UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
ÁREA DE CONCENTRAÇÃO EM BIOLOGIA CELULAR E MOLECULAR

PATRÍCIA BATISTA TRAVASSOS

# PARTICIPAÇÃO DA HIPOGLICEMIA E DA RAZÃO LACTATO: PIRUVATO COMO MARCADORES BIOLÓGICOS DO DESEMPENHO DURANTE A NATAÇÃO AGUDA E INTENSA EM RATOS EM JEJUM

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas (área de concentração - Biologia Celular e Molecular), da Universidade Estadual de Maringá para a obtenção do grau de Doutor em Ciências Biológicas.

**Orientador:** Prof. Dr. Roberto Barbosa Bazotte

Maringá
2018

Dados Internacionais de Catalogação na Publicação (CIP)
(Biblioteca Central - UEM, Maringá, PR, Brasil)

T779p

Travassos, Patrícia Batista
Participação da hipoglicemia e da razão lactato: piruvato como marcadores biológicos do desempenho durante a natação aguda e intensa em ratos em jejum / Patrícia Batista Travassos. -- Maringá, 2018.
56 f. : il., color., figs., tabs.

Orientador(a): Prof. Dr. Roberto Barbosa Bazotte.
Tese (doutorado) - Universidade Estadual de Maringá, Centro de Ciências Biológicas, Programa de Pós-Graduação Ciências Biológicas - Área de Concentração: Biologia Celular e Molecular, 2018.

1. Ácido lático. 2. Razão lactato:piruvato. 3. Exaustão física. 4. Neogliconêogenese. I. Bazotte, Roberto Barbosa, orient. II. Universidade Estadual de Maringá. Centro de Ciências Biológicas. Programa de Pós-Graduação Ciências Biológicas - Área de Concentração: Biologia Celular e Molecular. III. Título.

CDD 21.ed. 612.39
AHS-CRB-9/1065

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Aprovado em: 09/03/2018

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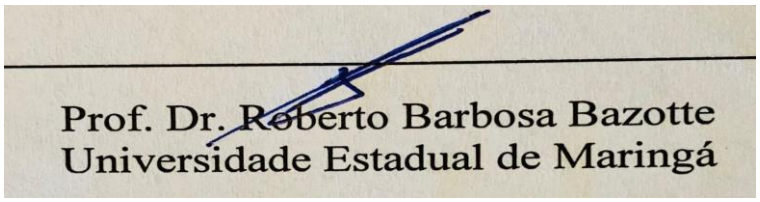


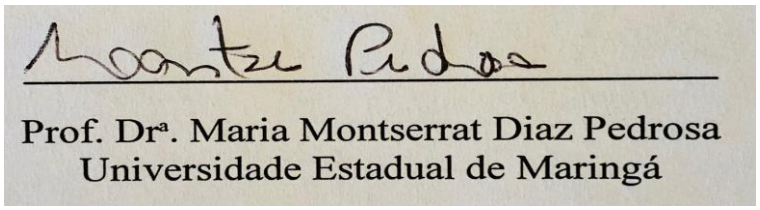


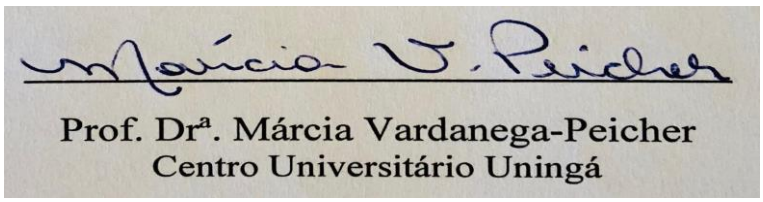


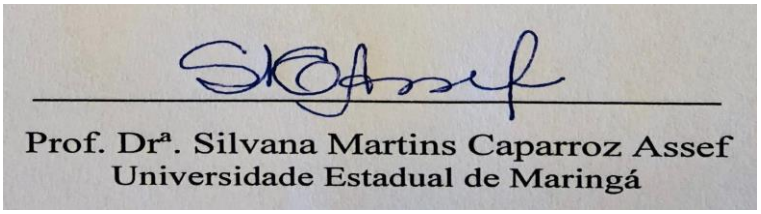


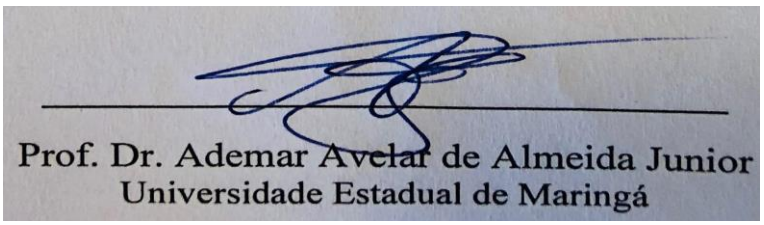

# BIOGRAFIA

**Patricia Batista Travassos** nasceu em Paranavaí/PR, em 10 de janeiro de 1991, onde cursou Ensino Fundamental e Médio. Possui graduação em Educação Física (Bacharel – 2012) pela Universidade Estadual de Maringá (UEM). Durante a graduação participou de projetos de pesquisa no Departamento de Ciências Fisiológicas da UEM. Realizou seu mestrado pelo Programa de Pós-graduação em Ciências Biológicas (PBC) da UEM no período de 2013 a 2015, desenvolvendo seus estudos no Laboratório de Investigação em Diabetes e Obesidade (LIDO) do Departamento de Farmacologia e Terapêutica (DFT) da UEM. Em 2016 iniciou sua segunda habilitação em Educação Física (Licenciatura), participando do grupo de estudo voltado à Educação Física Escolar: perspectivas e ações pedagógicas na atualidade. Atualmente é doutoranda no PBC/UEM. Participa de projetos do DFT/LIDO em andamento, onde parte de seus estudos contribuíram para o patenteamento (Número do Processo: BR 10 2017 013771 6) de um agente com o potencial de ser empregado no tratamento das hipoglicemias.

## AGRADECIMENTOS

À Deus, pelas bênçãos diárias e por estar cuidando de cada detalhe. Jesus misericordioso, eu confio em Vós.

Agradeço ao Prof. Dr. Roberto Barbosa Bazotte, que me recebeu em seu laboratório, me encaminhou na pesquisa, sendo um exemplo de profissional e pessoa.

À minha família, pelo apoio incondicional e amor eterno. A família é o bem mais precioso que podemos ter, eu amo muito vocês.

Ao meu marido Henry, tantos anos de companheirismo, amizade e amor. Esse trabalho não seria possível sem seu apoio e cuidado diário.

Aos técnicos de laboratório, Carlos Eduardo de Oliveira e Solidalva Caruso de Oliveira pela amizade, prontidão e carinho sempre.

Aos amigos de laboratório Lorena, Vanessa, Marina, Any, Tuane, Guilherme, Camila e Christiano pela ajuda contínua com o trabalho, amizade e risadas diárias.

E a todos que de alguma forma fizeram parte desse trabalho.

