UNIVERSIDADE ESTADUAL DE CAMPINAS

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EFEITO DA DURAÇÃO, REPETIÇÃO E INTENSIDADE DO ESTRESSE SOBRE INDICADORES METABÓLICOS EM RATOS.

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À minha família, por compreender

quanto vale um ideal ...

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RESUMO

O objetivo deste trabalho foi avaliar a mobilização metabólica em ratos submetidos a 50-60 minutos de natação de acordo com três protocolos: no grupo A os ratos foram submetidos a uma sessão de 50 minutos de natação; no grupo B o tempo total de natação foi dividido em três sessões diárias de 5, 15 e 30 minutos e no grupo C os ratos foram submetidos a cinco sessões de natação de 5, 15, 30, 45 e 60 minutos. Os conteúdos de glicogênio (GO) do fígado e dos músculos sóleo e gastrocnêmio foram depletados nos três grupos experimentais, mas a glicemia (GA) foi aumentada significativamente apenas no grupo B. Concentrações séricas de lactato (L) foram maiores que os controles nos grupos A e B. Houve aumento significativo nas concentrações dos ácidos graxos livres (AGL) nos três grupos experimentais. Como a lipólise basal (LB) estava aumentada em adipócitos isolados do grupo B, o aumento plasmático dos níveis de AGL poderia ser conseqüência da estimulação da lipólise pela adrenalina e noradrenalina. Adipócitos isolados de ratos dos grupos A e B foram supersensíveis à adrenalina, entretanto os do grupo C não foram. Conclui-se, portanto, que após cinco sessões de natação as alterações nos marcadores metabólicos foram menos pronunciadas que após uma sessão de natação com duração similar e que três sessões de natação não foram suficientes para causar adaptação. Em outro experimento, avaliamos o efeito da temperatura da água sobre a mobilização metabólica, em ratos submetidos a três sessões de natação de 5, 15 e 30 minutos a 35°C ou a 18°C. Após a sessão de natação a 35°C os ratos mantiveram sua temperatura corporal comparados ao controle, entretanto os ratos que nadaram a 18°C mostraram hipotermia. As concentrações de GO no fígado e nos músculos sóleo e gastrocnêmio foram depletadas nos

ratos que nadaram a 35°C. Nestes ratos as concentrações de GA, L e AGL foram significativamente maiores que o controle. Nos ratos que nadaram a 18°C as concentrações de GO no músculo gastrocnêmio diminuíram e a glicemia não foi alterada em comparação ao controle. As concentrações de L e AGL foram significativamente maiores que o controle e também foram maiores do que os ratos que nadaram a 35°C. A LB foi aumentada em adipócitos isolados de ambos os grupos, assim como a sensibilidade à adrenalina. Em ratos submetidos à natação em baixa temperatura ocorre bloqueio da mobilização de carboidratos e do metabolismo aeróbio da glicose.

ABSTRACT

The aim of this work was to evaluate metabolites mobilization in rats submitted to 50-60 minutes of swimming according to three protocols as follows: in the group A, rats were submitted to one 50 minutes swimming session; in the group B the total 50 minutes swimming time was divided in three daily sessions of 5, 15 and 30 minutes and in the group C, rats were submitted to five swimming sessions of 5, 15, 30, 45 and 60 minutes. The glycogen (GO) contents of liver, soleus and gastrocnemius muscles were depleted in the three experimental groups, but it was not altered in ventricle. The glycaemia (GA) was significantly increased only in the group B and it was not different from control in the rats from groups A and C. However, serum lactate (L) concentration was higher than control in the groups A and B, with no significant differences in the group C. There were significant increases in serum free fatty acids (FFA) concentration in all the three experimental groups, indicating that lipids mobilization had already started. Since basal lipolysis (BL) was altered in isolated adipocytes from group B but not A or C, the increase in plasma FFA levels might be a consequence of the lipolysis stimulation by epinephrine and norepinephrine. Moreover, adipocytes from rats submitted to swimming were supersensitive to epinephrine, in groups A and B, but not C. We conclude that after five swimming sessions the metabolic tracers alterations were less pronounced than after one session even though session durations were similar. The metabolic features of groups A and B are mostly similar suggesting that three swimming sessions were not enough to cause adaptation. To test this hypothesis, in other experiment, we evaluated the effect of water temperature, as a determinant of stress intensity, on metabolic markers in rats submitted to

three swimming sessions of 5, 15 and 30 minutes at 35°C or 18°C. Immediately after swimming sessions at 35°C rats were homeothermic compared to control whereas rats swimming at 18°C showed hypothermia. The GO content of liver, soleus and gastrocnemius muscles was depleted after swimming at 35°C. In these rats, GA, L and FFA concentrations were significantly higher than control. In rats swimming at 18°C the GO content of gastrocnemius muscle decreased and GA were not altered compared to control, whereas L and FFA concentrations were significantly higher than control and than rats swimming at 35°C. BL was increased in adipocytes isolated from both groups, as well as the sensitivity to epinephrine. We conclude that swimming at 35°C causes substrates mobilization and increases metabolism mostly guaranteed by the higher plasma levels of epinephrine, norepinephrine and corticosterone. These metabolic alterations allow the animal to keep homoeothermy and normal plasma glucose levels. However, swimming at low temperature impaired carbohydrates mobilization and glucose aerobic metabolism, then causing increased serum lactate and FFA levels although plasma levels of catecholamines were higher than in the other groups. Those alterations are not sufficient to avoid hypothermia.

I. INTRODUÇÃO

1.1 Aspectos neuroendócrinos do estresse

O equilíbrio orgânico pode sofrer variações diante do efeito de fatores do ambiente interno e/ou externo, denominados agentes estressores. Evolutivamente, os animais adquiriram a capacidade de resposta a estes agentes. A função da resposta de estresse é manter tal equilíbrio orgânico, por meio de alterações fisiológicas compensatórias, que permitem inicialmente, resistir ao agente estressor e, posteriormente, adaptar-se à sua presença, quando esta é mantida ou repetida.

Esta resposta está relacionada ao eixo hipotálamo-hipófise/adrenal e foi denominada "Síndrome Geral de Adaptação" por SELYE em 1936, que a descreveu em três estágios. O primeiro estágio configura uma "reação de alarme", representando a resposta inicial do organismo frente ao agente estressor, e ocorreria quando o organismo não estivesse adaptado ao estímulo recebido. Em seguida, sendo mantido o estímulo, ocorreria a fase de resistência, caracterizada pela ativação de mecanismos adaptativos. Não ocorrendo adaptação, desenvolver-se-ia o estágio de exaustão onde o organismo estaria susceptível a distúrbios (SELYE, 1936; VAN DE KAR et al., 1991).

Inicialmente, a Síndrome Geral de Adaptação foi descrita como uma reação geral e inespecífica (SELYE, 1936). A continuidade dos estudos sobre a resposta de estresse evidenciou seu caráter específico (MASON, 1968a; MASON, 1968b; KRULICH et al., 1974; HENNESSY et al., 1979; HERD, 1991) e a influência de estressores ambientais (MARPLES, 1972), do sexo (LESCOAT et al., 1970; ANISHCHENKO e GUDKOVA, 1992; PARÉ e REDEI, 1993), da idade (RIEGLE, 1973) e, principalmente, da percepção individual do agente estressor, o que depende das experiências vividas anteriormente e da novidade ou previsibilidade do estímulo (VOGEL e JENSH, 1998; GRIFFIN, 1989).

Na resposta de estresse, a alteração fisiológica inicial decorre da decodificação

da informação sensorial pelo sistema límbico-mesencefálico, em associação com áreas corticais do cérebro, e por transmissão neural emissora de sinais ativadores ou inibidores para o eixo hipotálamo-hipófise adrenal (ELIOT, 1992; BEIER et al., 2002).

Observa-se a ativação dos eixos sistema nervoso simpático-medula adrenal e hipotálamo-hipófise-córtex adrenal, causando respectivamente a elevação das concentrações plasmáticas de catecolaminas e de glicocorticóides (SELYE, 1956; U'PRICHARD e KVETNANSKY, 1980; AXELROD e REISINE, 1984; KONARSKA et al., 1989; DE BOER et al., 1989; KONARSKA et al., 1990; DE BOER et al., 1990; NONOGAKI, 2000).

As catecolaminas, por sua vez, podem causar elevação adicional nas concentrações de glicocorticóides, através de adrenoceptores localizados no hipotálamo, os quais estimulam a liberação de hormônio liberador da corticotrofina (CRH). Este último causa elevação das concentrações plasmáticas não apenas de corticotrofina (ACTH) (NOMURA et al., 1981; AL-DAMLUJI, 1988), como também de catecolaminas (BROWN e FISHER, 1984; 1985).

Outros eixos neuroendócrinos também podem ser alterados, como os relacionados ao hormônio do crescimento (KRULICH et al., 1974; LUGER et al., 1988), ao hormônio luteinizante (KRULICH et al., 1974; EUKER et al., 1975), ao hormônio folículo-estimulante (KRULICH et al., 1974), à prolactina (EUKER et al., 1975; KANT et al., 1983) e à tireotropina (MILLS, 1985).

Do aumento nas concentrações plasmáticas de catecolaminas e de glicocorticóides, decorrem alterações metabólicas no sentido de causar mobilização de substratos energéticos, a partir dos tecidos de armazenamento, como o hepático e o adiposo branco, liberando-os para a circulação sangüínea. O tecido adiposo marrom é ativado quando o agente estressor inclui o frio, uma vez que sua função se relaciona à termogênese (BERNE e LEVY, 2000).

Este redirecionamento metabólico disponibiliza maior quantidade de carboidratos e lipídios para a atividade celular e permite ajustes de sistemas como o

cardiovascular, o respiratório, o nervoso e o muscular. Tais adaptações contribuem para a manutenção da homeostase em situações de estresse, nas quais podem ser deflagrados comportamentos de luta ou fuga (SELYE, 1936; HERD, 1991).

1.2. Efeitos metabólicos dos hormônios do estresse

Os hormônios liberados durante a reação de estresse agem sobre tecidos como o adiposo, o hepático e o pancreático, modificando o metabolismo de lipídios e de carboidratos, com efeitos opostos aos da insulina (PITTNER et al., 1985a, b; BRINDLEY et al., 1988; LAFONTAN, 2001; RADZIUK e PYE, 2001).

Com relação ao metabolismo de carboidratos, as catecolaminas endógenas, principalmente a adrenalina, estimulam a glicogenólise e a gliconeogênese hepáticas (HIMMS-HAGEN, 1967; EXTON et al., 1972; HUE et al., 1978; YOREK et al., 1980; KNEER e LARDY, 1983; PILKIS et al., 1988; WATT et al., 2001).

Em tecido pancreático estas, através de adrenoceptores alfa, estimulam a liberação de glucagon pelas células alfa das ilhotas de Langerhans, enquanto inibem a liberação de insulina pelas células beta (PORTE JR., 1969; PORTE JR. e ROBERTSON, 1973), sendo que os adrenoceptores beta podem estimular a liberação de insulina pelas células beta-pancreáticas (YOSHIDA, 1992; ATEF et al., 1996).

A corticosterona influencia o metabolismo de carboidratos através de sua ação permissiva aos efeitos glicogenolíticos e gliconeogênicos das catecolaminas e do glucagon (EXTON et al., 1972). Como resultado destes efeitos ocorre aumento da glicemia (VERAGO et al., 2001; RETANA-MARQUEZ et al., 2003).

Em tecido adiposo branco de ratos, o glucagon e os agonistas beta-adrenérgicos estimulam a atividade lipolítica causando liberação de ácidos graxos livres e glicerol para o plasma (SLAVIN et al., 1994; LAFONTAN et al., 1995; 2001; 2002), enquanto os

glicocorticóides desempenham um papel permissivo na manutenção da resposta lipolítica às catecolaminas (FAIN e GARCÍJA-SÁINZ, 1983).

Aumentos dos níveis plasmáticos de ácidos graxos livres e de glicerol foram observados em cães e humanos após infusão endovenosa de catecolaminas (CONNOLLY et al., 1991). GALITZKY et al. (1993) observaram a elevação de ácidos graxos livres em plasma de cães, durante a infusão de noradrenalina, adrenalina, isoproterenol, BRL37344 e outros agonistas beta-adrenérgicos. DARIMONT et al. (1996) demonstraram *in situ*, maior liberação de glicerol pelo tecido adiposo de ratos, após infusão de isoproterenol. VERAGO et al. (2001) demonstraram *in vivo* que as concentrações plasmáticas de corticosterona em ratos aumentaram significativamente após cada sessão de choque nas patas; as de glicerol não se alteraram; as de triacilgliceróis aumentaram apenas após a 1ª sessão, e as concentrações plasmáticas de glicose aumentaram após as 2ª e 3ª sessões de choque nas patas.

O fígado é considerado o principal órgão responsável pela captação de glicerol plasmático (LIN, 1977), que pode ser utilizado como um dos substratos para a gliconeogênese, mas também para a síntese de triacilgliceróis (PITTNER et al., 1985a e b; BRINDLEY et al., 1988; NEWGARD et al., 2000).

A síntese hepática de triacilgliceróis a partir de ácidos graxos e de glicerol pode ser modulada pelas catecolaminas, pelo glucagon e pelos próprios ácidos graxos, principalmente através da estimulação da atividade enzimática da fosfatidato fosfohidrolase (PAULETTO et al., 1991; PITTNER et al., 1985a e b; BRINDLEY et al., 1988). Em hepatócitos, tanto a síntese, quanto a atividade desta enzima, são estimuladas por glicocorticóides (KNOX et al., 1979; PITTNER et al., 1985a e b; BRINDLEY et al., 1988).

Os triacilgliceróis, em células hepáticas, podem ser incorporados em lipoproteínas de muito baixa densidade (VLDL) e assim serem liberados para o plasma sangüíneo (PITTNER et al., 1985a e b; BRINDLEY et al., 1988). Entretanto, a velocidade desta liberação é dependente da função circulatória no tecido hepático, e esta também pode ser influenciada pela ação adrenérgica sobre os vasos sangüíneos (GARDEMANN et al., 1991; YAMAUCHI et al., 1998). Em cultura de hepatócitos de ratos, observou-se que a

dexametasona estimula a secreção de VLDL (MANGIAPANE e BRINDLEY, 1986).

Além disso, a concentração plasmática de triacilgliceróis também é dependente da velocidade de sua captação, mediada pela atividade da lipoproteína lipase em tecidos como o adiposo, o muscular e o cardíaco. Vários hormônios influenciam a atividade da lipoproteína lipase, incluindo a insulina, as catecolaminas e os glicocorticóides. Cada um destes hormônios pode estimular ou inibir sua atividade, de acordo com o tecido em que agem (ASHBY e ROBINSON, 1980; HULSMANN e DUBELAAR, 1986; DESHAIES et al., 1993; MORA-RODRIGUEZ e COYLE, 2000).

Em situações em que ocorre elevação da concentração plasmática das catecolaminas e dos glicocorticóides, como ocorre durante o estresse, espera-se um aumento do *turnover* dos elementos lipídicos do metabolismo, que pode ser acompanhado ou não da elevação de suas concentrações plasmáticas.

Em animais, o tecido adiposo marrom (TAM) parece especificamente organizado para desempenhar funções de termogênese. Suas grandes mitocôndrias são estimuladas por uma proteína de desacoplamento (*uncoupling protein* - UCP), a termogenina, que dissocia a produção de ATP da utilização do oxigênio na cadeia respiratória impedindo o funcionamento normal da ATP sintetase e a conversão do ADP em ATP. Como conseqüência, há produção de calor, sem produzir trabalho químico ou mecânico útil (calafrios). A síntese de termogenina é regulada por sinais do sistema nervoso simpático, isto é, através da noradrenalina interagindo com adrenoceptores β_3 (LAFONTAN e BERLAN, 1993; BERNE e LEVY, 2000). Recentemente demonstrou-se que a expressão do gene que codifica essa proteína mitocondrial está sob controle da triiodotironina (T_3) e da noradrenalina. Na ausência do hormônio tireoidiano, o gene permanece silencioso, enquanto que na presença de T_3 a taxa de transcrição gênica aumenta de 5 a 10 vezes, levando a aumento proporcional dos seus níveis de RNAm, da concentração mitocondrial dessa proteína desacopladora e da termogênese tecidual (BIANCO e KIMURA, 1999). Em 2000, HERNÁNDEZ e OBREGÓN demonstraram que a triiodotironina amplifica a estimulação adrenérgica da expressão da proteína de desacoplamento, em tecido adiposo marrom de ratos.

1.3. Estresse e sensibilidade dos tecidos às catecolaminas

Como conseqüência das modificações de natureza neural e hormonal ligadas à reação de estresse, além das modificações na dinâmica metabólica, descritas acima, podem ocorrer também alterações de sensibilidade adrenérgica em diferentes tecidos.

Dentre os fatores que determinam subsensibilidade ou supersensibilidade adrenérgica em um dado tecido, considera-se: a ação dos sistemas de metabolização, que limitam a meia vida do agonista na biofase, o número de receptores, a afinidade dos receptores aos agonistas e/ou o processo de acoplamento entre os receptores e os sistemas de segundos mensageiros.

Os mecanismos envolvidos nessas alterações podem variar de acordo com o tipo de agente estressor empregado e de fatores intrínsecos, como a espécie e o sexo do animal. No caso de ratas fêmeas, também dependem das fases do ciclo reprodutivo (POLLARD et al., 1975; RODRIGUES et al., 1995; MARCONDES et al., 1996; VANDERLEI et al., 1996; SPADARI-BRATFISCH et al., 1999; TANNO et al., 2002).

A equipe do Laboratório de Estudos do Estresse (LABEEST / UNICAMP) vem estudando a sensibilidade a agonistas adrenérgicos do tecido cardíaco e, mais recentemente, também de adipócitos isolados de ratos submetidos a estresse (MARCONDES et al., 1996; VANDERLEI et al., 1996; SPADARI-BRATFISCH et al., 1999; FARIAS-SILVA et al., 1999).

Como resultante dos mecanismos adaptativos associados à reação de estresse, vários autores demonstraram que ocorre subsensibilidade ou supersensibilidade às catecolaminas em átrios direitos isolados de ratos machos submetidos a diferentes agentes estressores (CALLIA e DE MORAES, 1984; BASSANI e DE MORAES, 1987a e b; 1988; SPADARI e DE MORAES; 1988; SPADARI et al., 1988; CAPAZ e DE MORAES, 1988).

Neste mesmo tecido, as alterações de sensibilidade às catecolaminas, observadas após uma ou três sessões de natação, estão relacionadas respectivamente, à inibição dos sistemas de metabolização das catecolaminas (SPADARI et al., 1988) e à alteração na constante de afinidade de antagonistas pelos receptores adrenérgicos (SPADARI e DE MORAES; 1988). Os autores propuseram que ambos os mecanismos

seriam dependentes do aumento dos níveis plasmáticos de corticosterona, induzidos pela exposição ao estímulo estressor, pois a adrenalectomia cirúrgica ou terapêutica impediu o aparecimento de tais alterações (SPADARI et al., 1988; SPADARI e DE MORAES; 1988), assim como o bloqueio dos receptores de glicocorticóides (RAHNEMAYE et al., 1992).

Posteriormente SANTOS e SPADARI-BRATFISCH, 2001 e SANTOS et al., 2002 demonstraram que átrios direitos isolados de ratos submetidos ao estresse por choque nas patas foram supersensíveis à isoprenalina e ao TA2005, agonista seletivo de adrenoceptores β_2, como conseqüência de aumento da expressão deste subtipo de adrenoceptores.

Em fêmeas, tanto MARCONDES (1995), utilizando estresse por natação, quanto VANDERLEI et al. (1996), que utilizaram estresse por choque nas patas, observaram que após três sessões de estresse, quando as ratas eram sacrificadas em diestro, o tecido atrial apresentou subsensibilidade aos efeitos cronotrópicos da noradrenalina. Contudo, quando o sacrifício ocorreu em estro, não foram observadas alterações de sensibilidade do tecido atrial às catecolaminas. Os autores concluíram que as alterações de sensibilidade do tecido atrial, observadas em ratas submetidas a estresse por natação ou choque nas patas, são dependentes das fases do ciclo estral, além de confirmarem que tais alterações ocorrem na vigência de níveis plasmáticos elevados de corticosterona (MARCONDES, 1995; VANDERLEI et al., 1996).

Vários outros autores também relataram alterações de sensibilidade adrenérgica em tecido cardíaco isolado de animais submetidos a estresse por frio, restrição alimentar, exercício físico, imobilização, natação ou choque nas patas (U'PRICHARD e KVETNANSKY, 1980; NOMURA et al., 1981; BASSANI e DE MORAES, 1987a e b; SPADARI e DE MORAES, 1988).

Com o prosseguimento dos trabalhos, e a compreensão de que o estresse determina alterações de sensibilidade adrenérgica em tecido cardíaco, as quais estão relacionadas com a resposta hormonal ao agente estressor, surgiu interesse em avaliar se alterações semelhantes ocorreriam em outros tecidos que, reconhecidamente, sejam alvos para a ação das catecolaminas.

Neste sentido, FARIAS-SILVA et al. (1999) demonstraram que as alterações de sensibilidade da resposta adrenérgica causadas por estresse ocorrem também em adipócitos

epididimais isolados de ratos submetidos a três sessões de choque nas patas. Nestas células adiposas ocorreu supersensibilidade ao isoproterenol e à adrenalina e subsensibilidade à noradrenalina e ao BRL37344 (FARIAS-SILVA et al., 1999). A resposta analisada foi a liberação de glicerol, a qual foi tomada como índice da lipólise que ocorre em resposta às catecolaminas.

Estes resultados conferiram importância fisiológica considerável ao fenômeno descrito neste modelo experimental, pois sugerem que as alterações de sensibilidade adrenérgica, induzidas por estresse, não se restringem ao tecido cardíaco, mas que se manifestam também em outros tecidos, e que as alterações cardiovasculares dele decorrentes podem ser acompanhadas de alterações endócrinas e metabólicas, com repercussão sistêmica.

Entretanto, restava demonstrar se as alterações de sensibilidade observadas *in vitro* poderiam também ser observadas *in vivo*. VERAGO et al. (2001) utilizaram ratos machos, alimentados e conscientes, após terem sido submetidos a três sessões diárias de estresse por choque nas patas, para estudar o efeito deste protocolo experimental sobre as concentrações plasmáticas de corticosterona, glicose, glicerol e triacilgliceróis. Aqueles autores mostraram que as concentrações plasmáticas de corticosterona aumentaram significativamente após cada sessão; as de glicerol não se alteraram; as de triacilgliceróis aumentaram apenas após a 1ª sessão, e as concentrações plasmáticas de glicose aumentaram após as 2ª e 3ª sessões de choques nas patas.

A ausência de alterações na concentração plasmática de glicerol apesar dos níveis elevados de corticosterona e, provavelmente, de catecolaminas endógenas que se segue ao estresse, foi curiosa, pois, se aceita que nestas condições, a lipólise está aumentada e que, como conseqüência, a liberação de glicerol estaria elevada. Assim, estes dados sugerem que poderia haver um aumento do *turnover* de glicerol em ratos submetidos a estresse. Por outro lado, como as concentrações de triacilgliceróis aumentaram após a 1ª sessão e as de glicose, após as 2ª e 3ª sessões, poder-se-ia sugerir que após a 1ª sessão de choques, o glicerol liberado estaria sendo utilizado para a síntese de triacilgliceróis pelas células hepáticas, mas que, com a repetição de estresse, a função hepática poderia estar sendo redirecionada para a liberação de glicose. Esta hipótese, entretanto, não foi testada.

Considerando que as alterações demonstradas em tecidos isolados de ratos

submetidos a estresse se mostraram específicas, e que o estresse por choque nas patas causa alterações fisiológicas que apresentam repercussões metabólicas e endócrinas possíveis de serem demonstradas *in vivo*, o objetivo deste trabalho foi o de analisar a mobilização metabólica em ratos submetidos a 50-60 minutos de natação de acordo com três protocolos, como se segue: no grupo A os ratos foram submetidos a uma sessão de 50 minutos de natação; no grupo B o tempo total de natação de 50 minutos foi dividido em três sessões diárias de 5, 15 e 30 minutos e no grupo C os ratos foram submetidos a cinco sessões de natação de 5, 15, 30, 45 e 60 minutos.

Em outro experimento, avaliamos o efeito da intensidade do estressor sobre a mobilização metabólica. Este parâmetro foi determinado pela temperatura da água no tanque de natação, 35 ou 18°C (KONARSKA et al., 1989; 1990).

Os resultados obtidos foram organizados em dois manuscritos, um já aceito para publicação e outro que será enviado. Ambos são apresentados a seguir.

II. CAPÍTULOS

II.1. Effect of Swimming Session Duration and Repetition on Metabolic Markers in Rats

Este trabalho foi aceito para publicação no periódico *Stress, The International Journal on the Biology of Stress.*

1: Stress 2003 Jun;6(2):127-32 Related Articles, Links

Effect of swimming session duration and repetition on metabolic markers in rats.

Sampaio-Barros MM, Farias-Silva E, Grassi-Kassisse DM, Spadari-Bratfisch RC.

The aim of this study was to investigate the profile of metabolites in male rats subjected to 50-60 min of swimming on three protocols: group A, a single 50 min swimming session; group B, one session a day for three days (5 min on day 1, 15 min on day 2 and 30 min on day 3); and group C, one session a day for 5 days, with increasing duration from 5 min on day 1, 15, 30, 45 and 60 min on consecutive days. The interval between sessions was 24 h. Measurements were made after the last swimming session. Controls did not swim. The glycogen content of liver and gastrocnemius and soleus muscle was depleted in the three groups that swam, but blood glucose concentration was significantly increased only in group B. Serum lactate concentrations were greater than the controls in groups A and B. There were significant increases in serum free fatty acid concentrations in all groups that swam. The increases in plasma free fatty acids may have resulted from lipolysis stimulated by endogenous catecholamines in groups A and C, since basal lipolysis measured in vitro was unchanged by swimming. The large increase in basal lipolysis in group B may have contributed to the rise in plasma free fatty acids. Adipocytes from rats in groups A and B were supersensitive to epinephrine, whereas those from group C were not. We conclude that the metabolic alterations were less pronounced after the last of five swimming sessions over 5 days than after a single session, even though session duration and the contribution of the physical component were similar. Glucose mobilization, but probably not utilization, was similar in the three groups that swam. The mechanisms of lipid mobilization from adipose tissue differed, depending on the stress paradigm. The metabolic changes in groups A and B indicated that three daily swimming sessions were insufficient to cause adaptation. The results contrast with previous findings for foot-shock stress, which leads to sensitization rather than adaptation in response to repeated stimuli.

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To Editor-in-Chief: Professor John A. Russell

Effect of Swimming Session Duration and Repetition on Metabolic Markers in Rats

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Running title: METABOLIC MARKERS AFTER SWIMMING STRESS

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ABSTRACT

The aim of this work was to investigate the profile of metabolites in rats submitted to 50-60 min of swimming in three protocols: group A, a single 50 min swimming session; group B, three sessions one on each day for three days (5 min on day 1, 15 min on day 2 and 30 min on day 3); and group C, one session a day, with increasing duration from 5 min on day 1 to 15, 30, 45 and 60 min on consecutive days. The interval between sessions was 24 h. The glycogen content of liver and gastrocnemius and soleus muscle was depleted in the three groups, but glycaemia was significantly increased only in group B. Serum lactate concentrations were higher than the controls in groups A and B. There were significant increases in the serum free fatty acid concentrations in all groups. The increases in plasma free fatty acids could be a consequence of lipolysis stimulated by endogenous catecholamines, at least in groups A and C, since basal lipolysis remained unchanged after swimming. The large increase in basal lipolysis in group B could contribute to the rise in plasma free fatty acids. Adipocytes from rats in groups A and B were supersensitive to epinephrine whereas those from group C were not. We conclude that the metabolic alterations were less pronounced after five swimming sessions than after one session, even though session duration and the contribution of the physical component were similar. The glucose mobilization, but probably not use, was similar in the three groups. The mechanisms of lipid mobilization from adipose tissue differed, depending on the stress paradigm. The metabolic changes in groups A and B indicated that three swimming sessions were not enough to cause adaptation, in contrast to foot-shock stress which leads to sensitisation rather than adaptation in response to repeated stimuli.

Keywords: adipocytes, free fatty acid, glycaemia, stress

INTRODUCTION

The cardiovascular system and metabolic pathways are important targets for several stress hormones. The consequences of stress in cardiac tissue depend on the type of stressor. Thus, swimming stress reduces the chronotropic responses mediated by β_1-adrenoceptors (Spadari and De Moraes, 1988; Marcondes *et al.*, 1996) in right atria from rats. In contrast, foot-shock stress enhances the responses mediated via β_2-adrenergic receptors as a consequence of increased β_2-adrenoceptor expression (Vanderlei *et al.*, 1996; Spadari-Bratfisch *et al.*, 1999; Santos and Spadari-Bratfisch, 2001; Santos *et al.*, 2002) and also decreases the response to norepinephrine that is mediated by β_1-adrenoceptors (Vanderlei *et al.*, 1996). Adipocytes from foot-shock stressed rats show enhanced and reduced lipolytic responses mediated by β_2- and β_1-adrenoceptors, respectively (Farias-Silva *et al.*, 1999). Foot-shock stress also causes important metabolic alterations which do not adapt to repeated stress (Verago *et al.*, 2001).

Since swimming stress appears to be milder than foot-shock stress based on the changes those stressors produce in cardiac function and in plasma corticosterone levels (Marcondes *et al.*, 1996), we hypothesized that the adaptive mechanisms triggered by swimming could be different from those triggered by foot-shock stress, and that the metabolites formed would vary according to the swimming stress paradigm.

To test this hypothesis, we evaluated several metabolic parameters in rats submitted to 50-60 min of swimming in three protocols. In group A, the rats were submitted to one 50 min swimming session; in group B, the total 50 min swimming time was divided into three sessions of 5, 15 and 30 min; in group C, the rats were submitted to five swimming sessions of 5, 15, 30, 45 and 60 min, once a day on five consecutive days.

MATERIALS AND METHODS

Animals

Forty-seven male Wistar rats (*Rattus norvergicus*) weighing 250-360 g at the beginning of the experiments were used. The animals were housed individually in cages (30 cm x 18 cm x 20 cm) at 22°C, on a 12 h light/dark cycle with lights on at 6:30 a.m. Standard laboratory chow and tap water were available *ad libitum*. During the experiments, the rats were cared for in accordance with the principles for the use of animals in research and education, as laid down in the Statement of Principles adopted by the FASEB Board. The institutional Committee for Ethics in Animal Experimentation approved the experimental protocols.

Experimental Groups

Rats were subjected to swimming sessions in a cylindrical plastic compartment (40 cm inner diameter and 60 cm high) filled to a height of 40 cm with water at 35 ± 0.5°C. The swimming sessions lasted from 5 to 60 min, according to three protocols: group A – a single 50 min swimming session (physical and emotional stress), group B - three sessions, one per day for three days, with sessions lasting 5 min on day 1, 15 min on day 2 and 30 min on day 3 (lower physical and emotional stress), and group C - one session per day, with increasing session duration from 5 min on day 1 to 15, 30, 45 and 60 min on consecutive days (low emotional stress but the same physical component as in group A). The increase in session duration was designed to increase the workload. The swimming sessions occurred between 7:30 a.m. and 11:00 a.m., and the interval between sessions was 24 hours. After each swimming session, the rats remained in a warm room until they were completely dry and were then returned to their cages. Rats not submitted to any swimming sessions were

used as controls.

Body and Tissue Weights

The rats were weighed immediately before the last swimming session. After swimming, the rats were sacrificed by a blow to the back of the head and exsanguinated. White adipose epididymal, perirenal and mesenteric pads and the adrenal glands were dissected and weighed.

Serum Glucose and Free Fatty Acid Levels

Blood was collected from trunk vessels into glass tubes and allowed to clot at room temperature. The serum was stored at -80°C until used. Serum glucose (Trinder, 1969) and free fatty acid (Regouw *et al.*, 1971) levels were determined and the results were expressed in mg/dL and mmol/L, respectively.

Hepatic and Muscle Glycogen Content

Samples of liver (L), soleus muscle (S), white (WG) and red (RG) portions of gastrocnemius muscle, and left ventricle apex (V) were obtained to determine the glycogen content according to Lo *et al.* (1970) with minor modifications. Following collection, the tissues were frozen in liquid nitrogen and then stored at -80°C. The liver and muscle samples were weighed and immersed in 30% KOH saturated with Na_2SO_4. Ninety-five percent ethanol was added to precipitate the glycogen from the alkaline digestion. The samples were centrifuged and the supernatants were carefully aspirated. The pellets were dissolved in a mixture of distilled water, phenol (5%) and concentrated H_2SO_4. The

absorbance was read in a Beckman DU®-640 spectrophotometer (Fullerton, CA, USA) and the results were expressed in mg of glycogen/100 mg wet weight of tissue.

Blood Lactate Determination

Blood samples were collected from conscious rats before and after each swimming session by an incision at the tip of the tail. The blood was collected in heparinized capillary tubes (32 μl) and transferred to BM-Lactate strips (Roche Diagnostics GmbH, D-68298; Mannheim, Germany). The blood lactate level (mmol/L) was determined in a reflection photometer (ACCUSPORTS, type 1488767, Boehringer, Mannheim, Germany).

Adipocyte Preparation and Lipolysis Measurements

Rats were sacrificed as described above and the epididymal white adipose tissue was removed. Lipolytic activity was studied in isolated fat cells obtained according to the method of Rodbell (1964), with minor modifications (Farias-Silva *et al.*, 1999, 2002). Krebs-Ringer bicarbonate buffer (KRBA) containing bovine serum albumin (3%), glucose (6 mM) and HEPES (10 mM), adjusted to pH 7.4 with 1 M NaOH immediately before use, was used. After treatment with collagenase (1 mg/ml), isolated fat cells were filtered through a nylon mesh, washed three times and the packed cells were brought to a suitable dilution with KRBA. The cells were incubated in plastic vials with gentle shaking in a water bath at 37°C for 60 min. Pharmacological agents at suitable dilutions were added to the cell suspension just before the beginning of the assay to obtain a final volume of 1 ml. Concentration-response curves for norepinephrine and epinephrine (0.1 nM - 10 μM) were obtained in isolated adipocytes. In each series of experiments, two

vials of cells received no lipolysis stimulant in order to determine basal lipolysis while two other vials received 1 mM dibutyryl cyclic AMP in order to determine maximum lipolysis (Morimoto *et al.*, 2001). After a 45 min incubation, the tubes were placed in an ice bath and 200 µl aliquots of the infranatant were taken for the enzymatic determination of glycerol (Wieland, 1957), which was used as an index of fat cell lipolysis. Total lipids were determined gravimetrically after extraction (Dole and Meinertz, 1960). The absorbances were read in a Beckman spectrophotometer and the results were expressed in µmol of glycerol released in 60 min/100 mg of total lipids.

The maximum response to agonists was obtained when two consecutive concentrations differing by 0.5 log units caused no additional lipolysis. The concentration of agonist causing 50% of the maximum lipolysis (EC_{50}) was calculated and expressed as pD_2 values (-log EC_{50}). Calculations were done by using Prism Graph Pad Software (San Diego, CA, USA) and Systat 5.03 for Windows (Evanston, IL, USA).

Drugs and Chemicals

Glycine, heptane, isopropanol and sulfuric acid were from Merck KgaA (Darmstadt, Germany). Adenosine 5′-triphosphate (ATP), bovine serum albumin (fraction V), collagenase type II from *Clostridium histolyticum*, dibutyryl cyclic AMP, epinephrine, α-glycerophosphate dehydrogenase type I from rabbit muscle, glycerokinase from *Geotrichum candidum*, β-nicotinamide adenine dinucleotide (β-NAD) and (-) norepinephrine, were from Sigma Chemical Company (St. Louis, MO, USA). Hydrazine hydrate was purchased from Vetec (Rio de Janeiro, RJ, Brazil). The serum glucose kit was purchased from Laborlab (Guarulhos, SP, Brazil) and the serum free fatty acid kit was

provided by Waco Chemicals Gmbh (Neuss, Germany). The chemicals used for buffer preparation were of analytical grade.

Statistical Analysis

The results are presented as the means ± SEM for the number of rats indicated. The data were analysed by one-way analysis of variance (ANOVA) followed by Fisher's test. Differences were considered significant at $p < 0.05$.

RESULTS

The body weight of control rats (360 ± 14 g, n= 9) was not significantly different from that of rats which had swum 50-60 min in one (group A, 331 ± 9 g, n=12) or three (group B, 347 ± 8 g, n=13) sessions or from rats that were adapted to swimming (group C, 337 ± 12 g, n=13). The wet weights of the epididymal, perirenal and mesenteric adipose pads and the adrenal glands were not altered by swimming (data not shown).

There was a significant decrease in the glycogen content of hepatic tissue and of gastrocnemius and soleus muscles in groups A, B and C compared to the controls. There were no changes in the left ventricle glycogen content (Table 1).

There was a significant increase in the blood glucose levels of rats in group B compared to the controls. In contrast, there were no significant changes in groups A and C (Table 2). The blood lactate levels increased significantly in groups A and B, but not in C, compared to the controls. In all three groups, serum free fatty acid levels were significantly higher after swimming (Table 2).

The basal lipolysis in adipocytes isolated from rats in groups A and C was not significantly different from the controls, but was higher than the controls in adipocytes from rats in group B. The lipolysis stimulated by dibutyryl cyclic AMP was similar in all groups (Table 3).

Dose-response curves to the endogenous catecholamines norepinephrine and epinephrine were obtained in adipocytes isolated from control and swimming rats. There were no differences in the sensitivity (pD_2 values) to norepinephrine among the groups or in the maximal lipolysis stimulated by any of the catecholamines (Table 4). However, adipocytes

from rats submitted to one 50 min swimming session (group A) or three sessions of 5, 15 and 30 min duration (group B) were supersensitive to epinephrine, with the dose-response curves to this non-selective adrenoceptor agonist being shifted to the left by around 15-fold in group A ($p<0.05$) and 9-fold in group B ($p<0.05$). The sensitivity to epinephrine of adipocytes from group C rats was not significantly different from that of control rats (Table 4).

DISCUSSION

As shown here, there were no significant changes in body weight, adipose tissue depots or adrenal gland weight after any of the swimming protocols used. Considering that food was available *ad libitum*, and that the rats were exercised for up to five days, the lack of change in body weight was not unexpected. Body weight alterations have been reported in rats with attached weights during swimming and submitted to 2-3 h swimming sessions for 7 to 11 weeks, in association with food restriction (Bukowiecki *et al.*, 1980; Walberg *et al.*, 1983; Wilmore, 1983; Goodpaster *et al.*, 1999).

The glycogen content of the liver, gastrocnemius muscle and soleus muscle decreased in the three experimental groups. This indicates that the slow (type I) fibres of soleus muscle as well as the fast (type IIa and IIb) fibres of red and white gastrocnemius muscle, respectively, were recruited. These data agree with those reported by Calil *et al.* (2002) who demonstrated that the glycogen content of liver, and of gastrocnemius and soleus muscles decreased significantly in rats after deep water swimming, such as used here. During the swimming sessions, the rats exhibited vigorous paddling behavior alternating with short periods of immobility. The soleus and gastrocnemius muscles were used in the legs movements needed to keep their heads above the water. In contrast, when the rats were made to swim in shallow water so that they could simultaneously touch bottom with their feet or tail in order to keep their heads above water without swimming, the liver and muscle glycogen content was unaltered (Calil *et al.*, 2002), probably because of the longer periods of immobility (Abel, 1994).

Whereas muscle glycogen is the major local source of carbohydrates in high intensity exercise, blood glucose is essential in low intensity exercise (Coyle *et al.*, 1991; Stanley

and Connett, 1991). Hepatic glucose release occurs immediately after the start of exercise (Dudley *et al.*, 1982; Wasserman, 1995) and depends on the intensity of muscle contraction (Christensen and Galbo, 1983; Yakovlev and Viru, 1985; Jansson *et al.*, 1986; Spriet *et al.*, 1988; Ren and Hultman, 1990). Although liver glycogen decreased to a similar extent in groups A, B and C compared to the controls, the blood glucose level increased significantly only in group B and was not different from the controls in rats from groups A and C. The serum levels of free fatty acids increased similarly in the three groups. However, in group B rats, the basal lypolisis in isolated adipocytes and the sensitivity to adrenaline were enhanced. Thus, the use of lipid metabolites by peripheral tissues would save glucose and lead to increased glycaemia in this group. This hypothesis and its underlying mechanisms remain to be confirmed.

The serum lactate concentration was higher than the controls in groups A and B, with no significant change in group C. Anaerobic metabolism predominates at the beginning of exercise and aerobic metabolism after 30 min of low intensity exercise (Wasserman *et al.*, 1973; Brooks, 1985; Ryan *et al.*, 1993; Gleeson, 1996). Hence, the longer duration of exercise in group C compared to group B would explain this difference. However, in groups A and C, the duration of exercise was similar but the blood lactate concentrations were different. In rats from group A, the stress of being in the water for the first time probably interfered with their metabolism and led to the accumulation of lactate in the blood. Previous work has shown that in rats made to swim for 30 minutes in the first session the plasma norepinephrine and epinephrine levels were 3- and 4.5-fold above basal values, respectively. In contrast, in rats made to swim for the 27th time, plasma catecholamine responses were reduced compared to the first session (Konarska *et al.*,

1990). The plasma corticosterone levels were also higher after the first than after the third swimming session (Spadari *et al.*, 1988), suggesting that the endocrine response to swimming stress can adapt to repetition.

During prolonged low intensity exercise, fat metabolism is greater than that of carbohydrate (Gollnick and Saltin, 1988; Holloszy, 1990; Ladu *et al.*, 1991). As shown here, there were significant increases in the serum free fatty acid concentrations in the three experimental groups, indicating that lipid mobilization had already started. The increases in serum free fatty acids could be a consequence of lipolysis stimulated by endogenous catecholamines, at least in groups A and C, since basal lipolysis remained unchanged after swimming. In group B, the large increase in basal lipolysis could contribute to the rise in serum free fatty acids. In addition, adipocytes from group A and B rats were supersensitive to epinephrine. These data indicate that the sensitivity of the response mediated by β_2-adrenergic receptors is enhanced in these groups since the affinity of β_2-adrenergic receptors is greater for epinephrine than for norepinephrine. In this context, glucocorticoids have been shown to differentially regulate β-adrenergic receptors in 3T3 cells by enhancing the expression of β_2-adrenergic receptors, but repressing that of β_1- and β_3-adrenergic receptors (Fève *et al.*, 1990; 1992; Strosberg and Pietre-Rouxel, 1996). Increased sensitivity to isoprenaline and adrenaline, as well as subsensitivity to norepinephrine, have been reported for adipocytes isolated from rats submitted to foot-shock stress (Farias-Silva *et al.*, 1999). The supersensitivity to isoprenaline was abolished by ICI118,551, a β_2-adrenergic receptor antagonist and by treating the rats with RU-37344, an antagonist of glucocorticoid receptors (Rahnemaye *et al.*, 1992). Although we have not investigated the mechanisms underlying the supersensitivity to epinephrine in groups A and B, an increase in the

expression of β_2-adrenergic receptors induced by the high corticosterone plasma levels (Spadari *et al.*, 1988) in these rats could be a plausible explanation.

In conclusion, our data show that the metabolic alterations were less pronounced after five swimming sessions than after one session, even though session duration and the contribution by the physical component were similar. The glucose mobilization, but probably not use, was similar in the three groups. The mechanisms of lipid mobilization from adipose tissue differ depending on the stress paradigm. The metabolic changes in groups A and B indicated that three swimming sessions were not enough to cause adaptation, in contrast to foot-shock stress which leads to sensitisation rather than adaptation in response to repeated stimuli.

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Table 1. Glycogen concentration (mg/100 mg) in hepatic tissue, soleus muscle, white (WG) and red (RG) portions of gastrocnemius muscle, and left ventricle of control rats and rats submitted to 50-60 min of swimming in one session (group A), in three sessions of 5, 15 and 30 min (group B) or after a period of adaptation in which the session duration was progressively increased from 5 to 60 min (group C).

	Groups			
Tissues	**Control**	**A**	**B**	**C**
Liver	5.21 ± 0.80	2.69 ± 0.68 *	3.32 ± 0.40 *	3.51 ± 0.24 *
Soleus muscle	0.37 ± 0.06	0.16 ± 0.05 *	0.24 ± 0.04 *	0.25 ± 0.03 *
RG	0.43 ± 0.06	0.11 ± 0.03 *	0.11 ± 0.01 *	0.076 ± 0.007 *
WG	0.56 ± 0.06	0.11 ± 0.04 *	0.15 ± 0.02 *	0.22 ± 0.03 *
Ventricle	0.18 ± 0.02	0.13 ± 0.01	0.18 ± 0.03	0.21 ± 0.02
N	11	7	7	7

The values are the mean ± S.E.M.; n – number of rats; * $p < 0.05$, compared to control (ANOVA plus Fisher's test).

Table 2. Plasma glucose (mg/dL), blood lactate (mmol/L) and serum free fatty acid (FFA, mmol/L) levels in control rats and in rats submitted to one 50 min swimming session (group A) or to three (group B) or five (group C) swimming sessions of 5, 15, 30, 45 and 60 min duration.

groups	glucose	Lactate	free fatty acid
control	118.8 ± 7.1 (6)	1.95 ± 0.24 (6)	0.62 ± 0.07 (5)
A	127.2 ± 6.7 (7)	2.93 ± 0.35 (4)*	1.10 ± 0.06 (4)*
B	159.9 ± 8.8 (7)*	2.88 ± 0.26 (8)*	1.03 ± 0.13 (4)*
C	114.9 ± 5.4 (7)	2.40 ± 0.26 (5)	1.20 ± 0.11 (4)*

The values are the mean ± S.E.M.; number of rats is shown in parentheses;

* $p < 0.05$, compared to control (ANOVA plus Fisher's test).

Table 3. Basal glycerol release and response to 1 mM dibutyryl-cAMP (μmol of glycerol/ 100 mg total lipids in 60 min) in adipocytes isolated from control rats and from rats submitted to 50-60 min of swimming in one session (group A), in three sessions of 5, 15 and 30 min (group B), or after a period of adaptation in which the session duration increased progressively (5, 15, 30, 45 and 60 min, group C).

	control	A	B	C
Basal	0.55 ± 0.13	0.44 ± 0.08	1.45 ± 0.19*	0.78 ± 0.23
	(5)	(12)	(5)	(12)
dibutyryl-cAMP	1.46 ± 0.34	1.38 ± 0.17	1.73 ± 0.14	1.03 ± 0.06
	(4)	(11)	(7)	(7)

The values are the mean ± S.E.M. of the number of rats in parentheses;

* $p < 0.05$, compared to the control group (ANOVA plus Fisher's test).

Table 4. The pD_2 values and maximum responses (MR) to norepinephrine and epinephrine in epididymal adipocytes isolated from control rats and from rats submitted to 50-60 min of swimming in one session (group A), in three sessions of 5, 15 and 30 min (group B), or after a period of adaptation in which the session duration was progressively increased (5, 15, 30, 45 and 60 min, group C).

	norepinephrine		**Epinephrine**	
	pD_2	**MR**	**pD_2**	**MR**
Control	6.55 ± 0.38	1.47 ± 0.27	5.56 ± 0.26	1.33 ± 0.19
	(7)	(7)	(8)	(8)
A	6.61 ± 0.27	1.62 ± 0.33	6.75 ± 0.14 *	1.63 ± 0.18
	(6)	(6)	(11)	(11)
B	6.67 ± 0.15	1.56 ± 0.09	6.50 ± 0.33 *	1.58 ± 0.12
	(6)	(7)	(6)	(7)
C	7.21 ± 0.26	1.18 ± 0.18	6.12 ± 0.27	1.36 ± 0.18
	(8)	(6)	(14)	(14)

MR was expressed in μmol of glycerol/100 mg total lipids in 60 min: the values are the mean ± S.E.M. of the number of rats in parentheses; * $p < 0.05$, compared to the control (ANOVA plus Fisher's test).

II. 2. Effect of Water Temperature on Metabolic Tracers of Rats Submitted to Swimming

Este trabalho será enviado para publicação.

EFFECT OF WATER TEMPERATURE ON METABOLIC TRACERS OF RATS SUBMITTED TO SWIMMING

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Running title: Water temperature on rat metabolic tracers

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Abstract

We evaluated the effect of water temperature, as a determinant of stress intensity, on metabolic markers in rats submitted to three swimming sessions of 5, 15 and 30 minutes at 35°C or 18°C. Immediately after swimming sessions at 35°C rats were homeothermic (37.6 ± 0.2°C) compared to control (37.1 ± 0.1°C) whereas rats swimming at 18°C showed hypothermia (24.1 ± 0.6°C). The glycogen content of liver, soleus and gastrocnemius muscles was decreased after swimming at 35°C. In these rats, blood glucose, lactate and free fat acids (FFA) concentrations were significantly higher than control. In rats swimming at 18°C the glycogen content of gastrocnemius muscle were decreased but not in liver, soleus muscle or ventricle. Glycaemia were not altered compared to control in these rats, whereas blood lactate and FFA concentrations were significantly higher than control and than rats swimming at 35°C. Basal lipolysis was increased in adipocytes isolated from both groups, as well as the sensitivity to epinephrine. Those two combined factors might contribute to the increase in plasma FFA levels considering that the plasma levels of epinephrine and norepinephrine were higher in rats swimming at 18°C than rats swimming at 35°C and than control rats. We conclude that swimming at 35°C causes substrates mobilization and metabolism mostly guaranteed by the higher plasma levels of epinephrine, norepinephrine and corticosterone. These metabolic alterations allow the animal to keep homeothermy and normal plasma glucose levels. However, swimming at low temperature impaired carbohydrates mobilization and glucose aerobic metabolism, then causing increased serum lactate and FFA levels although plasma levels of catecholamines were higher than in the other groups. Those alterations are not sufficient to avoid hypothermia.

Keywords: adipocytes, metabolic markers, cold-swimming stress.

INTRODUCTION

The cardiovascular and the metabolic systems are important targets of the catecholamines and glucocorticoids, being profoundly affected during stress.

We have previously reported that adipocytes isolated from the epidydimal depots of rats submitted to foot shock stress showed supersensitivity to isoprenaline due to an increase in the participation of the β_2-adrenoceptors in the lipolytic response to catecholamines as well as a decrease in the response mediated by β_1-adrenoceptors, since the sensitivity to norepinephrine was lower than control (Farias-Silva *et al.*, 1999). Additionally, the metabolic response to foot shock stress increased with stress repetition rather than habituate causing progressively increasing plasma levels of glucose (Verago *et al.*, 2001) together with adipocytes subsensitivity to insulin (Farias-Silva *et al.*, 2002).

On the other hand, adipocytes isolated from rats submitted to three swimming sessions were supersensitive to epinephrine, a non-selective β_1-β_2-adrenoceptor agonist, whereas the sensitivity to norepinephrine was not altered (Sampaio-Barros *et al.*, 2003 *in press*). Those results indicate that the alterations induced by foot shock stress are more pronounced than those induced by swimming stress suggesting that foot shock is a more intense stressor for rats than swimming, as previously proposed by Marcondes *et al.* (1996).

In order to test the above mentioned hypothesis, in this paper we evaluated metabolic markers in rats submitted to the same stressor, swimming, in two different intensities that were determined by the water temperature, 35 and 18°C. The use of swim stress was especially appropriate as changing the water temperature may modify the intensity of the stressor. Indeed, Konarska *et al.* (1990) showed that the increase in the plasma levels of

both catecholamines was higher when rats were submitted to swim in water temperatures of 18°C than 34°C.

MATERIAL AND METHODS

Animals

Male Wistar rats (*Rattus norvergicus*) weighing 250-360 g at the beginning of the experiments were used. The animals were housed individually in cages (30 cm x 18 cm x 20 cm) at 22°C, on a 12 h light/dark cycle with lights on at 6:30 a.m. Standard laboratory chow and tap water were available *ad libitum*. During the experiments, the rats were cared for in accordance with the principles for the use of animals in research and education, as laid down in the Statement of Principles adopted by the FASEB Board. The experimental protocols were approved by the institutional Committee for Ethics in Animal Experimentation.

Experimental Groups

Naive rats were used as controls and were not submitted to swimming. The stressed rats were submitted to one swimming session a day, in three consecutive days, in a cylindrical plastic compartment (40 cm wide and 40 cm high) containing water at 35 ± 0.5°C or at 18 ± 0.5°C. The swimming occurred between 7:30 a.m. and 11:00 a.m. and lasted 5, 15 and 30 minutes, respectively. After each swimming session, the rats colonic temperature was measured by introducing the tip of a tele-thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio, USA) in the animal rectus. After that, the rats remained in a warm room until they were completely dry and then returned to their cages.

Body and Tissue Weights

The rats were weighed immediately before the last swimming session. After swimming, the rats were sacrificed by a blow to the back of the head and exsanguinated. White adipose epididymal, perirenal and mesenteric pads and the adrenal glands were dissected and weighed.

Serum Glucose and Free Fatty Acid Levels

Blood was collected from trunk vessels into glass tubes and allowed to clot at room temperature. The serum was stored at -80°C until used. Serum glucose (Trinder, 1969) and free fatty acid (Regouw *et al.*, 1971) levels were determined and the results were expressed in mg/dl and mmol/L, respectively.

Blood Lactate Determination

Tail blood samples were collected from conscious rats before and after each swimming session. The blood was collected in heparinized capillary tubes (32 µl) and transferred to BM-Lactate strips (Roche Diagnostics GmbH, D-68298; Mannheim, Germany). The blood lactate level (mmol/L) was determined in a reflection photometer (ACCUSPORTS, type 1488767, Boehringer, Mannheim, Germany).

Hepatic and Muscle Glycogen Content

Samples of liver (L), soleus muscle (S), white (WG) and red (RG) portions of

gastrocnemius muscle and cardiac left ventricle acme (V) were obtained to determine the glycogen content according to Lo *et al.* (1970) with minor modifications. Following collection, the tissues were frozen in liquid nitrogen and then stored at -80°C. The liver and muscle samples were weighed and immersed in a 30% KOH saturated with Na_2SO_4. Ninety-five percent ethanol was added to precipitate the glycogen from the alkaline digestion. The samples were centrifuged and supernatants were carefully aspirated. The pellets were dissolved in a mixture of distilled water, phenol (5%) and concentrated H_2SO_4. The absorbance was red in a Beckman DU®-640 spectrophotometer (Fullerton, CA, USA) and the results were expressed in mg of glycogen/100 mg wet weight of tissue.

Adipocyte Preparation and Lipolysis Measurements

Rats were sacrificed by as described above and the epididymal white adipose tissue was removed. Lipolytic activity was studied in isolated fat cells obtained according to the method of Rodbell (1964), with minor modifications. Krebs-Ringer bicarbonate buffer (KRBA) containing bovine serum albumin (3%), glucose (6 mM) and HEPES (10 mM), adjusted to pH 7.4 with 1 M NaOH immediately before use, was used. After treatment with collagenase (1 mg/ml), isolated fat cells were filtered through a nylon mesh, washed three times and the packed cells were brought to a suitable dilution with KRBA. The cells were incubated in plastic vials with gentle shaking in a water bath at 37°C for 60 min. Pharmacological agents at suitable dilutions were added to the cell suspension just before the beginning of the assay to obtain a final volume of 1 ml. After a 45 min incubation, the tubes were placed in an ice bath, and 200 µl aliquots of the infranatant were taken for the enzymatic determination of glycerol (Wieland, 1957), which was used as an index of fat

cell lipolysis. Total lipids were determined gravimetrically after extraction (Dole and Meinertz, 1960). The absorbances were red in a Beckman spectrophotometer and results were expressed in μmol of glycerol/100 mg of total lipids in 60 min.

Drugs and chemicals

Glycine, heptane, isopropanol and sulfuric acid were purchased from Merck KgaA (Darmstadt, Germany). Adenosine 5'-triphosphate (ATP), bovine serum albumin (fraction V), collagenase type II from *Clostridium histolyticum*, dibutyryl cyclic AMP, epinephryne, α-glycerophosphate dehydrogenase type I from rabbit muscle, glycerokinase from *Geotrichum candidum*, β-nicotinamide adenine dinucleotide (β-NAD) and (-) norepinephryne, were purchased from Sigma Chemical Company (St. Louis, MO, USA). Hydrazine hydrate was purchased from Vetec (Rio de Janeiro, RJ, Brazil). The serum glucose kit was purchased from Laborlab (Guarulhos, SP, Brazil) and the serum free fatty acid kit was provided by Waco Chemicals Gmbh (Neuss, Germany). The chemicals used for buffer preparation were of analytical grade.

Statistical Analysis

The results are presented as the means ± SEM for the number of experiments (n) indicated in parentheses. The data were analysed by one-way analysis of variance (ANOVA) followed by Fisher's test. Differences were considered significant at $p < 0.05$.

RESULTS

The body weight of control rats (360 ± 14 g) was not significantly different from that of rats which had swum at 35°C (353 ± 8 g) or at 18°C (317 ± 20 g). The wet weights of the epididymal, perirenal and mesenteric adipose pads and the adrenal glands were not altered by swimming (data not shown). The deep body temperature of rats measured immediately after 30 min swimming in water at 35°C was not altered (37.6 ± 0.2°C $p>0.05$) but the colonic temperature of rats swimming at 18°C (24.1 ± 0.6°C, $p<0.05$, $n = 4$) was significantly lower than control (37.1 ± 0.1°C, $n = 4$).

There was a significant decrease in the glycogen content of hepatic tissue, soleus and gastrocnemius muscles from rats swimming at 35°C compared to the controls. However, the glycogen content was not altered in these same tissues isolated from rats that had swum at 18°C. The glycogen concentration was not altered in left ventricle from rats of both experimental groups (Table 1).

Rats that had swum at 35°C showed a significant increase in the blood glucose and lactate levels compared to the controls whereas glycaemia was not significantly altered in rats that had swum at 18°C (Table 2). Serum free fatty acid levels were significantly higher after swimming in both groups compared to the controls and they were even higher in rats swimming at 18°C than at 35°C (Table 2).

Moreover, in rats swimming at 35°C, the blood lactate levels significantly increased compared to the controls after each swimming session but the levels were higher after the first (4.38 ± 0.23 mmol/L) than the second (3.15 ± 0.31 mmol/L) and the third sessions (2.88 ± 0.26 mmol/L). However in rats swimming at 18°C, blood lactate increased

significantly more after the third (4.46 ± 0.49 mmol/L) than the first swimming session (3.63 ± 0.50 mmol/L).

The basal lipolysis was higher in adipocytes from rats swimming at 18°C or 35°C than in adipocytes isolated from the control rats, whereas the lipolysis stimulated by d-butyril-cyclic AMP was not altered by swimming (Table 3).

Dose-response curves to the endogenous catecholamines, norepinephrine and epinephrine, were obtained in adipocytes isolated from control and swimming rats. There were no differences among the groups in the maximal lipolysis stimulated by any of the catecholamines or in the sensitivity (pD_2 value) to norepinephrine (Table 4). However, adipocytes from rats submitted to swimming at 35°C were supersensitive to epinephrine, with the dose-response curves to this non-selective adrenoceptor agonist being shifted to the left by around 10-fold related to the control ($p < 0.05$). The sensitivity to epinephrine of adipocytes from rats swimming at 18°C was not significantly different from that of adipocytes from the control rats ($p > 0.05$; Table 4).

DISCUSSION

The main focus of these experiments was to analyse the effects of stressor intensity on the pattern of metabolites mobilization in rats submitted to intermittent stress. The use of swim stress was especially appropriate as changing the water temperature may modify the intensity of the stressor. Indeed, Konarska *et al.* (1990) showed that the increase in the plasma levels of both catecholamines was higher when rats were submitted to swim in water temperatures of 18°C than 34°C.

At the temperature of 18°C rats were not able to maintain their deep body temperature, which drastically fell, whereas rats swimming at 35°C were able to maintain homeothermy. The glycogen contents of the liver as well as soleus and gastrocnemius muscles were depleted in the rats swimming at 35°C, but it was altered in gastrocnemius muscles of rats swimming at 18°C.

It is well established that, during exercise, the oxidized carbohydrate to fat ratio increases with intensity (Rominjn *et al.*, 1993; Gilbert *et al.*, 2002).

In rats swimming at 35°C, there was a decrease in the glycogen concentration of the soleus and gastrocnemius muscles confirming that both muscles have been recruited, since these two muscles were used in the legs movements, essential to keep rats on water surface during swimming. The soleus muscle is classified as a fatigue resistant muscle because of their fibers oxidative capacity, with large mitochondria and high concentration of myoglobin and capillaries (Bottinelli *et al.*, 1994). The RG type IIa fibbers are intermediate between fast (type IIb) and slow (type I) muscular fibbers. Its oxidative capacity increases with training (Bonen *et al.*, 1990; Pette and Staron, 1990; Pereira *et al.*, 1992). White

muscular type IIb fibbers are not fatigue resistant, since they are poor in mitochondria and they have limited capacity for aerobic metabolism, being mostly anaerobic (McLane and Holloszy, 1979; Green, 1986; Pette and Spamer, 1986). Although swimming is mostly a low intensity aerobic exercise, those fibbers were probably recruited (Ryan *et al.*, 1993) since their glycogen stores were depleted.

Whereas muscular glycogen is the major local source of carbohydrates in high exercises intensity, blood glucose is essential in low intensity exercise (Coyle *et al.*, 1991; Stanley and Connett, 1991). It is known that hepatic glucose release occurs immediately after the exercise starts (Dudley *et al.*, 1982; Yamada *et al.*, 1993, Wasserman, 1995) and that it depends on muscular contraction intensity (Christensen and Galbo, 1983; Yakovlev and Viru, 1985; Jansson *et al.*, 1986; Spriet *et al.*, 1988; Ren and Hultman, 1990; Mora-Rodriguez and Coyle, 2000). Liver glycogen was decreased only in rats swimming at 35°C compared to control, whereas the glycaemia was not significantly different from control in the rats swimming at 18°C, on the contrary, it showed a tendency, although non-significant, to decrease.

In swimming both the swimming activity and oxygen uptake are directly related to the water temperature (Harri and Kuusela, 1986). It is reasonable to assume that rats swimming at 18°C are able to restrict their muscular work to the minimum during their repeated hypothermic swimming sessions in order to abolish hypothermia, because the primary factor affecting cooling rate in swimming rats is convection. This is most influenced by the movement of the rat (Dawson *et al.*, 1970).

Adaptation to cold include increased food intake and increase in both mass and metabolic activity of brown adipose tissue, leading to an increase capacity for non-shivering

thermogenesis, and maintenance of the stores of muscle glycogen and adrenal ascorbic acid during cold exposure. These changes were associated with improved resistance to cold (Harri and Kuusela, 1986; Prathima and Devi, 1999; Devi *et al.*, 2002).

Previous animal and human studies have recognized that both carbohydrate and fat oxidation are enhanced during cold exposure (for reviews see Jacobs *et al.*, 1994). In particular, muscle glycogen appears to be an important energy substrate during shivering thermogenesis, as evidenced by the 20% reduction in muscle glycogen during cold water immersion (Martineau and Jacobs, 1989). However, although Martineau and Jacobs (1988) established that intramuscular glycogen depletion is unlike to occur before the onset of hypothermia in man, these researchers hypothesized that depleting the shivering musculature of glycogen would adversely influence thermoregulation during cold exposure. Indeed, when the availability of glycogen was lowered in the large muscle groups using a combination of exhaustive exercise and a low-carbohydrate diet, there was a reduction in the initial hot production and a faster core cooling rate during subsequent cold water immersion, compared with a normal glycogen condition (Martineau and Jacobs, 1989). Those rats swimming at 18°C showed a similar condition since they were not able to mobilize their glycogen stores which were not depleted and as a consequence they were not able to keep homoeothermy.

The studies by Weller *et al.* (1998) contrast with those suggesting that carbohydrate is the main substrate oxidized in cold-exposed subjects. The reason for this difference is not immediately apparent and may be related to the severity of the cooling stimulus.

Serum lactate concentration was 0.9-fold higher in the rats swimming at 35°C than it was in control rats, and 2.3-fold higher than control in rats swimming at 18°C. Therefore, in rats

swimming at 18°C there was a predominance of anaerobic metabolism with a high production of lactate by the active muscles, the liver not being able to mobilize its glycogen stores and to increase gluconeogenesis in order to keep glucose homeostasis.

Concerning to the fat metabolism, our results have shown that there were significant increases in serum free fatty acids concentration in both experimental groups, indicating that lipids mobilization had already started. The increase in plasma FFA levels might be a consequence of the increased basal lipolysis as determined in isolated adipocytes as well as lipolysis stimulation by epinephrine and norepinephrine, which plasma levels are expected to be high after a period of swimming (Ostman-Smith, 1979; Vallerand *et al.*, 1999). Moreover, our results have show that adipocytes from rats submitted to swim are supersensitive to epinephrine. Kornaska *et al.* (1989) have reported that in rats exposed to swim stress at 18°C for the first time, plasma levels of norepinephrine and epinephrine peaked at levels that were 13-fold greater and 58-fold greater than baseline, respectively, at 15 minutes into the period of swim stress. Exposure to 34°C swim stress represented a relatively mild stressor for laboratory rats. In rats stressed for the first time, plasma norepinephrine peaked at 3-fold above basal values at 15 minutes poststress. In contrast, epinephrine peaked at 4.5-fold above basal values at 15 minutes of homeothermic swim stress.

The present results have shown that in adipocytes from rats submitted to swimming at 18°C the sensitivity to norepinephrine or to epinephrine was not altered than control. Moreover, as above cited those rats have higher plasma levels of norepinephrine and epinephrine. Both factors contributing for the increase in the lipids mobilization and increased plasma free fat acids levels.

Concluding, our results show that rats swimming at 18°C were not able to keep homeothermy probably because they are not able to mobilize carbohydrate substrates from the liver and active muscles. Moreover, the carbohydrate metabolism is mainly anaerobics producing high amounts of lactate. On the other hand, the fat mobilization is increased since the basal lipolysis is enhanced in those rats compared to control and to rats submitted to swimming at 35°C.

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Table 1. Glycogen concentration (mg/100 mg) in the liver, soleus muscle, white (WG) and red (RG) portions of gastrocnemius muscle, and left ventricle of control rats and rats submitted to three swimming sessions (5, 15, 30 minutes duration, respectively), at water temperatures of 35°C or 18°C.

	Groups		
Tissues	**Control**	**35°C**	**18°C**
Liver	5.21 ± 0.80	3.32 ± 0.40 *	4.98 ± 0.48
Soleus muscle	0.37 ± 0.06	0.24 ± 0.04 *	0.31 ± 0.01
WG	0.56 ± 0.06	0.15 ± 0.02 *	0.23 ± 0.06 *
RG	0.43 ± 0.06	0.11 ± 0.01 *	0.11 ± 0.02 *
Ventricle	0.18 ± 0.02	0.18 ± 0.03	0.20 ± 0.02
N	11	7	5

The values are the mean ± S.E.M., N – number of experiments; * $p < 0.05$, compared to control (ANOVA plus Fisher's test).

Table 2. Glucose (mg/dL plasma), lactate (mmol/L blood) and free fatty acid (FFA, mmol/L) levels in control rats and in rats submitted to three swimming sessions (5, 15, 30 minutes duration, respectively), at water temperatures of 35°C or 18°C.

	Control	35°C	18°C
Glucose	118.8 ± 7.1	159.9 ± 8.8 *	91.7 ± 16.9
	(6)	(7)	(4)
Lactate	1.95 ± 0.24	2.88 ± 0.26 *	4.46 ± 0.49 **
	(6)	(8)	(5)
FFA	0.62 ± 0.07	1.03 ± 0.13 *	1.68 ± 0.13 **
	(5)	(4)	(4)

The values are the mean ± S.E.M. The numbers of experiments are shown in parentheses;

* $p < 0.05$ compared to control (ANOVA plus Fisher's test).

Table 3. Basal glycerol release and response to 1 mM di-butyryl cAMP (μmol glycerol/100 mg total lipids in 60 min) in adipocytes isolated from control rats and rats subjected to three swimming sessions with 5, 15 and 30 minutes duration, respectively, at water temperatures of 35°C or 18°C.

	Control	35°C	18°C
Basal	0.55 ± 0.13 (5)	1.45 ± 0.19 * (5)	1.54 ± 0.24 * (7)
Dibut-cAMP	1.46 ± 0.34 (4)	1.73 ± 0.14 (7)	1.56 ± 0.13 (8)

The values are the mean ± S.E.M. of the number of experiments in parentheses;

* $p < 0.05$, compared to the control group (ANOVA plus Fisher's test).

Table 4. The pD_2 values and maximum responses (MR) to norepinephrine and epinephrine in epididymal adipocytes isolated from control rats and from rats subjected to three swimming sessions of 5, 15 and 30 minutes, respectively, at water temperatures of 35°C or 18°C.

	Norepinephrine		epinephrine	
	pD_2	**MR**	**pD_2**	**MR**
Control	6.55 ± 0.38 (7)	1.47 ± 0.27 (7)	5.56 ± 0.26 (8)	1.33 ± 0.19 (8)
35°C	6.67 ± 0.15 (6)	1.56 ± 0.09 (7)	6.50 ± 0.33 * (6)	1.58 ± 0.12 (7)
18°C	6.53 ± 0.50 (7)	1.29 ± 0.21 (8)	6.38 ± 0.46 (8)	1.52 ± 0.25 (7)

MR was expressed in μmol glycerol/100 mg total lipids in 60 min. The values are the mean ± S.E.M. of the number of experiments in parentheses; * $p < 0.05$ compared to the control group (ANOVA plus Fisher's test).

III. RESUMO DOS RESULTADOS E CONCLUSÕES

Em ratos submetidos à natação em água a 35°C nossos resultados mostraram que:

1. Não houve alteração estatisticamente significativa na massa corporal ou nas massas dos tecidos adiposos epididimal, perirenal e mesentérico, assim como das glândulas adrenais dos ratos que nadaram 50 minutos em sessão única (grupo A), três sessões (grupo B) ou dos ratos adaptados à natação (grupo C).

2. Houve diminuição do conteúdo de glicogênio hepático e dos músculos sóleo e gastrocnêmio nos grupos A, B e C comparados ao controle. Entretanto, não houve alteração no conteúdo de glicogênio no músculo cardíaco ventricular.

3. Houve aumento da glicemia nos ratos do grupo B comparados ao controle. A glicemia não foi alterada nos grupos A e C.

4. A concentração sangüínea de lactato aumentou nos grupos A e B mas não aumentou no grupo C, comparada com o grupo controle.

5. A concentração plasmática de ácidos graxos livres estava aumentada após a natação nos três grupos comparada com o controle.

6. A lipólise basal em adipócitos isolados de ratos dos grupos A e C não foi diferente do controle, mas estava aumentada no grupo B. Entretanto, a lipólise máxima estimulada pelo d-butiril AMPc ou por catecolaminas (noradrenalina ou adrenalina) foi similar ao controle nos três grupos.

7. Adipócitos isolados de ratos submetidos a uma sessão de natação de 50 minutos de duração (grupo A) e a três sessões (grupo B) foram supersensíveis à adrenalina, com

desvio à esquerda de 15 e 9 vezes, respectivamente, nas curvas dose-resposta. A sensibilidade à adrenalina em adipócitos isolados de ratos do grupo C não foi diferente do controle.

Estes resultados sugerem que as alterações em marcadores metabólicos induzidas por natação dependem da repetição das sessões. Após cinco sessões de natação as alterações nos indicadores metabólicos foram menos pronunciadas que após uma sessão, embora a duração fosse similar, o que indica que o processo de adaptação foi iniciado naqueles animais submetidos a cinco sessões de natação. As características metabólicas dos grupos A (uma sessão) e B (três sessões) foram similares, sugerindo que três sessões de natação não foram suficientes para causar adaptação. Estas respostas foram diferentes daquelas observadas em ratos submetidos a estresse por choques nas patas, indicando que a resposta metabólica ao estresse é específica a cada um destes diferentes estressores.

Em ratos submetidos à natação em água na temperatura de 18°C os resultados mostraram que:

1. Não houve alteração estatisticamente significativa na massa corporal ou dos tecidos adiposos epididimal, perirenal e mesentérico, assim como as massas das glândulas adrenais.

2. Houve situação de hipotermia nos ratos que nadaram a 18°C, em relação aos controles e àqueles que nadaram a 35°C.

3. Houve diminuição no conteúdo de glicogênio dos músculos gastrocnêmio vermelho e branco de ratos que nadaram a 18°C, embora a mesma tenha ocorrido também no conteúdo de glicogênio do fígado e dos músculos sóleo e gastrocnêmio nos ratos que nadaram a 35°C, comparados aos controles.

4. A glicemia não foi alterada nos ratos que nadaram a 18°C. Houve aumento da glicemia nos ratos que nadaram a 35°C comparados aos controles.

5. A concentração sangüínea de lactato aumentou em ambos os grupos comparados com o grupo controle, sendo que a 35°C houve menor aumento destes níveis que a 18°C.

6. A concentração sérica de ácidos graxos livres estava aumentada após a natação em ambos os grupos, comparados com o controle.

7. A lipólise basal em adipócitos isolados estava aumentada em ambos os grupos em relação ao controle. Entretanto, a lipólise máxima estimulada pelo d-butiril AMPc ou por noradrenalina ou adrenalina foi similar ao controle em ambos.

8. Adipócitos isolados de ratos submetidos a três sessões de natação em água a 35°C

foram supersensíveis à adrenalina, com desvio à esquerda de 9 vezes na curva dose-resposta a este agonista em relação ao controle. A sensibilidade à adrenalina em adipócitos isolados de ratos que nadaram a 18°C não foi diferente do controle.

Nossos resultados sugerem que as alterações em marcadores metabólicos induzidas por natação dependem da intensidade do estresse, aqui determinada pela temperatura da água. Em ratos submetidos à natação em temperatura baixa houve bloqueio da mobilização de carboidratos e do metabolismo aeróbio de glicose, causando aumento das concentrações plasmáticas de lactato e ácidos graxos livres, além de aumento da lipólise basal.

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V. TRABALHOS APRESENTADOS EM CONGRESSOS

FeSBE 99

25 a 28 de Agosto / Caxambu - MG

Certificamos que

O resumo n° 08.052, intitulado "Efeito comparativo do estresse por choque nas patas ou natação sobre a resposta lipolítica às catecolaminas.", de autoria: Sampaio-Barros, M.M.; Farias-Silva, E.; Ferreira, R. C.; Grassi-Kassisse, D. M.; Spadari-Bratfisch, R. C., foi apresentado na

XIV Reunião Anual da Federação de Sociedades de Biologia Experimental-FeSBE, realizada no Hotel Glória na cidade de Caxambu-MG, de 25 a 28 de agosto de 1999.

Comissão Organizadora

óxido nítrico participa do controle da temperatura corporal, mas pouco se sabe sobre os mecanismos envolvidos. O presente estudo tem por objetivo determinar os efeitos da inibição da nNOS na termoregulação.
Métodos e Resultados: Nós usamos 7-nitroindazole (7-NI), um inibidor seletivo da nNOS (1, 10 e 30 mg/kg peso corporal) injetado intraperitonealmente em animais normotérmicos e animais com febre induzida pela administração de lipopolissacarídeo (LPS), 100 µg/kg. Em todos experimentos a temperatura retal dos ratos foi medida por um período de 5 horas após a injeção intraperitoneal a cada intervalo de 15 minutos. Quando 1 mg/kg de 7-NI foi injetado observou-se um decréscimo não significativo da temperatura corporal, enquanto com 10 e 30 mg/kg de 7-NI foi observado um decréscimo significativo da temperatura corporal (P<0.05). Nós observamos que a injeção de 7-NI induz uma hipotermia dose-dependente por 3 horas após a injeção. Para se determinar se o 7-NI influencia o aumento da temperatura corporal induzida pela injeção intraperitoneal de LPS, nós co-injetamos 30mg/kg de 7-NI com 100 µg/kg de LPS. A co-injeção de LPS e 7-NI seguiu com significante hipotermia (P<0.02)
Conclusão: Estes achados sugerem a participação da isoforma nNOS na termoregulação e na gênese da febre induzida por LPS.
Apoio Financeiro: FAPESP e CNPq.

08.051

PARTICIPAÇÃO DO ÓXIDO NÍTRICO (NO) NA LIBERAÇÃO DO PEPTÍDIO NATRIURÉTICO ATRIAL (ANP) E OCITOCINA (OT) DURANTE O CHOQUE SÉPTICO EXPERIMENTAL. Leone, A.F.C.*, Antunes-Rodrigues, J. e Cárnio, E.C. Escola de Enfermagem de Ribeirão Preto/USP e Fac. de Medicina de Ribeirão Preto/USP.
Introdução: O choque séptico experimental caracteriza-se por uma acentuada vasodilatação, oligúria nas doze primeiras horas e natriurese nas doze horas seguintes. Sabe-se que o lipopolissacarídeo (LPS), um componente externo da membrana externa de bactérias gram-negativo, aciona mecanismos em cascata levando a uma produção excessiva de óxido nítrico (NO) e que o NO, além de suas ações diretas sobre a musculatura vascular lisa, age também no controle endócrino.
Objetivo: O objetivo desse trabalho é avaliar a liberação de ANP e OT durante o choque séptico experimental, induzido por LPS.
Metodologia: Os animais receberam a administração endovenosa de LPS (1,5 mg/kg) ou salina. Após 6 horas da administração os ratos foram decapitados e o sangue coletado para dosagem das concentrações plasmáticas de ANP e OT por radioimunoensaio.
Resultados: Verificamos que após seis horas de administração de LPS, os níveis plasmáticos de ANP eram significativamente (P<0.02) menores e as concentrações plasmáticas de OT significativamente maiores (P<0.05) nos ratos tratados com LPS quando comparados aos ratos controle.
Conclusão: Esses dados sugerem que a oligúria verificada durante as doze primeiras horas de choque podem ser decorrentes dessas alterações na liberação desses dois hormônios.
Apoio Financeiro: FAPESP e CNPq

08.052

EFEITO COMPARATIVO DO ESTRESSE POR CHOQUE NAS PATAS OU NATAÇÃO SOBRE A RESPOSTA LIPOLÍTICA ÀS CATECOLAMINAS. Sampaio-Barros, M.M.**, Farias-Silva, E.**, Ferreira, R.C.**, Grassi-Kassisse, D.M., Spadari-Bratfisch, R.C. Depto de Fisiologia e Biofísica, IB/ UNICAMP, Campinas, SP.
Objetivo: Comparar a resposta lipolítica a agonistas adrenérgicos em adipócitos brancos (AB) de ratos submetidos a estresse por choque nas patas ou natação.
Métodos: Ratos Wistar, machos, adultos foram submetidos a três sessões de choque nas patas ou natação em dias consecutivos. Após a última sessão de estresse, os animais foram sacrificados e o tecido adiposo epididimal removido. Nos AB foram obtidas curvas dose-resposta para isoprenalina (iso), BRL37344 salbutamol (salb), e a resposta a 1 mM de d-butiril-AMPc (d-but). A concentração de glicerol liberado (n mol glicerol/100 mg lipídeos totais/60 min) foi considerada como índice de lipólise e as diferenças significativas a 5% (testes: Anova / Fisher).
Resultados:
Tabela 1. Liberação basal e resposta ao d-but 1mM (média ± epm)

	Controle	Natação	Choque
basal	0.59 ± 0.07	0.99 ± 0.26	1.22 ± 0.09
d-but (1 mM)	1.67 ± 0.16	1.96 ± 0.15	2.72 ± 0.15

Tabela 2. Valores pD_2 (média ± epm).

Controle	Natação	Choque	
BRL	8.54 ± 0.18	8.44 ± 0.52	7.54 ± 0.21*
iso	7.11 ± 0.08	6.47 ± 0.23*	8.35 ± 0.07*
nor	6.98 ± 0.13	6.61 ± 0.19	6.59 ± 0.09*
adr	5.58 ± 0.15	6.48 ± 0.14	6.70 ± 0.32*
salb	5.19 ± 0.17	5.74 ± 0.35	6.36 ± 0.84*

Conclusões: Alterações da resposta lipolítica às catecolaminas em AB de ratos induzidas por estresse dependem do modelo de estresse utilizado sendo que o estresse por choque tem efeito mais pronunciado do que a natação.
Apoio Financeiro: FAPESP.

08.053

PAPEL DO LOCUS COERULEUS NA LIBERAÇÃO DE OCITOCINA DURANTE A EXPANSÃO DE VOLUME. [1]Rodovalho, G.V.; [1]Anselmo-Franci, J.A.; [2]Franci, C.R. Depto de Fisiologia, [1]FMRP-USP, [2]FORP-USP.
Objetivos: Dados de nosso laboratório demonstraram que a lesão do Locus Coeruleus (LC) reduz a secreção plasmática de ocitocina (OT) que ocorre após a hemorragia. O objetivo deste trabalho foi estudar a participação do LC na secreção de OT plasmática e seu conteúdo no núcleo paraventricular (PVN) após a expansão de volume (EV).
Métodos e Resultados: Ratos Wistar, 24 horas após manipulação cirúrgica (lesão do LC ou operação fictícia e canulação da veia jugular), foram submetidos à retirada de 10% do volume total de sangue (6,7% do peso corporal) aos 5 min antes e aos 5 e 10 min após a EV; imediatamente após as coletas, os mesmos volumes foram repostos com NaCl 0,9%. Aos 15 min após a EV, os ratos foram decapitados e uma quarta amostra colhida. O plasma foi separado e os cérebros removidos para dissecção do PVN. A OT plasmática e tissular foi dosada por radioimunoensaio. A concentração de OT plasmática aumentou 5 minutos após a expansão de volume. Este aumento foi ao redor de 6 vezes para todos os grupos experimentais, e não houve diferença estatisticamente significante entre eles. O conteúdo de OT no PVN também foi semelhante em todos os grupos estudados.
Médias e EPM das concentrações de OT plasmática (pg/ml)

Grupo	1ª amostra	2ª amostra	3ª amostra	4ª amostra
Controle (13)	343 ± 57	1794 ± 243	1269 ± 157	697 ± 84
Sham (9)	297 ± 88	1976 ± 261	1076 ± 260	804 ± 113
Lesão (10)	318 ± 86	2403 ± 365	1962 ± 380	1418 ± 289

Conclusões: A ativação dos neurônios do PVN e conseqüente aumento de OT, causada pela EV, diferentemente da hemorragia, não é mediada pelo LC.
Apoio Técnico: Rute M. F. Marcon e Sônia A. Z. Baptista.
Apoio Financeiro: FAPESP

08.054

SECREÇÃO DE CORTICOTROFINA (ACTH) EM RESPOSTA AO ESTRESSE AGUDO APÓS LESÃO SELETIVA DE NEURÔNIOS PARVOCELULARES DO NÚCLEO PARAVENTRICULAR (NPV) Caldeira, J.C**1, Franci, C.R2; 2Depto de Fisiologia da Fac. de Medicina de Ribeirão Preto -USP, 1 Depto de Biologia Molecular – FAMERP.
Objetivo: A secreção de ACTH em resposta ao estresse é bloqueada pela lesão eletrolítica do NPV mas recupera-se de quatro a seis semanas após a lesão por ação de outros fatores ou de hormônio liberador de objetivo foi verificar a secreção de ACTH em resposta ao estresse após a lesão seletiva de neurônios parvocelulares do NPV por ácido ibotênico (IBO).
Métodos e Resultados: Ratos Wistar (180-200g) foram submetidos a lesão seletiva do NPV pela microinjeção de IBO (1µg/0,2µl) ou veículo (salina tamponada pH 7,4) nos controles. Após três semanas, os animais foram submetidos ao estresse agudo pela exposição ao éter durante 1 minuto. O início dos experimentos foi às 17:00 horas. Amostras de sangue foram coletadas 30 minutos antes e 2, 5, 15, 30 e 60 minutos após ao estresse para dosagem de ACTH por radioimunoensaio. Não houve diferença na concentração basal de ACTH entre controles e lesados. A secreção de ACTH em resposta ao estresse nos animais lesados foi significativamente menor aos 2 minutos mas a seguir, manteve-se mais elevada que o controle até os 60 minutos.
Conclusões: A atividade do eixo Hipotálamo-Hipófise para a secreção de ACTH é acompanhada de uma hipersensibilidade ao estresse provavelmente pela supressão de algum outro fator de controle inibitório e ou potenciação de algum fator excitatório.
Apoio Técnico – Sônia A. Zanon Baptista.
Suporte Financeiro – CNPq, FAPESP, FINEP (PRONEX)

08.055

ETANOL CRÔNICO DIMINUI O "BINDING" DO [3H]PDBu E A PRODUÇÃO DE TESTOSTERONA ESTIMULADA COM ATIVADORES DA PK-C EM CÉLULAS DE LEYDIG ISOLADAS DE RATO. Xavier, M.M.B.**, Udrisar, D.P., Wanderley, M.I., Depto de Fisiologia e Farmacologia, UFPE.
Objetivos: Uma das consequências bem conhecidas do tratamento agudo ou crônico com etanol em ratos machos é a redução direta da esteroidogênese testicular. O envolvimento da via de tradução do sinal do diacilglicerol/proteína quinase C (DAG/PK-C) na ação do etanol adicionado diretamente às células de Leydig isoladas, foi previamente demonstrado em nosso laboratório. O propósito do presente estudo foi o de comparar os efeitos da administração crônica do etanol sobre o "binding" do [3H]PDBu e a produção de testosterona estimulada com ativadores da PK-C, em células de Leydig isoladas.
Métodos e Resultados: Os animais foram tratados com 2,5 g/kg de etanol, 25% v/v em salina, via i.p./7 dias. Nos experimentos de "binding" as células (6x106/0.5ml) foram incubadas com aproximadamente 40.000 dpm [3H]PDBu e com diferentes concentrações de PDBu durante 20 minutos a 37 C. Após a incubação as células foram centrifugadas (1.100g x 15 min, a 4 C) e o precipitado de células e sobrenadante separados para a contagem da radioatividade em contador de cintilação líquida. O etanol reduziu a ligação do [3H]PDBu em aproximadamente 38%. Para a produção de testosterona foram utilizadas 0,2 x 106 células/0,5 ml, as quais foram incubadas durante 3 hs com os seguintes ativadores da PK-C: PDBu (200 nM), LHRH (10-7 M) e L-propranolol (10-4 M), todos estimuladores da produção de testosterona. O etanol crônico inibiu em aproximadamente 30 % (estímulo – basal) o efeito estimulatório do PDBu e em aproximadamente 100 % (estímulo – basal) o efeito estimulatório do LHRH e do propranolol sobre a esteroidogênese.
Conclusão: O tratamento crônico com etanol reduziu a ligação do [3H]PDBu e a produção de testosterona em células de Leydig isoladas. A comparação desses resultados sugerem que a via de tradução de sinal do DAG/PK-C pode estar afetada, pelo menos em parte, pelo tratamento crônico com etanol.
Apoio Financeiro: CNPq, PROPESQ-UFPE

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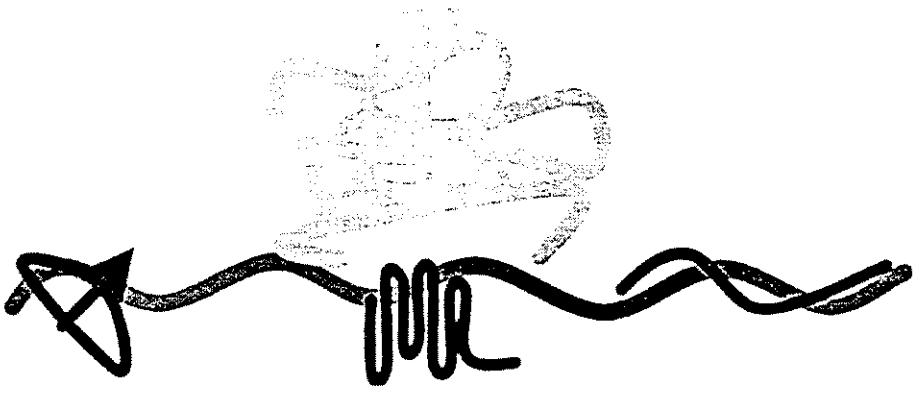

5 - ENDOCRINOLOGIA
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05.001

EVALUATION OF THE HYPOGLYCEMIC EFFECTS OF MYO-INOSITOL IN ASSOCIATION WITH MANGANESE IN DIABETIC RATS.

Almeida, M.Q., Pontes, C.K., Larner, J., Fonteles, M.C., Clinical Research Unit, UFC / UECe, Fortaleza, Ce, Brazil.

Introduction and Goals: D-chiro-inositol (DCI) and 3-O-methyl-DCI associated with Mn^{++} reduced glycemia similarly to insulin mediator (Horm. Metab. Res.: in press). Since myo-inositol, an epimer of DCI, is the nutritionally active form of inositol, we decided to evaluate the effects of myo-inositol and its interaction with Mn^{++} on reducing hyperglycemia. **Methods:** Wistar rats were injected with streptozotocin (45mg/kg). After 48h, animals were anaesthetized and hyperglycemia (341±21mg/dl) determined. NaCl 0.9% (n=7), myo-inositol 5mg/kg (n=6) and 15mg/kg (n=6) were injected i.v. as a bolus. In another group, $MnCl_2$ 8.3µg/min (n=6) was infused i.v. while myo-inositol 15mg/kg was injected as a bolus. