference was observed in lymphocyte counts, which rose 2.2–fold compared to the control group. This increase was prevented by arginine (Figure. 6A).

Monocytes rose more than twofold in response to exercise in the control group and did not show a significant increase in the Arg-supplemented group (Figure 6B).

We plotted the ammonia response to exercise against lymphocyte count to understand the relationships between ammonia levels, lymphocyte count, and exercise. The exercise-induced increase in both ammonia and lymphocyte count was highly correlated. The lymphocyte count increase was prevented by arginine supplementation (Figure 7A and 7B).

DISCUSSION

It is widely known that ammonia has a deleterious effect on many systems, including the CNS, and has been pinpointed as a potential cause of central fatigue. Blood ammonia is normally in the 20-100 µM range, and concentrations above this range have been correlated with the incidence of encephalopathy, coma and death. Surprisingly, during exercise, ammonemia levels can become greater than 350 µM without obvious symptoms(Bassini-Cameron, Monteiro *et al.*, 2008). Here we used a ketogenic diet to deplete glycogen stocks combined with a Brazilian Jiu-Jitsu fight as an experimental model to understand blood ammonia increase and changes in white blood cells following exercise.

In this study, blood ammonia concentrations increased four- to sixfold after a six-minute fight, reaching as much as 610 µM. These values are above literature averages, even if we consider other fight-based models(Degoutte, Jouanel *et al.*, 2003; Ravier, Dugue *et al.*, 2008), reinforcing this experimental protocol as a powerful short term metabolic stress inducer. The velocity of ammonia increase was partially (50%) retarded by previous arginine supplementation and the total ammonia was lower in the group receiving Arg supplementation. In addition, the analysis of individual ammonia clearance points to a greater velocity in the supplemented group.

An increase in blood ammonia depends on different factors, including glycogen stocks, amino acid deamination and glucose availability(Wagenmakers, Beckers *et al.*, 1991; Van Hall, Van Der Vusse *et al.*, 1995; Snow, Carey *et al.*, 2000). We used this knowledge as the rational for depleting glycogen stocks using a ketogenic diet. In our study, the blood glucose increased up to 30% in response to a fight and stayed at this heightened level until a final measurement ten minutes after the fight,

regardless of Arg supplementation. This finding rules out an Arg effect on ammonemia due to glucose production as a consequence of supplementation. The ammonemia curve shape suggests that arginine is helping to buffer against the ammonemia increase, probably increasing the clearance by higher levels of urea cycle intermediates as previously described(Schaefer, Piquard *et al.*, 2002).

We performed a biochemical pre-evaluation on our subjects to assess the integrity of their liver function. The liver function of the athletes was assessed based on their hepatic metabolic function and hepatocyte integrity, measured by the presence of intracellular hepatocyte enzymes in blood. Neither urea nor urate production showed any differences before or in response to exercise. This finding is acceptable because we measured the total production of both metabolites in the blood in a short time frame. Previous studies in our laboratory showed that long-term supplementation of both glutamine and alanine increased the resting levels of blood urea(Bassini-Cameron, Monteiro *et al.*, 2008). In this study, we did not find any differences in both urea and urate at rest between groups. Both groups had similar increased urea basal levels when compared with normal subjects due to ketogenic diet. This data reinforce the possibility that arginine is acting as a reservoir for increased ammonia detoxification instead of being used as a carbon skeleton donor.

It has been proposed that exercise has a biphasic effect on the immune function(Nieman, 1994) and that various immune cell functions are temporarily impaired following acute bouts of heavy exercise(Gleeson, 2007). In this study, we observed an increase in leukocytes after fighting. We did not find changes in either packed cell volume, an internal control of volemic changes, or thrombocytes (data not shown). We did not detect either a significant increase in both eosinophils and neutrophil counts in response to exercise or any change due to Arg supplementation.

In contrast, we found a significant effect of supplementation on basophils; monocytes and lymphocytes in response to exercise.

Distinct effects on white blood cells were reported. In a study on heavy-resistance exercise, (Kraemer, Noble *et al.*, 1987) reported a decrease in eosinophils, which was contradicted by later studies showing an increase in total leukocyte count without differences in differential leukocyte counts(Kraemer, Clemson *et al.*, 1996). Even with an increase of 50-70% in some athletes, neutrophils did not have a significant change in response to exercise in our experiment, as expected based on previously reports(Boyum, Ronsen *et al.*, 2002). Little is known about granulocytes in response to acute exercise. However some data suggest that there is an increase in neutrophils following acute exercise, similar to the neutrophil increase caused by trauma(Northoff, Berg *et al.*, 1998), and that high-intensity exercise decreases neutrophil and thrombocyte adhesion(Cuzzolin, Lussignoli *et al.*, 2000). These findings together can help to explain our results.

An increase in leukocytes after acute exercise has been extensively described in a review by (Gleeson, 2007). In our study, we found a 75-85% increase in leukocytes. This increase was mainly due to an increase in lymphocytes, in agreement with many previous reports(Ronsen, Pedersen *et al.*, 2001; Boyum, Ronsen *et al.*, 2002). We also found an interesting protection against leukocyte and lymphocyte increase in the arginine-supplemented group. A previous study showed that carbohydrate supplementation decreases both leukocyte and lymphocyte trafficking during exercise(Gleeson, Nieman *et al.*, 2004). Recently it has been show that carbohydrate supplementation attenuates lymphocytosis after acute exhaustive resistance(Carlson, Headley *et al.*, 2008). As previously discussed, our data can rule out a protective effect of Arg in leukocytosis due to changes in the glycemia.

Nitric oxide (NO) is an important defense molecule against infectious organisms and also regulates the functional activity, growth and death of many immune cells(Coleman, 2001). NO is synthesized from arginine by nitric oxide synthases and inhibits leukocyte adhesion to endothelial cells with anti-inflammatory activity(Wiesinger, 2001). A previous report by (Sureda, Tauler *et al.*, 2006) showed neutrophilia and lymphopenia after exhaustive exercise with maintained basal plasma concentrations of arginine and ornithine, but decreased citrulline levels. It has been shown that a 3 g/day arginine supplementation can increase the availability of arginine, ornithine and citrulline(Schaefer, Piquard *et al.*, 2002), and since we used 100 mg/kg/day (6.5-12.0 g/day) it is possible to postulate that the supplementation used in our experiments resulted in an increased reservoir of these urea cycle intermediates. The activation of guanylate cyclase by NO was also altered in lymphocytes from hyperammonemic rats(Corbalan, Montoliu *et al.*, 2002), and it has been proposed that arginine availability can increase intracellular NO synthesis(Van De Poll, Siroen *et al.*, 2007). Taking these findings together, it is possible that our results regarding Arg supplementation in leukocytes could be explained in terms of NO regulation, where NO synthesis depends on arginine availability, a control point for cellular NO production.

Hyperammonemia is widely proposed as the main cause of hepatic encephalopathy (reviewed in (Zwingmann, 2007)). In this study we showed a high correlation between increases in lymphocyte count and blood ammonia levels, both of which can be prevented by arginine. It was recently proposed in an elegant study by (Garg, Banerjee *et al.*, 2008) that T cells can act together with glia to protect neurons. This protection occurs via liberation of lactate and glutamate from T cells following astrocyte release of cysteine (a precursor of glutathione synthesis) to protect and

lactate to feed neurons. In addition, previous reports have shown a metabolic protection from lymphocytes in target tissues, including the maintenance of cognition(Moalem, Leibowitz-Amit *et al*., 1999; Moalem, Yoles *et al*., 2000; Kipnis, Cohen *et al*., 2004). In addition our data also show that blood globulins increase is affected by Arg supplementation. Given these data, we propose that an increase in lymphocytes could be a mechanism of metabolic protection from an ammonia increase in the blood, pointing to a possible role for lymphocytes in response to hyperammonemia.

PERSPECTIVE

Here we propose that lymphocytes increase in blood in response to acute exhaustive resistance exercise can be a parallel event to ammonemia raise and more that it is possible that lymphocytes could be a protective agent helping to metabolizes ammonia.

ACKNOWLEDGEMENTS

We wish to thank Dr. Mazon for his professional support during the performance tests.

REFERENCES

Bassini-Cameron, A., A. N. Monteiro, et al. Glutamine protects against blood ammonia increase in soccer players in an exercise intensity dependent way. Br J Sports Med, Nov 5. 2008.

Bassini-Cameron, A., E. Sweet, et al. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. Br J Sports Med, v.41, n.8, Aug, p.523-30; discussion 530. 2007.

Boyum, A., O. Ronsen, et al. Chemiluminescence response of granulocytes from elite athletes during recovery from one or two intense bouts of exercise. Eur J Appl Physiol, v.88, n.1-2, Nov, p.20-8. 2002.

Carlson, L. A., S. Headley, et al. Carbohydrate supplementation and immune responses after acute exhaustive resistance exercise. Int J Sport Nutr Exerc Metab, v.18, n.3, Jun, p.247-59. 2008.

Carvalho-Peixoto, J., R. C. Alves, et al. Glutamine and carbohydrate supplements reduce ammonemia increase during endurance field exercise. Appl Physiol Nutr Metab, v.32, n.6, Dec, p.1186-90. 2007.

Coleman, J. W. Nitric oxide in immunity and inflammation. Int Immunopharmacol, v.1, n.8, Aug, p.1397-406. 2001.

Colombani, P. C., R. Bitzi, et al. Chronic arginine aspartate supplementation in runners reduces total plasma amino acid level at rest and during a marathon run. Eur J Nutr, v.38, n.6, Dec, p.263-70. 1999.

Corbalan, R., C. Montoliu, et al. Altered modulation of soluble guanylate cyclase by nitric oxide in patients with liver disease. Metab Brain Dis, v.17, n.4, Dec, p.295-301. 2002.

Cuzzolin, L., S. Lussignoli, et al. Influence of an acute exercise on neutrophil and platelet adhesion, nitric oxide plasma metabolites in inactive and active subjects. Int J Sports Med, v.21, n.4, May, p.289-93. 2000.

Degoutte, F., P. Jouanel, et al. Energy demands during a judo match and recovery. Br J Sports Med, v.37, n.3, Jun, p.245-9. 2003.

Felipo, V. e R. F. Butterworth. Neurobiology of ammonia. Prog Neurobiol, v.67, n.4, Jul, p.259-79. 2002.

Field, C. J., I. Johnson, et al. Glutamine and arginine: immunonutrients for improved health. Med Sci Sports Exerc, v.32, n.7 Suppl, Jul, p.S377-88. 2000.

Garg, S. K., R. Banerjee, et al. Neuroprotective immunity: T cell-derived glutamate endows astrocytes with a neuroprotective phenotype. J Immunol, v.180, n.6, Mar 15, p.3866-73. 2008.

Gleeson, M. Immune function in sport and exercise. J Appl Physiol, v.103, n.2, Aug, p.693-9. 2007.

Gleeson, M., D. C. Nieman, et al. Exercise, nutrition and immune function. J Sports Sci, v.22, n.1, Jan, p.115-25. 2004.

Graham, T. E., L. P. Turcotte, et al. Effect of endurance training on ammonia and amino acid metabolism in humans. Med Sci Sports Exerc, v.29, n.5, May, p.646-53. 1997.

Guezennec, C. Y., A. Abdelmalki, et al. Effects of prolonged exercise on brain ammonia and amino acids. Int J Sports Med, v.19, n.5, Jul, p.323-7. 1998.

Kipnis, J., H. Cohen, et al. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc Natl Acad Sci U S A, v.101, n.21, May 25, p.8180-5. 2004.

Kraemer, W. J., A. Clemson, et al. The effects of plasma cortisol elevation on total and differential leukocyte counts in response to heavy-resistance exercise. Eur J Appl Physiol Occup Physiol, v.73, n.1-2, p.93-7. 1996.

Kraemer, W. J., B. J. Noble, et al. Physiologic responses to heavy-resistance exercise with very short rest periods. Int J Sports Med, v.8, n.4, Aug, p.247-52. 1987.