# APRESENTAÇÃO

Esta tese de doutorado foi redigida na forma de um primeiro artigo científico "**The performance during a strenuous swimming session is associated with high blood lactate: pyruvate ratio and hypoglycemia in fasted rats"**. Este estudo faz parte de uma das linhas de pesquisa (estudo dos mecanismos de regulação da glicemia) do Laboratório de Investigação em Diabetes e Obesidade (LIDO) da Universidade Estadual de Maringá (UEM). No presente estudo investigou-se o papel da glicose e da razão lactato:piruvato sanguínea no desencadeamento da exaustão durante a natação aguda e intensa em animais em jejum. O artigo foi redigido de acordo com as normas da revista "**Brazilian Journal of Medical and Biological Research**", sendo aceito para publicação em 09/01/2018. Ainda em consonância com as normas do Programa de Pós-graduação em Ciências Biológicas (PBC), o segundo artigo científico, intitulado: "**Investigation of the acute effects of dry extract of *glycine max* on postprandial glycemia in rats**" (**Brazilian Archives of Biology and Technology** v.59, p. e16150085, 2016), também apresenta resultados dentro da linha de pesquisa "Mecanismos de regulação da glicemia". Além disso, como parte integrante dessa tese, anexamos a patente desenvolvida e depositada (Número do Processo: BR 10 2017 013771 6) no período de vigência do doutorado, intitulada: **"Formulação contendo glicose associada ao 1,2,3-propanotriol para a prevenção e tratamento das hipoglicemias"**.

## RESUMO GERAL

**INTRODUÇÃO.** A maioria dos estudos que avaliam as alterações do metabolismo induzidas pelo jejum, são realizados em condições de repouso. No entanto, em condições naturais os mamíferos fazem exercícios aeróbicos intensos em situações inesperadas, quando são intensificados os mecanismos de mobilização de energia, para garantir a sobrevivência, mesmo no estado de jejum. Além disso, em geral, investigações sobre exercícios aeróbicos intensos em seres humanos e em animais experimentais são realizados no estado alimentado. Assim, há uma escassez de investigações sobre as mudanças metabólicas induzidas pelo exercício aeróbico intenso associado ao estado pós-absortivo. O exercício aeróbico intensifica a condição catabólica, desencadeada pelo jejum, quando há aumento da degradação do triacilglicerol intra miocelular, bem como maior oxidação de ácidos graxos e gliconeogênese hepática. Outra característica do exercício aeróbico intenso associado ao jejum é a redução da glicemia e elevação da lactacemia associada à exaustão. Além disso, a redução do desempenho no exercício tem sido observada em atletas em jejum em comparação com os não jejuados. Porém, a heterogeneidade na duração, intensidade do exercício, sexo e nível de treinamento dos participantes, limitam nossa capacidade de prever/discriminar a influência da glicemia e lactacemia em promover a exaustão. Assim, uma questão pode ser levantada: a grande variabilidade no tempo para alcançar a exaustão durante uma sessão de exercício extenuante poderia ser atribuída à redução da glicemia, elevação da lactacemia ou ambos? Para avaliar estas possibilidades, o principal objetivo deste estudo foi investigar a correlação entre o desempenho dos animais com a concentração sanguínea de glicose, lactato, piruvato, glicerol e razão lactato: piruvato. A captação de lactato e sua conversão a glicose no fígado proveniente de animais submetidos ao exercício e sua correlação com a exaustão também foram avaliadas. **MATERIAIS E MÉTODOS.** Utilizou-se ratos machos *Wistar* (210 - 240 g) jejuados durante 15 h. A sessão de natação foi realizada em tanques de água com capacidade de 30 litros (31 ± 1 °C). Durante a natação, os animais suportaram uma sobrecarga de 6% atada à cauda. A exaustão foi definida como a incapacidade de manter o nado em superfície, a perda de movimentos simétricos e a permanência em baixo da água por pelo menos 5 segundos. Para fins comparativos, incluímos um grupo de animais sem exercício (Controle), um segundo grupo de animais que nadaram (grupo Exe) e foram imediatamente removidos da água quando o terceiro grupo de animais atingiram o tempo de exaustão (grupo Exh). Os animais do grupo exercício sem exaustão (grupo Exe) foram incluídos para diferenciar as alterações metabólicas induzidas pelo exercício (grupo Exe *vs.* grupo Controle) das alterações metabólicas causadas pela exaustão (grupo Exe *vs.* grupo Exh). Na segunda série de experimentos, mantivemos um grupo controle. Antes de iniciar a sessão de

natação, 20 minutos, animais de dois grupos receberam solução salina oral (gavagem) ou glicerol + glicose. Os ratos que receberam solução salina (grupo salina) ou glicerol mais glicose foram forçados a nadar até a exaustão, independentemente do tempo necessário para que ela ocorresse. Os animais foram eutanasiados para obtenção do sangue, ou anestesiados para os experimentos de perfusão hepática. A normalidade dos dados foi avaliada usando o teste de Shapiro-Wilk. Os resultados foram analisados por One- way ANOVA (Tukey) ou Kruskal-Wallis (Dunn). As diferenças entre as duas médias foram analisadas utilizando Mann Whitney U para testes não paramétricos. Os resultados são apresentados como médias ± SEM, sendo $p<0,05$ utilizado para indicar a significância estatística. **RESULTADOS.** Houve redução ($p<0,05$) da glicemia após 5, 10, 15 ou 20 min de natação (grupos Exe ou Exh *vs*. Controle). Além disso, a redução ($p<0,05$) da glicemia foi mais pronunciada no grupo exaustão (grupo Exh) em comparação ao sem exaustão (grupo Exe). O grupo Exaustão apresentou maior ($p<0,05$) lactacemia em comparação ao grupo controle e também maior ($p<0,05$) lactacemia em comparação com o grupo Exe nos tempos de 5 e 15 min de natação. Em geral, os níveis de piruvato aumentaram ($p<0,05$) durante o exercício (grupo Exe *vs*. Controle) e diminuíram ($p<0,05$) quando a natação forçada atingiu exaustão (grupo Exh *vs*. Exe). A razão lactato: piruvato permaneceu inalterada durante o exercício (grupo Exe *vs*. Controle). No entanto, a razão lactato: piruvato foi maior nos animais que entraram em exaustão precocemente (5 e 10 min) em relação aos que atingiram a exaustão mais tardiamente (15 e 20 min). Observou-se uma correlação positiva entre o tempo de natação até a exaustão e a redução ($p = 0,0475$) da glicemia ou elevação ($p = 0,0017$) da lactacemia. No entanto, não houve correlação positiva entre a redução da glicemia ou elevação da lactacemia no grupo Exe. A captação de lactato, bem como a produção de glicose e piruvato no fígado foram semelhantes nos três grupos. Os ratos que receberam glicerol + glicose (Gly+Glu – Exh) via oral alcançaram exaustão mais tardiamente em comparação aos animais que receberam solução salina (Saline- Exh). Os grupos Salina - Exaustão ou Glicerol + Glicose – Exaustão apresentaram menor ($p<0,05$) glicemia em relação ao grupo Controle enquanto o grupo Glicerol + Glicose – Exaustão apresentou maior ($p<0,05$) glicemia em comparação ao grupo Salina- Exaustão. Os grupos Salina - Exaustão e Glicerol + Glicose – Exaustão apresentaram maiores ($p<0,05$) concentrações de piruvato e lactato sanguíneo em relação ao grupo controle, enquanto que não houve diferenças entre as concentrações de lactato e piruvato em comparação aos grupos Salina - Exaustão ou Glicerol + Glicose – Exaustão. Os animais do grupo Glicerol + Glicose – Exaustão apresentaram maiores ($p<0,05$) concentrações de glicerol em comparação ao grupo controle ou grupo Salina-Exaustão. **DISCUSSÃO**. A capacidade dos mamíferos em sobreviverem de condições de jejum prolongado depende da capacidade do fígado

