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Álisson de Carvalho Gonçalves

Efeito da suplementação com benfotiamina sobre parâmetros metabólicos e desempenho de *endurance* de camundongos submetidos a treinamento de natação.

UBERABA
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Tese apresentada ao Programa de Pós-graduação em Ciências da Saúde, área de concentração Patologia Experimental, da Universidade Federal do Triângulo Mineiro, como requisito parcial para obtenção do título de Doutor em Ciências da Saúde.

Orientador: Prof. Dr. Guilherme Vannucchi Portari

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Dedico este resultado aos meus pais e irmão, por todo incentivo, cuidado e amor a mim dedicados.

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“Se eu vi mais longe, foi por estar sobre ombros de gigantes.”

Isaac Newton

RESUMO

Introdução: Exercícios físicos aumentam a demanda energética, fazendo-se necessário o adequado consumo de nutrientes a fim de aperfeiçoar a produção de energia, possibilitar a melhor recuperação e otimizar os processos adaptativos. O difosfato de tiamina (TDP) é cofator essencial para o funcionamento das enzimas complexo piruvato-desidrogenase (PDH), α-cetoglutarato desidrogenase (OGDH) e transcetolase (TKT), fundamentais no metabolismo energético. A benfotiamina é um análogo sintético da tiamina, capaz de promover uma biodisponibilidade de TDP maior em comparação a outros sais de tiamina.

Objetivo: O objetivo deste estudo foi avaliar os efeitos da suplementação com benfotiamina sobre os parâmetros metabólicos e de desempenho em camundongos submetidos o treinamento físico de *endurance*.

Métodos: A pesquisa utilizou 25 camundongos BALB/c, machos com seis semanas de idade. Os animais foram separados em 4 grupos: Dieta padrão e sedentarismo (Pad-Sed); Dieta padrão com treinamento em natação (Pad-Tr); Dieta suplementada com benfotiamina e sedentário (Ben-Sed); Dieta suplementada com benfotiamina e treinamento em natação (Ben-Tr). A benfotiamina foi adicionada à ração AIN-93 (500mg/kg). Os animais foram submetidos a treinamento de natação durante 6 semanas. O teste de exaustão foi a última sessão de natação com sobrecarga de 5% da massa corporal. A concentração plasmática de lactato foi dosada antes e imediatamente após o teste de exaustão. As concentrações de substâncias reativas ao ácido tiobarbitúrico (TBARS), proteínas carboniladas, tióis totais e tióis não-proteicos foram analisadas no fígado, coração e músculo tibial anterior. As concentrações de tiamina nos eritrócitos e no músculo gastrocnêmio foram avaliadas por cromatografia líquida de alta eficiência. A expressão do RNAm dos genes PDHa1 e OGDH no músculo gastrocnêmio foi avaliada por Transcrição reversa-reação em cadeia da polimerase em tempo real (qRT-PCR). A concentração dos ácidos pirúvico, lático e hidroxibutírico foram quantificados no músculo por cromatografia gasosa acoplada ao espectrômetro de massas (GC-MS).

Resultados: Os animais suplementados apresentaram níveis mais elevados de tiamina livre, monofosfato de tiamina e difosfato de tiamina nos eritrócitos e nos músculos. No músculo tibial, a peroxidação lipídica foi maior no grupo Pad-Sed foi maior, enquanto no coração, a peroxidação lipídica nos grupos Pad-Sed e Ben-Tr foi maior do que no grupo Ben-Sed. A concentração de proteínas carboniladas no músculo foi maior no grupo Pad-Sed do que em

ambos os grupos suplementados. No fígado, a carbonilação de proteínas foi menor no grupo Ben-Sed do que no Pad-Sed. O nível de tióis totais foi menor no grupo Ben-Sed do que no Pad-Tr. No coração, o nível de tióis totais foi mais alto no grupo Ben-Sed do que no Ben-Tr. A concentração de tióis não-proteicos no músculo foi maior no grupo Ben-Sed do que no Ben-Tr, enquanto no coração, a concentração de tióis não-proteicos do grupo Pad-Tr foi menor do que no grupo Pad-Sed. Não houve diferença na expressão gênica entre todos os grupos. O músculo dos animais treinados suplementados apresentou maiores concentrações de ácido lático e de ácido hidroxibutírico que animais sedentários. A razão ácido lático:ácido pirúvico foi maior nos animais treinados. Não houve diferença na capacidade de *endurance* entre os grupos Pad-Tr e Ben-Tr. Da mesma forma, a concentração final de lactato também não foi diferente entre os grupos.

Conclusão: A suplementação oral com benfotiamina aumenta a concentração de tiamina e seus éteres nos eritrócitos e no músculo gastrocnêmio. A benfotiamina se mostrou um antioxidante eficiente contra o estresse oxidativo no músculo tibial anterior e no coração de animais submetidos ao treinamento de *endurance*. Entretanto, não é capaz de afetar a expressão dos genes de enzimas dependente de tiamina no músculo gastrocnêmio. A suplementação aumentou o catabolismo do piruvato no músculo dos animais treinados, mas não apresentou efeito antifadiga.

Palavras-chave: Exercícios físicos; Tiamina; Estresse Oxidativo; Suplementos Nutricionais; Resistência física.

ABSTRACT

Introduction: Physical exercises increase energy demand, making it necessary an adequate nutrients intake to improve energy production, aiming to promote better recovery and optimize adaptive processes. Thiamine diphosphate (TDP) is an essential cofactor for the functioning of the enzymes pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (OGDH), and transketolase (TKT), which are fundamentals in energy metabolism. Benfotiamine is a synthetic analogue of thiamine capable of promoting a greater bioavailability of TDP compared to other thiamine salts.

Objective: The objective of this study was to evaluate the effects of supplementation with benfotiamine on metabolic and performance parameters in mice, physical training of endurance.

Methods: Twenty-five male BALB/c mice were allocated to the following treatment groups: standard diet and sedentary activity (Sta-Sed), benfotiamine–supplemented diet and sedentary activity (Ben-Sed), standard diet and training activity (Sta-Tr) and benfotiamine–supplemented diet and training activity (Ben-Tr). Benfotiamine was added to the AIN-93 diet (500mg/Kg). The training comprised 6 weeks of endurance swimming training. The exhaustion test was the last swimming session with an overload of 5% of the body weight attached to the tail. The plasma lactate concentration was measured before and immediately after the exhaustion test. The concentration of thiobarbituric acid reactive substances (TBARS), carbonylated proteins, total thiols and non-protein thiols was analyzed in the liver, heart and tibialis anterior muscle. The thiamine concentration in erythrocytes and in gastrocnemius muscle was assessed using high performance liquid chromatography. The expression of the genes PDHa1 and OGDH in gastrocnemius muscle was evaluated by reverse transcriptase-Reaction in the polymerase chain in real time (qRT-PCR). Pyruvic, lactic and hydroxybutyric acids were quantified in muscle by gas chromatography coupled to the mass spectrometer (GC-MS).

Results: Supplemented animals showed higher levels of thiamine, thiamine monophosphate and thiamine diphosphate in the erythrocytes and in the muscle. In the tibialis muscle, lipid peroxidation was higher in the Sta-Sed group was higher while in the heart, lipid peroxidation in the Sta-Sed and Ben-Tr groups was higher than in the Ben-Sed group. The carbonyl content in the muscle was higher in the Sta-Sed group than in both supplemented groups. In liver, the carbonyl content was lower in the Ben-Sed group than in the Sta-Sed. The level of total thiols was lower in the Ben-Sed group than in the Sta-Tr. In the heart, the level of total thiols was

higher in the Ben-Sed group than in the Ben-Tr. The concentration of non-protein thiols in the muscle was higher in the Ben-Sed group than in the Ben-Tr, whereas in the heart, concentration of non-protein thiols of Sta-Tr group was lower than in the Sta-Sed. There was no difference in gene expression between all groups. The muscle of the Ben-Tr animals showed higher concentrations of lactic acid and hydroxybutyric acid than sedentary animals. The lactic acid: pyruvic acid ratio was higher in the trained animals. There was no difference in endurance capacity between the Pad-Tr and Ben-Tr groups. Similarly, the final lactate concentration was also no different between groups.

Conclusion: Oral supplementation with benfotiamine increases the concentration of thiamine and its ethers in the erythrocytes and in the gastrocnemius muscle. It is an efficient antioxidant against oxidative stress in the anterior tibial muscle and in the heart of animals submitted to endurance training. However, it is not able to affect the expression of thiamine-dependent enzyme genes in gastrocnemius muscle. The supplementation increased the pyruvate catabolism in muscle of trained mice, but did not affect endurance performance.

Keywords: Physical exercise; Thiamine; Oxidative stress; Nutritional supplementation; Endurance exercise.

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LISTA DE ABREVIATURAS

ADP: Adenosina difosfato

ATP: Adenosina trifosfato

Ben-Sed: Grupo suplementado com benfotiamina e sedentário

Ben-Tr: Grupo suplementado com benfotiamina e treinado

CDT: Cloridrato de dicetiamina

CoA: Coenzima A

DNA: Ácido desoxirribonucleico

DNAc: Ácido desoxirribonucleico complementar

DTTF: Dissulfureto de tiamina de tetrahidrofurfuril

FAD: Flavina adenina dinucleotídeo

KGDH: α-cetoglutarato desidrogenase

MC: Massa corporal

NAD: Nicotinamida adenina dinucleotídeo

Pad-Sed: Grupo dieta padrão e sedentário

Pad-Tr: Grupo dieta padrão e treinado

PDH: Piruvato desidrogenase

Pi: Fosfato inorgânico (Pi)

qRT-PCR: Transcrição Reversa-Reação em Cadeia da Polimerase em tempo real

RNA: Ácido ribonucleico

RNAm: Ácido ribonucleico mensageiro

TKT: Transcetolase

TMP: Monofosfato de tiamina

TPK-1: Tiamina pirofosfoquinase-1

TDP: Difosfato de tiamina

TTP: Trifosfato de tiamina

$VO_{2máx}$: Consumo máximo de oxigênio

VPF: Via das pentoses fosfato

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1. INTRODUÇÃO

A prática de exercícios físicos determina um aumento da demanda energética, fazendo-se necessário o adequado consumo de nutrientes a fim de aperfeiçoar a produção de energia e possibilitar a melhor recuperação, consequentemente, melhorando o desempenho físico de indivíduos engajados em programas de treinamento e competições. Desta maneira, uma dieta adequada está diretamente relacionada ao desempenho atlético (RODRIGUEZ *et al.*, 2009). A modalidade esportiva executada, a intensidade, o volume e a frequência do treinamento, a recuperação, a massa e composição corporal e o nível de aptidão física modificam as necessidades energéticas de cada indivíduo, alterando as recomendações nutricionais (ROMIJN *et al.*, 2000).

Para Williams e Devlin (1994), o desempenho em modalidades esportivas de alta intensidade e longa duração é geralmente limitado pela disponibilidade de carboidratos. Em contrapartida, alguns estudos apontam que a redução do desempenho durante o exercício pode não estar necessariamente relacionada à oferta de macronutrientes, mas também à disponibilidade de micronutrientes reguladores do metabolismo energético, dentre outros fatores (HORWITT, KREISLER, 1949; LUKASKI, 2004; VAZ, *et al.*, 2011).

A atividade física é um fenômeno que aumenta o consumo de energia do corpo acima do gasto energético de repouso. A quantidade de energia necessária para manter ou elevar o esforço durante o exercício físico é uma resposta à intensidade, duração e ao tipo de exercício, além da aptidão física individual (McARDLE; KATCH; KATCH, 2010). Desta maneira, garantir a integridade do funcionamento das vias bioenergéticas é fundamental para a manutenção ou o aumento do esforço físico durante a atividade esportiva.

Dentre os objetivos do treinamento físico, destaca-se o ajuste das vias metabólicas. Aperfeiçoar a capacidade de produzir energia pode representar uma significativa melhora no desempenho físico (LUNDBY, JACOBS, 2016). De fato, vários estudos demonstraram o aperfeiçoamento da função mitocondrial nos músculos esquelético de indivíduos submetidos a treinamento com exercícios físicos (TERBLANCHE *et al.,* 2001; JACOBS *et al.*, 2013; SCALZO *et al.,* 2014;). Estes

achados subsidiam a crença na necessidade da aplicação/utilização de recursos ergogênicos capazes de aumentar a produção e oferta de energia, na forma de adenosina trifosfato (ATP), sejam melhorando o trabalho das vias bioenergéticas ou reduzindo o gasto energético.

1.1. Metabolismo Energético

O suprimento da demanda energética imposta pela atividade física e manutenção da vida animal depende exclusivamente do catabolismo de macronutrientes. Séries de reações a partir do catabolismo dos substratos energéticos, i. e. glicose, ácidos graxos e proteínas, fornecem energia para a formação de ATP, a principal molécula capaz de fornecer energia química para estruturas e componentes do organismo vivo (NELSON, COX, 2018)**.**

O ATP pode ser gerado tanto por vias aeróbias quanto anaeróbias. As vias anaeróbias são aquelas que não envolvem a redução de oxigênio. O catabolismo da glicose pode fornecer energia para a formação de ATP tanto por via anaeróbia quanto aeróbias. Os demais substratos, i. e. ácidos graxos e aminoácidos, necessitam de moléculas de oxigênio como aceptoras finais de elétrons para formação do ATP necessário para manutenção das funções do organismo (WELLS, SELVADURAI, TEIN, 2009; NELSON, COX, 2018).

Os passos inicias do catabolismo dos substratos são, obviamente, distintos, uma vez que são moléculas diferentes em suas composições e estruturas químicas. Contudo, existem similaridades na cascata de reações necessária para produção de ATP por vias aeróbias. De fato, no metabolismo bioenergético aeróbio, todas as reações convergem para a formação da molécula de Acetil-CoA, a qual entra no ciclo de Krebs tem sua energia retida pelas moléculas NADH e $FADH_2$ (WELLS, SELVADURAI, TEIN, 2009; NELSON, COX, 2018). Na etapa final do metabolismo energético, conhecida como cadeia respiratória, estas moléculas entregam seus elétrons e prótons H^+ às moléculas de O_2, liberando significativa quantidade de energia, conservada como ATP (ACÍN-PEREZ *et al.*, 2008).

1.1.1. Glicólise

No organismo humano, as moléculas de glicose são armazenadas em forma de polímeros de hexose, o glicogênio. Em momentos de aumento na demanda energética, o glicogênio é degradado (glicogenólise) liberando as moléculas de glicose para sofrerem o catabolismo (glicólise). A glicólise trata-se da degradação da molécula de glicose em uma cascata de reações químicas no citoplasma catalisadas por diversas enzimas, gerando duas moléculas de piruvato em seu passo final (MASTER, REID, DOM, 1987; NELSON, COX, 2018).

A molécula de glicose é composta por seis átomos de carbono que sofrem uma sequência de reações em dez etapas até a formação de duas moléculas de três carbonos, o piruvato. Na primeira etapa, as enzimas hexoquinase ou glicoquinase catalisam a fosforilação do grupo hidroxil da glicose, formando a D-glicose-6-fosfato. Esta molécula é convertida a D-frutose-6-fosfato pela ação da enzima fosfo-hexose-isomerase (etapa 2). Na etapa seguinte, a frutose-6-fosfato é novamente fosforilada, com o auxílio da fosfofrutoquiinase, formando a D-frutose-1,6-bifosfato. Uma vez que o doador dos fosfatos são moléculas de ATP, até esta etapa, a glicólise consumirá 2 moléculas de ATP (COPELAND, TURNER, 1987; WELLS, SELVADURAI, TEIN, 2009; NELSON, COX, 2018).

Na quarta etapa, a aldolase catalisa a quebra da molécula única de D-frutose-1,6-bifosfato em uma molécula de gliceraldeído-3-fosfato e outra de di-hidroxiacetona-3-fosfato, sendo que esta última é transformada em gliceraldeído-3-fosfato na etapa 5, por ação da enzima tiosefosfato-isomerase (COPELAND, TURNER, 1987; WELLS, SELVADURAI, TEIN, 2009; NELSON, COX, 2018).

A partir da ação da enzima gliceraldeído-3-fosfato-desidrogenase, os gliceraldeído-3-fosfato são oxidados e fosforilados por fosfatos inorgânicos, formando duas moléculas de 1,3-bifosfoglicerato, formando $2NADH+2H^+$ (etapa 6). NA etapa seguinte, a formação de duas moléculas de 3-fosfoglicerato, a partir dos 1,3-bifosglicerato, produz duas moléculas de ATP, em uma reação catalisada pela fosfoglicerato-cinase. As moléculas de 3-fosfoglicerato são convertidas a 2-fosfoglicerato e em seguida a fosfoenolpiruvato, a partir da ação das enzimas fosfoglicerato-mutase e

enolase respectivamente (etapas 8 e 9). Na décima e última etapa da glicólise, a enzima piruvato-cinase catalisa a conversão de duas moléculas de fosfoenolpiruvato para duas moléculas de piruvato, com a formação de dois ATP's (COPELAND, TURNER, 1987; WELLS, SELVADURAI, TEIN, 2009; NELSON, COX, 2018).

Nota-se que, apesar de consumir duas moléculas de ATP, a glicólise produz quatro, além de $2NADH+2H^{+}$, apresentando um pequeno saldo energético. Contudo, a contribuição na produção de ATP pela glicose não é interrompida com a formação de piruvato. De fato, as moléculas de piruvato possuem um potencial energético, que pode ser extraído nos demais estágios da via bioenergética, i. e. ciclo de Krebs e fosforilação oxidativa (WELLS, SELVADURAI, TEIN, 2009).

Nos mamíferos, o piruvato formado na etapa final da glicólise pode ser dirigido a dois possíveis destinos. Em condições anaeróbias, o piruvato prioritariamente é convertido a lactato pela ação da enzima lactato desidrogenase. Já em condições de oferta normal de oxigênio (aeróbia), o piruvato tende a entrar na mitocôndria e a enzima piruvato desidrogenase promove sua conversão a acetil-CoA (NELSON, COX, 2018). O acetil-CoA é a molécula com potencial energético advindo dos macronutrientes, a qual prosseguirá para o ciclo de Krebs, sendo ela fundamental para o início deste ciclo (PIETROCOLA, *et al.*, 2015).

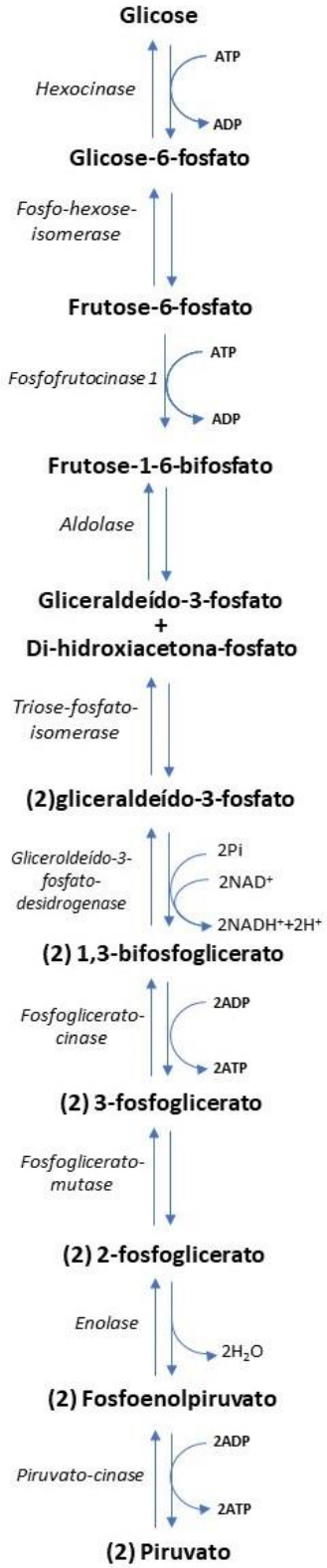

Figura 1: Representação esquemática das reações da glicólise. Adaptado de Nelson e Cox, 2018.

1.1.2. Oxidação dos ácidos graxos

Os ácidos graxos possuem uma quantidade de energia potencial maior que a glicose, entretanto, somente sua oxidação completa, ou seja, por vias aeróbias, pode formar ATP (NELSON, COX, 2018). Para isso, sucessivos ciclos de beta-oxidação formam moléculas de acetil-CoA, as quais suprirão o ciclo de Krebs, e a fosforilação oxidativa. A cada ciclo de beta-oxidação, a molécula de ácido graxo perde dois carbonos na forma de acetil-CoA. Sendo assim, a beta-oxidação de um ácido graxo de dezesseis carbonos formará oito moléculas de acetil-CoA, uma quantidade muito superior às duas moléculas formadas em uma via de glicólise. Diferentemente da glicólise, todo o processo de oxidação de ácidos graxos acontece no interior das mitocôndrias (SCHULS, 1991, KOMPARE, RIZZO, 2008).

No primeiro passo, a enzima acil-CoA-desidrogenase transfere dois hidrogênios do grupo acil-CoA do ácido graxo, para uma molécula de FAD^+, formando $FADH_2$ e trans-Δ^2 -enoil-CoA. Adicionando uma molécula de H_2O a este último, a enzima enoil-CoA-hidratase forma L-β-hidroxiacil-CoA, o qual transfere um H para uma molécula de NAD^+, formando $NADH+H^+$ e β-cetoacil-CoA, pela ação da enzima β-hidroxiacil-CoA-desidrogenase. Por último, a enzima tiolase catalisa a formação de acetil-CoA e outra cadeia carbônica com grupamento acil-CoA, a partir da reação do β-cetoacil-CoA com um CoA livre. O ciclo é repetido a partir da nova cadeia carbônica com o acil-CoA graxo, até que todos os carbonos sejam utilizados na formação de acetil-CoA (SCHULS, 1991; KOMPARE, RIZZO, 2008; NELSON, COX, 2018).

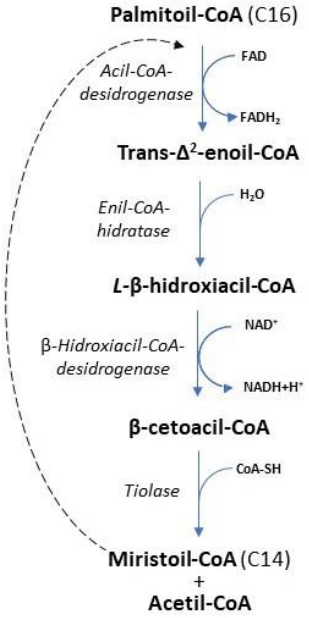

Figura 2: Representação esquemática das reações da beta-oxidação. Adaptado de Nelson e Cox, 2018.

1.1.3. Ciclo de Krebs

O ciclo de Krebs, ou ciclo do ácido cítrico, é a cadeia de reações que oxida as moléculas de acetil-CoA produzidas na glicólise, beta-oxidação e oxidação de alguns aminoácidos. De fato, o ciclo de Krebs é responsável por extrair o potencial energético do acetil-CoA, fornecendo elétrons para a cadeia fosforilativa. Sendo assim, a molécula precursora deste ciclo de oito etapas é o acetil-CoA. Entretanto, a presença do oxaloacetato é fundamental para que o ciclo se inicie (AKRAM, 2014; NELSON, COX, 2018).

Na primeira etapa do ciclo de Krebs, o oxaloacetato recebe o grupo acetil do acetil-CoA, numa reação catalisada pela enzima citrato-sintase. Visto que o oxaloacetato possui quatro carbonos, esta reação formará um composto de seis carbonos, o citrato. Este será transformado a isocitrato no passo subsequente, pela ação da enzima aconitase. A primeira perda de CO_2 e produção e NADH ocorre na conversão de isocitrato a alfa-cetoglutarato, em uma descarboxilação oxidativa catalisada pela isocitrato-desidrogenase (AKRAM, 2014; NELSON, COX, 2018).

No quarto passo, a enzima alfa-cetoglutarato-desidrogenase promove a conversão do alfa-cetoglutarato a succinil-CoA. Esta reação também extrai um CO_2 e um NADH. No passo seguinte, succinil-CoA é convertido a succinato, produzindo energia suficiente para a produção de uma molécula de guanosina trifosfato (GTP) a partir de uma molécula de guanosina difosfato (GDP). Na etapa seis, o succinato sofre uma desidrogenação, produzindo uma molécula de $FADH_2$ e fumarato. A enzima fumarase promove a conversão do fumarato a malato. Este é convertido a oxoloacetato pela ação da enzima malato-desidrogenase. Neste último passo, acontece a produção de mais uma molécula de NADH (AKRAM, 2014; NELSON, COX, 2018).

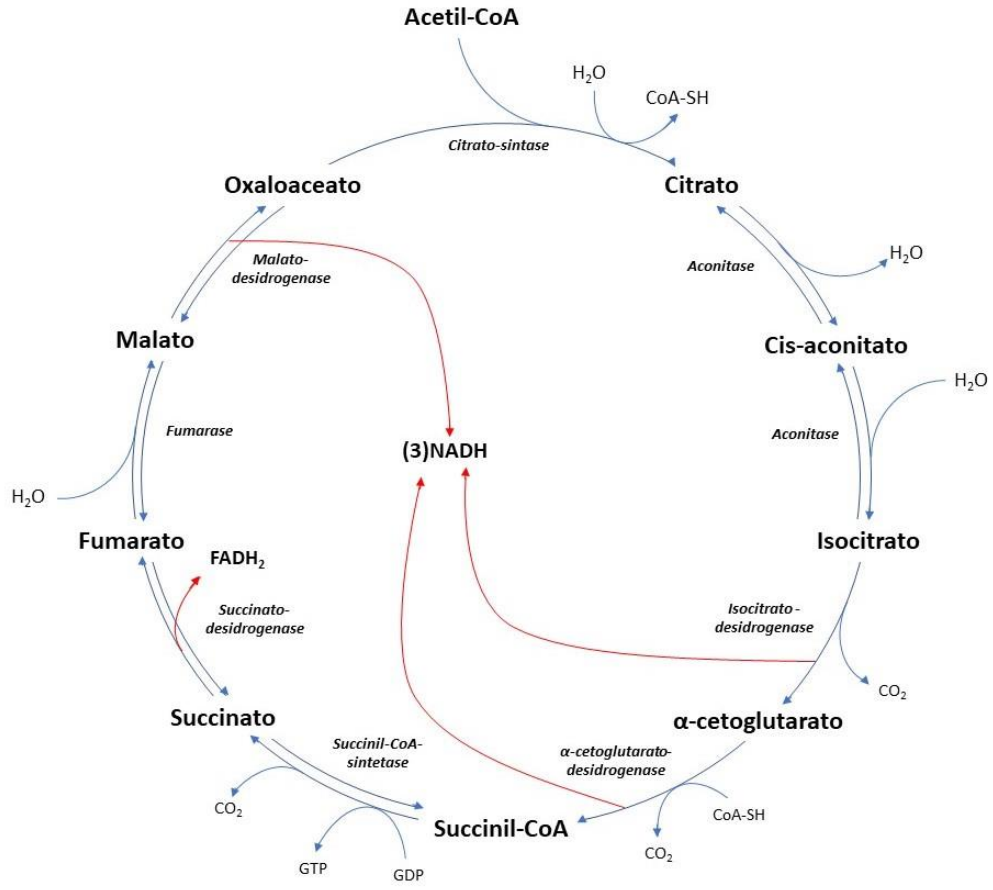

Figura 3: Representação esquemática das reações do Ciclo de Krebs. Adaptado de Nelson e Cox, 2018.

Apesar de não ser um grande produtor direto de ATP, as moléculas de NADH e $FADH_2$ produzidas durante o ciclo de Krebs fornecem significativa quantidade de

elétrons à fosforilação oxidativa. Assim, mesmo que cada acetil-CoA oxidado no ciclo de Krebs produza apenas um GTP neste ciclo, ele é um importante precursor de energia armazenada na forma de ATP (FERNIE, CARRARI, SWEETLOVE, 2004).

1.1.4. Fosforilação oxidativa

Todo catabolismo de macronutrientes converge para a cadeia fosforilação oxidativa, fazendo deste o estágio final do metabolismo bioenergético. Os elétrons produzidos nas reações de desidrogenases durante o catabolismo de glicoses, ácidos graxos e aminoácidos suprem a cadeia respiratória na membrana mitocondrial interna, que produzirá uma quantidade muito superior de ATP quando comparada aos passos que a antecedem no metabolismo energético (NELSON, COX, 2018).

A cadeia respiratória é uma série de carreadores aceptores/doadores de elétrons. Os carreadores de elétrons são complexos moleculares que se encontram dentro da membrana interna da mitocôndria. Na sequência de transferência de elétrons, existem os complexos I, II e II, o citocromo c e o complexo IV, através dos quais os elétrons são transferidos sequencialmente (FERNIE, CARRARI, SWEETLOVE, 2004; NELSON, COX, 2018).

O complexo I, composto por várias cadeias polipeptídicas grandes, estende uma de suas extremidades até a matriz mitocondrial. Ele é responsável por transferir um íon hidreto do NADH para a ubiquinona. Ainda, o complexo I transfere quatro prótons da matriz mitocondrial para o espaço intermembranas. Assim, a matriz se torna negativamente carregada pela saída de prótons, enquanto o espaço intermembranas é carregado positivamente. O complexo II, conhecido também como succinato-desidrogenase, é menor que o complexo I. Sua ação contribui para a formação de ubiquinona reduzida (FERNIE, CARRARI, SWEETLOVE, 2004; NELSON, COX, 2018).

Os elétrons do ubiquinol (QH_2), produzido pela redução da ubiquinona nos complexos I e II, são transferidos para o citocromo c pela ação do complexo III. Após ter recebido um elétron do complexo III, o citocromo c se desloca até o complexo IV para

entregar este elétron. Nesta etapa final, o complexo IV reduz as moléculas de oxigênio a H_2O, utilizando H^+ da matriz mitocondrial. Para cada elétron que passa por este complexo, um próton é bombeado para o espaço intermembranas (FERNIE, CARRARI, SWEETLOVE, 2004; NELSON, COX, 2018).

Tendo em vista que o fluxo de elétrons pelos carreadores é acompanhado pela transferência de prótons para o espaço intermembranas, ao final de um ciclo na cadeia respiratória (transferência de dois elétrons para o O_2) terão sido transferidos dez prótons para o espaço intermembranas, sendo quatro pelo complexo I, quatro pelo complexo III e dois pelo complexo IV. Assim, um gradiente eletroquímico é gerado a favor do espaço intermembranas em relação à matriz mitocondrial, em consequência da maior concentração de H^+. Estes prótons fluem a favor do seu gradiente eletroquímico, através do poro de prótons da ATP-sintase, disponibilizando a energia que será armazenada na forma de ATP, produzido pela ligação de adenosina difosfato (ADP) e fosfato inorgânico (Pi) (FERNIE, CARRARI, SWEETLOVE, 2004; NELSON, COX, 2018).

É importante destacar que uma pequena parcela do oxigênio consumido não é totalmente reduzida na cadeia fosforilativa. Cerca de 1-2% das moléculas de oxigênio são reduzidas parcialmente e formam moléculas de ânion superóxido (O_2^-) nos complexos I e III da cadeia transportadora de elétrons. O O_2^- é uma molécula extremamente instável e reativa, precursora de outras espécies reativas, como peróxido de hidrogênio e, subsequentemente, o radical hidroxila. Sendo assim, a presença e atividade eficiente de enzimas antioxidantes são fundamentais para manter o balanço redox nas mitocôndrias (TURRENS, 1997).

1.2. Exercício e metabolismo energético

O movimento humano intencional se dá a partir da aplicação coordenada de forças em um complexo sistema de alavancas. O ciclo contração-relaxamento dos músculos pode promover a redução ou expansão do ângulo das articulações, movendo o corpo e/ou seus segmentos no espaço (McARDLE; KATCH; KATCH, 2010). Assim, a premissa do movimento humano é a contração muscular, o que demanda por energia química a ser transformada em energia mecânica.

Toda contração muscular se inicia ou perpassa pelo sistema nervoso central. O potencial de ação gerado pelo sistema nervoso percorre o nervo motor até suas terminações nas fibras musculares. A despolarização do botão terminal do neurônio motor expõe as vesículas de acetilcolina, fazendo com que o neurotransmissor seja secretado na junção neuromuscular. A acetilcolina provoca a abertura de canais catiônicos na membrana das fibras musculares, permitindo assim a entrada de grande quantidade de íons sódio (Na^+) na membrana das fibras, causando uma despolarização local. A abertura de mais canais de Na^+ voltagem-dependentes inicia um novo potencial de ação, que causará uma despolarização na membrana muscular (HALL, GUYTON, 2011).

A despolarização da membrana é direcionada ao interior das fibras musculares, fazendo com que os túbulos T e, principalmente, os retículos sarcoplasmáticos liberem íons cálcio (Ca^2) no interior das fibras musculares. Os íons Ca^{2+} ativam as forças atrativas entre os miofilamentos actina e miosina, fazendo com que se deslizem em direções opostas, provocando o encurtamento da fibra muscular. Este processo de deslizamento só é possível na presença de energia, provida pela hidrólise de ATP e consequente formação de ADP e fosfato inorgânico (Pi). Assim, a contração muscular demanda grande quantidade de ATP, principalmente durante o esforço físico intenso (HALL, GUYTON, 2011).

Embora o movimento demande grandes quantidade de ATP, durante os períodos de treinamento com exercícios existem outros processos metabólicos que demandam energia provida pela clivagem das moléculas de ATP. A reparação dos danos promovidos pelo esforço, a recuperação da homeostase e os processos adaptativos ao exercício (recuperação e estocagem de substratos energéticos, produção de proteínas, processos inflamatórios) carecem de energia (BAHR, *et al.*, 1987; EGAN, ZIERATH, 2013). Sendo assim, a produção adequada de ATP pode garantir, além de um bom desempenho durante o esforço físico, a eficiência do treinamento.

Os consecutivos e frequentes ciclos de contração muscular, associados às exigentes demandas promovidas pelo treinamento com exercícios estimula a adaptação fisiológica. As adaptações promovidas pelo treinamento com exercícios de *endurance* abrangem os distintos sistemas fisiológicos, especialmente em níveis musculoesquelético, cardiovascular e endócrino-metabólico. Contudo, diante da

plasticidade dos músculos-esqueléticos, estes tecidos apresentam expressivo remodelamento e adaptação funcional (EGAN, ZIERATH, 2013). De fato, as adaptações musculoesqueléticas, principalmente mitocondriais, são objetivo constante dos programas de treinamento físico que buscam melhorar a capacidade aeróbia de um indivíduo (LUNDBY, JACOBS; 2016).

As mitocôndrias são organelas responsáveis por produzir a energia química, em forma de ATP, são abundantes nos músculos esqueléticos, especialmente naqueles compostos principalmente por fibras do tipo I, com características metabólicas prioritariamente aeróbias. Diante do treinamento corretamente aplicado, as mitocôndrias podem adaptar-se tanto em quantidade quanto em atividade, permitindo assim uma produção de energia mais eficiente e, consequentemente, prolongamento da manutenção ou intensificação de determinado esforço. (DAVIES, PAKER e BROOKS, 1981; TERBLANCHE *et al.*, 2001)

Em seu estudo clássico, Davies, Paker e Brooks (1981) mostraram também que a correlação entre a capacidade oxidativa das mitocôndrias musculares a e capacidade aeróbia de animais apresentava valores muito superiores quando comparados a correlação entre a capacidade oxidativa das mitocôndrias a potência aeróbia ($VO_{2máx}$). Este achado informa a importância do aperfeiçoamento do trabalho mitocondrial na melhora do desempenho em exercícios de *endurance*, uma vez que o desempenho neste tipo de esforço está fortemente relacionado aos marcadores capacidade aeróbia (FEUD, KINDERMANN e MEYER, 2009; IMPELLIZZERI *et al*., 2005; TANAKA *et al.,* 1983).

Enzimas do metabolismo energético apresentaram adaptações positivas ao treinamento de *endurance*. Estudo de Leblanc *et al*. (2004) submeteu indivíduos a oito semanas de treinamento em cicloergômetro, e observou um modesto aumento na expressão de RNAm de um dos genes do complexo enzimático PDH. Entretanto, foi observado um aumento de 1,3 vezes na concentração desta enzima e aumento de 31% em sua atividade (LEBLANC *et al*., 2004). Outro estudo apresentou o aumento da atividade da enzima α-cetoglutarato desidrogenase em indivíduos treinados em *endurance* (BLOMSTRAND *et al.,* 2011). Além destas, estudos mostraram adaptações em diversas enzimas, de diferentes etapas do metabolismo energético, tais como palmitoil carnitina oxidase citrato sintase, piruvato carboxilase, citocromo oxidase, malato desidrogenase,

glutamato desidrogenase (DAVIES, PAKER e BROOKS, 1981; LEBLANC et al., 2004; SIU et al., 2003).

É importante destacar que, o exercício físico, principalmente aqueles de alta intensidades de trabalho (volume x intensidade), promove alterações significantes no estado redox, e por vezes é capaz de desencadear um quadro de estresse oxidativo (POWERS e JACKSON, 2008). Como discutido anteriormente, a principal fonte de radicais livres me indivíduos saudáveis são as mitocôndrias. Uma vez que o metabolismo mitocondrial é exacerbado durante o esforço a fim de suprir a demanda energética, é normal que a produção de ânion superóxido seja aumentada (POWERS e JACKSON, 2008). Estudo recente mostrou que uma única sessão de esforço exaustivo aumenta a concentração de marcadores de peroxidação lipídica no plasma de corredores, reduz a concentração de moléculas antioxidantes plasmáticas e interfere na atividade de enzimas antioxidantes eritrocitárias (GONÇALVES et al., 2019).

1.3. Tiamina

No século XIX, o beribéri motivou a pesquisa e a descoberta da tiamina. Christiaan Eijkman observou que as galinhas alimentadas com arroz polido apresentaram sintomas semelhantes aos do beribéri humano, enquanto os animais alimentados com arroz não-polido não apresentaram tais sintomas. A partir dessas observações, Gerrit Grjins, Fraser e Stanton concluíram que o arroz polido não possuía um nutriente orgânico, que poderia ser extraído do arroz parboilizado ou farelo de arroz. Em 1926, Roger Williams extraiu cerca de 100 gramas de tiamina de 600 kg de farelo de arroz (ZEMPLENI *et al.,* 2013).

Conhecida como vitamina B_1, a tiamina é sintetizada apenas por plantas, fungos e bactérias, o que torna a sua ingestão essencial para mamíferos visto que possui papel fundamental no metabolismo energético destes animais (MANZETTI, ZANG, VAN DER SPOEL, 2014). O nome químico da tiamina é 5- (2-hidroxietil) -4-metiltiazólio cloreto de 3- cloridrato de (40-amino-20-metil-pirimidin-50-ilmetil) e a sua fórmula empírica é C12H17N4OSCl.HCl (ZEMPLENI *et al.,* 2013).

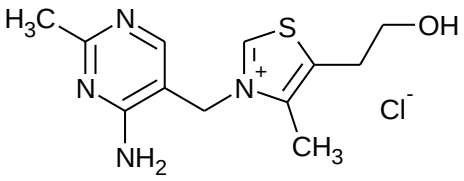

Figura 4: Estrutura química da tiamina.

A tiamina é absorvida na porção proximal do intestino delgado (jejuno). Visto ser uma vitamina hidrossolúvel, a participação de carreadores específicos é fundamental para que o composto atravesse a mucosa intestinal e então seja disponibilizado na corrente sanguínea. A tiamina pode ser encontrada no organismo humano em sua forma livre ou formas fosfoesterificadas: monofosfato de tiamina (TMP), difosfato de tiamina (TDP), trifosfato de tiamina (TTP) e trifosfato de adenosil de tiamina (PÁCAL, KURICOVÁ, KANKOVA, 2014). O transporte plasmático de tiamina livre e TMP exige que estas estejam ligadas à albumina. Os ésteres TDP e TTP são produzidos principalmente pelo fígado por fosforilação da tiamina, todavia, os demais tecidos também são capazes de fosforilar tiamina ou TMP com menor eficiência. A captação celular de tiamina é feita pelos seus transportadores THTR1 e THTR2. Dentro da célula, a tiamina é convertida para TDP pela enzima tiamina pirofosfoquinase-1 (TPK-1), que é então enviada ao interior das mitocôndrias com o auxílio de carreadores de TDP. A tiamina excedente na corrente sanguínea é filtrada pelo glomérulo e excretada na urina (BENDER, 2003).

Visto que a tiamina é uma vitamina solúvel em água, que não pode acumular-se no corpo, faz-se necessário sua ingestão diária. Para os adultos saudáveis, as Recomendações de Ingestão Diária (do inglês *Dietary Reference Intakes*) sugere a ingestão de 1,2 mg de tiamina diariamente (PADOVANI, 2006). As fontes de tiamina são carnes, especialmente suínas, gérmen de trigo, fígado, ovos, peixe, feijão, nozes e grãos integrais. Alguns alimentos, como café e alguns chás contêm polifenóis, substâncias capazes de inativar a tiamina ingerida (LONSDALE, 2006). O alcoolismo crônico ou ingestão nutricional inadequada também podem comprometer a absorção e a utilização de tiamina (SINGLETON; MARTIN, 2001).

A inadequada ingestão ou absorção de tiamina podem originar uma deficiência nos níveis de tiamina e seus ésteres, o que pode causar sintomas como dor de cabeça, náuseas e fadiga muscular. Período prolongado de deficiência desta vitamina está relacionado a doenças em distintos sistemas fisiológicos. A primeira e principal doença relacionada aos baixos níveis de tiamina foi o beribéri (CARPENTER, 2000). No entanto, esta deficiência também está correlacionada a outras desordens neurológicas, como a síndrome de Wernicke-Korsakoff (BUTTERWORTH, GIGUIRE, BESNARD, 1986; ZUBARAN, FERNANDES, RODNIGHT, 1997; BLASS, GIBSON, 1977), doença de Parkinson (BUTTERWORTH. BESNARD, 1990) e Alzheimer (KARUPPAGOUNDER, *et al.*, 2009; JIMÉNEZ-JIMENEZ, *et al.*, 1999). Além destas, algumas evidências têm apontado que a carência desta vitamina pode estar associada à insuficiência cardíaca (HANNINEN, *et al.*, 2006; KWOK, *et al.*, 1992).

A suplementação oral com tiamina ou sais derivados, i.e. tiamina tetrahidrofurfuril dissulfito, hidrocloreto de tiamina, tem sido amplamente aplicada a fim de corrigir a deficiência de tiamina e seus ésteres. Contudo, análogos sintéticos de tiamina, lipossolúveis, tem se mostrado mais eficientes em aumentar a biodisponibilidade da vitamina e seus ésteres em diversos tecidos (PORTARI; VANNUCHI; JORDÃO, 2013; XIE, *et al.*, 2014).

1.4. Tiamina e metabolismo energético

A tiamina não possui nenhum papel reconhecido nos passos iniciais do catabolismo dos macronutrientes. Contudo, a TDP, a forma biologicamente ativa da tiamina, é coenzima essencial para o funcionamento adequado de duas enzimas do metabolismo energético oxidativo, a piruvato desidrogenase e a α-cetoglutarato desidrogenase, além de ser coenzima da transcetolase, enzima chave do metabolismo não-oxidativo da via das pentoses fosfato (VPF) (ZEMPLENI *et al.*, 2013; MANZETTI, ZANG, VAN DER SPOEL, 2014).

O complexo piruvato-desidrogenase (PDH) é responsável por promover a descarboxilação oxidativa do piruvato e produzir Acetil-CoA. A PDH é composta por três componentes enzimáticos: piruvato desidrogenase (E1), dihidrolipoamida

acetiltransferase (E2); dihidrolipoamida desidrogenase (E3) (PATEL, KOROTCHKINA, 2006). Respeitando uma sequência de ação, estes três componentes são responsáveis pela descarboxilação do piruvato para formar Acetil-CoA, CO_2 e NADH(H). O componente E1 necessita da ação coenzimática da TDP, forma biologicamente ativa da tiamina, para a descarboxilação do piruvato e acetilação redutora dos grupos lipois ligados ao componente E2. O componente E2 transfere o Acetil para a CoA, formando a molécula de Acetil-CoA. O componente E3 carrega os elétrons do dihidrolipoato ligado ao componente E2 para moléculas de FAD, formando $FADH_2$ e em seguida para moléculas de NAD^+, formando NADH(H) (Figura 5) (PATEL, KOROTCHKINA, 2006; PATEL *et al.*, 2014). As moléculas NADH(H) descarboxilação do piruvato abastece a cadeia respiratória com seus elétrons, enquanto o CO_2 é eliminado pela troca gasosa através dos sistemas circulatório e respiratório. O Acetil-CoA sofre oxidação à CO_2 no ciclo de Krebs, tendo sua energia extraída através da cascata de reações descrita anteriormente (NELSON, COX, 2018).

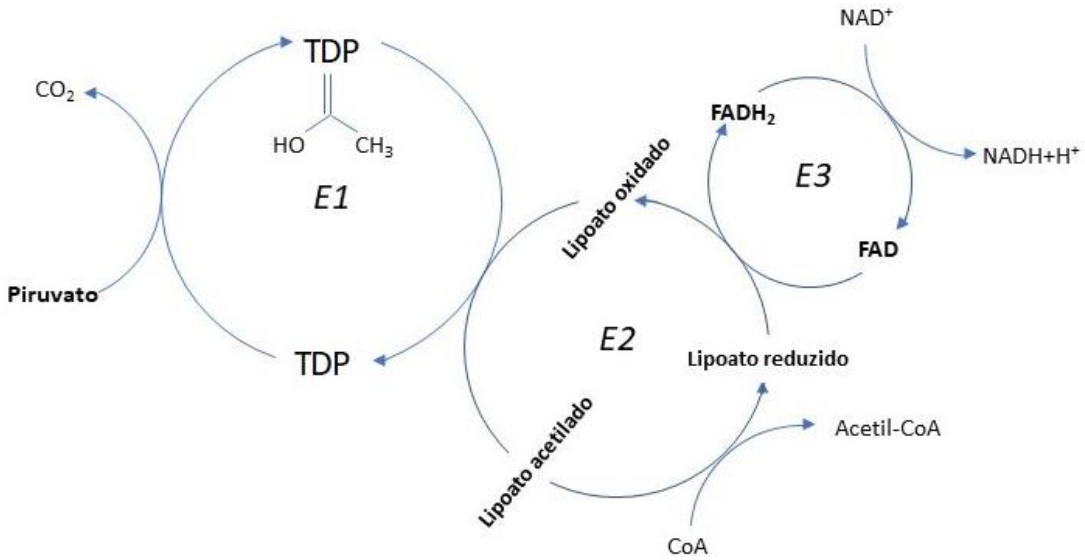

Figura 5: Representação esquemática das reações do complexo enzimático Piruvato-Desidrogenase. Adaptado de Patel *et al.*, 2014.

Dentro do ciclo de Krebs, o complexo enzimático α-cetoglutarato desidrogenase (KGDH) é responsável pela descarboxilação de α-cetoglutarato e formação e succinil-CoA (BUBBER, 2004; LUKASKI, 2004). O complexo KGDH funciona de maneira semelhante ao PDH, composto também por três componentes que trabalham

sequencialmente. Sua unidade E1k, a enzima α-cetoglutarado desidrogenase, catalisa a descarboxilação do α-cetoglutarato e libera uma molécula de CO_2, utilizando-se da TDP como coenzima. O grupo succinil é transferido ao lipoato oxidado no componente E2k. A reação entre o CoA-SH e o succinil forma o succinil-CoA e lipoato reduzido. Finalmente, no componente E3K as moléculas de NAD+ são reduzidas pelo $FADH_2$, formando NADH(H) (Figura 6) (GIBSON *et al.*, 2000; GIBSON *et al.*, 2005). O NADH(H) produzido pela ação do complexo KGDH é reciclado na cadeia respiratória, oferecendo seus elétrons para os processos de formação de ATP. As moléculas de CO_2 são transportadas pelo sangue até os pulmões e então eliminadas nos alvéolos (NELSON, COX, 2018).

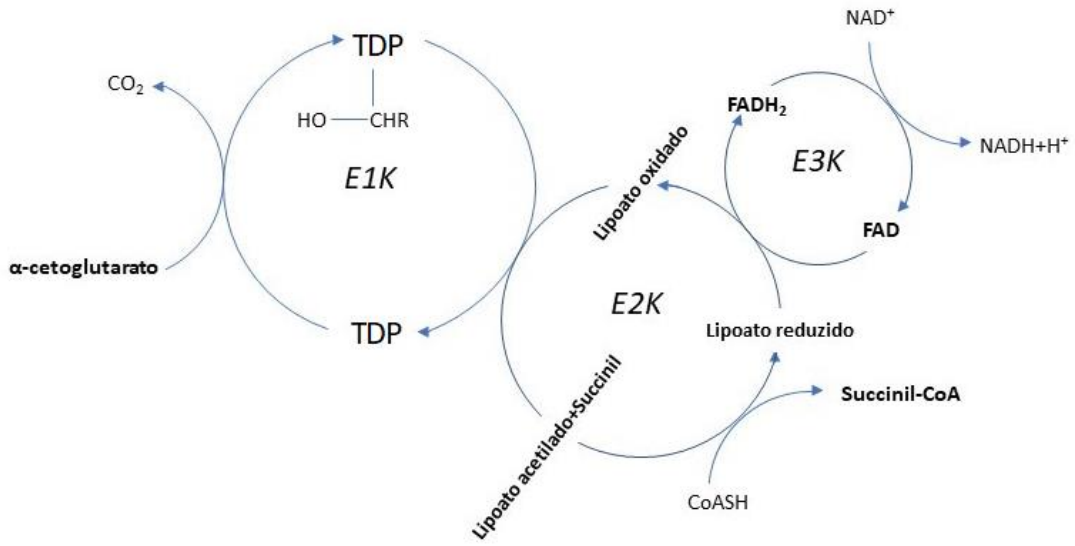

Figura 6: Representação esquemática das reações do complexo enzimático α-cetoglutarato-desidrogenase. Adaptado de Gibson et al., 2000.

Além disso, o TDP é importante para a atividade normal de transcetolase, uma enzima reguladora da VPF, essencial para recuperação e manutenção dos tecidos. Um dos principais produtos desta é a ribose, molécula essencial para a síntese de nucleotídeos e, consequentemente, ácidos nucleicos, i. e. DNA e RNA. A VPF produz ainda $NADPH^+$, molécula cofator em vários mecanismos de biossíntese, com potencial antioxidante direto e fundamental para o adequado funcionamento de antioxidantes endógenos (WOOD, 1986; BOLAÑOS *et al.*, 2008; YILMAZ *et al.*, 2015).

Gibson, Ke e Bubber (2014) constataram redução em mais de 20% na atividade da enzima α-cetoglutarato desidrogenase no cérebro de animais submetidos a 8 dias de restrição à tiamina. Por outro lado, Zhao et al. (2009) avaliaram a atividade das enzimas dependentes de tiamina e constataram que apenas a transcetolase apresentou redução significativa em sua atividade nas células neurais cultivadas em meio deficiente em tiamina. Além de seu papel essencial para a atividade das enzimas do metabolismo energético, evidências sugerem que a tiamina pode participar da regulação da expressão do gene destas enzimas. Singleton, Martin e Pekovich (1997) mostraram em cultura de células que a deficiência de tiamina reduz os níveis de mRNA das enzimas transcetolase e PDH, contudo não afeta a expressão gênica da KGDH.

Estudo de Tanaka *et al*. (2010) avaliou a atividade do complexo PDH no fígado de ratos obesos tratados com tiamina, e constataram que a suplementação foi eficiente em aumentar a atividade da enzima. Em uma cultura de células de tumor mamário humano, o tratamento com hidrocloreto de tiamina mostrou ser eficiente em aumentar tanto a atividade quanto a quantidade de PDH celular (Liu *et al*., 2018).

Diante a importância dos macronutriente no processo de produção e ATP, como os substratos energéticos, é comum supor que uma oferta inadequada de macronutriente limitaria o desempenho físico (BURKE *et al*., 2011). Realmente, Havemann *et al*., (2006) mostraram que uma dieta com baixa quantidade de carboidrato prejudicou o a capacidade dos atletas em manter um esforço de alta intensidade, associado ainda ao aumento da frequência cardíaca e da percepção de esforço.

O fato da baixa oferta de macronutrientes prejudicar o desempenho esportivo está principalmente associado à oferta insuficiente de energia potencial para produzir ATP. Assim, o treinamento com exercícios pode não gerar as adaptações almejadas, a fadiga durante o esforço pode se manifestar prematuramente, e/ou o incremento da intensidade pode não ser sustentável frente a baixa produção de ATP (CAMPBELL, WISNIEWSKI, 2017). Contudo, é importante ressaltar que micronutrientes também são fundamentais para os processos metabólicos, especialmente para o metabolismo bioenergético (CAMPBELL, WISNIEWSKI, 2017).

1.5. Tiamina e exercício

Embora esteja clara a participação fundamental da tiamina sobre o metabolismo energético, as recomendações de ingestão de tiamina não são específicas de acordo com os níveis de atividade física, gasto energético basal ou valor energético dos alimentos ingeridos. Apesar de algumas evidências indicarem que a suplementação com tiamina pode melhorar o desempenho do exercício, as recomendações direcionadas aos atletas, indivíduos com alto consumo diário de energia, são as mesmas que as destinadas aos indivíduos sedentários saudáveis. Várias avaliações dos níveis e consumo de tiamina mostraram não haver diferenças entre atletas e indivíduos saudáveis (FOGELHOLM, 1992; FOGELHOLM *et al.,* 1992; FOGELHOLM *et al.,* 1992).

Outros estudos sugerem que as necessidades e adaptações ao exercício aumentam a demanda de tiamina. Shibata e Fukuwatari (2013) encontraram redução nos níveis de tiamina em ratos treinados alimentados com as recomendações mínimas de vitamina B_1, mas não em ratos treinados alimentados com uma quantidade extra da vitamina. Corroborando, Kim, Choi e Cho (2015) demonstraram menor excreção urinária de tiamina por ratos exercitados quando comparados a animais sedentários.

Estudo da Kniazuk e Molitor (1943) investigou o reflexo da deficiência de tiamina sobre o desempenho no exercício. Para tanto, os pesquisadores ofereceram a ratos uma dieta deficiente em tiamina e submeteram os animais a testes de natação máxima, encontrando significativa redução no desempenho do exercício. Após recuperarem os níveis adequados de tiamina nos eritrócitos, novos testes de esforço máximo permitiram verificar a recuperação da aptidão física dos animais. Horwitt e Kreisler (1949) propuseram que a deficiência de tiamina pode aumentar a concentração de lactato por uma diminuição na atividade do complexo PDH. Os cientistas apontaram correlação positiva entre a lactacidemia e a fadiga muscular e afirmaram que a deficiência de tiamina poderia promover uma fadiga precoce.

Recentemente, Choi, Baek e Choi (2013) suplementaram atletas do sexo feminino com 10 mg/kg de dissulfureto de tiamina de tetrahidrofurfuril (DTTF) durante quatro semanas, e observaram um significativo efeito antifadiga. Nozaky *et al.* (2009) verificaram aumento nos níveis de ATP e ésteres de tiamina em vários órgãos de ratos

após suplementação com DTTF, e observaram redução na fadiga, evidenciando a melhora do metabolismo energético durante o teste de natação máximo. Os cientistas supuseram que o nível elevado de TDP, induzida pela suplementação DTTF, poderia melhorar a resíntese de ATP e glicogenólise no músculo esquelético exercitado, retardando assim a fadiga induzida pelo esforço. Concordando, Mehdi *et al*. (2013) mostraram que a suplementação com dose elevada de TDP (300 mg / dia) em indivíduos não atletas é mais eficiente para combater a fadiga, em comparação à suplementação com baixa dose (30 mg/dia).

Em um estudo *cross-over*, Suzuki e Itokawa (1996) concluíram que os atletas com níveis normais de tiamina, suplementadas com 100 mg/dia de DTTF tiveram menos aumento de glicose no sangue e menos queixas após a fadiga induzida pelo exercício. Um estudo similar mostrou que os indivíduos que consomem alta dose TDP têm menos glicose no sangue imediatamente após o exercício, quando comparado à suplementação com baixa dosagem de TDP ou placebo, indicando uma participação de tiamina no metabolismo da glicose (MEHDI *et al.*, 2013).

Bautista-Hernandez *et al*. (2008) administraram, por via intravenosa, TDP ou solução placebo durante 30 dias em 27 voluntários participantes de um estudo duplo-cego, randomizado e *cross-over*. Em relação ao grupo placebo, o grupo suplementado apresentou menores níveis de lactato sérico e frequência cardíaca e maior consumo máximo de oxigênio ($VO_{2máx}$) após a atividade física aeróbica. Outros estudos não encontraram resultados semelhantes. Por exemplo, a suplementação com DTTF não influenciou o desempenho de exercícios de alta intensidade e os seus marcadores em um teste contra-relógio em bicicleta ergométrica (WEBSTER *et al*., 1997). Pesquisas que utilizaram outro derivado de tiamina, com melhor absorção, mostrou que a suplementação com alitiamina foi ineficiente em aperfeiçoar o desempenho durante o exercício (WEBSTER, 1998; DOYLE, WEBSTER, ERDMANN, 1997).

Shimizu *et al*. (2010) investigaram o papel antifadiga de outro derivado de tiamina, o cloridrato de dicetiamina (CDT). Eles submeteram os ratos a estresse e teste de natação máxima durante cinco dias, um modelo que combina estresse físico e mental. Os animais suplementados por via oral com CDT suportaram nadar mais tempo do que os animais que ingeriram a vitamina B_1 ou veículo. O estudo mostrou que apenas os

animais com incremento nos níveis de tiamina livre, os níveis de TMP e TDP melhoraram sua resistência ao nada forçado, levando à conclusão que níveis aumentados de tiamina e/ou seus ésteres possuem algum efeito contra a fadiga induzida pelo esforço físico. Outro achado importante foi o aumento dos níveis de tiamina livre, TMP e TDP no cérebro, músculo da coxa e no sangue, promovidos pela ingestão de CDT, enquanto a vitamina B_1 não mostrou o mesmo efeito. Este resultado revela que a substância suplementada pode interferir significativamente na biodisponibilidade de tiamina e seus ésteres nos tecidos, influenciando assim na oxidação de substrato e produção energética, e consequentemente, alterando o desempenho esportivo.

1.6. Benfotiamina

Os compostos lipofílicos derivados da tiamina foram descobertos nas plantas do gênero Allium, como cebola, cebolinha e alho-poró, por este motivo são chamados allitiaminas. A benfotiamina (S-benzoyltiamina O-monofosfato) tem se apresentado eficiente em aumentar a biodisponibilidade de tiamina em tecidos biológicos. Isto porque apresenta um anel tiazol aberto em sua estrutura química, o que a torna lipossolúvel (Balakumar et al., 2010), capaz de atravessar facilmente as membranas biológicas. A fórmula molecular da benfotiamina é C19H23N4O6PS com peso molecular 466,5 g/mol (EFSA, 2008).

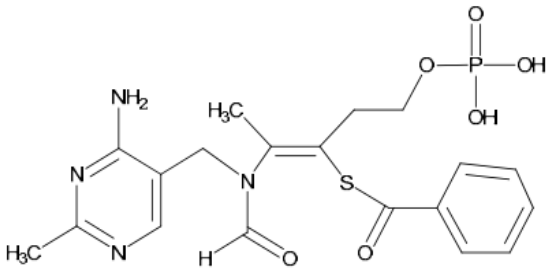

Figura 7: Estrutura química da benfotiamina.

Embora a tiamina tenha função essencial no metabolismo oxidativo dos carboidratos, a suplementação com a vitamina não tem se mostrado tão eficiente quanto alguns de seus análogos para aumentar os níveis sanguíneos de tiamina, TMP e TDP (PORTARI; VANNUCHI; JORDÃO, 2013; XIE *et al.*, 2014). Desta maneira, possivelmente as inconsistências dos estudos em relação ao efeito da suplementação com tiamina sobre o desempenho esportivo, estejam relacionadas aos distintos suplementos ofertados. Os vários derivados e análogos da tiamina, i. e. benfotiamina, alitiamina,

DTTF, CDT, hidrocloreto de tiamina, promovem diferentes aumentos na biodisponibilidade da vitamina em distintos tecidos.

Ao alcançar a mucosa intestinal, a benfotiamina ingerida é defosforilada por fosfatases inespecíficas, formando a S-benzoiltiamina. A benzoiltiamina é lipossolúvel e facilmente absorvida pela mucosa intestinal, atravessando as membranas intestinal e endotelial por difusão passiva e atingindo a corrente sanguínea de maneira mais rápida e econômica quando comparada a outros sais de tiamina (PORTARI; VANNUCHI; JORDÃO, 2013). No interior das células, a molécula de benzoiltiamina sofre redução catalítica por compostos sulfidrila intracelulares e/ou desbenzoilação enzimática, tendo seu anel tiazol fechado, formando assim a tiamina ativa utilizada pela célula ou liberada na corrente sanguínea, tendo o ácido benzóico como subproduto (EFSA, 2008).

Portari, Vannuchi e Jordão (2013) mostraram que a suplementação com benfotiamina aumentou a biodisponibilidade de vitamina B_1 nos eritrócitos de ratos 21 vezes mais que a suplementação com hidrocloreto de tiamina. Comparando as mesmas substâncias, Xie *et al.* (2014) observaram que a suplementação oral com benfotiamina promoveu um aumento 195% maior na biodisponibilidade de TDP nos eritrócitos de humanos saudáveis. O estudo mostrou ainda um aumento nos níveis plasmáticos de tiamina livre 12 vezes maior nos indivíduos que ingeriram benfotiamina.

Estudos têm observado um papel antioxidante da benfotiamina diante diferentes situações sabidamente pró-oxidantes, tais como alcoolismo e exercício. Estudo de Portari et al. (2016) mostrou que a suplementação com a benfotiamina foi eficiente em conter os danos oxidativos produzidos no fígado de animais submetidos a um modelo de alcoolismo crônico. Gonçalves et al. (2019) mostraram que a suplementação com benfotiamina melhorou os marcadores de estresse oxidativo no músculo gastrocnêmio de animais submetidos ao treinamento de *endurance*.

1.7. Justificativa

Considerando as evidências, é possível que a suplementação com benfotiamina aumente os níveis de tiamina e seus ésteres nos tecidos, e promova incremento na função

do metabolismo bioenergético aeróbio e consequentemente, incremento do desempenho físico.

Até o momento, existem poucas informações científicas sobre a influência da tiamina em nível biomolecular, e como este micronutriente pode regular a expressão e atividade de moléculas das vias bioenergéticas. Embora já seja esclarecido a maior biodisponibilidade da benfotiamina em relação às outras formas de tiamina, são escassos os experimentos sobre sua relação com o desempenho físico, tanto em humanos quanto em animais.

Considerando a alta demanda energética, é possível que indivíduos constantes submetidos a altas intensidades de esforço físico demandem por mais tiamina, uma vez que este micronutriente é cofator de enzimas do metabolismo energético. Uma vez que as recomendações de ingestão diária de tiamina não são diferentes para atletas e pessoas sedentárias, faz-se necessário a revisão das informações que embasam tal recomendação, bem como a experimentação com análogos de tiamina ainda não testados.

A alta demanda energética imposta pelo esforço físico promove um aumento na produção de espécies reativas, especialmente em tecidos metabolicamente mais solicitados durante o exercício. Uma vez que estudos têm demonstrado o papel antioxidante da benfotiamina em diferentes situações sabidamente pró-oxidantes, a suplementação com benfotiamina pode contribuir para a manutenção do balanço redox nos tecidos de indivíduos engajados em programas de treinamento físico de *endurance*.

Sendo assim, os achados deste estudo poderão elucidar a atletas e treinadores sobre a necessidade de uma dieta altamente específica a fim de melhorar o desempenho esportivo.

2. OBJETIVO

2.1. Objetivo Geral

Avaliar os efeitos da suplementação de benfotiamina em parâmetros metabólicos e de desempenho de camundongos submetidos ao treinamento físico de *endurance* em natação.

2.2. Objetivos específicos

- Avaliar o efeito da suplementação com benfotiamina sobre as enzimas do metabolismo energético.

- Avaliar o efeito da suplementação com benfotiamina sobre o desempenho aeróbio dos animais submetidos ao treinamento de *endurance* em natação;

- Avaliar o efeito da suplementação com benfotiamina sobre o estresse oxidativo em diferentes tecidos de animais submetidos a treinamento de *endurance* em natação.

3. MÉTODOS

3.1. Animais e aspectos éticos

De caráter experimental, a pesquisa utilizou 25 camundongos da linhagem BALB/c, machos com seis semanas de idade. Esta quantidade de animais se faz necessário para a formação de grupos com número suficiente para comparações estatísticas confiáveis. Os animais foram mantidos sob regime circadiano invertido de 12 horas de claro/escuro com água e ração *ad libitum*.

Este projeto foi submetido à avaliação ética pela Comissão de Ética no Uso de Animais e aprovado em reunião de 22/05/2015 sob protocolo número 343/2015.

3.2. Delineamento Experimental

Após um período de 7 dias de adaptação às condições do Laboratório de Nutrição Experimental, da Universidade Federal do Triângulo Mineiro, os animais foram separados em 4 grupos de acordo com a dieta ofertada (**Tabela 1**) e a prática de exercício físico, formando assim os grupos descritos abaixo e representados na **Figura 2**:

- **Padrão Sedentário (Pad-Sed):** 6 animais que receberam dieta AIN-93 e não farão exercício;

- **Padrão treinado (Pad-Tr):** 6 animais que receberam dieta AIN-93 e farão o protocolo de exercício aeróbio;

- **Benfotiamina Sedentário (Ben-Sed):** 6 animais que receberam dieta suplementada sem fazer o exercício;

- **Benfotiamina Treinado (Ben-Tr):** 7 animais que receberam dieta suplementada e farão o protocolo de exercício.

A semana 1 (sem1) foi destinada a aclimatação ao ambiente e ciclo circadiano. A partir da segunda semana (sem 2) os animais iniciaram os treinamentos como descrito posteriormente.

Tabela 1 – Quantidade, em gramas, dos Ingredientes para 1Kg de dieta AIN-93 padrão e suplementada com benfotiamina.

Ingredientes	Controle	Suplementada
Caseína (ptn > 85%) (g)	200	200
Óleo de soja (g)	70	70
Amido de milho dextrinizado (g)	132	132
Sacarose (g)	100	100
Fibra (g)	50	50
Mix mineral (g)	35	35
Mix vitamínico (g)	10	10
L-Cistina	3	3
Colina	2,5	2,5
Benfotiamina (mg)	6	100
Amido de milho (q.s.p.) (g)	397,5	397,4
Aporte calórico (kcal)	3948	3948

Foi realizada a pesagem da ração uma vez por dia, pela manhã, e a pesagem dos animais uma vez por semana, durante os dias de repouso.

3.3. Protocolo de Treinamento Físico

Os animais foram submetidos a um treinamento de natação, seguindo protocolo de treinamento adaptado de Chen *et al.* (2014). Os animais treinaram em grupo, em um container de plástico de 60 cm de diâmetro e 40 cm de profundidade preenchida com água de torneira, mantida a 34°C (±1°C) regulada por aquecedor com termostato automático (Hopar SA-333, Zhong Shan, China).

No primeiro dia os animais ficaram por 30 minutos em água rasa (profundidade de 3 cm). No segundo dia os animais treinarão natação por 30 minutos, no terceiro dia por 45 minutos e nos dois dias seguintes as sessões de treinamento tiveram duração de 60 minutos. A primeira semana de treinamento acorreu sem incremento de carga, sendo essa a semana de adaptação ao treinamento dos animais. Na segunda semana de treinamento (Semana 3) foi utilizado um incremento de carga com 1% da massa corporal do animal e da terceira à sexta semana os camundongos treinaram com sobrecarga de 2% da massa corporal (CHEN *et al.*, 2014).

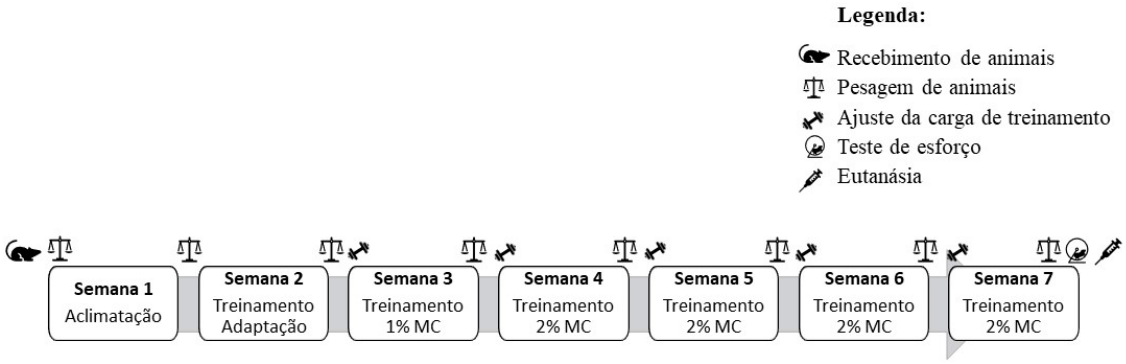

Figura 8: Representação esquemática da experimentação com animais. *MC= massa corporal.*

Sendo assim, os camundongos foram submetidos a sessões de treinamento físico por natação em uma frequência de cinco sessões por semana, com duração de 60 minutos a sessão, durante cinco semanas consecutivas. Os animais foram treinados em grupo a fim de aumentar a intensidade do exercício. Os camundongos dos grupos sedentários foram mantidos em tanques com água a aproximadamente 1,5 centímetros de profundidade por tempo igual à duração do treinamento dos animais treinados, a fim de mimetizar algum possível efeito estressante da exposição à água. A sobrecarga foi realizada com peso de metal fixado com fita adesiva na porção proximal da cauda do animal.

3.4. Protocolo de exaustão

No último dia do protocolo, os animais foram submetidos a um treinamento de exaustão que consiste em sua última sessão de natação com sobrecarga de 5% da massa corporal presa à cauda. A exaustão foi determinada pela incapacidade do animal manter o focinho na superfície da água por aproximadamente 8 segundos (CHEN *et al*, 2014).

3.5. Determinação do Lactato Plasmático

A concentração de lactato plasmático foi aferida antes e imediatamente após o protocolo de exaustão. Foram coletados 40 µL de sangue a partir de pequena incisão na cauda do animal, utilizando capilares de vidro heparinizados e previamente calibrados. O sangue coletado foi depositado em microtubos com 8,5 µL de fluoreto de potássio/EDTA

e centrifugados a 4000 rpm durante 15 minutos. Foram então retirados 10 μL de plasma para a análise da concentração de lactato por kit comercial (Intertek-São Paulo, Brasil) seguindo as instruções do fabricante.

3.6. Eutanásia e Necropsia.

Após jejum durante 12 horas subsequentes ao protocolo de exaustão, os animais foram anestesiados com Cloridato de Cetamina-Quetamina (90 mg/kg) e Cloridato de Xilazina-Xilazina 2% (12 mg/kg) via intraperitoneal e após a confirmação do estado anestésico por ausência de reflexos caudal e palpebral, foi realizada toracolaparotomia para punção cardíaca e exsanguinação do animal. Todos os procedimentos foram realizados de acordo com as normas do Conselho Nacional de Medicina Veterinária (CRMV) e as recomendações da Lei nº 11.794, que regulamenta o inciso VII do §1º do art. 225 da Constituição Federal, estabelecendo procedimentos para o uso científico de animais. O sangue extraído por punção cardíaca em tubos com EDTA foi centrifugado a 3000 rpm por 10 minutos. Eritrócitos e plasma foram depositados separadamente em microtubos e congelados. Os músculos gastrocnêmios foram imediatamente excisados e lavados em solução fisiológica gelada. Alíquota de um músculo gastrocnêmio foi imerso em solução conservante para análises de RNA mensageiro (RNAm). A porção restante foi armazenada imersa em nitrogênio líquido. O outro músculo gastrocnêmio foi submerso em nitrogênio líquido para pronto congelamento e estocado a -20°C para posteriores análises.

3.7. Dosagem de tiamina e ésteres de tiamina

Alíquota da amostra de eritrócito foi diluída em água destilada (1:1 vol/vol). Em seguida, a amostra foi rapidamente congelada em nitrogênio líquido e descongelado três vezes consecutivas para que sofresse hemólise. A amostra foi desproteinizada com ácido perclórico 0,8M (1:1 vol/vol) e centrifugada a 5000 rpm por 10 minutos a 4° C. Foram adicionados 50 μL de hexacianoferrato de potássio 30 mM (K3Fe (CN) 6) e 50 μL de 0,8

M de hidróxido de sódio (NaOH) a 80 µL do sobrenadante e agite brevemente em vórtex para derivatização. A solução foi misturada a 20 mL de metanol.

Foram injetados 20 mL da solução final no cromatógrafo (Shimadzu LC-10AT - Shimadzu Instruments, Kyoto, Japão). As análises foram realizadas na fase móvel composta por 70 volumes de tampão fosfato 25 mM (pH 7,0), metanol e acetonitrila (7: 2: 1 vol/vol/vol), coluna cromatográfica C18 (Ascentis, Supelco) e fluxo a 1,0 mL/min. O detector fluorimétrico (Shimadzu RF-20A - Shimadzu Instruments) foi ajustado em 365 nm para excitação e 435 nm para emissão (Portari *et al*., 2013). A quantificação foi normalizada pela concentração total de hemoglobina na amostra.

3.8. Marcadores de Estresse Oxidativo.

A capacidade antioxidante foi inferida a partir da concentração de tióis totais e tióis não-proteicos. A análise de tióis totais no músculo tibial, coração e fígado foi realizada por método colorimétrico a partir da concentração de grupos sulfidrila. Foi adicionado 1 ml de KCl 1,15% a aproximadamente 200 mg de tecido para homogeneização em ultraturrax. Foram adicionados a 500 µL de homogenato, 1,5 mL de solução de tampão Tris 0,2M-EDTA 0,02M pH 8,2, 1 mL de 5,5′-ditiobis (2-ácido nitrobenzóico) (DTNB) 0,01M e 7,9 mL de metanol com agitação subsequente. A solução foi incubada ao abrigo de luz durante 15 minutos, sendo agitada a cada 5 minutos. Em seguida, a solução foi centrifugada por 15 minutos a 3000 rpm. Alíquota do sobrenadante foi submetida à leitura espectrofotométrica em comprimento de onda de 412 nm.

Para quantificar a concentração de tióis não-proteicos, alíquotas dos homogenatos dos tecidos foram desproteinizados com ácido tricloroacético (10% vol) e centrifugados a 5000 rpm durante 10 minutos. Posteriormente, 400 mL de tampão Tris (0,4 M, pH 8,9) e 10 mL de DTNB 0,01M foram adicionados a 200 mL do sobrenadante. Após incubação durante 5 min, a absorbância foi lida em espectrofotômetro em comprimento de onda de 412 nm.

A concentração de grupos sulfidrila foi calculada utilizando o coeficiente de extinção molar de 13100 $M^{-1}cm^{-1}$ (SEDLAK; LINDSAY, 1968). As concentrações de

tióis totais e tióis não-proteicos foram apresentadas em relação a concentração de proteínas totais do homogenato do tecido.

A peroxidação lipídica foi inferida a partir da concentração de TBARS no tecido, (BUEGE; AUSTI, 1978). Aproximadamente 100 mg de músculo tibial, fígado e coração foram homogeneizados em ultraturrax com 250 µl de KCl 1,15%. Em seguida, 500 µL do reagente TCA-TBA-HCl foi adicionado ao homogenato e agitado por 2 minutos. A solução permaneceu incubada em banho-maria à 90-100°C durante 15 minutos. Após o resfriamento em água, a solução foi centrifugada por 10 minutos a 10000 rpm. A absorbância do sobrenadante foi lida em espectrofotômetro em comprimento de onda a 535 nm. A concentração de TBARS da amostra foi calculada usando uma curva de calibração previamente estabelecida com malondialdeído (MDA) e os resultados foram expressos em relação à concentração de proteína total no tecido.

O reagente TBA-TCA-HCL trata-se de uma solução com 15% m/v de ácido tricloroacético TCA, 0,375% m/v de ácido tiobarbitúrico diluídos em ácido clorídrico 0,25 N.

O conteúdo carbonílico de proteínas foi utilizado como marcador de dano oxidativo em proteínas. A concentração de proteínas carboniladas foi avaliada no músculo tibial, coração e fígado. Para tanto, foram adicionados 500 µL de KCl 1,15% a 50 mg de tecido para subsequente homogeneização em ultraturrax. Foram adicionados 500 µl de ácido tricarboxílico (TCA) 1% para precipitação das proteínas. O sobrenadante foi descartado e ao conteúdo proteico foram adicionados 500 µl de solução de dinitrofenilhidrazina (DNPH) 10 mM. Após agitação de 2-3 minutos, a solução foi mantida em temperatura ambiente, ao abrigo de luz, durante 60 minutos. Em seguida, foram adicionados 500 µl de TCA 20% e centrifugado a 3000 rpm, a 4ºC por 10 minutos. O conteúdo precipitado foi lavado com etanol/acetato de metila (1:1) a fim de remover DNPH e lipídeos. Após lavagem, o precipitado foi solubilizado em 500 µl de guanidina 6M e mantido em banho-maria a 37ºC durante 60 minutos. Após centrifugação, o sobrenadante foi recolhido para leitura em espectrofotômetro em 370 nm. A concentração de grupos carbonila foi determinado utilizando o coeficiente de extinção molar 22000 $M^{-1}cm^{-1}$ (MEKRUNGRUANGWONG *et al*., 2012). Os valores foram expressos em relação à concentração de proteína total no tecido.

3.9. Avaliação de Expressão Gênica

Foi avaliado a expressão de RNAm do componente E1, subunidade α do complexo enzimático piruvato desidrogenase (Pdha1) e do componente E1 do complexo α-cetoglutarato desidrogenase (Ogdh), através da técnica de Transcrição Reversa-Reação em Cadeia da Polimerase quantitativa (qRT-PCR) em tempo real.

Para extração do RNA total, foram utilizados 50mg de músculo gastrocnêmio dos animais dos diferentes grupos em estudo, utilizando método de extração fenol-clorofórmio descrito por Chomczynski & Sacchi (1987), a partir de 1,0 mL de Trizol (Invitrogen, Carlsbad, CA, EUA).

Sendo assim, 50mg de tecido foram homogeneizadas em 1 mL de trizol. Em seguida, foram adicionados 0,2 mL de clorofórmio. Após centrifugação por 15 minutos, a 13000 rpm e a 4°C, a fase aquosa superior foi coletada. Foram adicionados 0,5 mL de isoprapanol, e após incubação de 10 minutos, o material foi centrifugado por 10 minutos, a 13000 rpm e a 4°C. O sobrenadante foi descartado e o pellet de RNA foi lavado com 1 mL de etanol 75%. Após centrifugação por 5 minutos, a 13000 rpm e a 4°C, o etanol foi completamente descartado e o pellet de RNA foi resuspendido em 15 µL de água livre de nucleases.

Para a síntese de cDNA a partir do RNA total, foi utilizado o *kit* High Capacity RNA-to-cDNA (Applied Biosystem) seguindo as instruções do fabricante.

As Reações em Cadeia da Polimerase em tempo real (qPCR) foram realizadas em duplicata, utilizando como método de detecção o TaqMan®. Foram utilizados os ensaios inventariados TaqMan Assay dos genes PDHa1 (Mm00468675_m1), OGDH (Mm00803119_m1) e o gene RNA18s (Mm03928990_g1) como gene constitutivo. Foi usado o *master mix* TaqMan da Applied Biosystem, segundo as instruções do fabricante. Para a análise da qPCR, foi aplicado o método ΔΔCt a fim de realizar comparação com o grupo controle (calibrador) (BUSTIN, 2000).

3.10. Quantificação de Ácidos Orgânicos do Ciclo de Krebs

A análise de valores dos intermediários do ciclo de Krebs será realizada em alíquota do músculo gastrocnêmio utilizando cromatografia a gás acoplada à espectrometria de massas (GC-MS) com detecção tanto no modo de varredura como no modo de monitoramento de íons selecionados (SIM) em um GC-MS Shimadzu QP-5000, segundo metodologia descrita por Hur *et al.* (2014).

Para a derivação das amostras será adicionado 500 μL de água destilada a 50mg tecido e homogeneizado sob banho de gelo com um homogeneizador tipo Potter. Em seguida, água destilada (500 μL), acetonitrila (500 μL) e padrão interno (0,2 μg) serão adicionados à 100 μL de homogenato, e a mistura será submetida a agitação em vórtex (2 min) e centrifugadas a 14.000 rpm, por 10 min, para precipitação de proteínas.

O sobrenadante será ajustado a pH 12 com NaOH 5,0 M. Os grupos carbonila serão convertidos em derivados metoxima, por reação com hidrocloreto de metoxiamina (1,0 mg) a 60ºC durante 30 min. Em seguida, mistura de reação deve ser acidificada a pH 1-2 com solução de ácido sulfúrico a 10% saturada com cloreto de sódio e então extraída com 3 mL de éter dietílico, seguido por 2 mL de acetato de etila.

Após a adição de 5 μL de trietilamina, os extratos combinados serão evaporados totalmente sob nitrogênio gasoso em *dry block* (40ºC). Então serão adicionados então 20 μL de tolueno e 20 μL de N-metil-N-(tert-butildimetillsilil) trifluoroacetamida (MTBSTFA)+1% tert-butildimetilclorosilano (20 μL) como reagente de sililação seguido de aquecimento a 60ºC durante 30 min para formar derivados metoxima/terc-butildimetilsililados para análise no GC-MS.

As amostras serão depositadas em tubos do tipo *vial* e será manualmente injetado 1 μL em injetor do tipo *split*.

3.11. Análise Estatística

Todos os dados foram tabulados em planilhas e analisados utilizando as ferramentas estatísticas do *software* GraphPad Prism versão 8.0. Para verificar a

homogeneidade da variância, foi utilizado teste de Levene. Para verificar a distribuição dos dados da curva gausiana, foi aplicado o teste de *Shapiro-Wilk*. Após a constatação da normalidade na distribuição dos dados, foi utilizando análise de variância de um fator (ANOVA *one way*) e *post hoc* de Tukey para comparar os resultados de estresse oxidativo e concentração de tiamina. Nas comparações de concentração de lactato e tempo de exercício até a exaustão foi aplicado teste t de *Student*. Nos resultados de expressão gênica foi aplicado teste de Kruskal Wallis com *post hoc* de *Dunn*. Foram adotados níveis de significância de 95% ($p<0,05$).

4. RESULTADOS

Artigo 1: *Benfotiamine supplementation prevents oxidative stress in anterior tibialis muscle and heart.*

Situação: Publicado

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Benfotiamine supplementation prevents oxidative stress in anterior tibialis muscle and heart

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Abstract

Objective: This study aimed to evaluate the influence of oral supplementation with benfotiamine on oxidative stress in the liver, heart and muscles of endurance-trained mice.

Methods: Twenty-five male BALB/c mice were allocated to the following treatment groups: standard diet and sedentary activity (Sta-Sed), benfotiamine-supplemented diet and sedentary activity (Ben-Sed), standard diet and training activity (Sta-Tr) and benfotiamine-supplemented diet and training activity (Ben-Tr). The training comprised 6 weeks of endurance swimming training. The concentration of thiobarbituric acid reactive substances (TBARS), carbonylated proteins, total thiols and non-protein thiols were analyzed in the liver, heart and tibialis anterior muscle.

Results: In the muscle, TBARS concentration was higher in the Sta-Sed group than in other groups; in the heart, TBARS concentration in the Sta-Sed and Ben-Tr groups was higher than in the Ben-Sed group. The carbonyl content of the muscle tissues was higher in the Sta-Sed group than in both supplemented groups. In liver, the carbonyl content was lower in the Ben-Sed group than in the Sta-Sed group. The level of total thiols was lower in the Ben-Sed group than in the Sta-Tr group. In the heart, the level of total thiols was higher in the Ben-Sed group than in the Ben-Tr group. The concentration of non-protein thiols in the muscle was higher in the Ben-Sed group than

in the Ben-Tr group, whereas in the heart, concentration of non-protein thiols of Sta-Tr was lower than Sta-Sed.

Conclusion: The results show that benfotiamine is an efficient antioxidant for the anterior tibialis muscle and heart; however, swimming training did not alter redox status.

Keywords: Benfotiamine; Thiamine; Endurance exercise; Oxidative stress

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1. Introduction

Endurance exercise requires high levels of adenosine triphosphate production through oxidative metabolism. As the mitochondria are the greatest generator of reactive oxygen species (ROS), endurance exercise increases the production of ROS and, consequently, increases oxidative stress in several tissues [1,2]. An increase in ROS production and/or a reduction in the antioxidant capacity in cells causes an imbalance in redox status, a paradigm known as oxidative stress. Oxidative stress can promote oxidative damage to cellular macromolecules and promote cell death or dysfunction [3].

Some studies have shown that exercise increases ROS production and oxidative stress. Elevated levels of serum ROS and lipid peroxidation were reported in men subjected to exhaustive exercise before and after endurance training [4]. In rats, lipid peroxidation markers in muscle tissue increased in response to an exercise training protocol [5]. Therefore, the study showed a decrease in the reduced glutathione/oxidized glutathione ratio (GSH/GSSG), which is an indicator of greater pro-oxidant activity [5]. Several studies, *in vitro* and *in vivo*, have shown a relationship between muscle contraction and oxidative damage [6–8].

The preventative roles of some vitamins against oxidative stress in endurance exercise has been shown, and this is reflected in aerobic performance. The antioxidant role of vitamins C and E is well studied [9–11]. Also, the antioxidant capacity of thiamine (vitamin B1) has been reported in other situations [12–14]. Thiamine can efficiently transfer ions to reactive species [15]. In addition, this vitamin participates in metabolic reactions that contribute to the recycling of antioxidant molecules [16,17].

Thiamine is absorbed in the intestinal epithelium. It crosses the biological membranes using saturable carriers. Thus, high doses of thiamine hydrochloride may not result in greater bioavailability. Indeed, studies have shown that benfotiamine, a synthetic thiamine analog, is more bioavailable than other thiamine salts [14,18]. This is due to the fat-soluble characteristic of its metabolite, the S-benzoylthiamine, which eliminates the need for saturable carriers, and is able to cross the biological barriers through passive diffusion [18].

Based on current evidence, in the present study we hypothesized that benfotiamine supplementation would improve antioxidant systems and prevent oxidative damage in several tissues, especially those that have more pronounced oxidative metabolism during endurance exercise. In the present study we measured the effect of oral supplementation with benfotiamine on markers of oxidative damage and antioxidant activity in the muscle, heart and liver of endurance-trained mice.

2. Materials and methods

2.1. Animals

Twenty-five 6-week-old BALB/c male mice were housed individually in plastic cages, under an inverted circadian cycle (12/12 h dark:light), at 22 °C ± 1 °C and 55% ± 5% humidity, with *ad libitum* access to feed and tap water. The animals were randomly placed into four groups, according to the proposed intervention. The standard diet and sedentary activity group (Sta-Sed, $n = 6$) received AIN-93 growth standard rodent diet and did not train; the standard diet and training group (Sta-Tr, $n = 6$) was subjected to endurance swimming training. The benfotiamine and sedentary activity group (Ben-Sed,

n = 6) received AIN-93 supplemented with benfotiamine and did not train; the benfotiamine and training group (Ben-Tr, *n* = 7) received AIN-93 supplemented with benfotiamine and was subjected to swimming training.

The experimental protocol lasted 7 weeks. The first week was set aside for acclimation to the laboratory environment, AIN-93 standard diet and isolated cages. The experimental protocol was authorized by the Ethics Committee of Animal Use of the Federal University of Triangulo Mineiro, under protocol number 343/2015.

2.2. Diet and supplementation protocol

Animals received AIN-93 growth standard rodent diet, with or without benfotiamine supplementation (500 mg/kg). The diet was prepared in accordance with the protocol stablished by Reeves et al. [19]. All components (in powder) were mixed, homogenized and pelletized under pressure and water vapor. Benfotiamine was incorporated into the diet during the homogenization process. The standard and supplemented diets were isocaloric.

All four groups received AIN-93 growth standard diet during the first and second weeks, which were used for environment acclimation and training adaptation. The supplemented groups (Ben-Sed and Ben-Tr) received AIN-93 diet supplemented with benfotiamine from the third week through the end of the experiment. The feed intake was measured daily by weighing food on digital scale. Benfotiamine intake was evaluated by using food intake data.

2.3. Training protocol

The animals were subjected to endurance swimming training following the protocol used by Chen et al. [20]. Animals swam in a group, in plastic containers (40 cm in diameter by 60 cm in height), with a tap water depth of 40 cm, maintained at 32 (± 1) °C, controlled by a heater with an automatic thermostat (HOPAR SA-333 Zhong Shan, China).

The training adaptation period started with exposure to shallow water on the first day and finished with a 60-minute swim (without external load) on the fifth day. In the second week, the animals were forced to swim with an external load (metal ring) fixed to the proximal portion of the tail, comprising 1% of body weight, for 60-minute for five sessions per week. The load was increased to 2% of body weight for the following 4 weeks, and the duration and frequency of training was maintained. The sedentary groups were exposed to shallow water at a frequency and duration similar to the training protocol of the other groups [20].

2.4. Tissue preparation

Twenty-four hours after the final swimming session, the animals were anesthetized with ketamine/xylazine and euthanized by cardiac puncture exsanguination. The tibialis anterior muscle, heart and liver were excised and immediately frozen in liquid nitrogen. Aliquots of tissues were homogenized in a phosphate buffer pH 7.8 (1:150 w/v). The content of oxidative damage biomarkers was measured in tissue homogenates. The results were expressed relative to the total protein content in grams, which was determined with a commercial kit (Labtest, São Paulo, Brazil).

2.5. Thiobarbituric acid reactive substances

The concentration of thiobarbituric acid reactive substances (TBARS) was used as a lipid peroxidation marker. TBARS concentration was measured in tissues according to the methods of Buege and Aust [21]. Thiobarbituric acid reagent was added to the tissue homogenate and heated in boiling water, after which the absorbance of the supernatant was measured at 535 nm. TBARS concentration was calculated using a calibration curve made by performing similar reactions with a commercial malondialdehyde solution.

2.6. Carbonyl protein

The carbonyl content of proteins, used as a marker of oxidative damage, was measured by using a reaction with 2,4-dinitrophenylhydrazine, following the protocol of Mekrungruangwong et al. [22]. The carbonyl content in the sample was determined spectrophotometrically at 370 nm and was calculated using the molar extinction coefficient of 22,000 mol/Lcm.

2.7. Thiols

The total thiol content of the muscles was determined by a colorimetric assay, which was operated through the reaction of a sulfhydryl group from 5,50-dithiobis (2-nitrobenzoic acid) (DTNB) with an aliquot of muscle homogenate. First, 200 mL of 0.2 mol/L Tris/ ethylenediamine tetraacetic acid buffer, pH 8.2, 300 mL DTNB and 1.6 mL methanol were added to 100 mL of the muscle homogenate. After incubation for 15 min, the solution was centrifuged for 15 min at 1500 g. The absorbance of the supernatant was read at 412 nm. The total thiol concentration was calculated using the molar extinction coefficient of 13,100 mol/(Lcm) [23].

To quantify the non-protein thiol content, the tissue homogenates were deproteinized using 10% trichloroacetic acid (TCA). Subsequently, 400 µL of buffered Tris (0.4 mol/L, pH 8.9) and 10 µL of DTNB were added to 200 µL of the homogenate supernatant. After incubation for 5 min, the absorbance was read at 412 nm. The non-protein thiol concentration was calculated using the molar extinction coefficient of 13,100 mol/Lcm [23].

2.8. Statistical analysis

The data are presented as the mean ± standard deviation. The results were analyzed using SPSS software (Version 20.0). Levene's test and Shapiro-Wilk's test were used to test the equality of variances and data homogeneity, respectively. Benfotiamine intake was compared by Student's *t*-test. The results of oxidative stress were compared by one-way analysis of variance followed by post-hoc Tukey's test. The data for TBARS

concentration in heart tissue were compared using the Kruskal-Wallis test and Dunn's test as a post-hoc test. A significance level of 95% ($P < 0.05$) was adopted.

3. Results

3.1. Diet consumption and benfotiamine intake

All groups had a similar food intake and body weight (Table 1). The benfotiamine consumption was not different between the Ben-Sed and Ben-Tr groups (2.16 ± 0.12 mg/d *versus* 2.09 ± 0.09 mg/d).

Table 1. Body weight and food intake.

Group	n	*Initial body weight (g)*	*Final body weight (g)*	*Food intake (g/d)*
Sta-Sed	6	26.41 ± 1.06	30.58 ± 1.24	4.47 ± 0.27
Ben-Sed	6	26.16 ± 0.75	30.16 ± 1.77	4.33 ± 0.25
Sta-Tr	6	25.58 ± 2.31	29.83 ± 2.56	4.24 ± 0.30
Ben-Tr	7	25.42 ± 1.41	29.64 ± 1.57	4.17 ± 0.18

Data are presented as the mean ± standard deviation. Sta–Sed: standard diet and sedentary activity group; Ben–Sed: benfotiamine and sedentary activity group; Sta–Tr: standard diet and training group; Ben–Tr: benfotiamine and training group.

3.2. Oxidative stress markers in the tibialis muscle

TBARS concentration in the tibialis muscle was higher in the Sta-Sed group (53.57 ± 14.86 nmol/g) than in the other groups. The TBARS content in the Sta-Tr, Ben-Sed, and Ben-Tr groups was 33.31 ± 1.63 nmol/g, 27.08 ± 8.93 nmol/g and 26.84 ± 6.83 nmol/g, respectively (Fig. 1A). The Sta-Sed group showed a higher carbonyl concentration (2.31 ± 0.46 µmol/g) than the Ben-Sed (1.60 ± 0.24 µmol/g) and Ben-Tr (1.84 ± 0.14 µmol/g) groups ($P < 0.05$). The carbonyl content of proteins was higher in the Sta-Tr group (2.13 ± 0.23 µmol/g) than the Ben-Sed group ($P < 0.05$; Fig. 1B).

The total thiol concentration was lower in the Sta-Tr group (108.50 ± 19.65 nmol/g) than in the Ben-Sed group (160.82 ± 31.22 nmol/g; $P < 0.05$). The Sta-Sed (136.80±35.55 nmol/g) and Ben-Tr (136.72 ± 32.49 nmol/g) groups were not significantly

different in thiol content (Fig. 1C). The level of non-protein thiol was lower in the Ben-Tr group (1.64 ± 0.76 nmol/g) than the Ben-Sed group (3.09 ± 1.25 nmol/g). The non-protein thiol concentration was not different in the Sta-Sed and Sta-Tr groups (2.37 ± 0.59 nmol/g and 1.93 ± 0.57 nmol/g, respectively; Fig. 1D).

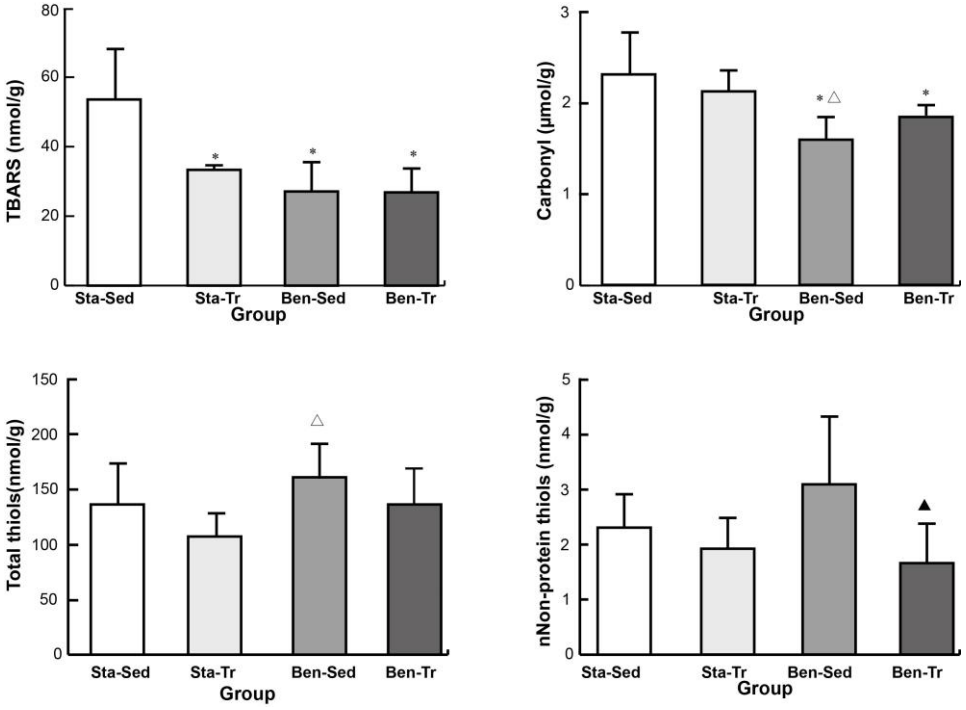

Fig. 1. Oxidative stress markers in tibialis anterior muscle. A: Thiobarbituric acid reactive substance (TBARS) concentration; B: Carbonyl content; C: Total thiol concentration; D: Non-protein thiol concentration (n = 6 in Sta-Sed; n = 6 in Sta-Tr; n = 6 in Ben-Sed; n = 7 in Ben-Tr). $^{*}P < 0.05$, *vs* Sta-Sed; $^{\triangle}P < 0.05$, *vs* Sta-Tr; $^{\blacktriangle}P < 0.05$ *vs* Ben-Sed.

3.3. Oxidative stress markers in the heart

TBARS concentration in the heart tissue was lower in the Ben-Sed (2.30 ± 1.31 nmol/g) group than in the Sta-Sed (13.36 ± 6.43 nmol/g) and Ben-Tr (9.09 ± 4.57 nmol/g) groups, but the Sta-Tr group (6.75 ± 6.20 nmol/g) was not significantly different from any of the groups (Fig. 2A). The carbonyl content was different among Sta-Sed (724.18

± 215.41 µmol/g), Sta-Tr (1039.36 ± 274.85 µmol/gPt), Ben-Sed (1014.11 ± 261.57 µmol/g) and Ben-Tr (1122.04 ± 488.92 µmol/g) treatment groups (Fig. 2B).

The total thiol concentration was lower in the Ben-Tr group (39.10 ± 5.40 nmol/g) than the Ben-Sed group (65.42±18.00 nmol/g). The concentration in the Sta-Sed (54.64 ± 9.62 nmol/g) and Sta-Tr (42.25 ± 22.11 nmol/g) groups was not significantly different (Fig. 2C). The Sta-Sed group showed a higher non-protein thiol concentration than the Sta-Tr group (7.30 ± 1.43 *vs.* 3.45 ± 2.11 nmol/g). The non-protein thiol concentrations in the Ben-Sed and Ben-Tr groups were 6.04 ± 1.60 nmol/g and 5.89 ± 2.00 nmol/g, respectively (Fig. 2D).

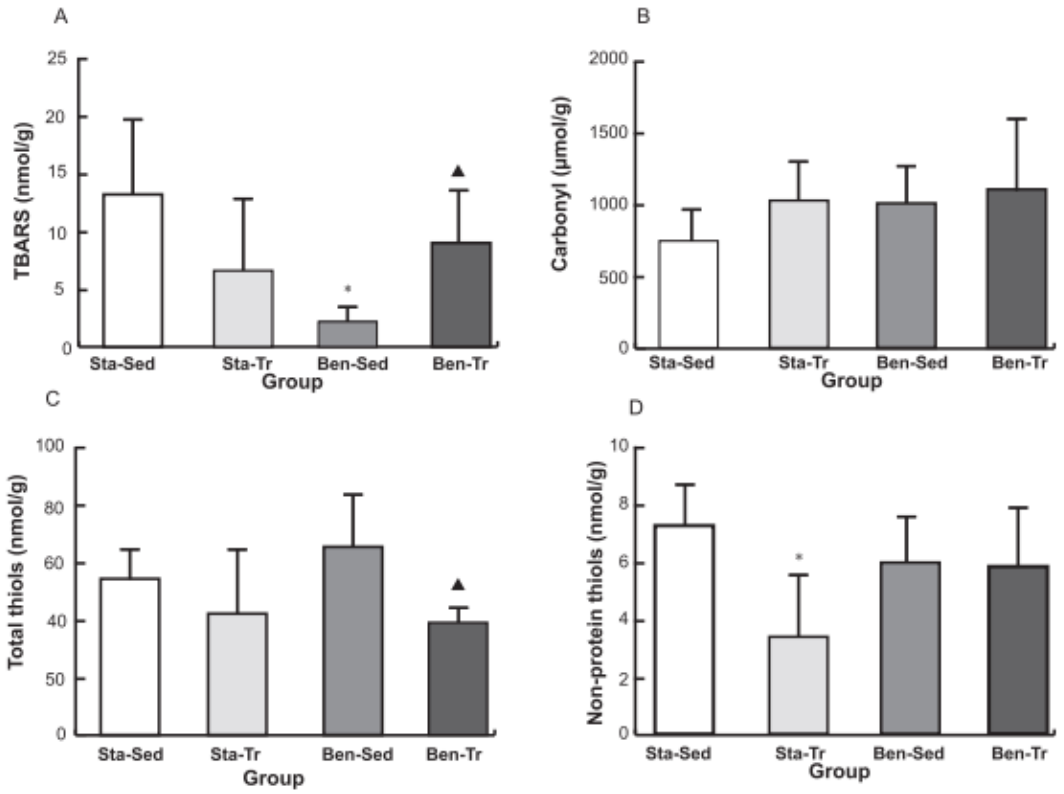

Fig. 2. Oxidative stress markers in the heart. (A) thiobarbituric acid reactive substance (TBARS) concentration; (B) carbonyl content; (C) total thiol concentration; (D) nonprotein thiol concentration. n = 6 in Sta–Sed; n = 6 in Sta–Tr; n = 6 in Ben–Sed; n = 7 in Ben–Tr. *P < 0.05, vs Sta–Sed; ▲P < 0.05, vs Ben–Sed. Sta–Sed: standard diet and sedentary activity group; Ben–Sed: benfotiamine and sedentary activity group; Sta–Tr: standard diet and training group; Ben–Tr: benfotiamine and training group.

3.4. Oxidative stress markers in the liver

Concentration of TBARS in the liver did not differ between the four groups, with values of 10.65 ± 3.88 nmol/g, 12.73 ± 4.14 nmol/g, 8.88 ± 3.62 nmol/g and 11.86 ± 2.30 nmol/g, in the Sta-Sed, Sta-Tr, Ben-Sed and Ben-Tr groups, respectively (Fig. 3A). The Ben-Sed group had lower carbonyl content than the Sta-Sed group (158.66 ± 58.75 *vs.* 270.17 ± 76.53 µmol/g). The carbonyl concentration of the Sta-Tr and Ben-Tr groups was not different (261.91 ± 76.92 and 177.04 ± 60.93 µmol/g, respectively; Fig. 3B).

Both the total thiol and non-protein thiol analyses revealed no differences between the groups. The total thiol concentration in the Sta-Sed, Sta-Tr, Ben-Sed and Ben-Tr groups was 313.02 ± 86.40 nmol/g, 308.80 ± 38.28 nmol/g, 319.94 ± 66.86 nmol/g and 350.16 ± 34.87 nmol/g, respectively (Fig. 3C), whereas the non-protein thiol concentration was 31.83 ± 15.55 nmol/g, 33.64 ± 7.32 nmol/g, 28.93 ± 9.56 nmol/g and 43.21 ± 8.91 nmol/g, respectively (Fig. 3D).

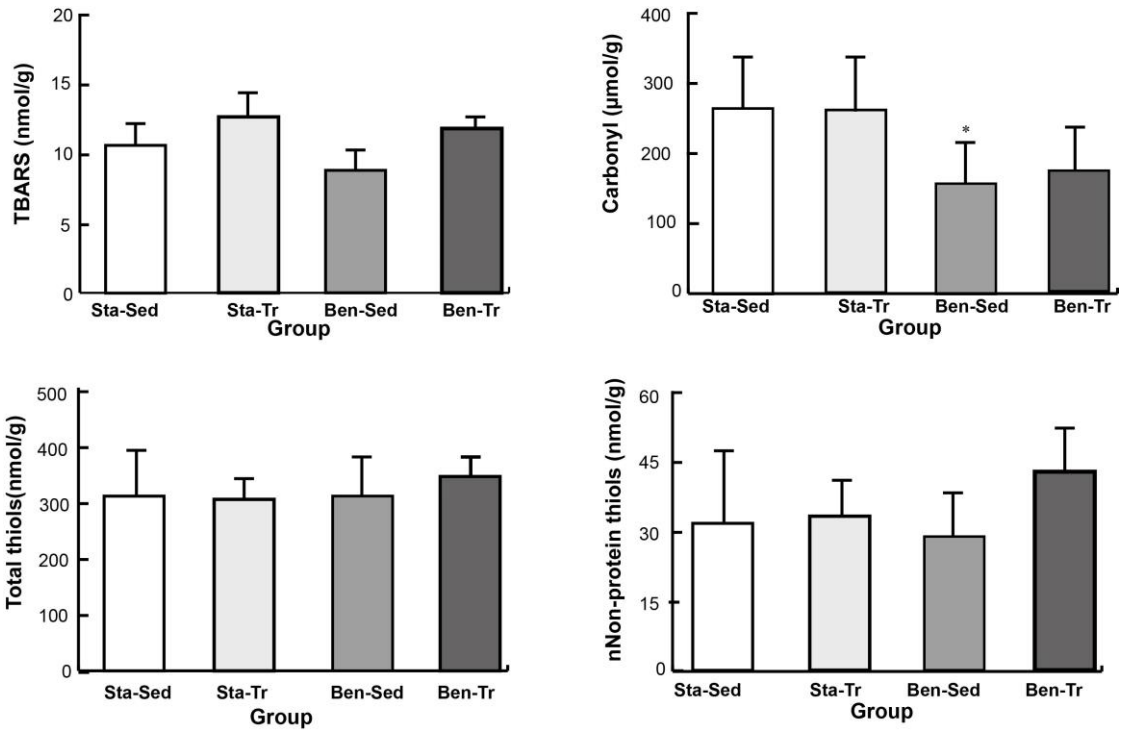

Fig. 3. Oxidative stress markers in the liver. (A) thiobarbituric acid reactive substance (TBARS) concentration; (B) carbonyl content; (C) total thiol concentration; (D) nonprotein thiol concentration. n = 6 in Sta–Sed; n = 6 in Sta–Tr; n = 6 in Ben–Sed; n = 7 in Ben–Tr. *P < 0.05, vs Sta–Sed. Sta–Sed: standard

diet and sedentary activity group; Ben–Sed: benfotiamine and sedentary activity group; Sta–Tr: standard diet and training group; Ben–Tr: benfotiamine and training group.

4. Discussion

The results of the present study revealed the antioxidant effect of oral supplementation with benfotiamine in tissues that have increased metabolic activity during exercise. As thiamine plays an antioxidant role, it was hoped that benfotiamine supplementation would enhance antioxidant defenses and reduce oxidative damage. This would be an important feature, as ROS activity is related to premature fatigue and the enhancement of the antioxidant system could aid fatigue resistance [6,8].

The main oxidative damage markers, TBARS and carbonyl content, in the tibialis muscle were higher in the Sta-Sed group than in the other groups. Thus, benfotiamine reduced lipid peroxidation and protein damage in the muscles of supplemented animals; however, the swimming training did not promote oxidative damage. In practice, endurance training promoted some defenses against oxidative damage. In support of the present data, Miyazaki et al. [4] showed that endurance training prevents lipid peroxidation, reporting higher serum TBARS concentration before a training period. Increased oxidative stress during endurance training triggers a cascade of biomolecular events that induce adaptation against oxidative damage [10].

The thiol concentration data support the antioxidant potential of benfotiamine. Of the studied groups, animals in the Ben-Sed group showed higher levels of antioxidants, confirming that benfotiamine supplementation may increase the thiol concentration in muscle; however, the high production of ROS during exercise increases antioxidant consumption. According to Leeuwenburgh et al. [24], GSH levels, the main component of thiols, are dependent on its utilization and recycling during exercise. Bejma and Ji [5] reported reduced GSH concentration and increased GSSG in rats subjected to endurance exercise, confirming an increase in pro-oxidant activity during exercise.

Lipid peroxidation mainly affects cell membranes, whereas protein damage can impair enzyme function and damage protein structure. Thus, increased levels of TBARS

and carbonyls in muscle indicates significant cellular damage that may affect cell and/or organelle function. As mitochondrial metabolism is the main source of ROS during endurance exercise, mitochondrial structures may be subject to the most damage. If damage occurs in these organelles, energy production will be impaired, which is harmful to exercise performance, recovery and adaption [25]. In addition, damage to proteins can lead to the disruption of cell signaling events that are involved in the processes of cell death and survival [26]. Indeed, Abadi et al. [9] showed that supplementation with antioxidant vitamins preserved mitochondrial function and running performance in mice.

As exercise increases metabolic demand in the myocardium, the results observed in the heart were not greatly different to those found in the muscle tissue. Unexpectedly, the animals in the Ben-Sed group that were supplemented without training showed lower levels of lipid peroxidation markers. These results reinforce the antioxidant potential of benfotiamine, which surpassed the antioxidant adaptations promoted by training. Although the concentration of TBARS showed favorable results in the supplemented animals, the damage to proteins in the myocardium was not different among groups.

The results of the total thiol and non-protein thiol concentrations in the heart were favorable after supplementation with benfotiamine. The trained groups presented lower levels of thiols than their respective sedentary groups, which demonstrated that, similar to the muscle, exercise increases the demand for antioxidants in the myocardium. However, the Ben-Sed group presented a greater concentration of total thiols, supporting the capacity of benfotiamine to increase or preserve antioxidants.

A study by Gioda et al. [12] showed that thiamine deficiency increased the concentration of oxidative damage markers in myocytes; the authors also indicated the involvement of disturbances in the redox state and cardiac dysfunction [12]. The heart has a critical role in supplying oxygen and nutrients to the muscles during physical activity, thus preserving adequate cardiac function is indispensable for the maintenance or optimization of athletic performance [27]. Indeed, one of the main objectives of endurance training is to improve the heart pump [28]. As such, it is fundamental to preserve the cellular structures in the myocardium that combat oxidative damage in individuals engaging in endurance training programs.

In contrast to the results obtained in the tibialis muscle and heart, the data for the liver revealed no significant effects, either in response to training or benfotiamine supplementation. The results showed that oxidative damage to proteins could be reduced, in animals receiving supplement but not training. In situations that are known to increase in oxidative stress in the liver, oral supplementation with vitamin B1 analogs has also shown efficacy in ameliorating damage to macromolecules [14,29].

During exercise, the oxidative metabolic demand on the liver is lower than in skeletal muscle or the myocardium. In addition, hepatic tissue has a greater concentration of antioxidants, which may contribute to the lack of changes in the redox state [30]. Studies that evaluated the hepatic response to acute exercise demonstrated only small effects from exercise on oxidative stress markers [31,32]. Furthermore, hepatic antioxidant adaptations due to endurance training were not very pronounced. Silva et al. [33] demonstrated that 4 weeks of endurance training did not promote antioxidant adaptations in hepatic tissue, although training for 8 weeks increased the activity of the enzyme superoxide dismutase and the total thiol concentration.

The oral intake of benfotiamine has advantages compared to thiamine hydrochloride intake. In the intestine, benfotiamine is converted to S-benzoylthiamine, a fat-soluble thiamine analog [34]. Although thiamine salts require saturable transporters to cross biological membranes, S-benzoylthiamine reaches the bloodstream by passive diffusion. In several tissues, enzymatic and non-enzymatic reactions form thiamine and Thiamine diphosphate (TDP) from the S-benzoylthiamine, increasing the bioavailability of thiamine and its esters [34]. Portari et al. [18] showed that benfotiamine supplementation increased the TDP levels by 25-fold in erythrocytes, whereas the supplementation with thiamine hydrochloride increased only four-fold.

Recent studies of thiamine and exercise have focused on the evaluating its effects on endurance performance. However, several studies have shown the antioxidant effects of thiamine. Thiamine can act directly against reactive species, participate in the recycling of antioxidant molecules [15,35] and share electrons with pro-oxidant species, recovering the redox balance [15].

Thiamine molecules also can participate in metabolic processes that recycle or produce antioxidant species [36,37]. TDP is the coenzyme of transketolase, an enzyme that regulates the activity of the pentose phosphate pathway (PPP). The PPP produces nicotinamide adenine dinucleotide phosphate, which plays a role in GSH and catalase recycling [36,37]. In addition, a high TDP concentration can reduce p53 activity. Normally, the p53 protein inhibits the expression of genes for antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase [38]. Therefore, an increased level of TDP, promoted by benfotiamine supplementation, can reduce p53 activity and increase the gene expression of antioxidant enzymes.

Benfotiamine has shown antioxidant potential in several pro-oxidant situations in both *in vitro* and *in vivo* experiments [14,16,29,39]. Bozic *et al.* [16] reported the significant antioxidant role of benfotiamine in cell culture. The authors showed that cells treated with benfotiamine had increased GSH content and enhanced activity and expression of the main antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase. Other studies showed that supplementation with thiamine analogs reduced oxidative damage in the liver of rats subjected to alcoholism [14,29,39].

The present results show the antioxidant potential of benfotiamine. However, swimming training did not promote expression changes in the redox state. Therefore, further investigations are needed to confirm the results. Prolonged supplementation protocols, as well as more refined training protocols, may potentiate responses and adaptations to oxidative stress. In addition, other tissues should be evaluated, especially muscle tissues with a predominance of aerobic metabolism, as the anterior tibial muscle is composed primarily of glycolytic fibers.

5. Conclusion

The present study shows that benfotiamine is an efficient antioxidant in the anterior tibialis muscle and heart of mice. However, we were unable to induce oxidative damage with endurance swimming training; thus, antioxidant supplementation was unnecessary. Benfotiamine supplementation can be considered as an adjuvant nutritional

strategy to mitigate the negative effects of situations that induce oxidative stress but needs further testing in animal model that successfully induces oxidative stress.

Acknowledgments

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Declarations of interest

The authors declare that they have no competing interests.

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Artigo 2: The B-complex vitamins related to energy metabolism and their role in exercise performance: a narrative review.

Situação: Aceito para publicação no periódico *Sports & Science* (ISSN:07651597).

De: Guilherme Vannucchi Portari
Enviado:domingo, 22 de novembro de 2020 19:49
Para: Álisson Gonçalves
Assunto: En: SCISPO - Your Submission

------ Mensagem encaminhada ------
De: "Science & Sports" <em@editorialmanager.com>
Para: "Guilherme Vannucchi Portari" <guilherme.portari@uftm.edu.br>
Enviado(s): 20/11/2020 14:12:32
Assunto: SCISPO - Your Submission

Ms. Ref. No.: SCISPO-D-20-00340R1
Title: The B-complex vitamins related to energy metabolism and their role in exercise performance: a narrative review.
Science et Sports

Dear Dr Guilherme Vannucchi Portari,

I am pleased to confirm that your paper "The B-complex vitamins related to energy metabolism and their role in exercise performance: a narrative review." has been accepted for publication in Science et Sports.

Thank you for submitting your work to this journal.

If you are available to continue to work with the journal as reviewer, we invite you to go to the website http://ees.elsevier.com/scispo, clic on « change details » and « available as a reviewer », and precise your specialty. The editorial board thank you in advance.

With kind regards,

Jean-Frédéric Brun, MD, PhD, Hab Dir Res
Editor / Redacteur
Science et Sports

Comments from the Editors and Reviewers:

The B-complex vitamins related to energy metabolism and their role in exercise performance: a narrative review.

Les vitamines du complexe B liées au métabolisme énergétique et leur rôle dans la performance physique: revue narrative.

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Abstract

Objective: The present review aims to clarify the relationship of thiamine, riboflavin, and niacin to exercise performance.

News: The B vitamins thiamine (vitamin B_1), riboflavin (vitamin B_2), and niacin (vitamin B_3) are hydrophilic vitamins that play a fundamental role as coenzymes in enzymatic reactions of energy metabolism. The increase in energy demand during physical exercise can alter the requirements for these vitamins.

Prospects and projects: This study is a narrative review which selected and analyzed studies that investigated how the vitamins thiamine, riboflavin, and niacin can affect sports performance in both humans and experimental animal models.

Conclusion: The research shows that supplementation with these vitamins does not necessarily produce ergogenic effects. Despite their fundamental roles in anabolic pathways, only the intake of thiamine-derivative substances showed some positive effects on exercise performance and fatigue. Isolated riboflavin supplementation did not show positive effects, while there is evidence that high niacin intake can harm exercise performance. Some studies found an increase in excretion and a decrease in the blood levels of these vitamins after exercise, but the results are

inconsistent. However, different pharmacokinetics, doses or dosing frequency, and administration route of the supplemented substances can influence the results. There are few studies of B-vitamins and exercise, and most of them are not recent. New studies, with systematically controlled protocols, are necessary to elucidate the real effects of thiamine, riboflavin, and niacin in exercise performance.

Keywords: thiamine, riboflavin, niacin, athletic performance, exercise.

Résumé

Objectifs: La présente revue vise à clarifier la relation entre la thiamine, la riboflavine et la niacine avec la performance physique.

Actualité: Les vitamines B, la thiamine (vitamine B1), la riboflavine (vitamine B2) et la niacine (vitamine B3) sont des vitamines hydrophiles qui jouent un rôle fondamental en tant que coenzymes dans les réactions enzymatiques du métabolisme énergétique. L'augmentation de la demande d'énergie pendant l'exercice physique peut modifier les besoins de ces vitamines.

Perspectives et projets: Cette étude est une revue narrative, qui a sélectionné et analysé des études qui ont examiné comment les vitamines thiamine, riboflavine et niacine peuvent affecter les performances sportives dans le modèle expérimental humain et animal.

Conclusion: La recherche montre que la supplémentation en ces vitamines ne montre pas nécessairement d'effets ergogéniques. Malgré leur rôle fondamental dans les voies anabolisantes, seule la prise de substances dérivées de la thiamine a montré des effets positifs sur les performances physiques et la fatigue. La supplémentation en riboflavine isolée n'a pas montré d'effets positifs, alors qu'il existe des preuves qu'une consommation élevée de niacine peut nuire aux performances de l'exercice. Certaines études ont révélé une augmentation de l'excrétion et une diminution des taux sanguins de ces vitamines, mais les résultats sont incohérents. Cependant, différentes pharmacocinétiques, doses ou fréquences et voies d'administration des substances supplémentées peuvent interférer

dans les résultats. Il existe peu d'études sur les vitamines B et l'exercice, et la plupart d'entre elles ne sont pas récentes. De nouvelles études, avec des protocoles systématiquement contrôlés, sont nécessaires pour élucider l'effet réel de la thiamine, de la riboflavine et de la niacine dans la performance de l'exercice.

Mots clés: la thiamine; riboflavine; niacine; performance physique; exercice.

1. Background

Vitamins are essential organic compounds necessary in small amounts (micro or milligrams) per day to maintain the metabolic integrity of organisms. Although vitamins cannot be transformed into energy directly, some are fundamental to converting macronutrients to energy. For example, the B vitamins thiamine (vitamin B_1), riboflavin (vitamin B_2) and niacin (vitamin B_3) play essential roles as cofactors in enzymatic reactions of anabolic pathways to produce energy in the Krebs cycle and electron transport chain during metabolism of carbohydrates, lipids, and amino acids[1].

It is known that exercise increases the energy demand of the body, according to the intensity, duration, and type of effort. Because thiamine, riboflavin, and niacin have fundamental roles in energy metabolism, it is plausible that changes in the levels of these vitamins could interfere with physical performance. If deficiency of these vitamins interferes with the metabolism of macronutrients and harms energy production, then it is possible that vitamin supplementation could enhance energy metabolism during exercise and increase performance. Thus, this review aims to clarify the relationship of thiamine, riboflavin, and niacin to exercise performance. For this purpose, we first review some basic characteristics and metabolic functions of these three B vitamins. Then, we present our analyses of published studies that investigated the effects of supplementation with these vitamins and/or their analogs on physical performance, as well as the levels of these vitamins and their products measured in different tissues of individuals engaged in physical exercise training programs.

2. Thiamine

In the nineteenth century, a growing prevalence of beriberi in Asia motivated research into the disease and the discovery of thiamine. Dr. Christiaan Eijkman observed that chickens fed polished rice showed symptoms similar to human beriberi, while animals fed unpolished rice did not show such symptoms. From these observations, Gerrit Grijns and Fraser and Stanton concluded that polished rice lacks an organic nutrient, and this could be extracted from parboiled rice or rice bran. In 1926, Roger Williams extracted about 100 grams of thiamine from 600 kg of rice bran[2].

Known also as vitamin B_1, thiamine is synthesized in plants, fungi, and bacteria but its dietary intake is essential for the energy balance of mammals[3]. The chemical name of thiamine is 3-(40-amino-20-methyl-pyrimidinyl-50-methyl)-5-(2-hydroxyethyl)-4-methylthiazoliumchloride hydrochloride and its empirical formula is C12H17N4OSCl.HCl[2].

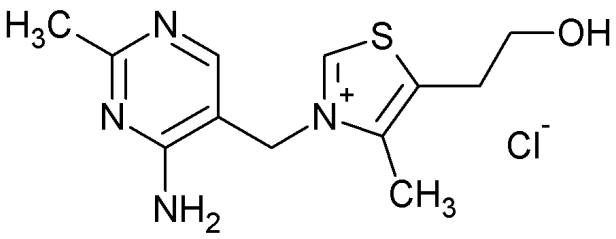

Figure 1: Thiamine structural formula.

Once absorbed by the intestine, thiamine is phosphorylated in cells by thiamine phosphokinase and converted to thiamine diphosphate (TDP). TDP is the biologically active form and a key cofactor for the proper function of certain enzymes in energy metabolism[4].

The pyruvate dehydrogenase complex (PDH) depends on TDP to promote the oxidative decarboxylation of pyruvate and produce acetyl-CoA, essential for the Krebs cycle. TDP is also a cofactor for α-ketoglutarate dehydrogenase activity, which promotes

the decarboxylation of α-ketoglutarate within the Krebs cycle, favoring adenosine triphosphate (ATP) production by glucose oxidation[5,6]. Furthermore, thiamine is important for normal transketolase activity, a regulatory enzyme of the pentose phosphate pathway[7].

Thiamine is a water-soluble vitamin which does not accumulate in the body, thus requiring daily intake[4]. For healthy adults, the Recommended Dietary Allowance (RDA) suggests 1.2 mg/day for healthy male adults[8]. Thiamine sources are meats, especially pork, wheat germ, liver, poultry, eggs, fish, beans, peas, nuts, and whole grains. Coffee and some teas contain polyphenols that can inactivate ingested thiamine[4].

Chronic alcoholism or inadequate nutritional intake can impair absorption and utilization of thiamine[9]. The first disease correlated with thiamine deficiency was beriberi[10]. However, thiamine deficiency is also correlated with Wernicke–Korsakoff syndrome[11–13], Alzheimer's disease, and Parkinson's disease [14–16]. Some evidence has associated thiamine deficiency with heart failure [17,18].

3. Riboflavin

In 1879, the English chemist A. Wynter Blyth isolated from cow milk a yellow-orange substance he called lactochromo. Subsequently, other scientists isolated the same substance from numerous sources and named the factor responsible for the fluorescent property riboflavin. They also established the vitaminic roles of riboflavin, but it was not until 1935 that Karrer and Kuhn synthesized it. Theorell in 1937 and Warburg and Christians in 1938 established the chemical structure of the coenzymes formed from riboflavin, flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD), respectively[19].

Riboflavin, or vitamin B_2, is a water-soluble vitamin composed of an isoalloxazine ring, referred to as 7,8 dimethyl-10-(-1-D-ribityl). Free riboflavin is not the active form, but it is the precursor of both FMN and FAD, the coenzymatically active forms. FMN is formed in an ATP-dependent flavokinase reaction. FAD is formed from FMN in a reaction catalyzed by FAD synthetase [20,21]. The biosynthesis of the flavin coenzymes from riboflavin is controlled by thyroid hormones[22].

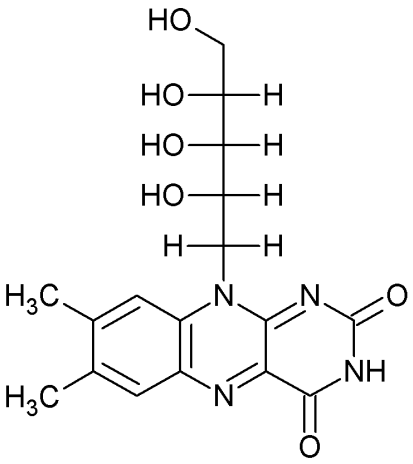

Figure 2: Riboflavin structural formula.

The two flavin coenzymes participate in redox reactions for intermediary metabolism. They accept or donate one or two electrons in reactions catalyzed by dehydrogenases, oxidases, and monooxygenases[20,22]. Within the Krebs cycle, FAD is a key coenzyme in the succinate dehydrogenase reaction, in which succinate is converted to fumarate, and FAD is reduced to $FADH_2$. In the electron transport chain, $NADH_2$ is oxidized into NAD by the FMN-dependent NADH-dehydrogenase. FAD is also a coenzyme in the first step of the β-oxidation in which acyl-CoA is converted to enoyl-CoA[23].

The absorption of flavins occurs in the upper gastrointestinal tract. This process involves a dephosphorylation–rephosphorylation mechanism, a sodium-dependent system[24–26]. In human blood, the flavins are transported bound to albumin and several globulins. There is little free riboflavin in erythrocytes but the cells do contain large amounts of FMN and FAD. Riboflavin is present in urine, whereas FMN and FAD are not [21,27].

Plants and some microorganisms can synthesize riboflavin. However, it is an essential nutrient for human health and is normally supplied by the diet. The RDA committee suggests for healthy adults an intake of 1.3 and 1.1 mg per day for men and women, respectively[8]. Sources of riboflavin are milk, other dairy products, eggs, lean meat, and leafy vegetables. Riboflavin deficiency can result in dry skin and fissuring of

the lips (cheilosis) and injury of the mouth (angular stomatitis), painful desquamation of the tongue, and seborrheic dermatitis. In addition, it can cause conjunctivitis and may progress to cataracts[20].

4. Niacin

The early studies of niacin were propelled by efforts to treat pellagra. This disease was correlated with corn consumption, because of the high incidence of pellagra in countries with corn as the staple food[28]. In 1937, it was discovered that nicotinic acid, a substance missing from corn, relieves pellagra symptoms in dogs[29]. Other scientists had suggested that tryptophan present in some proteins could prevent the disease, and tryptophan is a precursor of niacin[30].

Niacin, formerly vitamin B_3, is a water-soluble vitamin. The term “niacin” can describe both nicotinic acid and nicotinamide. They share a basic structure of pyridine-3-carboxylic acid. Free niacin is not the active form but serves as a precursor of nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), the coenzymatically active forms[1,2].

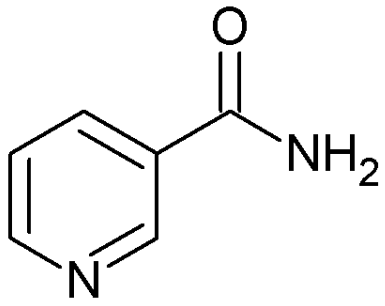

Figure 3: Nicotinamide structural formula.

Niacin coenzymes have a fundamental role in energy metabolism. NAD and NADP are involved in reduction reactions in glycolysis, β-oxidation, pentose shunt, Krebs cycle, and the electron transport chain. In glycolysis, NAD is an acceptor of electrons in a reaction catalyzed by 3-phospho-glyceraldehyde-dehydrogenase. In β-

oxidation, NAD is an acceptor of two H+ in reactions catalyzed by β-hydroxyacyl-CoA dehydrogenase[31]. In the Krebs cycle, the niacin coenzymes accept electrons from four reactions and transfer H^+ to the electron transport chain. NAD and NADP also participate in fat and protein synthesis[1,2,23].

Due to the key role of niacin in intermediary metabolism, the RDA guidelines suggest 6.6 mg/1000 kcal consumed. Tryptophan intake is a good way to obtain niacin, and the RDA guidelines propose 400 mg/1000 kcal be consumed when there is no niacin intake. The principal dietary sources of niacin and tryptophan are meat, fish, cereals, leguminous plants, and seeds[8].

Although niacin is quickly absorbed in the small intestine, it also can be absorbed in the stomach. In small concentrations, niacin is absorbed by sodium-dependent facilitated diffusion, whereas high concentrations can be absorbed by passive diffusion[23].

5. Thiamine, riboflavin, and niacin in exercise performance

As mentioned earlier, thiamine, riboflavin, and niacin are key coenzymes in energy metabolism. They participate in numerous reactions such as glycolysis, β-oxidation, pentose shunt, Krebs cycle, and the electron transport chain[32]. In humans, the principal aim of these metabolic pathways is to produce ATP to supply chemical energy[33].

Physical activity is a phenomenon that increases the body's energy demand. The amount of energy necessary to maintain or elevate effort is regulated by the intensity, duration and type of exercise, and individual fitness[34]. Many strategies have been applied to optimize energy production and consumption, especially in competitive sports. Diet manipulation, ergogenic clothes or footwear, and dietary supplements are legal ways to improve performance[35]. But interest in the ergogenic effects of vitamins and how they can enhance athletic performance is increasing[36].

In a study[37] which investigated how thiamine deficiency can influence exercise performance, scientists fed rats a thiamine-deficient diet and subjected the animals to maximal swimming tests, and found a significant reduction in exercise performance.

After restoring the thiamine levels, the rats promptly recovered their physical fitness[37]. Some authors proposed that thiamine deficiency can increase the concentration of lactate by decreasing pyruvate dehydrogenase activity[38]. Studies showed a positive correlation between lactate and muscle fatigue and affirmed that thiamine deficiency can promote early fatigue[39–41].

A study[42] in which female athletes were supplemented with 10 mg/kg of thiamine tetrahydrofurfuryl disulfide (TTFD) during four weeks showed an anti-fatigue effect of the thiamine derivative. Another study[43] in a rat model found that TTFD supplementation increased thiamine ester levels and ATP production in several organs and reduced fatigue during a forced maximal swimming test. The authors hypothesized that the elevated level of TDP, induced by TTFD supplementation, could enhance ATP re-synthesis and glycogenolysis in exercised skeletal muscle, thus delaying exercise-induced fatigue[43]. Huang et al.[44] showed that TTFD supplementation for four weeks increased the exhaustive swimming time and the forelimb grip strength in male mice. The TTFD supplementation was associated with decreased serum creatine kinase activity and increased glycogen levels in muscle and liver[44].

In humans, a cross-over study concluded that athletes with normal thiamine levels, supplemented with 100 mg/day of TTFD, had a lower increase in blood glucose and fewer complaints after exercise-induced fatigue[45]. In a similar study, the authors showed that individuals who received high-dose TDP had lower blood glucose immediately after thiamine consumption compared to the TDP low-dose supplementation and placebo groups, demonstrating thiamine participation in glucose metabolism[46]. In fact, TDP high-dose supplementation (300 mg/day) in non-athletes was found to be more effective in preventing fatigue versus low-dose supplementation (30 mg/day) or placebo[46].

When TDP or placebo was given intravenously to athletes for 30 days in a double-blind, randomized, cross-over study, the TDP-supplemented group showed lower levels of serum lactate, lower heart rates, and higher maximal oxygen consumption (VO_2max) after aerobic physical activity than the placebo group[47]. However, three studies that used different thiamine derivates and oral supplementation found no benefit. In the first, another randomized, double-blind, cross-over study, TTFD supplementation did not influence high-intensity exercise performance and its markers in a time trial test on a

cycle ergometer[48]. Similarly, two studies that used another thiamine derivative with better absorption showed that allithiamine supplementation was ineffective at improving exercise performance[49,50].

In an investigation of the anti-fatigue effect of another thiamine derivative, dicethiamine hydrochloride (DCET), rats were submitted to fatigue stress during five days and to a maximal swimming test at the end — a fatigue model which combines mental and physical stress[51]. The animals that received oral DCET swam for a longer time than animals given vitamin B_1 or placebo. The same study also showed that DCET ingestion increased free thiamine, TMP and TDP levels in the brain, thigh muscle, and blood, whereas vitamin B_1 did not show the same effect. The authors concluded that thiamine and/or thiamine esters have an anti-fatigue effect[51].

Thiamine intake recommendations are not specific to athletes. Although some evidence indicates that thiamine supplementation can improve exercise performance, the recommendations followed by athletes, who have high daily energy consumption, are the same as those for healthy non-athletes. Numerous assessments of thiamine status found no reduced intake and/or lower thiamine levels in athletes compared with healthy individuals[52–54]. Furthermore, a study demonstrated lower thiamine urinary excretion in exercised rats compared to sedentary animals, suggesting that the exercise adaptations increase thiamine requirements[55].

The published evidence for an ergogenic effect of thiamine and its analogs does not reach a scientific consensus. Heterogeneity of the experiments and the number of different thiamine analogs tested contribute to the lack of consensus. Moreover, we found no meta-analysis or systematic review about “thiamine and exercise performance” in our literature search. This may reflect a paucity of evidence in support of this physiological link. Five studies can be considered as level 2 in terms of evidence, and another six studies can be considered as level 3, according to the Oxford Centre for Evidence-Based Medicine[56]. Several studies with human participants used a randomized, cross-over, and double-blind design, which contributes to the trustworthiness of the information. It is important to consider that six studies used animal experimental models in seeking to clarify the physiological mechanisms related to the therapeutic/ergogenic potential of thiamine and its derivatives.

Riboflavin is a key cofactor of oxidative enzymes and therefore considered important for aerobic performance[57]. In rats subjected to hypoxic conditions, riboflavin supplementation improved energy metabolism. Under hypoxia without riboflavin supplementation, rats experienced increases in plasma pyruvate, lactate, β-hydroxybutyrate, and urea, and decreases in plasma carnitine and liver riboflavin levels; however, with riboflavin supplementation, reduced levels of plasma pyruvate, lactate, and β-hydroxybutyrate were observed[58]. Riboflavin's participation in energy metabolism and its role in physical exercise, including its potential benefits in athletic performance, have been investigated but the results are inconclusive [7,23,59].

In an evaluation of 14 older women during 10 weeks of training and riboflavin supplementation, VO_2max and anaerobic threshold were measured. The results suggest that riboflavin supplementation does not improve endurance performance[60]. Similar results were found in swimmers: the athletes received 60 mg/day riboflavin supplementation, but no significant differences in swim performance, VO_2max and anaerobic threshold in a treadmill test were observed between the supplemented and placebo groups[61].

Although some studies have demonstrated that riboflavin supplementation is ineffective in improving aerobic performance[60,62,63], one study confirmed that exercise training increases the requirement for riboflavin[64]. Six adult men deficient in riboflavin intake were submitted to additional exercise for 18 days and showed a significant reduction in riboflavin status, suggesting an increase in riboflavin demand[64]. However, another study[52] did not find alterations in riboflavin status after a 24-week fitness-type exercise program. Rats submitted to an 8-week endurance exercise regimen on a treadmill did not show an increase in their dietary riboflavin requirement[65]. In accordance, a more recent study found no difference in riboflavin urinary excretion between trained and sedentary rats[55].

There are few investigations about the ergogenic effects of riboflavin supplementation. None involved a controlled trial, and there is no systematic review. All six studies with humans can be considered as level 3 evidence, according to the classification of the Oxford Centre for Evidence-Based Medicine[56]. Moreover, only two studies used an animal model and no riboflavin derivatives were tested, which is

limiting in terms of data about physiological and biochemical responses. Differences in the type of exercise, the exercise training level of the participants, and the duration of exercise training applied in these studies do not permit a consensus view about the effect of riboflavin on exercise performance. Thus, further and stronger scientific investigations are necessary to improve our knowledge of the role of riboflavin in sports performance and its importance for individuals engaged in physical training programs.

NAD^+ participates in the generation of 28 of the 36 ATPs produced in the complete oxidation of 1 molecule of glucose. Due to its key role in energy metabolism, niacin is commonly associated with exercise performance[32]. Although only a few studies have investigated the matter, they showed that niacin supplementation is not effective for improving athletic performance.

One study[66] subjected ten individuals to nicotinic acid (NA) and carbohydrate/electrolyte (CE) supplementation during a 120-minute cycling exercise bout at 68% VO_2max followed by a 3.5-mile (5.6-km) performance task. The performances in the 3.5-mile task in the NA supplementation-, NA/CE supplementation-, and placebo groups were not different. Blood sample analysis indicated an alteration in hormonal responses to exercise due to a blunting in free fatty acids (FFA)[66]. Likewise, another study showed a reduction in lipolysis in runners submitted to a submaximal treadmill test after NA treatment. Their respiratory exchange ratio was higher after NA supplementation, while the FFA and glycerol levels were lower[67]. These data suggest there is inhibition in fat utilization during exercise caused by NA supplementation. However, the studies on the effect of niacin supplementation on exercise performance are insufficient to substantiate a role of niacin in exercise metabolism. The two studies that investigated the issue can be considered as level 3 in terms of evidence[56]. The available data fall short for establishing a study with a higher level of evidence, such as a systematic review, thus reinforcing the need for further investigations.

Few studies have investigated the role of various vitamins and minerals alongside physical performance. Van der Beek et al.[68] exposed adults to a low intake of thiamine, riboflavin, and vitamins B_6 and C for 8 weeks and found a significant decrease in VO_2max and an increase in blood lactate levels, suggesting that insufficient vitamin intake can reduce athletic performance[68]. Another study[69] restricted thiamine, riboflavin, and

vitamin B_6 intake and found a decrease in VO_2max and an earlier onset of blood lactate accumulation, which indicate a reduction in aerobic power and endurance, respectively. The authors[69] correlated the results to the important role of these vitamins in the oxidation of fatty acids. The discussions of both studies [68,69] emphasized the important role of thiamine and riboflavin in the adequate function of carbohydrate metabolism, β-oxidation, and the respiratory chain, and a deficiency of these vitamins can promote premature lactate accumulation and reduce mitochondrial efficiency.

A coformulation of propionyl-L carnitine, coenzyme Q10, nicotinamide, riboflavin, and pantothenic acid was administered orally to Wistar rats and their motor functions were tested. The results showed that the velocity of horizontal movement of the supplemented group was about 20% superior to that of the control group. However, in their discussion, the authors gave special attention to the possible effects of propionyl-L carnitine and coenzyme Q10 and did not elaborate on the role of nicotinamide in aerobic metabolism[70].

A beverage containing multiple micronutrients showed an ergogenic effect in Indian children. The participants ingested 40 g/day of a powder that provided thiamine, riboflavin, niacin, vitamins A, B_6, B_{12}, C and D, folate, biotin, pantothenic acid, calcium, iron, copper, iodine, magnesium, and zinc or a placebo beverage for 120 days. The study reported that the supplement had an ergogenic effect in the 20-m shuttle test and in predicted VO_{2max}, but not in handgrip, 40-m sprint, visual reaction, and static endurance tests[71].

6. Conclusion and prospects

Thiamine, riboflavin, and niacin, evidently play an important role in energy production during physical exercise. However, supplementation with these vitamins has not shown ergogenic effects, despite trained individuals having shown lower levels of some of these vitamins. The inconclusive results may be related to the pharmacokinetics, doses or dosing frequency, and routes of administration of the supplemented substances. Furthermore, interactions with other substances could hide the true effect of the vitamins in a maximum effort bout or exercise performance.

Only a few studies have investigated the relationship between riboflavin or niacin and exercise performance, whereas many studies have studied thiamine and exercise; nonetheless, the conclusions are inconsistent. In addition, most studies are not recent, and few studies investigated the effect of different analogs of the same vitamin, such as benfotiamine (a thiamine derivative). Furthermore, studies using isolated forms of niacin and riboflavin are scarce, so more research is needed in this area.

Overall, the level of evidence is low, since there is no systematic review or randomized trial with a meaningful number of participants. It is important to consider that the available data about the effects of riboflavin and niacin on exercise performance are not enough to support any study with a high level of evidence, such as a systematic review study. Thus, new studies, especially with human participants, with a systematically controlled protocol, such as an individualized randomized trial, are necessary to elucidate the real effect of these vitamins (thiamine, riboflavin, and niacin) on exercise performance.

Disclosure of interest: The authors declare that they have no competing interest.

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Artigo 3: Oral benfotiamine supplementation increases thiamine and thiamine phosphates in erythrocyte and gastrocnemius muscle but have no anti-fatigue effect in endurance-trained mice.

Situação: finalizado e formatado para submissão no periódico Applied Physiology, Nutrition and Metabolismo.

Oral benfotiamine supplementation increases thiamine and thiamine phosphates in erythrocyte and muscle but have no anti-fatigue effect in endurance-trained mice.

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Abstract

Objective: This study aimed to analyze the effects of oral benfotiamine supplementation on the function of the Krebs cycle in the muscle of endurance-trained mice and on its effects on endurance performance.

Methods: Twenty-five male BALB/c mice were allocated to the following treatment groups: standard diet and sedentary activity (Sta-Sed), benfotiamine-supplemented diet and sedentary activity (Ben-Sed), standard diet and training activity (Sta-Tr) and benfotiamine-supplemented diet and training activity (Ben-Tr). The training comprised six weeks of endurance swimming and it was applied an exhaustive exercise test at the end. Plasma lactate concentration was evaluated before and after the exhaustive test. The concentration of free thiamine, thiamine monophosphate, and thiamine diphosphate were analyzed in erythrocytes and in gastrocnemius muscle. It was analyzed the gene expression of pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase components in gastrocnemius muscle. The concentration of pyruvic, lactic and hydroxybutyric acid in gastrocnemius muscle was analyzed.

Results: The supplemented groups showed high levels of free thiamine, thiamine monophosphate, and thiamine diphosphate than standard-diet animals, in both erythrocytes and muscle tissues. There was no difference in the gene expression. Ben-Tr group showed higher concentration of muscle lactic acid than sedentary groups. The lactic: pyruvic acid ratio was higher in trained groups than Sta-Sed. The concentration of hydroxybutyric acid was higher Ben-Tr animals than Sta-Sed. There was no difference in plasma lactate concentration and in endurance capacity.

Conclusion: Oral benfotiamine supplementation is an efficient strategy to increase the concentration of thiamine and thiamine phosphates in erythrocyte and muscle but does not affect the gene expression pattern of thiamine-dependent enzymes. The benfotiamine supplementation increases the pyruvate catabolism in trained mice, but does not affect endurance performance.

Keywords: benfotiamine; thiamine; endurance exercise; energy metabolism.

Introduction

Physical exercise affects the energy demand by the cells, mainly in the active muscle tissues. Thus, adequate nutrient intake is essential to enhanced energy metabolism and tissue recuperation(Rodriguez et al. 2009). Besides the scientific literature that has focused on the effects of macronutrient intake on endurance performance, evidence suggests that micronutrient deficiency can harm exercise performance and the supplementation with some vitamins or minerals could increase the endurance capacity(Lukaski 2004; Vaz et al. 2011).

Thiamine, know also to vitamin B1, plays an important role in energy metabolism. It could be found in mammal organisms in its free form or the three esterified form, i. e. thiamine monophosphate (TMP), thiamine diphosphate (TDP), and thiamine triphosphate (TTP)(Pácal et al. 2014). In the Krebs cycle, the TDP is a coenzyme of pyruvate dehydrogenase complex (PDH) and α-ketoglutarate dehydrogenase (KGDH) enzymes(Manzetti et al. 2014). It is known that thiamine deficiency can cause neurological disorders (i.e. beriberi, Wernicke Korsakov Syndrome) which are related to default in energy metabolism in neuronal cells(Zubaran et al. 1997; Carpenter 2000).

Besides there are many studies about the issue, there is no scientific consensus about the effects of thiamine supplementation on exercise performance. Study in the which animals were submitted to a thiamine-deficient diet showed a decrease in endurance capacity of the animals and the subsequent supplementation with thiamine restore the thiamine levels and increase the endurance performance(KNIAZUK et al. 1944). The studies with humans have shown that the supplementation with thiamine derivatives could have an anti-fatigue effect and increases endurance performance(Choi et al. 2013). However, studies that supplemented athletes with thiamine analogous with better absorption (i.e. allithiamine) did not show any positive effect(Doyle et al. 1997; Webster 1998).

Benfotiamine is a synthetic analog of vitamin b1, with greater bioavailability than other thiamine salts. Benfotiamine is converted to s-benzoyl-thiamine in the intestinal mucosa and absorbed by passive diffusion, unlike thiamine salts which need specific carriers to cross the intestinal barriers(Portari et al. 2013; Xie et al. 2014). A study showed

that oral benfotiamine supplementation increases 21-times the TDP concentration in erythrocytes compared to thiamine hydrochloride supplementation(Portari et al. 2013).

The evidence suggests that benfotiamine can increase the TDP levels in several tissues(Portari et al. 2013; Xie et al. 2014). Since the TDP is an essential cofactor in energy metabolism, oral supplementation with benfotiamine could increase the TDP concentration in muscle tissue and modulates the function of the energy production pathway, especially in tissues with high energy demand and production. Thus, this study aimed to analyze the effects of oral benfotiamine supplementation on the function of the Krebs cycle in the muscle of endurance-trained mice and on its effects on endurance performance.

Methods

Animals

Twenty-five 6-week-old BALB/c male mice were housed individually in plastic cages and kept under an inverted circadian cycle (12/12 h dark:light), at 22 °C ± 1 °C and 55% ± 5% humidity, with ad libitum access to feed and tap water. The animals were randomly placed into four groups. The standard diet and sedentary group (Sta–Sed,n=6) received AIN-93 growth standard rodent diet and did not train; the standard diet and trained group (Sta–Tr, n = 6) received AIN-93 growth standard rodent diet (Reeves et al. 1993) and was subjected to endurance swimming training. The benfotiamine and sedentary group (Ben–Sed, n = 6) received AIN-93 supplemented with benfotiamine and did not train; the benfotiamine and trained group (Ben–Tr, n = 7) received AIN-93 supplemented with benfotiamine and was subjected to swimming training.

The experimental protocol lasted 7 weeks. The first week was set aside for acclimation to the laboratory environment, AIN-93 standard diet, and isolated cages. The experimental protocol was authorized by the Ethics Committee of Animal Use of the Federal University of Triangulo Mineiro, under protocol number 343/2015.

Training protocol

Animals swam in groups, in a plastic container of 40 cm diameter and 60 cm height, with a tap water depth of 40 cm, maintained at 32 ° C (± 1 °C) controlled by a

heater with automatic thermostat (HOPAR SA-333 Zhong Shan, China) (Chen et al. 2014).

The first training week was an adaptation period, starting with exposure to shallow water on the first day and finished with a 60-min swim on the fifth day. In the second week, the mice swam with an external metal load (1% of body weight) fixed to the proximal portion of the tail, during 60-min/session in 5 sessions/week. The load was increased to 2% of body weight in the following four weeks, and the duration and frequency of training were maintained. The sedentary groups were exposed to shallow water with similar frequency and duration to the training protocol of the trained groups(Chen et al. 2014).

The last training session was destined for the exhaustive exercise test. The mice of the trained groups were forced to an individual swimming session with an external metal load (5% of body weight) attached to the proximal portion of the tail. Animals were considered to be exhausted when they were unable to support the snout on the surface of the water for 8 seconds(Chen et al. 2014).

Determination of lactate concentration

The lactate concentration in peripheric blood was analyzed 5 minutes before and immediately after the exhaustive exercise test. A little incision was made in the distal portion of the tail and 40 μL of blood was collected with a heparinized tube. It was added 8.5 μL of potassium fluoride/EDTA to blood. The mixture was centrifugated at 4000 rpm, for 5 minutes at 4°C. then 10 μL of plasma was used for the analysis of lactate concentration by a commercial kit (Intertek) following the manufacturer's instructions.

Euthanasia and tissue preparation

The animals were euthanized by cardiac puncture exsanguination after being anesthetized with ketamine/xylazine, 24-h after the exhaustive effort test. The blood was collected in K3/EDTA tubes and immediately centrifugated at 5000 rpm and 4°C for 5 minutes. The erythrocyte was washed 3-times with saline solution and kept frozen at -20°C. Right gastrocnemius muscle was excised, washed in saline solution. An aliquot of the muscle was excised and storage in RNA latter solution and the remaining tissue was immediately frozen by liquid nitrogen immersion. The gastrocnemius muscle was kept under liquid nitrogen immersion until the analyses.

Thiamine quantification

The thiamine concentration was analyzed in erythrocytes and in gastrocnemius muscle. To erythrocytes, it was added distilled water (1:1 vol) in a cryotube. The solution was hemolyzed by quick-freeze in liquid nitrogen and defrost three consecutive times. Then, it was added 0.8M perchloric acid (1:1 vol) and centrifugated at 5000 rpm for 10-min at 4°C for deproteinization. It was collected 80 μL of the supernatant and added 50 μL of 30 mM potassium hexacyanoferrate (K3Fe(CN)6) and 50 μL of 0.8 M sodium hydroxide (NaOH) for derivatization. The solution was mixed with 20 μL of methanol, and 20 μL were injected into the chromatograph (Shimadzu LC-10AT - Shimadzu Instruments, Kyoto, Japan). The analyses were carried by a mobile phase composed of 70 volumes of 25 mM phosphate buffer (pH 7.0), methanol, and acetonitrile (7:2:1 vol), C18 chromatographic-column (Agilent, Sigma-Aldrich), and flow at 1.0ml/min. The fluorometric detector (Shimadzu RF-20A - Shimadzu Instruments) was set at 365 nm for excitation and 435 nm for emission (Portari et al. 2013). The concentration of thiamine and its esters (TMP and TDP) was normalized by the total hemoglobin concentration in the sample.

To gastrocnemius muscle, 50 mg of tissue were homogenized in 500 μL of 50 mM sodium phosphate buffer (pH 7.0) and centrifuged at 5000 rpm for 10 min. Total protein concentration was analyzed in supernatant. Aliquot of the supernatant was homogenized with 0.8 M perchloric acid (1:1 vol) and centrifuged for 10 min at min at 4°C for deproteinization. Them, 200 μL of the supernatant was mixed with 20 μL of 30 mM potassium hexacyanoferrate diluted in 15% sodium hydroxide (NaOH) and the solution was inject into the chromatograph system (Shimadzu LC-10AT - Shimadzu Instruments, Kyoto, Japan). The mobile phase was phosphate buffer (25 mM, pH 7.0) + Methanol (8:2 vol), and the stationary phase was an Ascentis RP-amide chromatographic column (Supelco). The fluorometric detector (Shimadzu RF-20A - Shimadzu Instruments) was set at 365 nm for excitation and 435 nm for emission(Batifoulier et al. 2005). The concentration of thiamine and its esters was normalized by the total protein concentration in the muscle supernatant.

Gene expression analyses

It was analyzed the expression of the gene of the component E1-subunit alpha of the PDH complex (PDHa1) and of the gene of the component E1 of KGDH (OGDH) using qRT-PCR. Total RNA was extracted from 50mg of gastrocnemius muscle (red portion) homogenized with 1 mL of Trizol (Invitrogen) according to manufacturer's instructions. Total RNA was converted to cDNA by High-Capacity cDNA-to-RNA kit (Applied Biosystems) according to the manufacturer's protocol. PCR was run using 2 μL cDNA, 10 μL of TaqMan® Fast Advanced Master Mix (Applied Biosystems), 7 μL of nuclease free water and 1 μL of TaqMan® Gene Expression Assay. Reaction was run in a 7900HT Real Time PCR Instrument. The gene expression level was calculated using the ΔΔCT method(Livak and Schmittgen 2001) with reference to the CTL animals.

Organic acids quantification

The quantification of pyruvic, lactic and hydroxybutyric acids was performed in an aliquot of the gastrocnemius muscle using gas chromatography coupled to mass spectrometry (GC-MS) with detection in both scanning mode and selected ion monitoring mode (SIM)(Hur et al. 2014).

It was added 500 μL of distilled water to 50 mg tissue and homogenized under an ice bath with a Potter homogenizer. Then, distilled water (500 μL), acetonitrile (500 μL) and internal standard (0.2 μg) were added to 100 μL of homogenate and, after vigorous vortexing (2 min), it was centrifuged at 14000 rpm, for 10 min for protein precipitation. The supernatant was adjusted to pH 12 with 5.0 M NaOH. After adding 1 mg of methoxyamine hydrochloride, the solution was incubated at 60ºC for 30 min. The solution was then acidified to pH 1-2 with 10% sulfuric acid and saturated with sodium chloride. The analytes were extracted with 3 ml of diethyl ether, followed by 2 ml of ethyl acetate. After adding 5 μL of triethylamine, the combined extracts were completely evaporated under a dry block (40ºC) argon gas flow. The precipitate was resuspended with 20μL of toluene and 20 μL of N-methyl-N- (tert-butyldimetillsylil) trifluoroacetamide (MTBSTFA) + 1% tert-butyldimethylchlorosilane (20 μL) and heated for 30 min at 60ºC. The final solution was collected and 1 μL was manually injected in a split type injector(Hur et al. 2014).

Statistical analysis

The data are presented as the mean ± standard deviation. The results were compared and graphs were built by GraphPad Prism software (Version Prism 8.0.1). Levene's test and Shapiro-Wilk's test were used to test the equality of variances and data homogeneity, respectively. The results of thiamine and its phosphates concentration were compared by one-way analysis of variance followed by post-hoc Tukey's test. The data of gene expression and organic acids concentration were compared using the Kruskal-Wallis test and Dunn's test as a post-hoc test. It was applied Student's T-test to analyzed endurance capacity and plasma lactate concentration. A significance level of 95% ($p < 0.05$) was adopted.

Results

There was no difference in benfotiamine intake between the supplemented groups (Ben-Sed and Ben-Tr). Supplemented animals showed high levels of TDP in erythrocyte than standard-diet animals in both erythrocytes (figure 1A) and gastrocnemius muscle (figure 2A). Interestingly, the Ben-Tr group showed high erythrocyte TDP levels also than the Ben-Sed group (figure 1A). In both tissues, there was no difference in the TDP concentration of non-supplemented groups. The TMP in the erythrocyte and muscle of supplemented groups was higher than in non-supplemented groups (figures 1B and 2B). Free thiamine concentration in the Ben-Sed group was higher than in both non-supplemented groups while the Ben-Tr animals showed erythrocyte thiamine levels only higher than the Pad-Tr group (figure 1C). However, free thiamine in the gastrocnemius muscle of Ben-Tr was higher them non-supplemented animals (figure 2C).

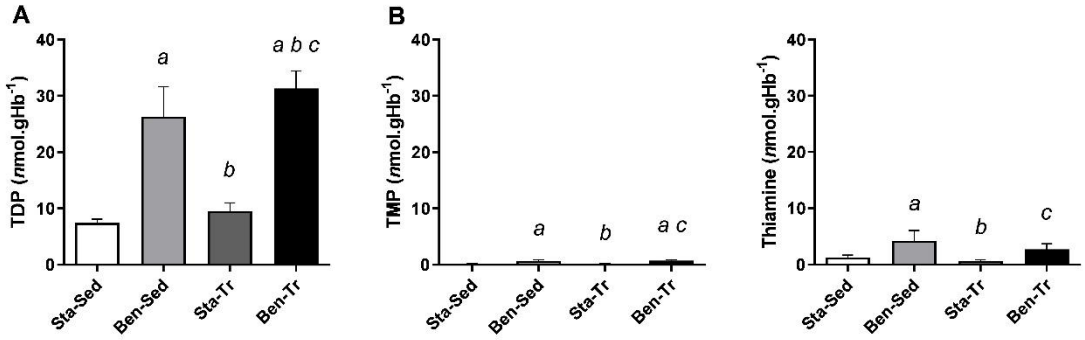

Figure 1: Concentration of thiamine and thiamine phosphates in the erythrocytes. **A:** Thiamine diphosphate; **B**: Thiamine monophosphate; **C**: Free thiamine. a: p<0,05 in relation to Sta-Sed. b: p<0,05 in relation to Ben-Sed. c: p<0,05 in relation to Sta-Tr.

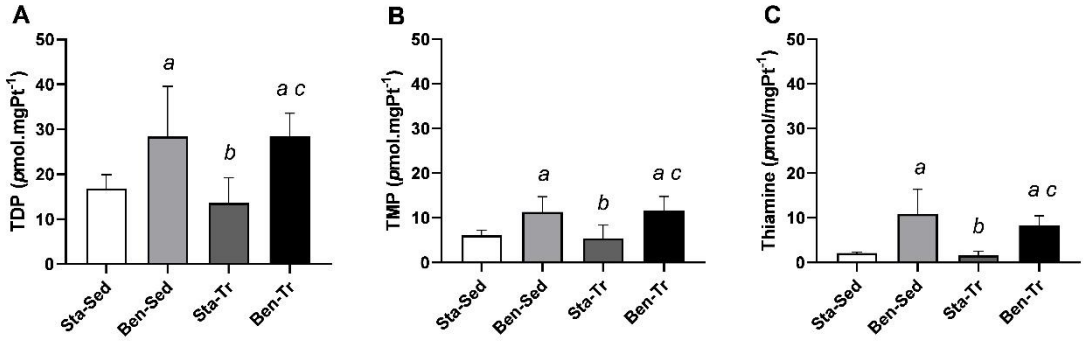

Figure 2: Concentration of thiamine and thiamine phosphates in the gastrocnemius muscle. A: Thiamine diphosphate; B: Thiamine monophosphate; C: Free thiamine. a: p<0,05 in relation to Sta-Sed. b: p<0,05 in relation to Ben-Sed. c: p<0,05 in relation to Sta-Tr.

There was no significant difference in gene expression of analyzed genes. In both PDHa1 and OGDH genes, the values did not show any difference between the groups Figure 3).

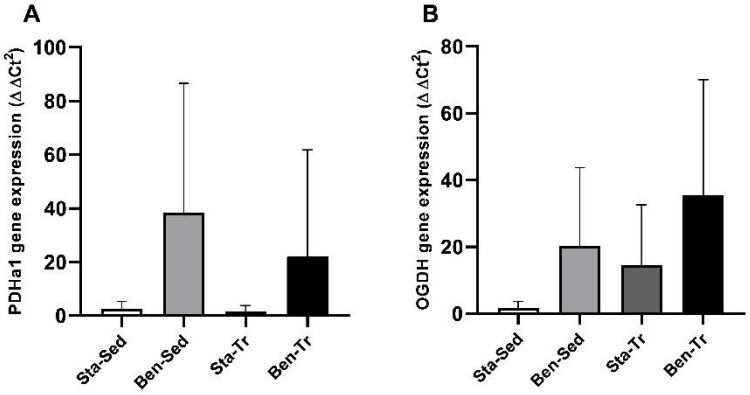

Figure 3: Target genes expression. **A**: PDHa1 gene expression; **B**: OGDH gene expression.

There was no difference in pyruvic acid concentration in gastrocnemius muscle tissue (Figure 4A). The concentration of lactic acid was higher in Ben-Tr group relative to Sta-Sed and Ben-Sed groups (Figure 4B). The lactic acid: pyruvic acid ratio in Sta-Sed animals was lower than in both trained groups (Figure 4 C). Muscle tissue of Ben-Tr group showed higher concentration of hydroxibutiric acid compared to Sta-Sed (Figure 4D).

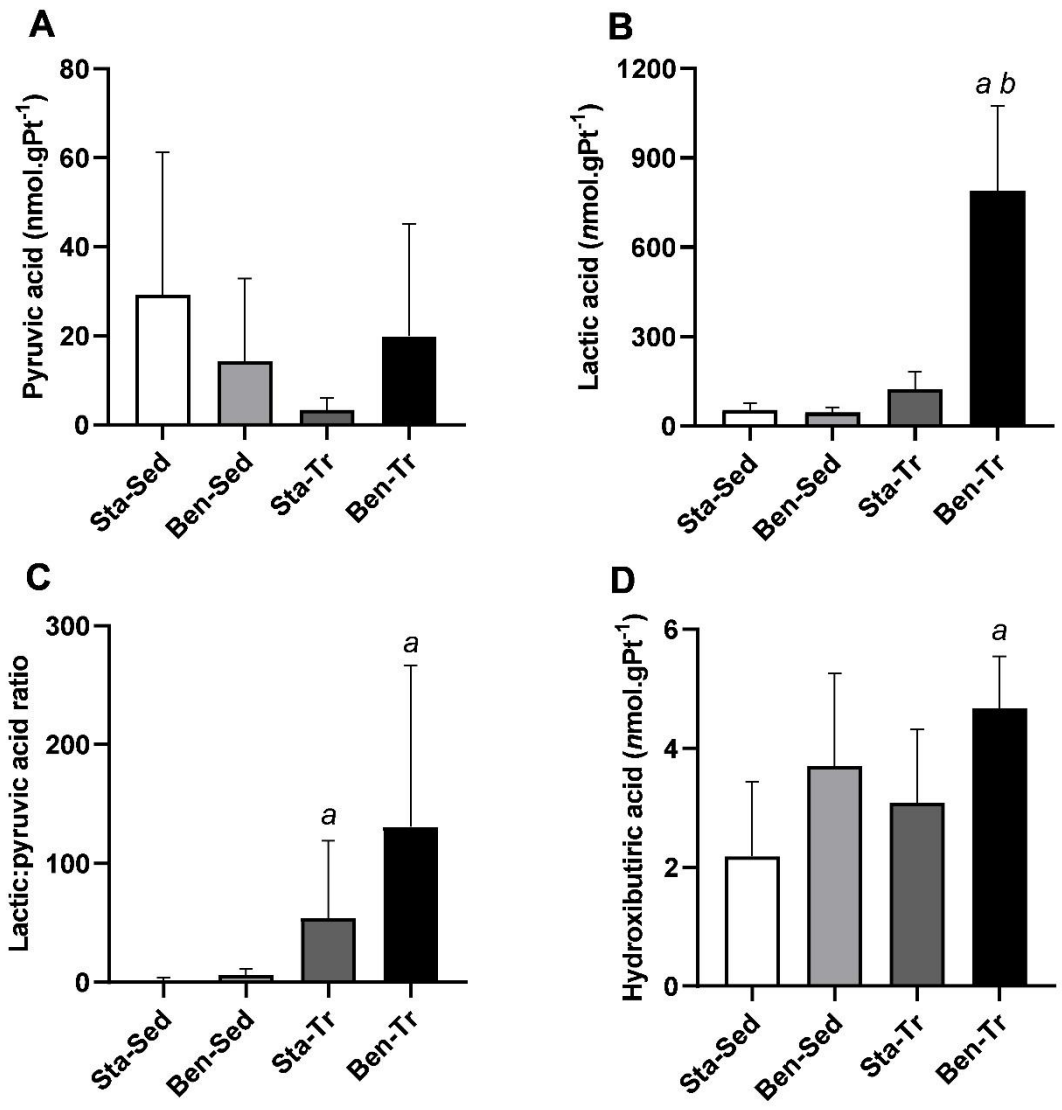

Figure 4: Concentration of organic acids produced in energy metabolism. A: Concentration of pyruvic acid; B: Concentration of lactic acid; C: Lactic acid: pyruvic acid ratio. D: Hydroxybutyric acid. a: $p<0,05$ in relation to Sta-Sed. b: $p<0,05$ in relation to Ben-Sed.

The results of the exhaustive test did not show significant difference between the performance of the tested groups. In addition, there was no difference in the lactate concentration between the groups but was higher after the exhaustive test in relation to pre-test values in both groups (Figure 5).

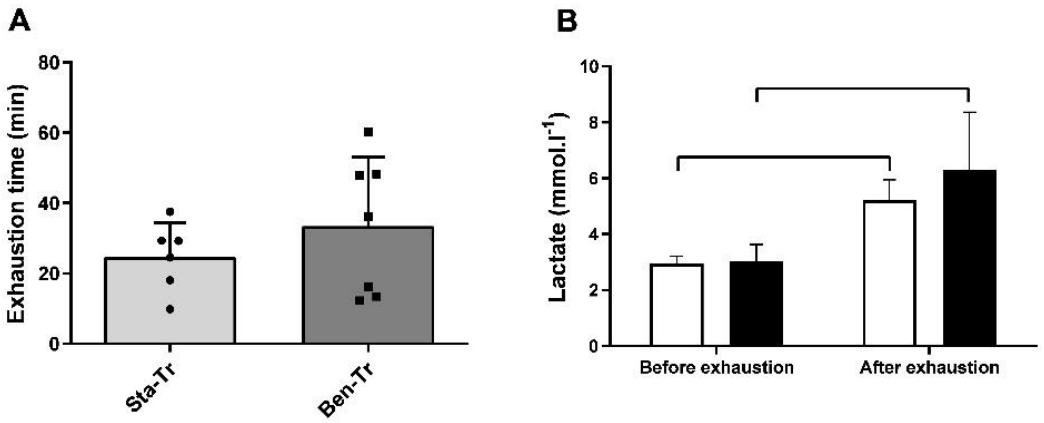

Figure 5: Endurance performance. **A**: Exercise time until the exhaustion; **B**: Concentration of plasma lactate. White bar: Sta-Tr group; Black bar: Ben-Tr group. Connectors: $p<0,05$.

Discussion

The present study aimed to investigate the effect of oral supplementation with benfotiamine on the energy metabolism components related to thiamine and its esters. Moreover, this study aimed to evaluated if benfotiamine supplementation could affects the endurance performance of trained mice.

The results showed that oral benfotiamine supplementation is efficient to increases free thiamine, TMP, and TDP in the erythrocytes. It is known that approximately 80% of the TDP in whole blood is transported by the erythrocytes. Despite of the increase in the level of thiamine phosphates in erythrocyte, this could not be efficient to promote the increase in TDP in tissues. However, the present study found similar results in the concentration of thiamine phosphates in muscle gastrocnemius. The benfotiamine intake increased the TDP, TMP and free thiamine also in muscle. The present results are corroborating with other studies, which showed that benfotiamine supplementation increases the concentration of thiamine and its esters in erythrocyte, in whole blood, in

neural tissues, and in liver (Portari et al. 2013; de Moraes et al. 2020). It was not found any study that evaluated the alterations in the concentration of thiamine esters in a muscle tissue in response to benfotiamine supplementation. Interestingly, the animals of the cited studies(Portari et al. 2013; de Moraes et al. 2020) received the benfotiamine by gavage, a stressful invasive method. In the present study, the substance was added to the food, that represents a less stressful method to offer the benfotiamine.

The TDP, the biologically active form of the thiamine, is a coenzyme of PDH complex and KGDH complex in the Krebs cycle(Pácal et al. 2014). PDH complex acts converting the pyruvate molecules to acetyl-CoA, the precursor molecules of the Krebs cycle. The KGDH catalyzes the conversion of α-ketoglutarate to succinil-CoA(Akram 2014). In both enzyme complexes, TDP acts in the component E1(Gibson et al. 2005; Patel et al. 2014). The gene expression of PDHa1 and OGDH, which are related to E1 components of the PDH and KGDH respectively, was not different between the four groups, despite oral benfotiamine supplementation have increased in approximately 3-fold the TDP levels in gastrocnemius muscle. Thus, an increase in TDP concentration seems not affects the gene expression of the thiamine-dependent enzymes.

Few studies have been dedicated to investigating the role of thiamine supplementation in the gene expression of enzymes of the Krebs cycle. It was not found any study that investigated the gene expression of PDH or KGDH genes in response to thiamine supplementation. However, a study (Pekovich et al. 1998) showed that thiamine deficiency reduces RNAm levels of enzyme transketolase and the component E1-subunit beta of the PDH, but was not observed alteration in PDHa1 and OGDH genes in three human cell types. The authors(Pekovich et al. 1998) suggest that thiamine does not affect the gene expression of all the components of thiamine-dependent enzymes. Other study showed that there was no alteration in PDHa1 expression in muscle of humans after 1 and 8 weeks of endurance training(LeBlanc et al. 2004). In accordance, the present study did not find any alteration in gene expression caused by exercise training.

Although the benfotiamine has positively affected the levels of TDP in muscle tissue, the pyruvic acid concentration was not altered. The thiamine-dependent enzyme, PDH complex, is responsible for converting pyruvate molecules to acetyl-CoA, a substrate of the Krebs cycle (Spriet and Heigenhauser 2002). Therefore, it was expected

to decrease pyruvic acid concentration in muscle. However, endurance training also did not affect pyruvic acid concentration in gastrocnemius muscle, despite the exercise known to increase the pyruvate demand. Thus, it seems that pyruvic acid concentration in muscle is not affected by pyruvate demand or an increase in PDH activity. In agreement, an study showed an expressive increase in PDH activity but no decrease in intramuscular pyruvate concentration during an isokinetic exercise (Putman et al. 1995).

In anaerobic glycolysis, the pyruvic acid is converted to lactic acid by the action lactate dehydrogenase enzyme(Young et al. 2020). It is important to consider that the trained animals were submitted to an exhaustive-exercise test, which increases significantly the utilization of the anaerobic metabolism compared to a sedentary or rest condition. A study showed that endurance cycling increases the lactate concentration in muscle of humans(Putman et al. 1995). In the present study, only the Ben-Tr group showed higher levels of intramuscular lactic acid. This result could indicate a combined effect of training and benfotiamine supplementation in muscle lactate metabolism. However, the results about lactic: pyruvic acid ratio show that the pyruvate-to-lactate conversion seems to be affect by the training, but not by supplementation. The higher lactic: pyruvic acid ratio indicates that anaerobic metabolism was more requested in the trained animals.

Both glycolysis and β-oxidation can provide acetyl-CoA to the Krebs cycle. The acetyl-CoA molecules that does not enter in Krebs cycle are converted to ketoacids, like hydroxibutiric acid(Laffel 1999; Stojanovic and Ihle 2011). The Ben-Tr group showed high concentration of intramuscular hydroxibutiric acid, which indicates excess of acetyl-CoA. It is possible that the higher levels of TDP improves PDH activity, as has already been showed in brain and heart of mice(Ikeda et al. 2016). The increased activity of PDH complex enhances the oxidative decarboxylation of the pyruvate and acetyl-CoA formation. In addition, endurance exercise causes an increase in glycolysis and fat acid oxidation(Hargreaves and Spriet 2020). These factors could increase expressively the acetyl-CoA concentration, above the levels of oxaloacetate, which would limit the acetyl-CoA entry in Krebs cycle. The excess of acetyl-CoA is converted to acetoacetate and them, hydroxybutyrate(Laffel 1999; Stojanovic and Ihle 2011).

The higher levels of lactate in the muscle of Ben-Tr animals also can be a result of an increased production of acetyl-CoA. The excess of acetyl-CoA molecules could inhibit the PDH activity to avoid the formation of more acetyl-CoA from pyruvate. The acetyl-CoA molecules increase the activity of pyruvate-dehydrogenase kinase (PDK), an enzyme inhibits the PDH activity (Pettit, Pelley and Reed, 1975). Thus, more lactate is produced from pyruvate by the action of the lactate-dehydrogenase.

The increase in the plasma lactate concentration after the exhaustive exercise test in both groups, without difference between them, showed that the animals reached the same intensity of effort, possibly the exhaustion. A study has proposed that thiamine deficiency increases the lactate concentration by decreasing pyruvate dehydrogenase activity(Horwitt and Kreisler 1949). A TDP deficiency impairs the PDH activity, but the TDP excess does not increase the enzyme activity at a rate that could affect the production:clearance ratio of serum lactate after the exhaustive exercise.

Another study showed, in humans, that 30 days of TDP infusion can reduce serum lactate concentration after a submaximal exercise test (Bautista-Hernández et al. 2008). However, the rate of lactate production in an exhaustive-exercise test is expressively higher than in a submaximal exercise, because the anaerobic metabolism is hardly requested in the final stages of the exhaustive-exercise bout(Gaitanos et al. 1993). Thus, it seems that benfotiamine supplementation cannot alter the PDH activity to the point that it be able to reduce the lactate production in a maximal-endurance exercise.

The benfotiamine supplementation was not able to improve the endurance capacity of trained mice. The results did not show any alteration in the capacity to delay the fatigue in supplemented and trained animals compared to trained animals. The evidence about the effects of thiamine supplementation and endurance performance is inconsistent. Several studies have shown that oral supplementation with different thiamine analogs can improve exercise performance in human and animals(Doyle et al. 1997; Webster 1998; Nozaki et al. 2009; Choi et al. 2013; Huang et al. 2018). However, no study tested the effects of benfotiamine supplementation on exercise performance.

Recently, a study showed that TTFD supplementation increased the exhaustive swimming time in male mice. Besides, the supplementation increased the glycogen levels

in muscle and liver, that could improve the submaximal-endurance performance(Huang et al. 2018). In another study, the TTFD supplementation did not improve the high-intensity exercise performance in humans(Webster et al. 1997). Allithiamine, a thiamine derivative with high bioavailability, also did not show an effect on endurance exercise performance in humans(Doyle et al. 1997). TDP applied intravenously for 30 days increased the maximal oxygen consumption (VO_2max) in humans submitted to an aerobic exercise session(Bautista-Hernández et al. 2008). In another study, rats supplemented orally with dicethiamine hydrochloride (DCET) swam a longer time than animals that ingested thiamine hydrochloride or placebo(Shimizu et al. 2010).

The anti-fatigue effect of thiamine and its analogs is not clear in the scientific literature. There are several differences in thiamine derivatives used, administration routes, the concentration of the thiamine derivatives, and time of the protocol, that contribute to the lack of scientific consensus about the issue. The present results show that the supplementation protocol offered does not represent an efficient anti-fatigue strategy. It is important to consider that the effects on submaximal-aerobic exercise performance can be different since exhaustive endurance exercise requires expressive participation of the anaerobic metabolism to maintain power output(Gaitanos et al. 1993). Although it is the aerobic metabolism the principal source of adenosine triphosphate (ATP) during an endurance exercise bout, the performance in an exhaustive-exercise test is delimited by the anaerobic metabolism since its capacity is expressively more limited than aerobic metabolism(Gastin 2001).

Thus, more studies with higher evidence levels are necessary to clarify the effects of thiamine supplementation on exercise performance. Studies with humans, with an expressive number of participants and exercise tests able to reflect the endurance performance, must be done, aimed to show the real necessity of a highly specific diet in thiamine and/or its derivatives. In addition, basic studies could improve the understanding of the biomolecular pathways in which thiamine acts to affect energy metabolism during exercise.

Conclusion

The oral benfotiamine supplementation increases the free thiamine and thiamine phosphate forms in erythrocytes and muscle tissue. Benfotiamine supplementation do not affect the gene expression of the component E1-alpha of the pyruvate dehydrogenase and of the component E1 of the alpha-ketoglutarate dehydrogenase complex. However, higher TDP levels seems to be related to an enhancement in pyruvate catabolism in muscle of trained animals, but had no anti-fatigue effect in endurance trained mice submitted to exhaustive-exercise test.

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8. COMENTÁRIOS

A tiamina foi amplamente estudada após a descoberta da sua relação com o funcionamento adequado do sistema nervoso. Assim sendo, os primeiros estudos sobre a vitamina focaram suas investigações para as desordens neurológicas relacionadas à deficiência de tiamina. Enquanto coenzima de complexos enzimáticos do metabolismo energético, principalmente do catabolismo da glicose, alguma atenção foi dada para a influência da tiamina no controle e prevenção do diabetes melitos.

Diante de sua participação fundamental no metabolismo energético, pesquisadores voltaram seus esforços para investigar os efeitos da deficiência e da suplementação com tiamina e seus derivados no desempenho físico, e embora tenham obtido resultados favoráveis a suplementação, a literatura científica não alcançou um consenso sobre o papel da tiamina no desempenho esportivo. Tanto que as recomendações de ingestão diária de tiamina não se distinguem de acordo com o gasto energético, idade, massa corporal ou outro fator que possa interferir no metabolismo desta vitamina.

Neste sentido, os resultados do presente estudo contribuem para o debate científico sobre o assunto, e acrescenta fatores antes ignorados nesta discussão, como o efeito antioxidante da benfotiamina em tecidos que, embora menos recrutados que os músculos agonistas do movimento esportivo, são altamente solicitados nos processos de manutenção do esforço, recuperação e adaptação ao exercício. Além disso, os levantamentos e revisões bibliográficas, realizados anteriormente e durante a execução da presente pesquisa, não encontraram estudo algum que tenha investigado os efeitos da suplementação com benfotiamina no desempenho físico em qualquer tipo de esforço.

Embora vários estudos tenham se dedicado a investigar os efeitos da suplementação com análogos de tiamina sobre desempenho físico, tanto em humanos quanto em animais, pouco se sabe sobre seus mecanismos de ação. Os achados sobre a concentração de tiamina e seus ésteres, expressão gênica de componentes de enzimas dependentes de tiamina e concentração de ácidos orgânicos produzidos pelo catabolismo dos macronutrientes, oferecem informação fundamental para o entendimento sobre o modo que a ingestão extra de tiamina ou algum de seus análogos interfere no funcionamento do metabolismo energético.

Contudo, o presente estudo possui limitações metodológicas que devem ser superadas em futuros experimentos. Para os próximos estudos, sugere-se a execução do protocolo de treinamento em esteira, a fim de proporcionar um melhor controle e ajuste da carga de treinamento. Além disso, a aplicação do teste de esforço antes e após o protocolo de treinamento proporcionaria melhor controle da carga de treinamento e aferição das alterações no desempenho físico. Investigações sobre outros parâmetros relacionados a tiamina ainda são necessários, tais como a expressão e atividade da enzima transcetolase, ação e expressão de transportadores de tiamina e difosfato de tiamina bem como a taxa de produção de ATP.

9. CONCLUSÃO

A suplementação oral com benfotiamina é eficiente em aumentar a concentração de tiamina livre, difosfato de tiamina e monofosfato de tiamina nos eritrócitos e no músculo gastrocnêmio. A benfotiamina aumenta a capacidade antioxidante e combate o dano oxidativo no músculo tibial e no coração dos camundongos submetidos ao treinamento de endurance. Apesar de aumentar expressivamente a concentração de difosfato de tiamina, a benfotiamina não afeta a expressão dos genes dos componentes dependentes do difosfato de tiamina das enzimas piruvato desidrogenase e α-cetoglutarato desidrogenase. A suplementação não foi capaz de promover melhora na função da enzima piruvato desidrogenase a ponto de reduzir o acúmulo plasmático de lactato durante o exercício exaustivo, contudo parece ser capaz de aumentar o catabolismo de piruvato e aumentar a oferta de acetil-CoA intramuscular nos animais treinados. Apesar disso, a suplementação não apresenta efeito antifadiga em camundongos submetidos a treinamento de endurance em natação.

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ANEXO

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CERTIFICADO

Certificamos que o projeto intitulado "**Avaliação do efeito da suplementação com benfotiamina no exercício físico: desempenho e expressão gênica de enzimas do ciclo de Krebs**", protocolo nº **343**, sob a responsabilidade de Guilherme Vannucchi Portari – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794 de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle e Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal do Triângulo Mineiro, em reunião de 22/05/2015.

Vigência do projeto	22/05/2015 a 22/07/2015
Espécie/Linhagem	Camundongo Isogênico BALB/c
Nº de animais	28
Peso/idade	50 gramas/ 1 mês
Gênero	Machos
Origem	Empresa ANILAB Animais de Laboratório, Paulínia-SP

Prof. Dr. Carlo José Freire de Oliveira
Vice-Coordenador da CEUA