Meneguello, M. O., J. R. Mendonca, et al. Effect of arginine, ornithine and citrulline supplementation upon performance and metabolism of trained rats. Cell Biochem Funct, v.21, n.1, Mar, p.85-91. 2003.

Moalem, G., R. Leibowitz-Amit, et al. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nat Med, v.5, n.1, Jan, p.49-55. 1999.

Moalem, G., E. Yoles, et al. Autoimmune T cells retard the loss of function in injured rat optic nerves. J Neuroimmunol, v.106, n.1-2, Jul 1, p.189-97. 2000.

Morris, S. M., Jr. Arginine: beyond protein. Am J Clin Nutr, v.83, n.2, Feb, p.508S-512S. 2006.

Munoz, M. D., P. Monfort, et al. Hyperammonemia impairs NMDA receptor-dependent long-term potentiation in the CA1 of rat hippocampus in vitro. Neurochem Res, v.25, n.4, Apr, p.437-41. 2000.

Natale, V. M., I. K. Brenner, et al. Effects of three different types of exercise on blood leukocyte count during and following exercise. Sao Paulo Med J, v.121, n.1, Jan 2, p.9-14. 2003.

Nieman, D. C. Exercise, infection, and immunity. Int J Sports Med, v.15 Suppl 3, Oct, p.S131-41. 1994.

Northoff, H., A. Berg, et al. Similarities and differences of the immune response to exercise and trauma: the IFN-gamma concept. Can J Physiol Pharmacol, v.76, n.5, May, p.497-504. 1998.

Nybo, L., M. K. Dalsgaard, et al. Cerebral ammonia uptake and accumulation during prolonged exercise in humans. J Physiol, v.563, n.Pt 1, Feb 15, p.285-90. 2005.

Nybo, L. e N. H. Secher. Cerebral perturbations provoked by prolonged exercise. Prog Neurobiol, v.72, n.4, Mar, p.223-61. 2004.

Ohtani, M., M. Sugita, et al. Amino acid mixture improves training efficiency in athletes. J Nutr, v.136, n.2, Feb, p.538S-543S. 2006.

Paddon-Jones, D., E. Borsheim, et al. Potential ergogenic effects of arginine and creatine supplementation. J Nutr, v.134, n.10 Suppl, Oct, p.2888S-2894S; discussion 2895S. 2004.

Pedersen, B. K. e L. Hoffman-Goetz. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev, v.80, n.3, Jul, p.1055-81. 2000.

Pedersen, B. K. e D. C. Nieman. Exercise immunology: integration and regulation. Immunol Today, v.19, n.5, May, p.204-6. 1998.

Ramel, A., K. H. Wagner, et al. Acute impact of submaximal resistance exercise on immunological and hormonal parameters in young men. J Sports Sci, v.21, n.12, Dec, p.1001-8. 2003.

Ravier, G., B. Dugue, et al. Impressive anaerobic adaptations in elite karate athletes due to few intensive intermittent sessions added to regular karate training. Scand J Med Sci Sports, Aug 5. 2008.

Ronsen, O., B. K. Pedersen, et al. Leukocyte counts and lymphocyte responsiveness associated with repeated bouts of strenuous endurance exercise. J Appl Physiol, v.91, n.1, Jul, p.425-34. 2001.

Ruel, M., R. S. Beanlands, et al. Concomitant treatment with oral L-arginine improves the efficacy of surgical angiogenesis in patients with severe diffuse coronary artery disease: the Endothelial Modulation in Angiogenic Therapy randomized controlled trial. J Thorac Cardiovasc Surg, v.135, n.4, Apr, p.762-70, 770 e1. 2008.

Schaefer, A., F. Piquard, et al. L-arginine reduces exercise-induced increase in plasma lactate and ammonia. Int J Sports Med, v.23, n.6, Aug, p.403-7. 2002.

Shao, A. e J. N. Hathcock. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regul Toxicol Pharmacol, v.50, n.3, Apr, p.376-99. 2008.

Simonson, S. R. e C. G. Jackson. Leukocytosis occurs in response to resistance exercise in men. J Strength Cond Res, v.18, n.2, May, p.266-71. 2004.

Snow, R. J., M. F. Carey, et al. Effect of carbohydrate ingestion on ammonia metabolism during exercise in humans. J Appl Physiol, v.88, n.5, May, p.1576-80. 2000.

Sureda, A., P. Tauler, et al. Blood cell NO synthesis in response to exercise. Nitric Oxide, v.15, n.1, Aug, p.5-12. 2006.

Van De Poll, M. C., M. P. Siroen, et al. Interorgan amino acid exchange in humans: consequences for arginine and citrulline metabolism. Am J Clin Nutr, v.85, n.1, Jan, p.167-72. 2007.

Van Eeden, S. F., J. Granton, et al. Expression of the cell adhesion molecules on leukocytes that demarginate during acute maximal exercise. J Appl Physiol, v.86, n.3, Mar, p.970-6. 1999.

Van Hall, G., G. J. Van Der Vusse, et al. Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. J Physiol, v.489 (Pt 1), Nov 15, p.251-61. 1995.

Wagenmakers, A. J., E. J. Beckers, et al. Carbohydrate supplementation, glycogen depletion, and amino acid metabolism during exercise. Am J Physiol, v.260, n.6 Pt 1, Jun, p.E883-90. 1991.

Wiesinger, H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. Prog Neurobiol, v.64, n.4, Jul, p.365-91. 2001.

Wu, G. e S. M. Morris, Jr. Arginine metabolism: nitric oxide and beyond. Biochem J, v.336 (Pt 1), Nov 15, p.1-17. 1998.

Zwingmann, C. The anaplerotic flux and ammonia detoxification in hepatic encephalopathy. Metab Brain Dis, v.22, n.3-4, Dec, p.235-49. 2007.

TABLES

Table 1 – Age and anthropometric measurements in Brazilian Jiu-Jitsu fighters assigned to groups PG (placebo group) and RG (arginine supplemented group).

	Ctl	Range	Arg	Range
Age (years)	25.2 (0.4)	21 - 28	26.2 (0.6)	23 - 29
Weight (Kg)	82.2 (1.8)	70 - 103	79.2 (3.2)	65 - 120
Height (cm)	177.9 (1.0)	170 - 188	175 (1.4)	170 - 190

Values are mean (SEM) and range. No statistically differences were detected ($p>0.05$).

Table 2 – Muscle and liver injury markers measured before (PRE) and at the first minute after fight (POST). PG, placebo group; RG, arginine group.

	PG		RG	
	PRE	**POST**	**PRE**	**POST**
CK (U/L)	737.0(187.2)	1051.2(401.9)	559.1(128.3)	625.1(148.8)
CKMB (U/L)	13.6(4.1)	36.0(13.5)	12.2(2.0)	17.6(3.2)
LDH (U/L)	390.0(41.9)	402.8(29.6)	354.6(18.4)	388.3(17.9)
γGT (U/L)	21.7(2.4)	21.7(2.7)	27.4(4.2)	30.2(4.4)
ALP (U/L)	80.8(11.8)	88.1(12.0)	67.6(7.7)	74.6(7.4)
ALT (U/L)	23.0(3.8)	26.2(3.2)	30.1(5.2)	29.9(5.1)
AST (U/L)	52.7(17.9)	68.2(21.2)	36.0(3.7)	45.2(5.8)
Albumin (g/L)	43.3(0.2)	46.0(0.2)	45.9(0.2)	50.2(0.2)
Globulins (g/L)	32.5(0.1)	38.0(0.1)#	31.1(0.1)	34.6(0.1)#

Values are mean (SEM) and range. No statistically differences were detected ($p>0.05$), except for globulins response to exercise and supplementation.

FIGURE CAPTIONS

Figure 1. Experimental design. Before the experiment, the athletes were submitted to a three-day ketogenic diet as described in the Materials and Methods. Blood was collected (PRE) before the athletes received supplementation. Warm up and exercise protocols were carried out, followed by blood collections (POST) at 1, 3, 5, 7 and 10 min after exercise.

Figure 2. Blood ammonia concentration increases after a Jiu-Jitsu fight in an arginine supplementation-dependent way. A six-minute Jiu-Jitsu fight was performed after a three-day ketogenic diet and athletes received either arginine (RG, Δ) or placebo (PG, ●). Blood was collected before and after exercise and treated as described in the Materials and Methods. Control, n = 23; Arginine, n = 16. (*) denotes that average ± SE is different between pre- and post-exercise; (#) denotes a difference between the two experimental groups. The calculated area under the curve was 3397 $\mu mol \cdot L^{-1} \cdot min^{-1}$ for placebo or for 2366 $\mu mol \cdot L^{-1} \cdot min^{-1}$ arginine. The inset shows data normalized to pre-exercise values.

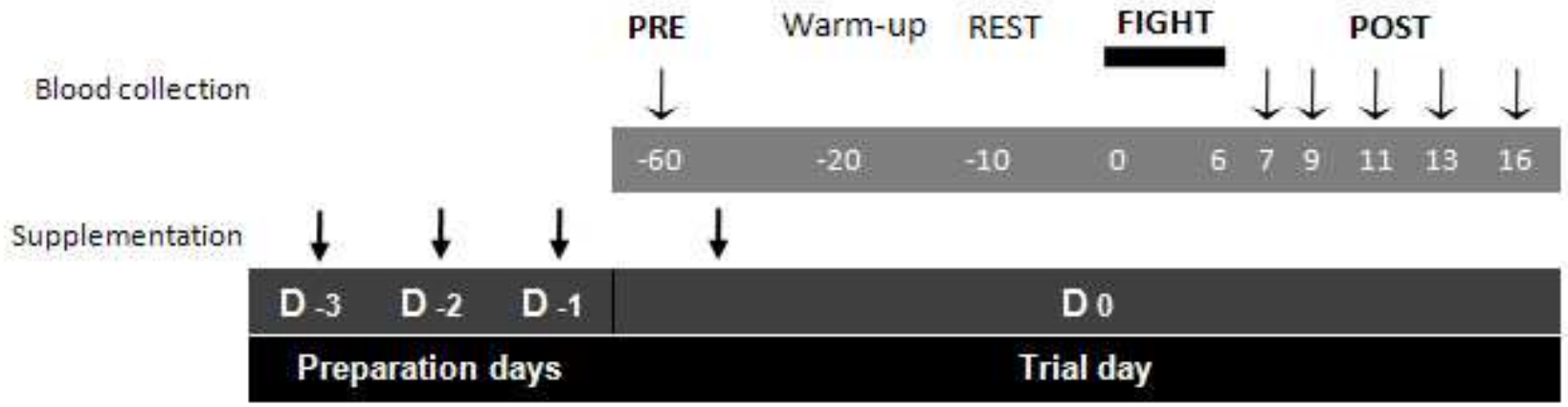
Figure 3. Glucose increases in response to exercise without supplementation dependence (A). Either supplementation or exercise can change both urea (B) and urate (C) after fight. Control, n = 23 (PG, ●) or Arginine, n = 16 (RG, Δ). Average ± SE is different from pre-exercise (*).