em produzir energia oxidando ácidos graxos e utilizando parte desta energia para produzir glicose. Estes processos metabólicos são acelerados se o jejum estiver associado a um exercício físico aeróbio intenso, uma vez que existe uma intensificação da lipólise no tecido adiposo e da glicólise e proteólise muscular. Com o aumento da oferta de precursores de glicose para o fígado, particularmente o lactato, a gliconeogênese hepática torna-se uma importante fonte de glicose durante o exercício. No exercício aeróbico de alta intensidade associado ao jejum quando o conteúdo de glicogênio hepático é baixo, a captação de glicose e a produção de lactato pelos músculos esqueléticos excede a captação de lactato e sua conversão em glicose no fígado. Nesse contexto, verificou-se que há uma correlação entre a hiperlactacemia ou hipoglicemia com o tempo de exaustão durante uma sessão de natação extenuante. Porém, há uma sequência temporal na participação da glicose e do lactato sanguíneo em determinar o desempenho da natação. Para ratos que atingiram a exaustão em 5 min ou 10 min de natação, o tempo de exaustão foi melhor associado à maior razão lactato: piruvato. Por outro lado, para ratos que atingiram a exaustão em 15 min ou 20 min de natação, a exaustão foi melhor associada à hipoglicemia. Durante uma intensa sessão de exercícios aeróbicos, a lactacemia aumentou abruptamente, resultando em elevação da razão lactato: piruvato. No entanto, observou-se que a natação extenuante está associada à hipoglicemia em ratos em jejum. Estudos anteriores demostraram que durante a hipoglicemia aguda induzida pelo exercício, existe um déficit de disponibilidade de glicose a curto prazo para os astrocitos e, consequentemente, um déficit de lactato para os neurônios que não podem ser compensados pela elevação da lactacemia. Um ponto importante a ser enfatizado é que a lactacemia e a produção de glicose no fígado a partir do lactato desempenham um papel central na manutenção da glicemia e da lactacemia durante o exercício. No entanto, a captação de lactato e a produção de glicose a partir do lactato no fígado foram mantidas durante o exercício extenuante (grupo de exercícios e grupo exaustão). Assim, o fígado impede a elevação da lactacemia e a redução da glicemia durante uma seção intensa de natação através da captação de lactato e sua conversão em glicose. Uma limitação dos experimentos em fígado isolado foi o uso de uma concentração única de lactato, isto é, 2 mM, considerando que a concentração de lactato no sangue exibiu grande variabilidade durante o exercício. **CONCLUSÃO.** Há uma sequência temporal na participação da razão lactato:piruvato e da hipoglicemia no desempenho durante uma seção de natação aeróbica intensa em ratos em jejum. Além disso, o fígado teve uma participação importante em prevenir a hiperlactacemia e hipoglicemia durante a natação através da captação de lactato e sua conversão em glicose.

**PALAVRAS-CHAVE:** Ácido lático; Razão lactato:piruvato; Exaustão física; Neogliconêogenese.

## ABSTRACT

**INTRODUCTION.** The majority of studies evaluate alterations of metabolism induced by fasting in resting conditions. However, mammals in nature face intense aerobic exercise in unexpected conditions and mechanisms for energy mobilization to ensure survival are activated even in the fasting state. Moreover, in general, the studies on intense aerobic exercise in humans and in experimental animals are performed in the fed state. However, there is a paucity of investigations about the metabolic changes induced by intense aerobic exercise associated with post-absorptive state. Aerobic exercise intensifies the catabolic condition of fasting where there is increased intramyocellular triacylglycerol breakdown, fatty acids oxidation and liver gluconeogenesis. Another characteristic of the short-term intense aerobic exercise in the fasted state is a reduction in glycemia and elevation in lactatemia associated with a shorter time to exhaustion. Moreover, the reduction in the performance has been observed in fasted athletes in comparison with fed ones. However, the heterogeneity (such as exercise duration time, exercise intensity, sex of the participants, training level of the participants), limit our capability to predict/discriminate the influence of blood glucose or lactate levels to promote exhaustion. A question was then raised: the great variability in the time to exhaustion during a strenuous exercise session could be attributed to the reduction of glycemia, the elevation of blood lactate level or both? To evaluate these possibilities, the main purpose of this study was to investigate the correlation between the performance of the animals with the blood levels of glucose, lactate, pyruvate, glycerol and lactate: pyruvate ratio. In addition, lactate uptake and its conversion to glucose in livers from animals submitted to exercise and their association with exhaustion were evaluated. **MATERIALS AND METHODS.** 15-h fasted male Wistar rats (210-240 g) were used. The swimming session was performed in water tanks of 30 litters capacity (31±1 °C). During swimming, the rats had a 6% extra body weight lead stone tied to the tail. Exhaustion was defined as the incapacity to stay at the water surface, the loss of symmetrical movements during swimming or remaining underwater for more than five seconds. For comparative purpose, we included not only a one rat without exercise (Control group), but also a second rat that was immediately removed from the water when the third rat reached exhaustion (Exhaustion group). The exercised rat (Exe group) without exhaustion was included in order to differentiate the metabolic changes induced by the exercise (Exe group *vs.* Control group) to the metabolic alterations caused by the exhaustion (Exe group *vs.* Exhaustion group). In second set de experiments, we maintained a control group, 20 min before starting the swim session two animals received oral saline (gavage) or glycerol + glucose. The rats which

received saline (saline group) or glycerol plus glucose were forced to swim until exhaustion regardless the time required for exhaustion. All rats were killed by decapitation the blood was centrifuged and the serum was obtained or anesthetized for liver perfusion experiments. The results were assessed for normality using the Shapiro-Wilk test. The data were analyzed by one-way ANOVA (post hoc Tukey test) or Kruskal–Wallis (post hoc Dunn test). Differences between two means were analyzed using Mann Whitney U for nonparametric tests. The results are presented as means ± SEM. P <0.05 was used to indicate statistical significances. **RESULTS.** Blood glucose levels were reduced ($p<0.05$) after 5, 10, 15 or 20 min of the swimming (Exe or Exhaustion groups *vs.* Control group). In addition, the reduction ($p<0.05$) of glycemia was more pronounced in the Exhaustion group as compared with the Exe group. Exhaustion group had higher ($p<0.05$) blood levels of lactate in comparison with the control group. The Exhaustion group also had higher ($p<0.05$) blood concentrations of lactate in comparison with the Exe group for 5 and 15 min. In general pyruvate levels were increased during exercise (Exe *vs.* Control group) and decreased when forced swimming reached exhaustion (Exhaustion *vs.* Exe group). Lactate: pyruvate ratio remained unchanged during exercise (Exe *vs.* Control group). However, lactate: pyruvate ratios were higher from the group that exhibited exhaustion earlier (5 and 10 min) in relation to the group that reached exhaustion later (15 and 20 min). There was a positive correlation between the time to achieve exhaustion and the reduction ($p = 0.0475$) of glycemia or elevation ($p = 0.0017$) of lactatemia. However, there was no positive correlation between the reduction of glycemia or elevation of lactatemia for Exe group. The liver lactate uptake and the hepatic glucose and pyruvate production from lactate were similar in the three groups. Rats that received oral glycerol + glucose (Gly+Glu – Exh) reached exhaustion later in comparison with rats that received oral saline (Saline-Exh group). The Saline Exhaustion and Glycerol + glucose - Exhaustion groups had lower ($p<0.05$) glycemia in comparison with the control group while Glycerol + glucose - Exhaustion group had higher ($p<0.05$) glycemia in comparison with the Saline-Exhaustion group. The Saline Exhaustion and Glycerol + glucose - Exhaustion groups had higher ($p<0.05$) blood pyruvate and lactate levels in comparison with the control group whereas there were no differences for lactate and pyruvate levels as compared with the Saline-Exhaustion and Glycerol + glucose - Exhaustion groups. The Glycerol + glucose - Exhaustion groups had higher ($p<0.05$) glycerol levels in comparison with the control group or saline-Exhaustion group. **DISCUSSION.** The capability of mammals to survive in conditions of prolonged fasting depends on the ability of the liver to produce energy by oxidizing fatty acids and to use part of this energy to produce glucose. These metabolic processes are accelerated if fasting is associated with an intense aerobic physical exercise since there is an

intensification of adipose tissue lipolysis and muscle glycolysis/proteolysis. With the increased supply of glucose precursors, particularly lactate, liver gluconeogenesis becomes a significant source of glucose during exercise. In high-intensity aerobic exercise associated with fasting, condition in which hepatic glycogen content is low, the glucose uptake and lactate production by the skeletal muscles exceeds lactate uptake and its conversion to glucose in the liver. In this context, our results showed that there are association between hyperlactatemia and hypoglycemia with time to exhaustion in a strenuous swimming session. However, there is a time sequence in the participation of blood glucose and lactate for determining swimming performance. For rats that reached physical exhaustion with 5 min or 10 min, the time to exhaustion was better associated with higher lactate:pyruvate ratio. On the other hand, for rats that reached exhaustion with 15 min or 20 min, the exhaustion was better associated with hypoglycemia. During an intense aerobic exercise session, the lactatemia increased abruptly, resulting in elevation of the lactate:pyruvate ratio. However, we also reported that strenuous swimming is associated with hypoglycemia in fasted rats. Previous studies showed that during acute exercise-induced hypoglycemia there is a short-term deficit of glucose availability to the astrocytes and consequently a deficit of lactate to the neurons that cannot be compensated by the elevation of blood lactate levels is described. To confirm that energy supply influences performance during physical exercise in fasted rats, glycerol plus glucose was used as energy precursors. It must be emphasized that blood lactate levels and liver glucose production from lactate play a central role for glycemia and lactatemia maintenance during exercise. However, liver lactate uptake and glucose production from lactate remained unchanged during strenuous exercise (Exercise group and Exhausted group). The liver prevents the plasma lactate level elevation and blood glucose level decrease during an acute intense swimming section through lactate uptake and its conversion to glucose. A limitation of the experiments in isolated perfused liver is the use of a unique concentration of lactate, i.e., 2 mM, whereas blood lactate concentration exhibited great variability during the exercise. In conclusion, there is a time sequence in the participation of lactate: pyruvate ratio and hypoglycemia determining the performance during an intense aerobic swimming section in fasted rats. Furthermore, the liver had an important participation in order to prevent hyperlactatemia and hypoglycemia during swimming through lactate uptake and its conversion to glucose.

**KEYWORDS:** Lactic acid; Lactate:pyruvate ratio; Exercise tolerance; Exhaustion; Gluconeogenesis.

# Performance during a strenuous swimming session is associated with high blood lactate: pyruvate ratio and hypoglycemia in fasted rats

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***ABSTRACT***

The aim of this study was to investigate the effect of lactatemia elevation and glycemia reduction on strenuous swimming performance in fasted rats. Three rats were placed in a swimming tank at the same time. The first rat was removed immediately (control group) and the remaining ones were submitted to a strenuous swimming session. After the second rat was exhausted (Exh group), the third one was immediately removed from the water (Exe group). According to the period of time required for exhaustion, the rats were divided into four groups: low performance (3 – 7 min), low-intermediary performance (8 – 12 min), high-intermediary performance (13 – 17 min) and high performance (18 – 22 min). All rats were removed from the swimming tank and immediately killed by decapitation for blood collection or anesthetized for liver perfusion experiments. Blood glucose, lactate, and pyruvate concentrations, blood lactate: pyruvate ratio, and liver lactate uptake and its conversion to glucose were evaluated. Exhaustion in low and low-intermediary performance were better associated with higher lactate: pyruvate ratio. On the other hand, exhaustion in high-intermediary and high performance was better associated with hypoglycemia. Lactate uptake and glucose production from lactate in livers from the Exe and Exh groups were maintained. We concluded that there is a time sequence in the participation of lactate: pyruvate ratio and hypoglycemia in performance during an acute strenuous swimming section in fasted rats. The liver had an important participation in preventing hyperlactatemia and hypoglycemia during swimming through lactate uptake and its conversion to glucose.

**KEYWORDS:** Lactic acid; Lactate:pyruvate ratio; Exercise tolerance; Exhaustion; Gluconeogenesis.

**RUNNING TITLE**: Physical performance during fasting

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## INTRODUCTION

Most studies evaluate metabolism alterations induced by fasting in resting conditions. However, mammals in nature face intense aerobic exercise in unexpected conditions and mechanisms for energy mobilization to ensure survival are activated even in the fasting state. On the other hand, studies on intense aerobic exercise in humans and in experimental animals are mostly performed in the fed state. However, there is a paucity of investigations about the metabolic changes induced by intense aerobic exercise associated with post-absorptive state. After an overnight fast, fatty acids from adipose tissue and glucose from liver gluconeogenesis are the main energy sources. Aerobic exercise intensifies the catabolic condition of a fasting state in which there is increased intramyocellular triacylglycerol breakdown [(1)], fatty acids oxidation [(2)], and liver gluconeogenesis [(3)]. Aerobic exercise performed in a fasted state, increases further the rate of fat oxidation up to 24 h after the effort [(4,5)]. Another characteristic of the short-term intense aerobic exercise in a fasted state is a reduction in glycemia [(6)] and elevation in lactatemia [(7)] associated with a shorter time to exhaustion. The reduction in performance is observed in fasted athletes during an acute physical exercise in comparison with fed ones [(8,9)]. However, the high heterogeneity of these studies (such as exercise duration time, exercise intensity, sex of the participants, and training level of the participants), limit our capability to predict/discriminate the influence of blood glucose or lactate levels to promote exhaustion. We previously reported that fasted rats, but not fed ones, submitted to an intensive forced swimming had the exhaustion time associated with hypoglycemia and hyperlactatemia [(3)]. We also described that rats submitted to a strenuous exercise session have a great variability in time for exhaustion [(3,10)]. A question was then raised: could this variability in time to exhaustion during a strenuous swimming exercise session be attributed to the reduction of glycemia, the elevation of blood lactate level or both? To evaluate these possibilities, fasted rats were divided into four groups according to the variability in time for exhaustion: low performance (3 – 7 min), low-intermediary performance (8 – 12 min), high-intermediary performance (13 – 17 min), and high performance (18 – 22 min). From the comparison of these four groups, the main purpose of this study was to investigate the correlation between the performance of the animals with blood levels of glucose, lactate, pyruvate, lactate: pyruvate ratio, and glycerol. Liver lactate uptake and its conversion to glucose during exercise and association with exhaustion were also evaluated.

## *MATERIALS AND METHODS*

### *Animals*

Male Wistar rats weighing 210 – 240 g were used in the experiments. The rats were maintained in a 22 ± 1°C room with automatically controlled photoperiod (12 h light/12 h dark) and free access to water and commercial chow (Nuvilab®, Brasil) until the day before the experiment when the animals were fasted for 15 h.

The swimming session was performed in cylindrical water tanks (60 cm height × 30 cm diameter) of 30 L capacity and water temperature at 31 ± 1°C. The rats were kept in individual tanks. During swimming, the rats had a lead stone of 6% body weight tied to the tail. Exhaustion was defined as the incapacity to stay on the water surface, the loss of symmetrical movements during swimming or remaining underwater for more than 5 s [(11)].

The experimental protocol was approved by the ethical committee of the Universidade Estadual de Maringá and followed the international regulations on the protection of animals. For comparative purpose, we included a second rat without exercise (Control group), and a third rat that was immediately removed from the water when the second rat reached exhaustion (Exh group). The exercised rat (Exe group) without exhaustion was included in order to differentiate the metabolic changes induced by the exercise (Exe group *vs.* Control group) to the metabolic changes caused by the exhaustion (Exe group *vs* Exh group).

### *Experimental design*

*First set of experiments* (Figure 1). Three rats were placed into the individual swimming tanks at the same time (total = 62 rats). The first rat was immediately removed (control group, n=5 – 8) and the remaining ones were left to swim. When the second rat was exhausted (Exh group), the third rat was also immediately removed from the water (Exe group). The rats that swam for less than 3 min or more than 22 min were excluded. The Exh group was subdivided into four subgroups: exhaustion between 3 and 7 min (Exh 5 min: low performance, n = 5 – 8), exhaustion between 8 and 12 min (Exh 10 min: low-intermediary performance, n = 4), exhaustion between 13 and 17 min (Exh 15 min subgroup: high-intermediary performance, n = 4 – 8), or exhaustion between 18 and 22 min (Exh 20 min subgroup: high performance, n = 4 – 8). The Exe group was subdivided in four subgroups: rats that swam between 3 and 7 min (Exe 5 min subgroup, n = 5 – 7), 8 and 12 min (Exe 10 min subgroup, n = 4), 13 and 17 min (Exe 15 min subgroup, n = 4 – 8), or 18 and 22 min (Exe 20 min subgroup, n = 4 – 7).

*Second set of experiments* (Figure 1). Three rats were placed into the individual swimming tank at the same time (total = 12 rats). The first rat was removed immediately (control group, n = 6) and the remaining two animals received oral (gavage) saline (1 mL) or glycerol (0.63 g/kg) + glucose (0.25 g/kg) 20 min before starting the swimming session. The rats that received saline (saline group, n = 4 – 6) or glycerol plus glucose (n = 5 – 6) were forced to swim until exhaustion.

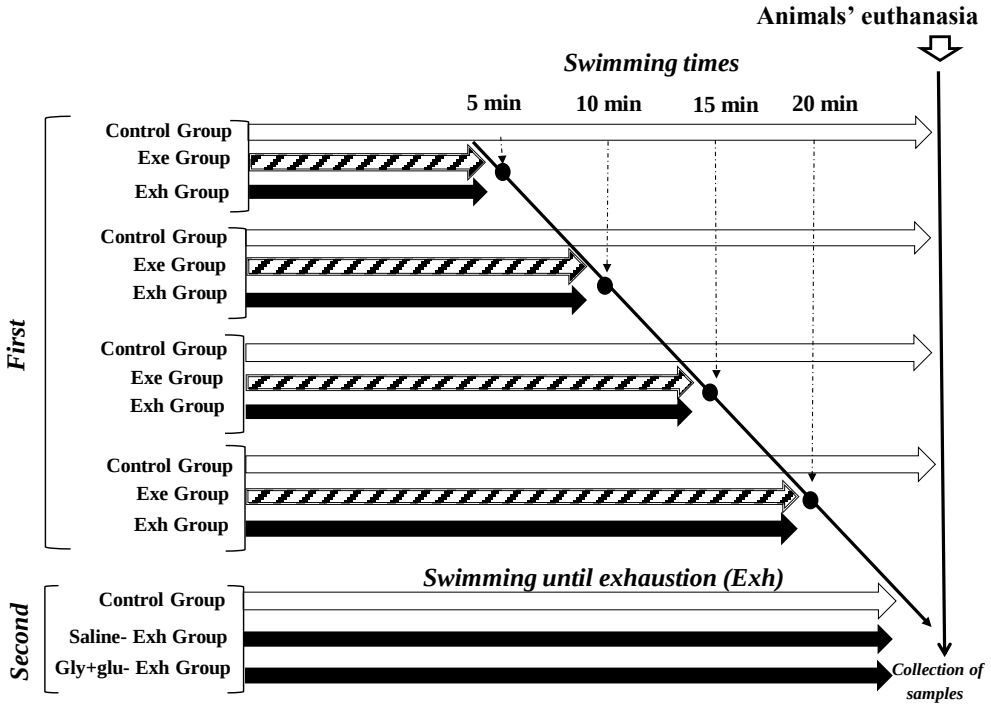


**Figure 1.** Design of the first and second experimental protocols. The control group was immediately removed from the tank. The exhausted (Exh) group was left until exhaustion, and the Exe group was immediately removed from the water at the same time as Exh. In the second experiment, the animals received oral (gavage) saline (1 mL) or 0.63 g/kg b.w. glycerol (Gly) + 0.25 g/kg b.w. glucose (glu), 20 min before starting the strenuous swimming session.

### *General procedure for all animals*

All rats were removed from the swimming tank and immediately killed by decapitation for blood collection or anesthetized for liver perfusion experiments.

### *Blood samples and analysis*

Blood was collected in tubes containing sodium EDTA and centrifuged at 1700 g for 10 min at 5 °C. Plasma levels of glucose, pyruvate, lactate, and glycerol were measured as previously described [12].

***Liver perfusion experiments to measure lactate uptake and glucose or pyruvate production from lactate***

Livers from the control (n = 4 – 5), Exe (n = 4 – 5) or Exh (n = 4 – 5) groups were compared after hemoglobin-free liver perfusion. The rats were anesthetized with intraperitoneal ketamine (100 mg/kg b.w., União Química Farmacêutica Nacional S/A, Brasil) plus xylazine (10 mg/kg b.w., Bayer S/A, Brasil). After laparotomy, livers were perfused in situ without recirculation with Krebs–Henseleit buffer containing lactate (2 mM), pH 7.4, saturated with an $O_2/CO_2$ mixture (95/5%) pumped through a temperature regulated (37°C) membrane oxygenator prior to entering the liver via a cannula inserted into the portal vein [12,13]. The whole procedure took about 10 min and the results obtained from these experiments reflect the in vivo condition immediately before the anesthesia, as previously described [3,10,12–14].

After 10 min of perfusion, lactate (2 mM) was dissolved in the perfusion fluid and infused between the 10 – 30 min of the perfusion period, followed by a period of post-infusion (10 min) to allow the return to basal levels. The activation of glucose and pyruvate production was measured as the difference between the rates of these metabolites released during (10 – 30 min) and before (0 – 10 min) the infusion of lactate. The differences allowed us to obtain and compare the areas under the curves (AUC). Liver lactate uptake was determined by the difference in the lactate concentrations during (10 – 30 min) and before (0 – 10 min) the infusion of lactate.

***Statistical analysis***

Graph-Pad Prism program (GraphPad Software, USA) was used for statistical analysis. The data were assessed for normality using the Shapiro-Wilk test. The normally distributed data were analyzed using one-way ANOVA with the *post hoc* Tukey's test for comparisons. The data that were not normally distributed were analyzed using the Kruskal-Wallis test with the Dunn test for *post hoc* comparisons. Differences between two means were analyzed using Mann Whitney-U for nonparametric tests. The Pearson product moment correlation was used to quantify the relationship among all variable estimated. Results are reported as means ± SE. $P<0.05$ was used to indicate statistical significance.

## *RESULTS*

### Blood levels of glucose, lactate, pyruvate, and lactate: pyruvate ratio

Blood levels of glucose, lactate, pyruvate, and lactate: pyruvate ratio after 5, 10, 15, or 20 min of forced swimming (Exe) or forced swimming until to exhaustion (Exh) are reported in Figure 1 (first set of experiments) and summarized in Table 1.

Blood glucose levels were lower ($P<0.05$) after 5, 10, 15 or 20 min of the forced swimming (Exe or Exh groups *vs.* Control group). In addition, except for 10 min of swimming, the reduction of glycemia was more pronounced ($P<0.05$) in the Exh group compared to the Exe group.

Except for 15 min of swimming, Exe group had higher ($P<0.05$) blood levels of lactate in comparison with the control group. The Exh group also had higher ($P<0.05$) blood concentrations of lactate in comparison with the Exe group for 5 and 15 min. In general, pyruvate levels were increased ($P<0.05$) during exercise (Exe *vs.* Control group) and decreased ($P<0.05$) when forced swimming reached exhaustion (Exh *vs.* Exe group).

Lactate: pyruvate ratio remained unchanged during exercise (Exe *vs.* Control group). However, lactate: pyruvate ratios were progressively lower from the group that reached exhaustion earlier (5 min) in relation to the group that reached exhaustion later (20 min).

**Table 1.** Blood levels of glucose, lactate, pyruvate and lactate: pyruvate ratio in 15 h fasted rats submitted to swimming and that reached exhaustion in 5, 10, 15, and 20 min.

| | | **5 min** | | **10 min** | | **15 min** | | **20 min** | |
|---|---|---|---|---|---|---|---|---|---|
| *Glucose (mmol/L)* | Control | 4.74 ± 0.11 | (*n* = 8) | 4.97 ± 0.34 | (*n* = 4) | 5.18 ± 0.29 | (*n* = 8) | 5.30 ± 0.25 | (*n* = 7) |
| | Exe | 3.14 ± 0.16[a] | (*n* = 7) | 2.28 ± 0.14[a] | (*n* = 4) | 2.53 ± 0.17[a] | (*n* = 8) | 2.33 ± 0.18[a] | (*n* = 7) |
| | Exh | 1.76 ± 0.17 [a,b] | (*n* = 8) | 2.32 ± 0.25[a] | (*n* = 4) | 1.28 ± 0.08[a,b] | (*n* = 8) | 1.33 ± 0.15[a,b] | (*n* = 8) |
| *Lactate (mmol/L)* | Control | 2.67 ± 0.75 | (*n* = 5) | 2.27 ± 0.13 | (*n* = 4) | 2.31 ± 0.21 | (*n* = 4) | 2.61 ± 0.49 | (*n* = 4) |
| | Exe | 7.69 ± 0.79[a] | (*n* = 5) | 7.82 ± 0.97[a] | (*n* = 4) | 4.65 ± 0.27 | (*n* = 4) | 6.44 ± 0.70[a] | (*n* = 4) |
| | Exh | 13.3 ± 0.42[a,b] | (*n* = 5) | 10.5 ± 1.35[a] | (*n* = 4) | 9.54 ± 1.26[a,b] | (*n* = 4) | 7.90 ± 0.85[a] | (*n* = 4) |
| *Pyruvate (mmol/L)* | Control | 0.11 ± 0.02 | (*n* = 5) | 0.15 ± 0.05 | (*n* = 4) | 0.11 ± 0.02 | (*n* = 4) | 0.12 ± 0.03 | (*n* = 4) |
| | Exe | 0.49 ± 0.08[a] | (*n* = 5) | 0.51 ± 0.08[a] | (*n* = 4) | 0.40 ± 0.08[a] | (*n* = 4) | 0.34 ± 0.08 | (*n* = 4) |
| | Exh | 0.20 ± 0.04[b] | (*n* = 5) | 0.21 ± 0.02[b] | (*n* = 4) | 0.23 ± 0.06 | (*n* = 4) | 0.25 ± 0.05 | (*n* = 4) |
| *Lactate: pyruvate ratio* | Control | 25.1 ± 4.7 | (*n* = 5) | 23.5 ± 3.1 | (*n* = 4) | 21.9 ± 2.9 | (*n* = 4) | 25.4 ± 5.9 | (*n* = 4) |
| | Exe | 19.7 ± 2.6 | (*n* = 5) | 19.4 ± 5.8 | (*n* = 4) | 13.4 ± 6.6 | (*n* = 4) | 23.3 ± 6.9 | (*n* = 4) |
| | Exh | 74.9 ± 4.9[a,b] | (*n* = 5) | 55.9 ± 15.2 | (*n* = 4) | 39.0 ± 14.5 | (*n* = 4) | 33.9 ± 6.3 | (*n* = 4) |

The control groups were placed into the water and removed immediately before starting swimming. Exhaustion (Exh) groups swam until exhaustion. Exercise (Exe) groups were removed from the water at the same time as the Exh animals Data are reported as means±SE. [a]$P<0.05$ compared to the control group; [b]$P<0.05$ compared to the Exe group (ANOVA).

**Correlations analysis**

There was a correlation between the time to achieve exhaustion and the reduction of glycemia (P=0.0475) or elevation of lactatemia (P=0.0017). However, there was no correlation between forced swimming (Exe group) and the reduction of glycemia (P=0.1721) or elevation of lactatemia (P=0.0543) (Table 2).

**Table 2-** Correlation analysis between forced swimming (Exe group) or forced to swim up to exhaustion (Exh group) and glycemia or lactatemia.

| **Correlation** | **$R^2$** | **Confidence Interval [CI 95%]** | P |
|---|---|---|---|
| *Glycemia vs. Time Exh* | -0.50 | [-0.79 to – 0.00] | 0.0475 [a] |
| *Lactatemia vs. Time Exh* | -0.71 | [-0.89 to – 0.34] | 0.0017 [a] |
| *Glycemia vs. Time Exe* | -0.35 | [-0.72 to 0.16] | 0.1721 |
| *Lactatemia vs. Time Exe* | -0.48 | [-0.79 to 0.00] | 0.0543 |

[a] P<0.05.

**Liver lactate uptake and hepatic glucose and pyruvate production from lactate – first set of experiments**

The liver lactate uptake and the hepatic glucose and pyruvate production from lactate (2 mM) were similar in the Control, Exe and Exh groups (Figure 2).

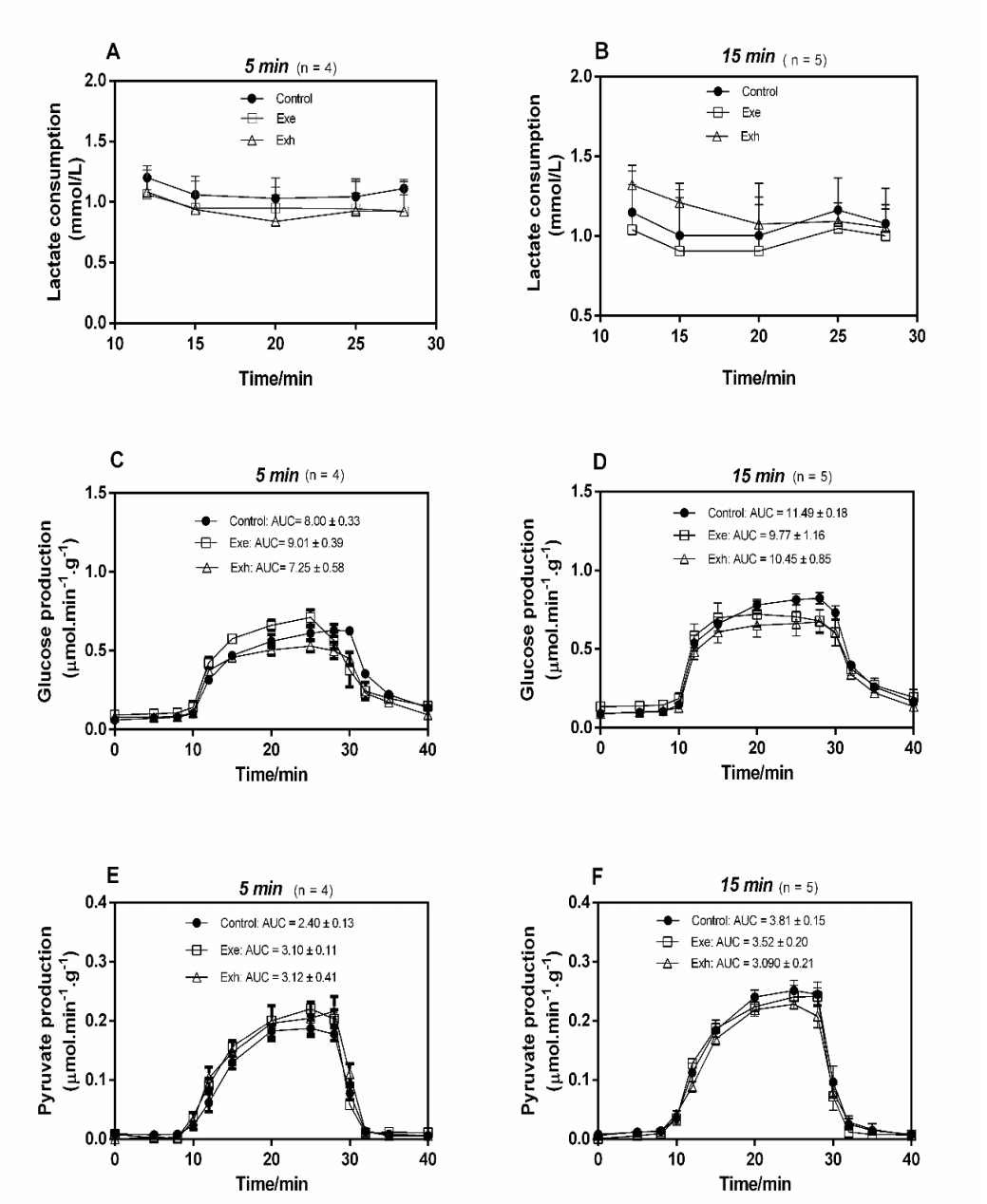


**Figure 2**. Lactate consumption (A and B), glucose (C and D) and pyruvate production (E and F) from lactate (2 mM) infused between 10 and 30 min, in the livers of 15-h fasted rats submitted to 5 or 15 min of forced swimming (Exe) or exhausted (Exh) after 5 or 15 min of forced swimming. The control group was placed in the water and removed immediately before starting swimming. Area under curves (AUC) is reported as mmol/g. Data are reported as means ± SE.

**Time of swimming and blood glucose, lactate, pyruvate and glycerol levels- second set of experiments**

Rats that received oral glycerol+glucose reached exhaustion later (P<0.05) in comparison with rats that received oral saline. The Saline Exh and Gly+glu-Exh groups had lower (P<0.05) glycemia in comparison with the sedentary control group while Gly+glu-Exh group had higher glycemia (P<0.05) in comparison with the Saline-Exh group. The Saline Exh and Gly+glu-Exh groups had higher (P<0.05) blood pyruvate and lactate levels in comparison with the control group whereas there was no difference for lactate and pyruvate levels compared to the Saline-Exh and Gly+glu-Exh groups. The Gly+glu-Exh groups had higher glycerol levels (P<0.05) in comparison with the control group or saline-Exh group (Table 3).

**Table 3-** Swimming time and blood glucose, lactate, pyruvate, and glycerol levels in 15 h fasted rats that received oral (gavage) saline (1 mL) or glycerol (0.63 g/kg) + glucose (0.25 g/kg), 20 min before the onset of forced swimming to exhaustion (Exh).

| **Parameters** | **Control** | **Saline Exh** | P | **Gly + glu Exh** | P |
|---|---|---|---|---|---|
| *Time Swimming (min)* | – | 12.6 ± 1.7 (n = 6) | | 19.0 ± 1.0 [b] (n = 6) | 0.0238 |
| *Glucose (mmol/L)* | 5.05 ± 0.13 (n = 6) | 2.00 ± 0.09[a] (n = 6) | [a] 0.0001 | 2.54 ± 0.09 [a,b] (n = 6) | [a] 0.0001<br>[b] 0.0103 |
| *Lactate (mmol/L)* | 1.32 ± 0.20 (n = 6) | 5.47 ± 0.55[a] (n = 4) | [a] 0.0001 | 6.3 ± 0.41 [a] (n = 5) | [a] 0.0001 |
| *Pyruvate (mmol/L)* | 0.07 ± 0.01 (n = 6) | 0.18 ± 0.01[a] (n = 5) | [a] 0.0002 | 0.22 ± 0.01 [a] (n = 5) | [a] 0.0001 |
| *Glycerol (mmol/L)* | 0.12 ± 0.013 (n = 6) | 0.19 ± 0.011 (n = 6) | | 98.2 ± 22.49 [a,b] (n = 6) | [a,b] 0.0002 |

The control groups were placed in water and removed immediately before starting swimming. Data are reported as means±SE. [a]P<0.05 compared to the control group; [b]P<0.05 compared to the saline group (ANOVA).

## DISCUSSION

The capability of mammals to survive in conditions of prolonged fasting depends on the ability of the liver to produce energy by oxidizing fatty acids and use part of this energy to produce glucose. These metabolic processes are accelerated if fasting is associated with an intense aerobic physical exercise since there is an intensification of adipose tissue lipolysis and muscle glycolysis/proteolysis. As a consequence, blood concentrations of free fatty acids, glycerol, amino acids, and lactate are increased. With the increased supply of glucose precursors, particularly lactate, liver gluconeogenesis becomes a significant source of glucose during exercise.

In high-intensity aerobic exercise associated with fasting, a condition in which hepatic glycogen content is low, the glucose uptake and lactate production by skeletal muscles exceed lactate uptake and its conversion to glucose in the liver [15,16]. In agreement with these studies, we found a correlation between hyperlactatemia and hypoglycemia with time to exhaustion in a strenuous swimming session. However, there is a time sequence in the participation of blood glucose and lactate for determining swimming performance. For rats that reached physical exhaustion between 3 and 7 min (Exh 5 min: low performance) or 8 and 12 min (Exh 10 min: low-intermediary performance), the time to exhaustion was better associated with higher lactate:pyruvate ratio. On the other hand, for rats that reached exhaustion between 13 and 17 min (Exh 15 min: high-intermediary performance) or 18 and 22 min (Exh 20 min: high performance), the exhaustion was better associated with hypoglycemia.

During an intense aerobic exercise session, lactatemia increases abruptly [17,18], resulting in elevation of the lactate: pyruvate ratio (indicative of the redox state – NADH:$NAD^+$ ratio), as a consequence of the reduction of pyruvate to lactate through lactate dehydrogenase [19]. In this condition, the brain could receive a considerable lactate supply. However, there is a limitation in the transport of lactate through the blood-brain barrier and the elevated blood lactate levels cannot supply the brain lactate deficit due to hypoglycemia [20]. In fact, the energy homeostasis in the brain depends on the metabolic cooperation between astrocytes and neurons. Thereby, the energy metabolism in astrocytes is predominantly glycolytic and lactate produced from glucose is released and then used by the neurons [21]. Thus, the hypothesis that during acute exercise-induced hypoglycemia there is a short-term deficit of glucose availability in astrocytes and consequently a lactate deficit in neurons that cannot be compensated by the elevation of blood lactate levels is confirmed.

Stressful conditions, with increased sympathetic activity, are commonly associated with hyperglycemia [22]. However, we reported that strenuous swimming is associated with hypoglycemia in fasted rats. Hypoglycemia associated with intensive exercise is also reported in diabetic patients receiving insulin [23].

To confirm that energy supply influences performance during physical exercise in fasted rats, glycerol plus glucose was used as energy precursors. Animals that received oral glycerol plus glucose before swimming had better performance, i.e., the exhaustion occurred later in comparison with animals that received saline before exercise. The contribution of glycerol to performance may be attributed not only to its hepatic conversion to glucose during exercise [3], but also by the fact that glycerol is directly used by skeletal muscle and its uptake increases by many folds during exercise [24]. It must be emphasized that blood lactate levels and liver glucose production from lactate play a central role for glycemia and lactatemia maintenance during exercise [3,25]. Lactate is converted into glucose through various steps in the liver gluconeogenesis pathway.

During liver perfusion experiments, infused lactate crosses the cell membrane and is then converted to pyruvate in the cytosol. Pyruvate then enters into the mitochondria where it is carboxylated and then leaves the mitochondria as malate. In cytosol, malate is converted to oxaloacetate and then to phosphoenolpyruvate and, after various steps, is converted by microsomal glucose-6-phosphatase to glucose, which is released from the liver to the blood.

Liver lactate uptake and glucose production from lactate remained unchanged during strenuous exercise (Exercise group and Exhausted group). The liver prevents plasma lactate elevation and blood glucose decrease during an acute intense swimming section through lactate uptake and its conversion to glucose, corroborating studies by others [26,27].

A limitation of the experiments in isolated perfused liver is the use of a unique concentration of lactate, i.e. 2 mM, whereas *in vivo* plasma lactate concentration exhibits great variability in the sedentary condition and in the strenuous exercise associated with exhaustion [28].

Glucose from liver gluconeogenesis is used for ATP production in skeletal muscle through glycolysis, generating lactate and pyruvate [29]. During skeletal muscle contraction, the activation of the AMP-activated protein kinase (AMPK) in response to the increased ATP demand [30] favors the translocation of type 4 glucose transporter (GLUT 4) to the plasma membrane increasing glucose uptake [31-34], which is associated with decreased glycemia and the consequent hypoglycemia [35].

In conclusion, there was a time sequence in the participation of lactate: pyruvate ratio and hypoglycemia determining the performance during an intense aerobic swimming section in fasted

rats. Furthermore, the liver had an important participation in the prevention of hyperlactatemia and hypoglycemia during swimming through lactate uptake and its conversion to glucose.

***ACKNOWLEDGMENTS***

CNPq, CAPES and PRONEX/Araucaria Foundation supported this study.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

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# ANEXO 1

**1)** Investigation of the acute effects of dry extract of *glycine max* on postprandial glycemia in rats. *Brazilian Archives of Biology and Technology,* v.59, p. e16150085, 2016.

# ANEXO 2

## 2) Depósito de Patente

“Formulação contendo glicose associada ao 1,2,3-propanotriol para a prevenção e tratamento das hipoglicemias”. (Número do Processo: BR 10 2017 013771 6).