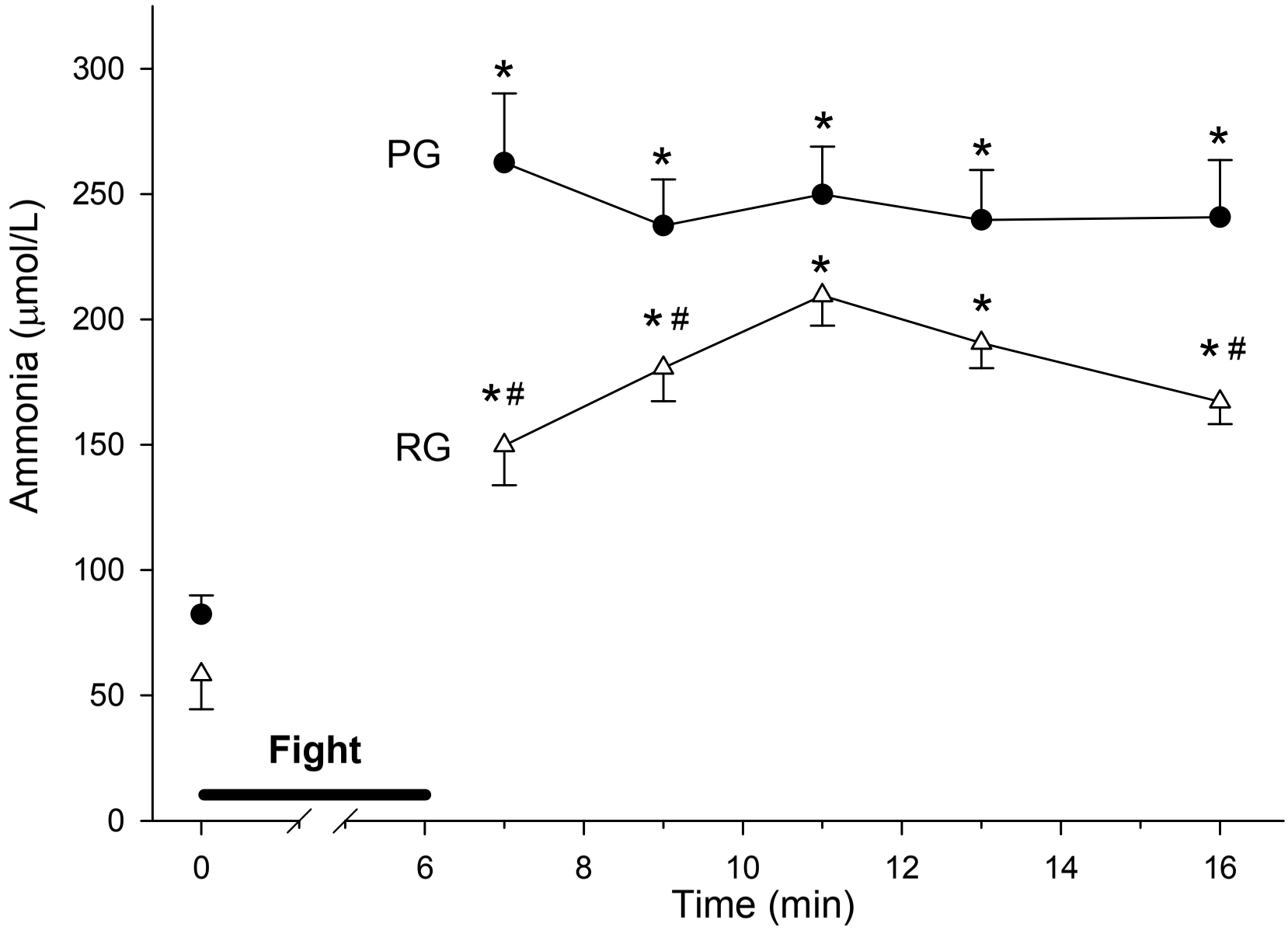
Figure 4. White blood cell counts increase (A) after a six-minute fight without packed cell volume change (B). Control, n = 23 (PG, ●) or Arginine, n = 16 (RG, Δ). Average ± SE is different from pre-exercise (*); Absolute pre-exercise for WBCs are PG 5.9 ± 0.2 cells x 10^9/L; RG 6.4 ± 0.5 cells x 10^9/L and for packed cell volume are PG 47.5 ± 0.6 %; RG 46.6 ± 0.6 %.

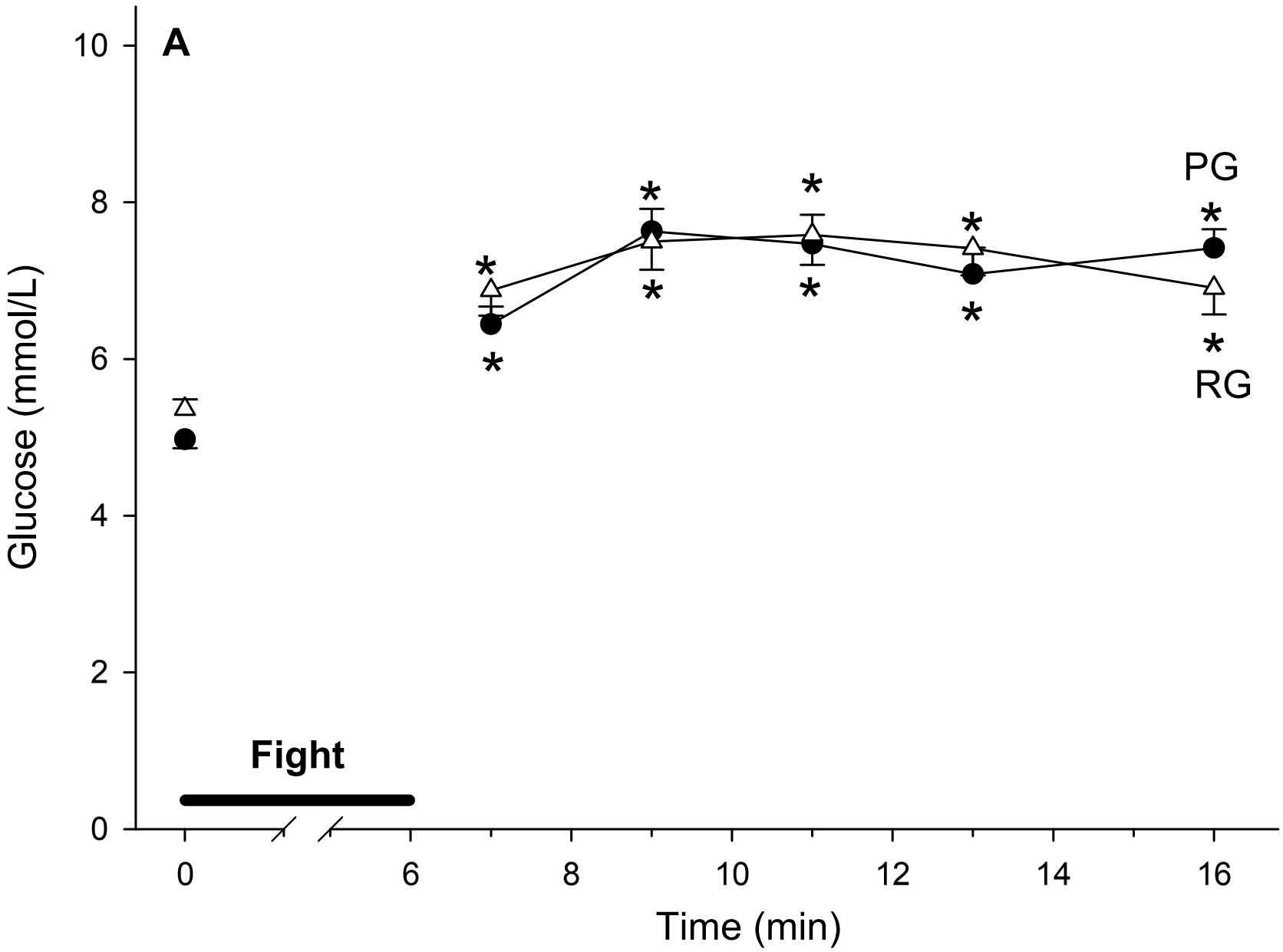
Figure 5. Granulocyte counts in response to exercise and supplementation. Basophils (A); Eosinophils (B); Neutrophils (C). Control, n = 23 (PG, ●) or Arginine, n = 16 (RG, Δ). Average ± SE is different from pre-exercise (*); Difference between experimental groups (#); Absolute pre-exercise values for basophils are PG 2.6 ± 0.4 cells x 10^7/L; RG 1.9 ± 0.9 cells x 10^7/L, for eosinophils are PG 1.8 ± 0.3 cells x 10^8/L; RG 2.0 ± 0.5 cells x 10^8/L and for neutrophils are PG 3.1 ± 0.2 cells x 10^9/L; RG 2.7 ± 0.4 cells x 10^9/L.

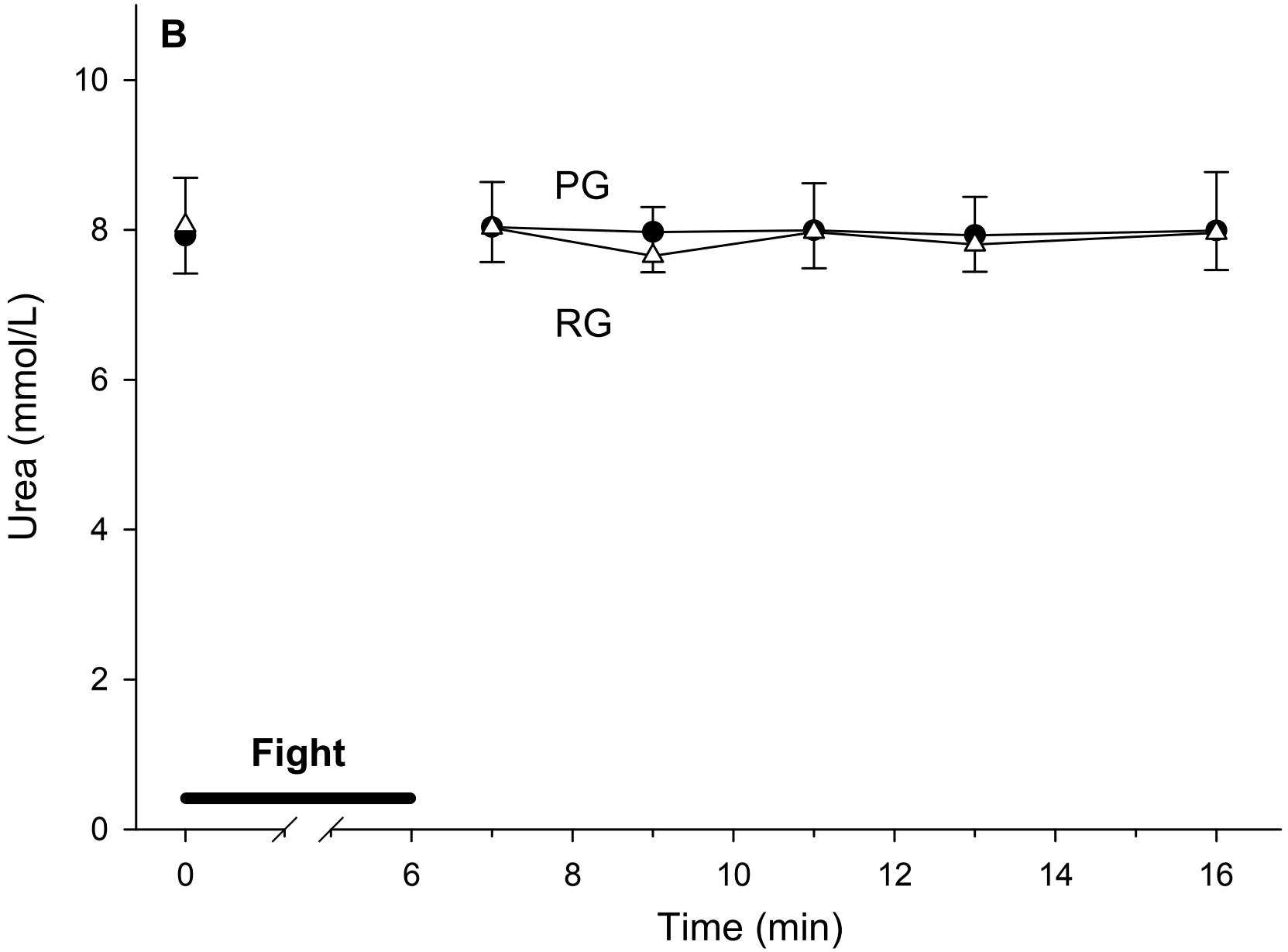
Figure 6. Exercise induces an increase in both lymphocyte (A) and monocyte (B) in an arginine supplementation-dependent way. Control, n = 23 (PG, ●) or Arginine, n = 16 (RG, Δ). Average ± SE is different from pre-exercise (*); Absolute pre-exercise values are shown within the graphs. Absolute pre-exercise for lymphocytes are PG 2.2 ± 0.1 cells x 10^9/L; RG 2.9 ± 0.3 cells x 10^9/L (no statistical difference, p = 0.07) and for monocytes are PG 4.2 ± 0.2 cells x 10^8/L; RG 5.5 ± 0.5 cells x 10^8/L.

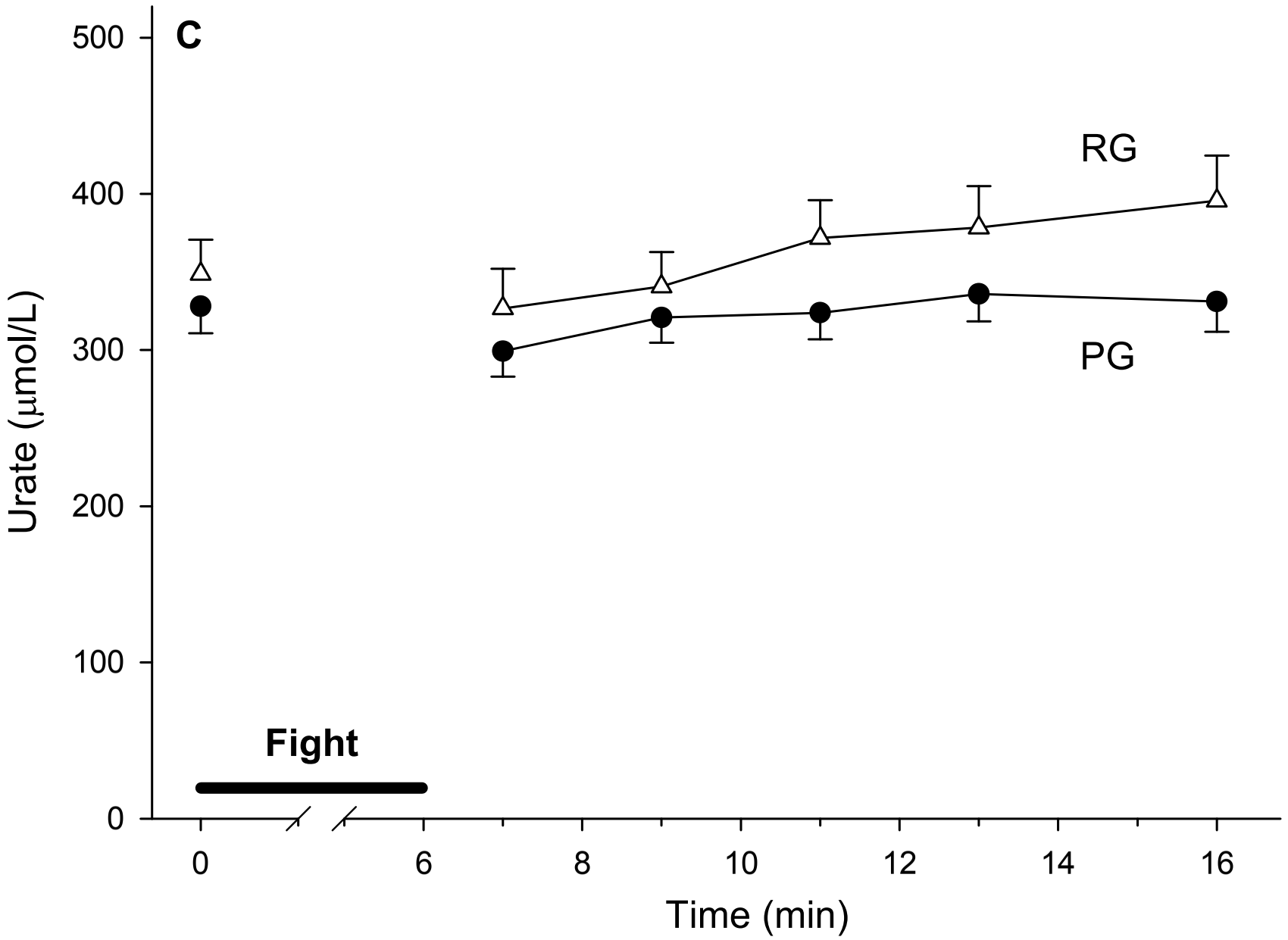
Figure 7. Ammonemia increase is related to lymphocytes and leukocytes blood count. White blood cells and lymphocytes were plotted against ammonemia (A). Average ± SE is different from pre-exercise (*); difference between experimental groups (#). Pearson correlation and P values are shown within the graph. Normalized leukocytes increase (B). Control, n = 23 (PG, ●) or Arginine, n = 16 (RG, Δ). Normalized lymphocytes increase (C). Control, n = 23 (Ctl, ■) or Arginine, n = 16 (RG, ◊).

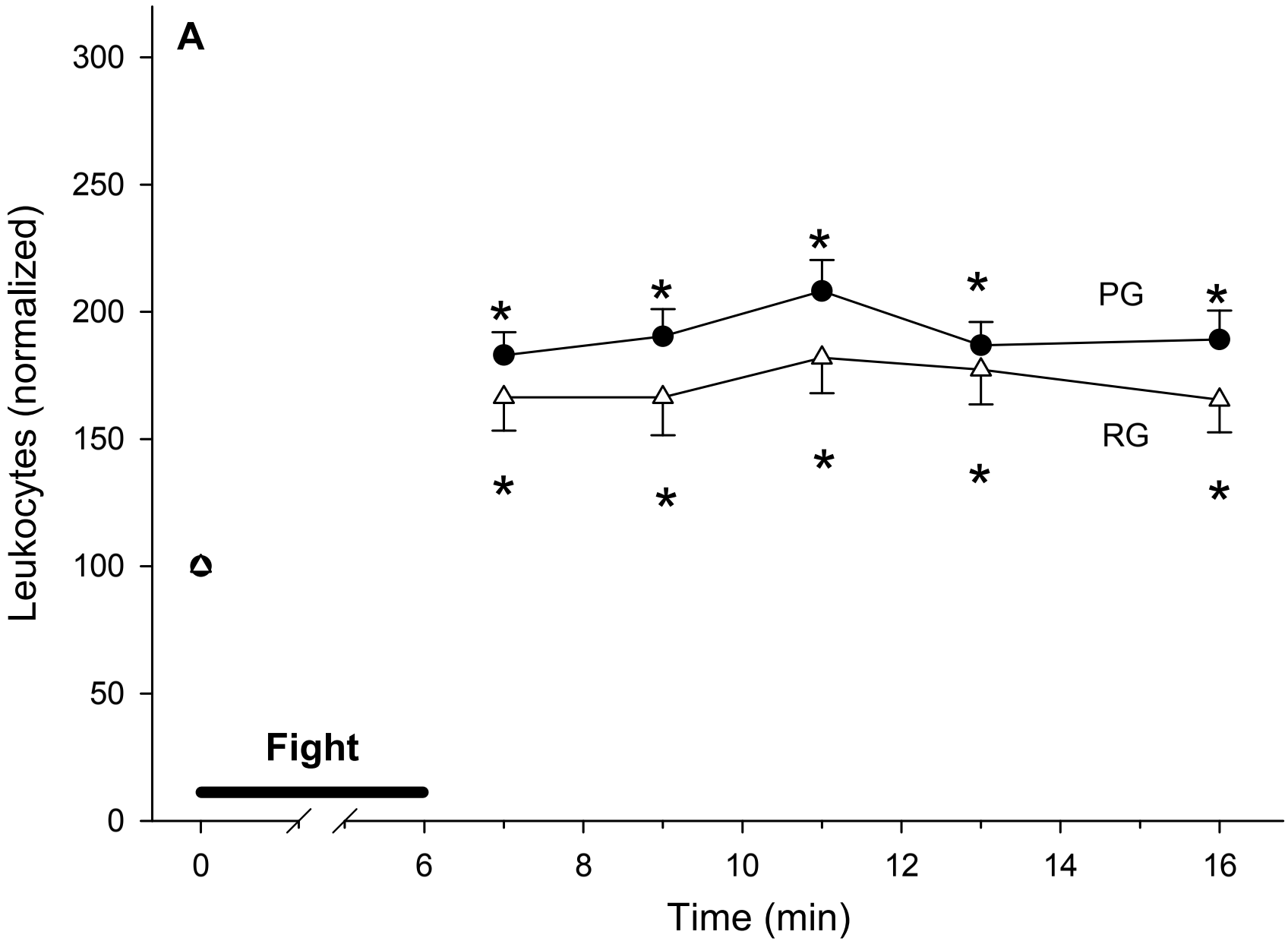

PRE
Warm-up
REST
FIGHT
POST
Blood collection
-60
-20
-10
0
6
7
9
11
13
16
Supplementation
D -3
D -2
D -1
D 0
Preparation days
Trial day

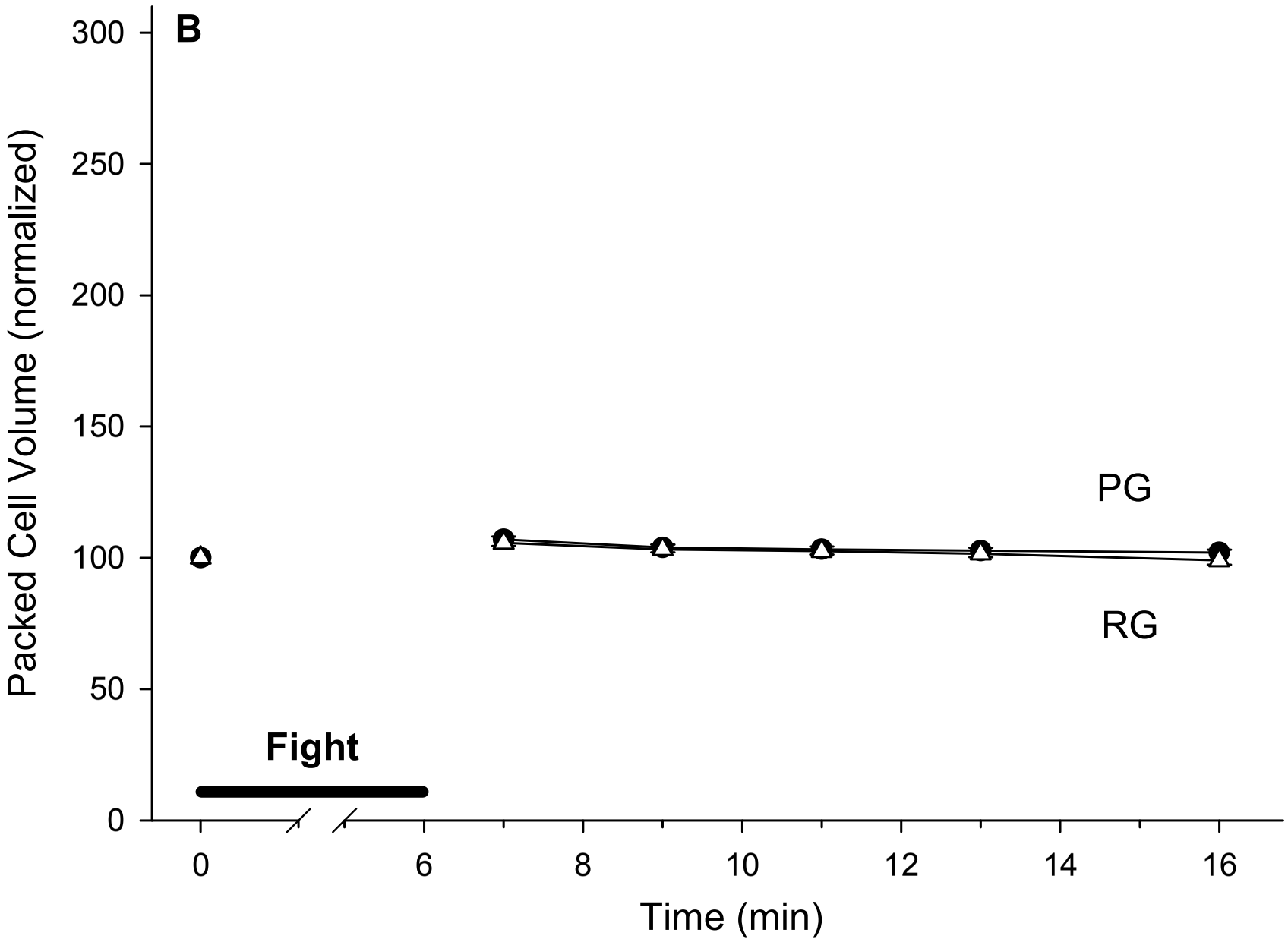

Ammonia (µmol/L)
300
250
200
150
100
50
0
PG
RG
*
*#
Fight
0
6
8
10
12
14
16
Time (min)

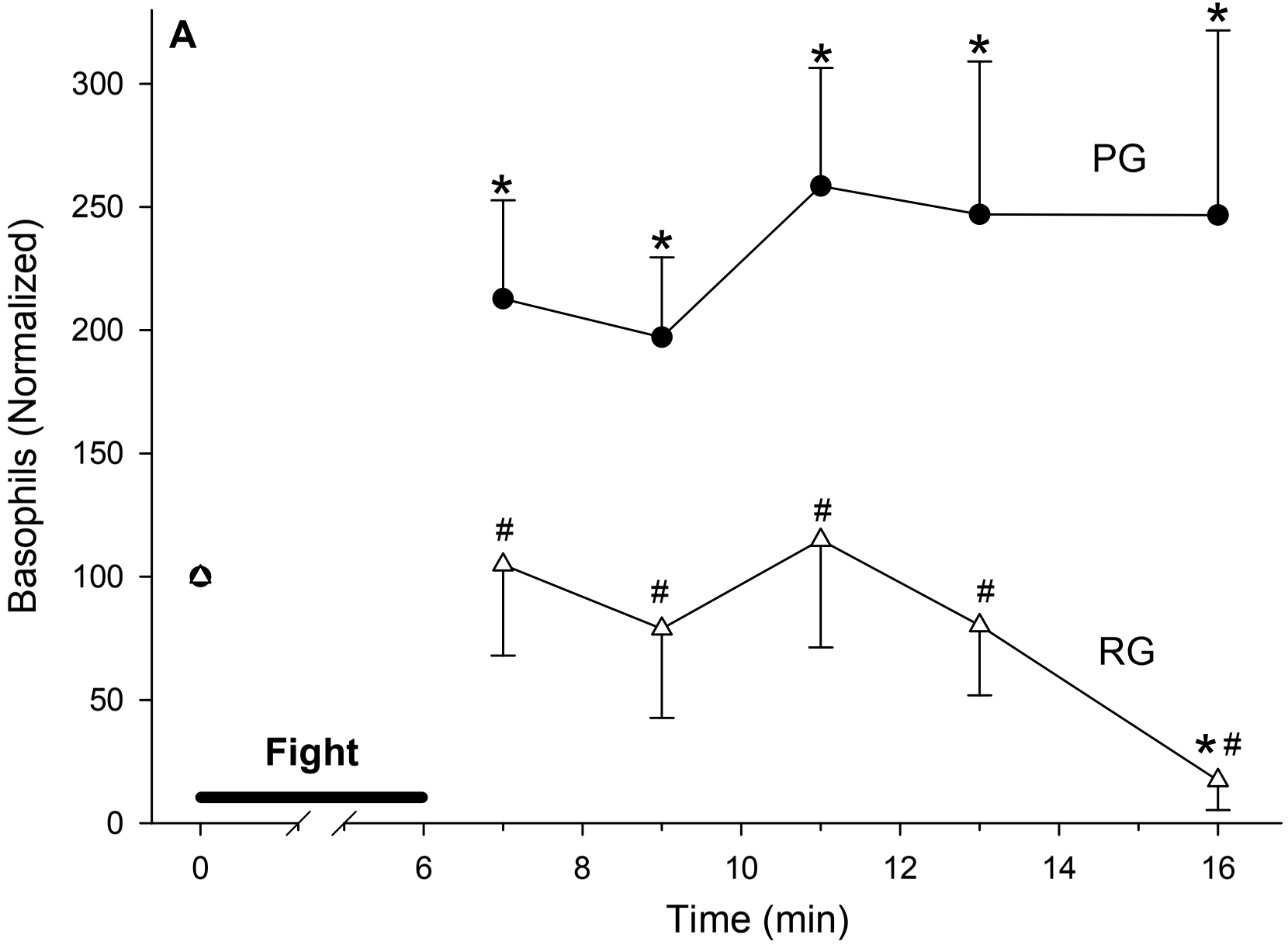

A
Glucose (mmol/L)
10
8
6
4
2
0
Fight
0
6
8
10
12
14
16
Time (min)
PG
RG

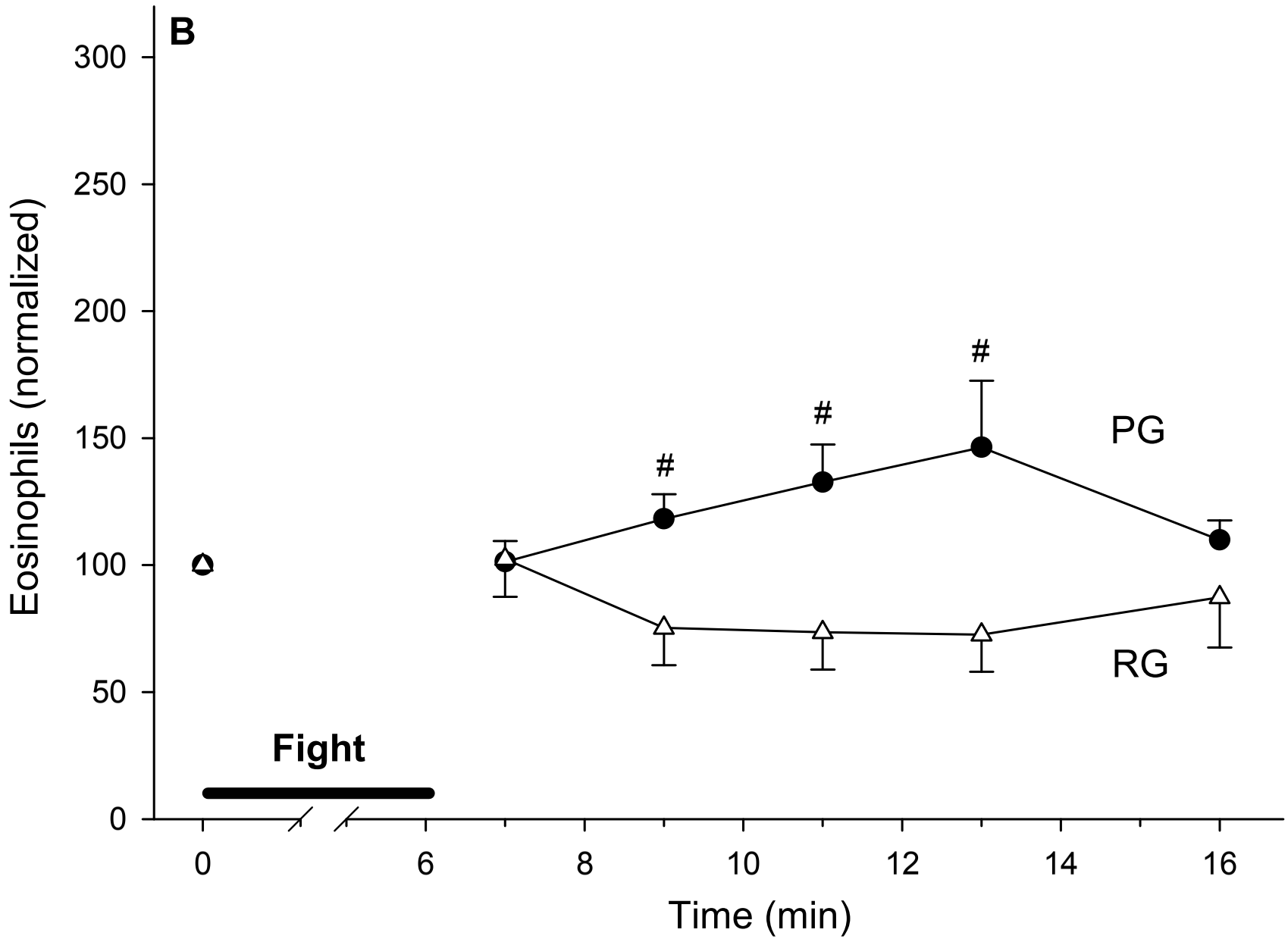

B
PG
RG
Fight
Urea (mmol/L)
Time (min)
0
2
4
6
8
10
0
6
8
10
12
14
16

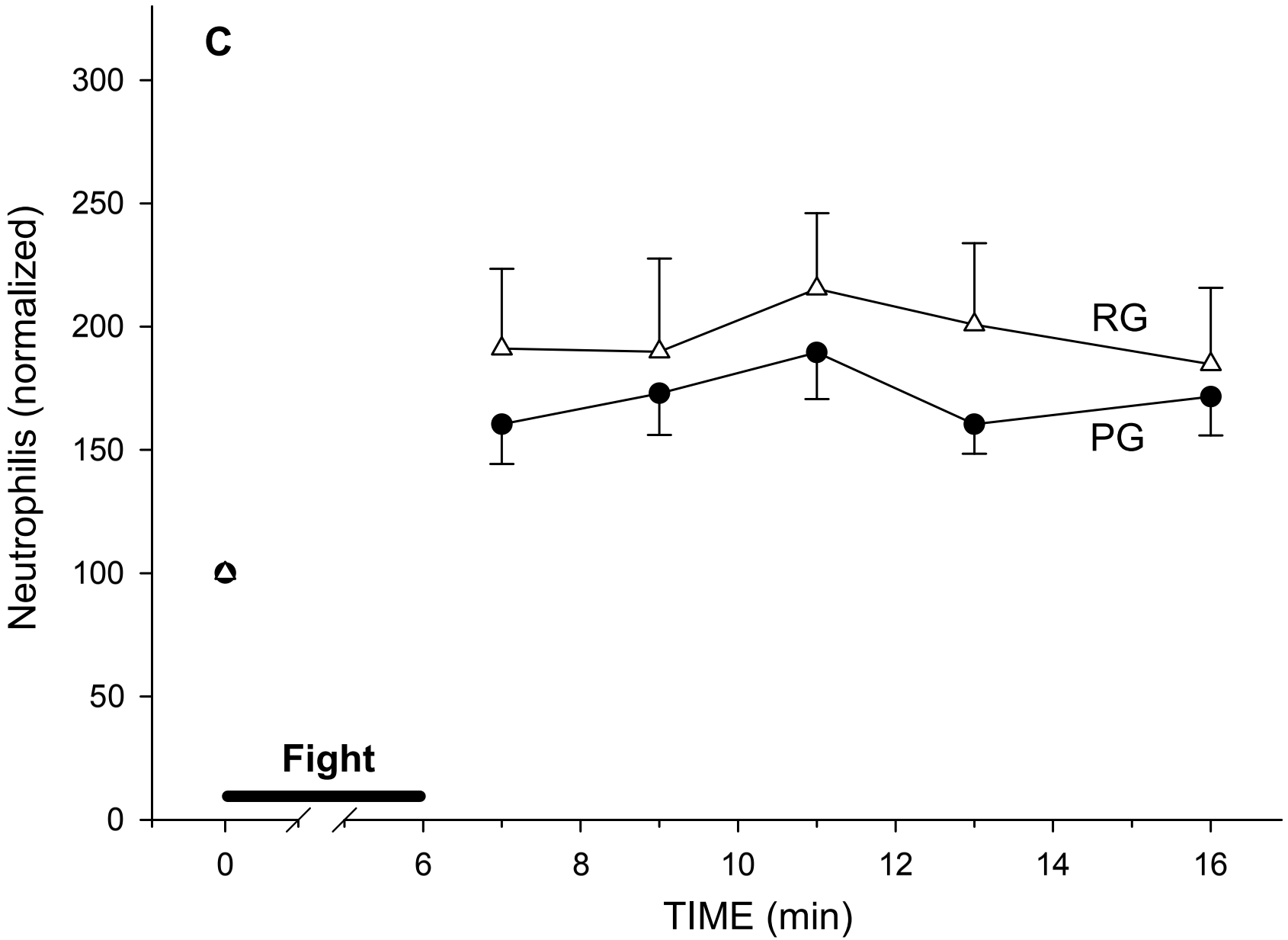

C
500
400
300
200
100
0
Urate (μmol/L)
RG
PG
Fight
0
6
8
10
12
14
16
Time (min)


A
Leukocytes (normalized)
300
250
200
150
100
50
0
PG
RG
Fight
0
6
8
10
12
14
16
Time (min)

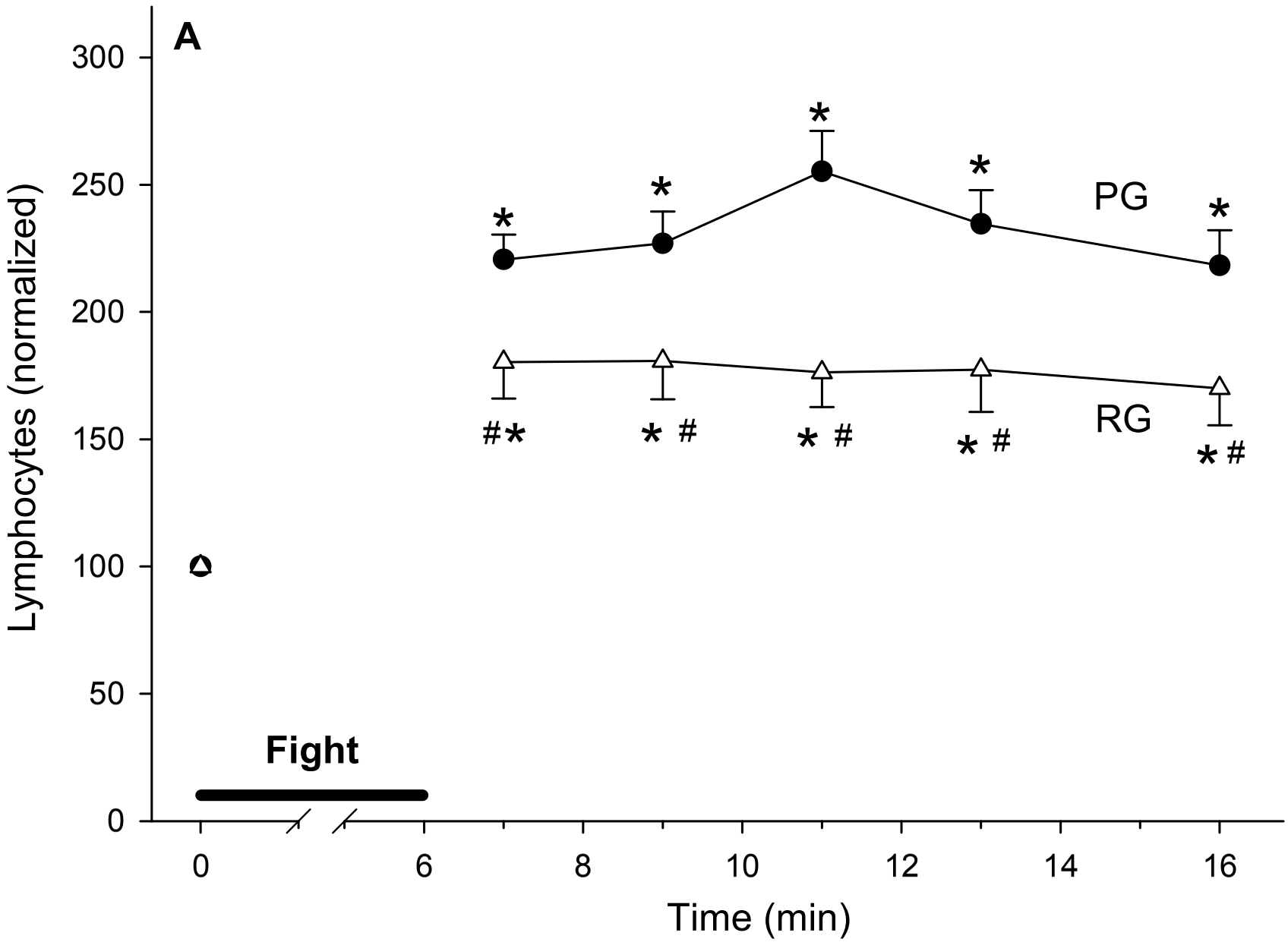

B
Packed Cell Volume (normalized)
300
250
200
150
100
50
0
PG
RG
Fight
0
6
8
10
12
14
16
Time (min)

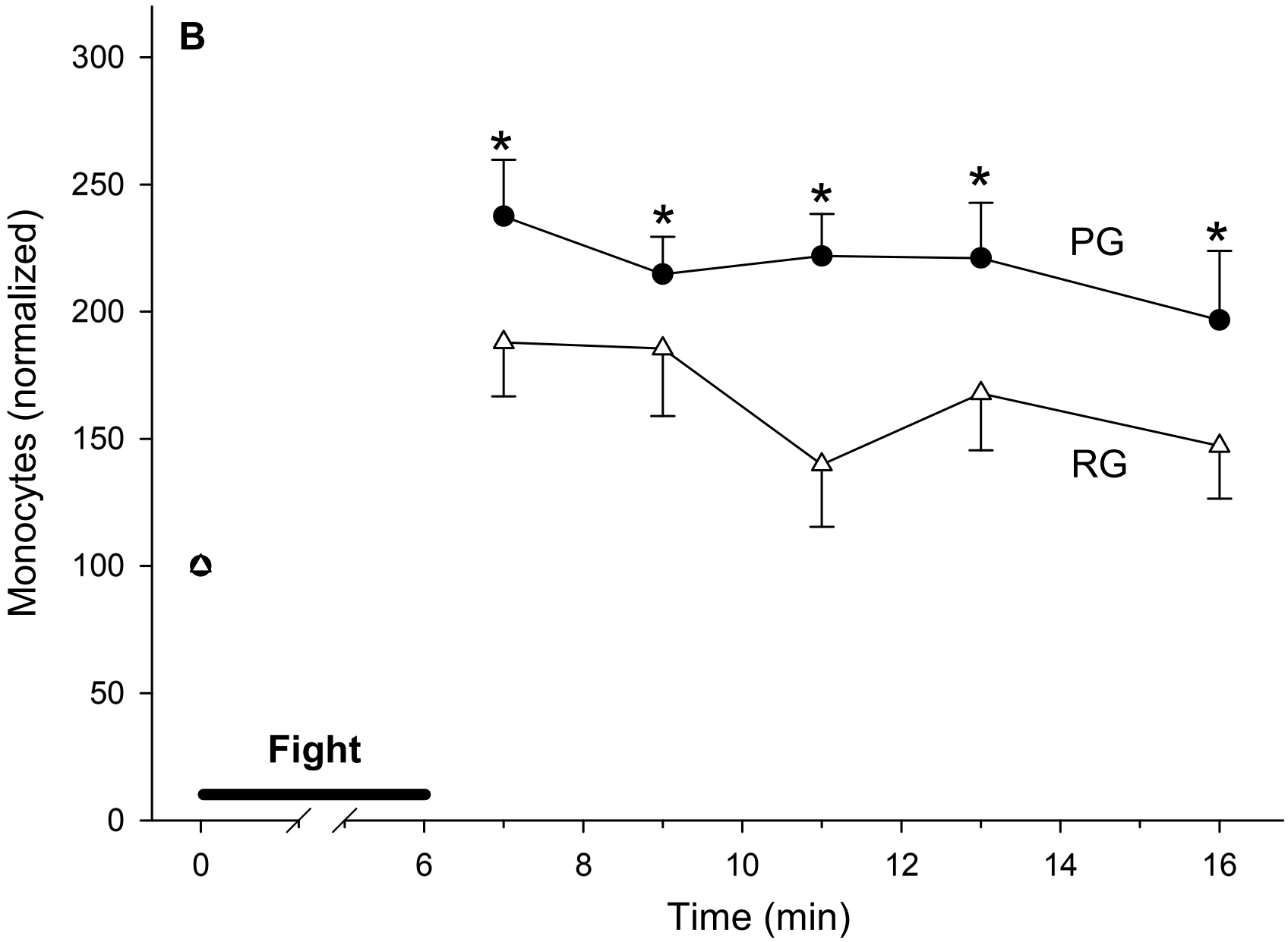

A
Basophils (Normalized)
300
250
200
150
100
50
0
PG
RG
Fight
0
6
8
10
12
14
16
Time (min)

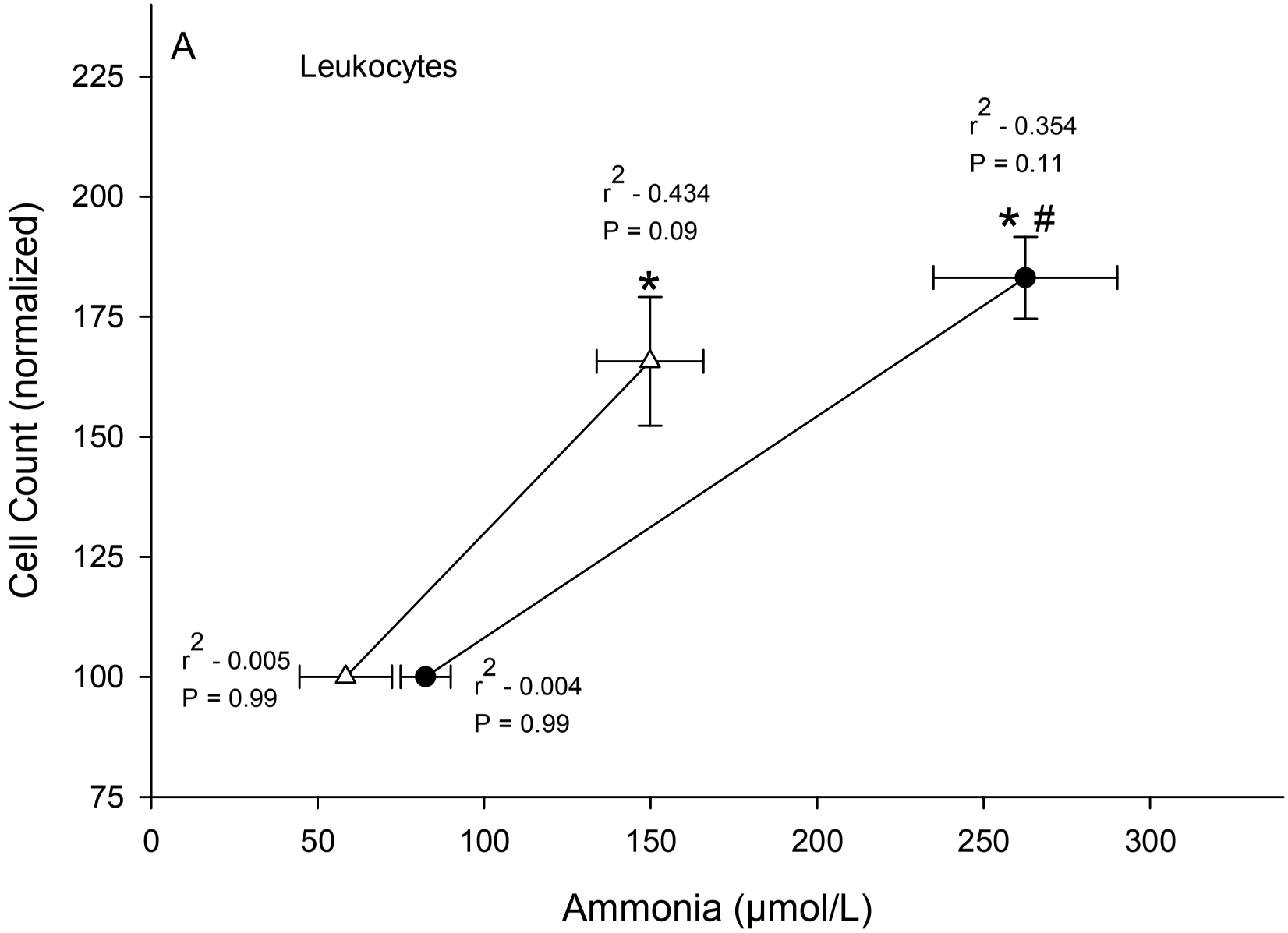

B
Eosinophils (normalized)
300
250
200
150
100
50
0
#
#
#
PG
RG
Fight
0
6
8
10
12
14
16
Time (min)

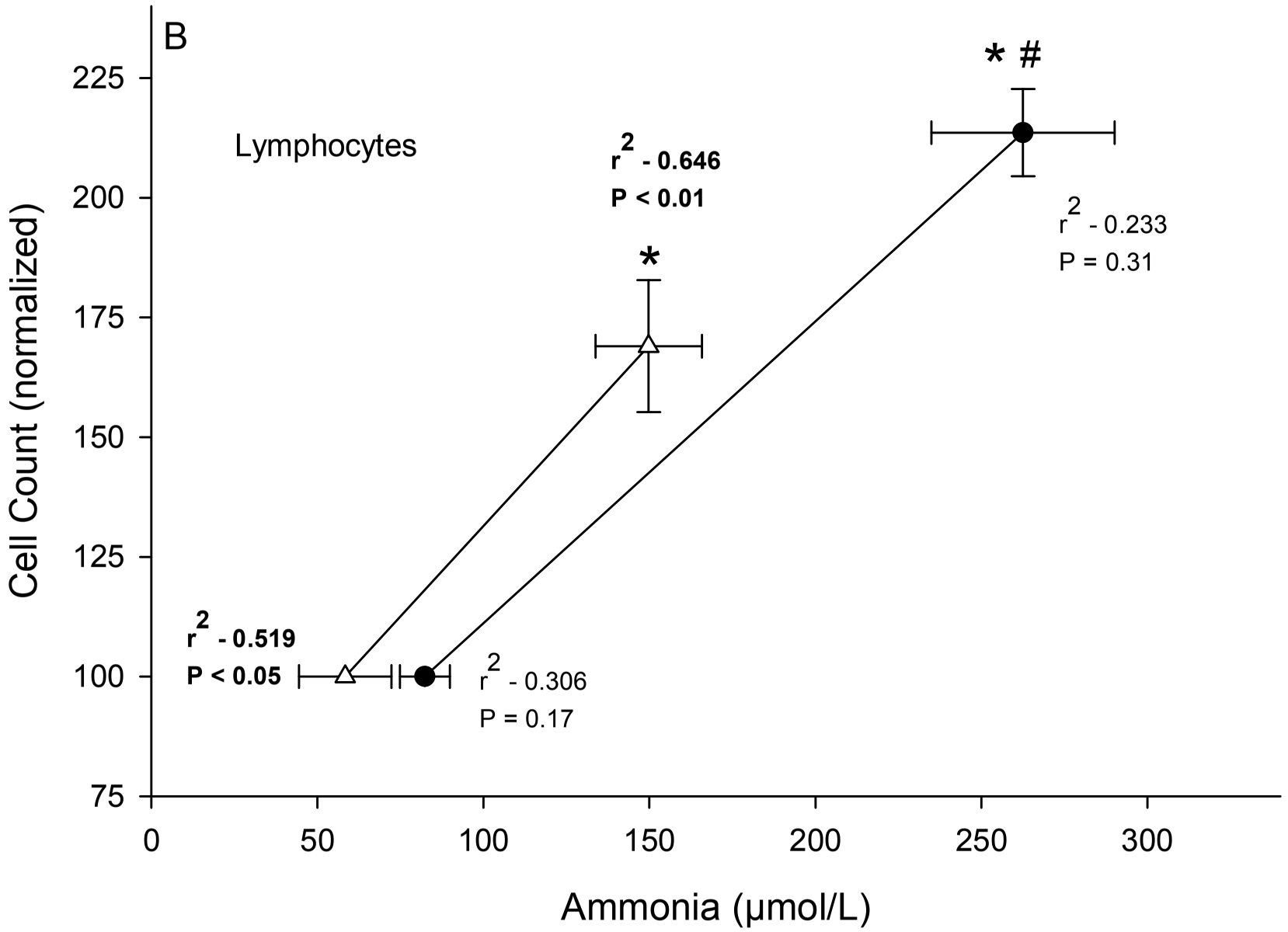

C
300
250
200
150
100
50
0
Neutrophilis (normalized)
RG
PG
Fight
0
6
8
10
12
14
16
TIME (min)

205


A
Lymphocytes (normalized)
300
250
200
150
100
50
0
PG
RG
Fight
0
6
8
10
12
14
16
Time (min)


B
Monocytes (normalized)
300
250
200
150
100
50
0
PG
RG
*
Fight
0
6
8
10
12
14
16
Time (min)


A
Leukocytes
Cell Count (normalized)
225
200
175
150
125
100
75
0
50
100
150
200
250
300
Ammonia (µmol/L)
r2 - 0.005
P = 0.99
r2 - 0.004
P = 0.99
r2 - 0.434
P = 0.09
*
r2 - 0.354
P = 0.11
* #


B
Lymphocytes
r2 - 0.646
P < 0.01
r2 - 0.519
P < 0.05
r2 - 0.306
P = 0.17
r2 - 0.233
P = 0.31
*
* #
Cell Count (normalized)
225
200
175
150
125
100
75
0
50
100
150
200
250
300
Ammonia (µmol/L)

CAPITULO IX
O exercício como modelo para estudo do metabolismo de aminoácidos e amônia

CAPITULO IX

O exercício como modelo para estudo do metabolismo de aminoácidos e amônia

DISCUSSÃO GERAL

Diversos estudos sobre metabolismo do exercício tem sido realizados em sujeitos aparentemente saudáveis, sem análise prévia de sua resposta fisiológica (Angus, *et al.*, 2002; Kato, *et al.* 2004; Kimber, *et al.* 2003). A investigação metabólica inicial é fator indispensável para a seleção de sujeitos já que distúrbios metabólicos subclínicos podem levar a conclusões errôneas e contraditórias. Nos estudos citados na sessão anterior avaliamos clinicamente e metabolicamente todos os sujeitos antes da experimentação. Este fato nos permitiu verificar a coerência e similaridade metabólica entre eles, além de evitar que indivíduos considerados em hegemonia clínica, antropométrica e em outros parâmetros como idade, atividade e sexo fossem considerados similares metabolicamente (devido a estes achados excluímos portadores de: de hepatite C silenciosa, resistência insulínica, lesão muscular grave/*overtraining* e intoxixação por APAP).

Nossa triagem inicial geralmente consta com 85 análises bioquímicas além das antropométricas e clínicas. Este tipo de anamnese nos permite selecionar grupos com características antropométricas; *status* alimentar; treinamento; metabolismo de carboidrato, lipídios e proteínas; capacidade de transporte de oxigênio; sinalizadores do anabolismo e catabolismo de macronutrientes; sem infecções ou infestações; em equilíbrio hídrico preservados e com integridade hepática e renal. Possibilitando diagnósticos metabólicos que excluem sujeitos com disfunções clínicas ou subclínicas pudessem interferir nos resultados ou dificultar sua interpretação. A randomização e divisão dos sujeitos nos possibilita grupos antropométricamente homogêneos, saudáveis e estatisticamente equivalentes.

A análise hematológica dos setores vermelho e branco dos grupos foi realizada para detectar possíveis alterações metabólicas causadas por diferenças no transporte de gases, infecções e/ou infestações silenciosas e possibilidade de

desvios nutricionais. Os achados hematológicos foram normais dentro dos parâmetros estudados, não havendo diferença entre os grupos nos trabalhos supra-citados. Ademais, além de termos para cada trabalho um (ou mais) grupo(s) controle os dados dos eritrócitos funcionam como controle interno dos indivíduos possibilitando que tenhamos análise mais precisa dos resultados, descartando assim mudanças volêmicas (Bassini-Cameron, *et al.*; 2007; Bassini-Cameron, *et al.* 2008; Bessa, *et al.* 2007).

O uso de marcadores bioquímicos indicadores do metabolismo e de lesão muscular podem ser de extrema importância para a prescrição e acompanhamento do treinamento (Tsintzas, *et al.*, 2001; Schulz & Heck, 2003). A AST (596 kDa) pôde ser utilizada como controle interno para medirmos os valores basais de CK em jogadores de futebol da primeira divisão do Campeonato Brasileiro. Os dados não mostrados deste controle interno no artigo de Lazarim *et al.*, 2007 estão na sessão do anexo do capítulo II. Nota-se que os valores de AST estão dentro da faixa de normalidade, o que nos possibilita inferir que o aumeto plamático de CK seja proveniente da microlesão muscular.

As medidas clínicas utilizadas mundialmente para a dosagem de enzimas marcadores de lesão muscular e hepática, foram popularizadas há décadas atrás com o uso de kits dosadores de atividade enzimática. Embora de uso simples este modelo não mede a quantidade de enzima e sim sua atividade. Para isso, estabelecemos uma faixa de referência para os valores normais em repouso destes jogadores que varia de 132 – 1338 (95% de intervalo de confiança) com média de 493.5 ± 315 U/L. A Tabela 2 do anexo do capítulo II nos demonstra que a população estudade é homogênea. Numa comparação entre alguns destes jogadores da primeira divisão com um outro time da terceira do Brasileirão.vimos que os atletas de elite tem maior capacidade de transportar gases, menor lesão muscular e melhor status nutricional (Monteiro *et al.* 2005).

Um outro controle interno de cada indivíduo submetido ao experimento é o valor de $D_{INICIAL}$ (anamnese) em comparação ao $D_{TESTE\ PRE}$, onde podemos avaliar a intervenção da dieta e/ou do treinamento (vide Bassini-Cameron, *et al.* 2007; Bassini-Cameron, *et al.* 2008 Bessa, *et al.*,-submetido)

Depois de fazermos nossas análises de rotina pré-experiemto, os valores séricos de GGT e demais marcadores de hepatotoxicidade dos nos chamaram atenção

sendo verificado que este atleta estava fazendo uso de APAP e iniciando uma sindrome de *overtraining*. Foi prescrito NAC, Met e Cys durante 15 dias e na segunda avaliação o indivíduo apresentava valores similares ao da equipe (artigo 2 desta Tese).

Para estudar os efeitos do exercício como um estresse metabólico causador de hiperamoniemia, necessitamos primeiramente entender as consequencias das intensidades dos diferentes modelos, e para tal fizemos investigações durante o exercício contínuo, endurance, intermitente e alta intensidade tomando como base nossos resultados prévios descritos em Bassini-Cameron *et al.* 2007.

O stress induzido pelo exercício durante o jogo de futebol foi grande mas precisavamos de um protocolo mais potente na indução da hiperamoniemia transitória. Então usamos de exercício de longa duração e endurance (HIU), modelo que parecia ser mais consistente e reproduzivel.

Devido a grande dificuldade na coleta de dados durante a corrida, nós avaliamos um pequeno número de indivíduos. Isto somente foi possível devido as analises prévias dos parâmetros bioquímicos e hematológicos que mostraram similaridade entre os sujeitos. Durante os 800 km de prova foram medimos marcadores inflamatórios, de lesão muscular, metabolismo de nitrogênio e macronutrientes. Este foi o primeiro estudo a mensurar respostas bioquímicas e hematológicas durante o HIU com todos os fatores internos e externos que motivam o atleta *in situ*. Diferente das outras provas de endurance o atleta tem tempo para se recuperar parcialmente depois de cada sesão de exercício.

Neste estudo, a LDH (~140 kDa) teve um aumento hiperbólico tendendo a saturação em 100Km. como esperado, proteína com menor peso, por exemplo CK (~86 kDa) chegou aos 300% com n de Hill ~1.8. isto representa um comportamento cooperativode aparecimento no sangue, provavelmente porque mais CK esta ligada a linha M. Nossos resultados de marcadores de lesão estão de acordo com os de 200Km de ultramaratona (Kim, *et al.*, 2007). Fato interessante é que pudemos mostrar aumento de CK e LDH antes dos tradicionamente descritos na literatura (Chen & Hsieh, 2001; Milias, *et al.*, 2005).

Comparando os dados do nosso laboratório podemos observar que o aumento destas enzimas vem primeiramente da lesão muscular e depois do fígado (Bassini-Cameron, *et al.* 2007; Bessa, *et al.* 2008).

No intuito de verificar os possíveis mecanismos protetores a hiperamoniemia aguda, induzimos situações de stress metabólico através do exercício de moderada-alta intensidade e utilizamos intermediários metabólicos como sondas. Em nosso laboratório, Carvalho-Peixoto, *et al.* (2007) testaram CHO associados ou não a Gln e observaram proteção a subida da amonemia a partir dos 60min de exercício contínuo.

O exercício extenuante mimetiza estados de hiperamoniemia e situações de caquexia que induzem ao maior catabolismo de aminoácidos com liberação de amônia e espécies reativas de oxigênio (Bachini *et al.* 2006). A dieta cetogênica acarreta a oxidação incompleta das gorduras aumentando a cetonemia. O SNC utiliza os corpos cetônicos, provenientes da degradação dos ácidos graxos, diminuindo sua dependência por glicose. Dietas com baixos teores de carboidratos aumentam a concentração de amônia no plasma em função dos baixos estoques de glicogênio muscular e hepático (Walser, 1979; Jahn, *et al.*1992). Utilizamos um modelo combinado de exercício com dieta cetogênica para com êxito para amplificar estresse metabolico causador de elevação acentudada da amonemia.

O *status* nutricional influência diretamente na produção de amônia e a dieta cetogênica não exaceba esta concentração em humanos normais em repouso. Podemos observar estes dados comparando os valores de D_0 com D_{TESTE} , contudo não é visto na literatura trabalhos que utizem este modelo de dieta para indução da hiperamoniemia.

É interessante ressaltar que em nossos estudos não observamos diferença através da utilização da Gln, CHO, Ala, Arg e cafeína na concentração de lactato plamático nas intensidades estudadas. Estudos em co-cultura de neurônio-glia o lactato tem sido demonstrado como um importante substrato metabólico ao SNC e que a glia exporta lactato para a nutrição dos neurônios principalmente durante a acidose sanguínea (Korf, 1996; Cairns, 2006)

Felipo e colaboradores (2004) mensuraram que a hiperamoniemia aguda não afeta a distribuição e a quantidade de NMDA no neurônio ou sua ativaçãoção pelas proteínas kinases. Recentemente foi demonstrado que linfócitos atuam como neuroprotetores durante a excitotoxicidade induzida por Glu. Achados em nosso laboratório mostraram que a linfocitose durante o exercício está relacionada à subida

da amônemia e descrevemos um modelo para a gênese e estudo da hiperamônemia em humanos (Garg, *et al.* 2008; Kipnis, *et al.* 2008).

Pouco se sabe, sobre o mecanismo de ação ou mesmo sobre o papel tamponante da Arg na amonemia. Embora a Arg não seja o transportador principal de nitrogênio, desempenha um papel importante no seu metabolismo como um intermediário no ciclo de uréia. Campebel *et al.* (2006) mediu a conversão de Arg em α-Ketoglutarato durante o exercío e verificou que a Arg tem seu pico na corrente sanguínea em 60min após ingestão. Estudos anteriores mostraram um efeito protetor da Arg no aumento das concentrações de amônia durante o exercício e que o indivíduos treinados tem menor produção que não treinados (Denis, 1991) fato também verificado em nosso estudo.

Liu *et al.* (2008) avaliou judocas treinados em lutas de 6min. com 6g/dia durante 3 dias de suplementação com Arg e não observou diferença em relação ao grupo controle para as concentrações séricas de lactato, amônia e NO. Vale ressaltar que os autores não observaram diferença nas concentrações séricas de Arg e citrulina (Cit) além de referirem trabalhar com um modelo de exercício intermitente. Em nosso estudo, para que pudessemos manter a intensidade alta da luta foi utilizado a cada 3 min. um oponente diferente e que estava em repouso, também não era permitido finalização. Em nosso modelo a suplementação é feita associada a dieta cetogênica. Comparando a curva de amônia dos dois trabalhos observa-se que a Arg protege da hiperamoniemia no primeiro e no décimo minuto pós luta. Liu e colaboradores também mediram a amônia pré e seu clearance pós luta e não observaram diferença.

A cafeína tem sido utilizado em nosso laboratório como modulador do metabolismo. Demonstramos que seu uso aumenta a chance de microlesões e que está correlacionada com a leucocitose (Bassini-Cameron *et al.* 2007). Estudos anteriores mostraram efeitos da cafeína em enzimas do ciclo da uréia (Colombatto, *et al.* 1989; Nikolic, *et al.*, 2003; Ofluoglu, *et al.* 2008). Demonstramos aqui as modificações causadas pela xantina no pool de AAs plasmáticos e de sua disponibilidade durante o exercício. Nossos achados demonstraram que a cafeína modifica o *pool* de AAs plasmáticos aumentando as concentrações de Arg, Cit e Orn. O aumento da amonemia nos atletas suplementados permitiu postular que a ação

inibitória da cafeína nas enzimas do ciclo da uréia modifica a disponibilidade do pool de AAs sanguíneos (artigo 6 desta Tese).

CONCLUSÃO GERAL

O exercício de moderada a alta intensidade são bons modelos de estudo para indução da hiperamoniemia, mas o protocolo de luta pareceu ser mais eficaz;

A suplementação de Glu, Ala, Arg, cafeína e a dieta cetogênica parecem proteger o SNC por induzirem o aumento da neoglicogênese quando combinados ao exercício;

A regeneração do hepatócito foi acelerada pela suplementação com NAC é Met;

REFERENCIAS BIBLIOGRÁFICAS

- Angus, D.J.; Febbraio, M.A.; Hargreaves, M. Plasma glucose kinetics during prolonged exercise in trained humans when fed carbohydrate. Am J Physiol Endocrinol Metab. 2002; 283:E573-7.
- Bassini-Cameron, A.; Monteiro, A.; Gomes, A.; Werneck-de-Castro, J.P.; Cameron, L.C. Glutamine protects against increases in blood ammonia in football players in an exercise intensity-dependent way. Br J Sports Med. 2008; 42:260-6.
- Bassini-Cameron, A.; Sweet, E.; Bottino, A.; Bittar, C.; Veiga, C.; Cameron, L.C. Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions.Br J Sports Med. 2007; 41:523-30.
- Bessa, A.; Nissenbaum, M.; Monteiro, A.; Gandra, P.G.; Nunes, L.S.; Bassini-Cameron, A.; Werneck-de-Castro JP, de Macedo, D.V.; Cameron, L.C. High-intensity ultraendurance promotes early release of muscle injury markers. Br J Sports Med. 2008; 42:589-93.
- Cairns, S.P. Lactic acid and exercise performance: culprit or friend? Sports Med. 2006; 36:279-91.
- Carvalho-Peixoto, J.; Alves, R.; Cameron, L.C. Glutamine and carbohydrate supplements reduce ammonemia increase during endurance field exercise. Appl Physiol Nutr Metab. 2007; 32:1186-90.
- Chen, T.C.; Hsieh, S.S. Effects of a 7-day eccentric training period on muscle damage and inflammation. Med Sci Sports Exerc. 2001; 33:1732-8.
- Milias, G.A.; Nomikos, T.; Fragopoulou, E. Athanasopoulos S, Antonopoulou S. Effects of eccentric exercise-induced muscle injury on blood levels of platelet activating factor (PAF) and other inflammatory markers. Eur J Appl Physiol. 2005; 95:504-13.
- Bachini, F.; Bassini-Cameron, A.; Cameron, L.C. Nutrition, Metabolism and Exercise in Chronic Obstructive Pulmonary Disease. Braz J Invest Pathol Morp Morph. 2006; 1: 7-14.
- Denis, C.; Dormois, D; Linossier, M.T.; Eychenne, J.L.; Hauseux, P.; Lacour, J.R. Effect of arginine aspartate on the exercise-induced hyperammoniemia in

humans: a two periods cross-over trial. Arch Int Physiol Biochim Biophys. 1991; 99:123-7.

- Colombatto, S.; Fasulo, L.; Mondardini, A.; Malabaila, A.; Grillo, M.A. Effect of caffeine on ornithine metabolism in rat brain, liver and kidney. J Biochem. 1989; 38:75-82.
- Garg, S.K.; Banerjee, R.; Kipnis, J. Neuroprotective immunity: T cell-derived glutamate endows astrocytes with a neuroprotective phenotype. J Immunol. 2008; 180:3866-73.
- Kato, T.; Matsumura, Y.; Tsukanaka, A.; Harada, T.; Kosaka, M.; Matsui, N. Effect of low oxygen inhalation on changes in blood pH, lactate, and ammonia due to exercise. Eur J Appl Physiol. 2004; 91:296-302.
- Kimber, N.E.; Heigenhauser, G.J.F.; Spriet, L.L.; Dyck, J.D. Skeletal muscle fat and carbohydrate metabolism during recovery from glycogen-depleting exercise in humans. J Physiol. 2003; 548:919-27.
- Kipnis, J.; Derecki, N.C.; Yang, C.; Scrable, H. Immunity and cognition: what do age-related dementia, HIV-dementia and 'chemo-brain' have in common? Trends Immunol. 2008; 29:455-63.
- Korf, J. Intracerebral trafficking of lactate in vivo during stress, exercise, electroconvulsive shock and ischemia as studied with microdialysis. Dev Neurosci. 1996; 18:405-14.
- Lazarim, F.L.; Antunes-Neto, J.M.; da Silva, F.O.; Nunes, L.A.; Bassini-Cameron, A.; Cameron, L.C.; Alves, A.A.; Brenzikofer, R.; de Macedo, D.V. The upper values of plasma creatine kinase of professional soccer players during the Brazilian National Championship. J Sci Med Sport. 2009; 12:85-90.
- Campbell, B.; Roberts, M.; Kerksick, C.; Wilborn, C.; Marcello, B.; Taylor, L.; Nassar, E.; Leutholtz, B.; Bowden, R.; Rasmussen, C.; Greenwood, M.; Kreider, R. Pharmacokinetics, safety, and effects on exercise performance of L-rginine alpha-ketoglutarate in trained adult men. Nutrition. 2006; 22:872-81.
- Liu, T.H.; Wu, C.L.; Chiang, C.W.; Lo, Y.W.; Tseng, H.F.; Chang, C.K. No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. J Nutr Biochem. 2008.

- Monteiro, A.; Bassini, A.; Cameron, L.C. Perfil hematológico das reservas de macronutrients em jogadores de futebol em fase de pré-temporada. F & P J. 2006; 5:129-33.
- Nikolic, J.; Bjelakovic, G.; Stojanovic, I. Effect of caffeine on metabolism of L-arginine in the brain. Mol Cell Biochem. 2003; 244:125-8.
- Ofluoglu, E.; Pasaoglu, H.; Pasaoglu, A. The Effects of Caffeine on L: -Arginine Metabolism in the Brain of Rats. Neurochem Res. 2009; 34:395-9.
- Schulz, H. & Heck, H. Glycogen depletion as indication for ammonia determination in exercise testing. Eur J Sport Sci. 2003; 3:1-9.
- Tsintzas, K.; Williams, C.; Constantin-Teodosiu, D.; Hultman, E.; Boobis, L.; Clarys, P.; Greenhaff, P. Phosphocreatine degradation in type I and type II muscle fibres during submaximal exercise in man: effect of carbohydrate ingestion. J Physiol. 2001; 537:305-11.
- Walser, M.; Mitch, W.E.; Collier, V.U. The effect of nutritional therapy on the course of chronic renal failure. Clin Nephrol. 1979; 11:66-70.

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo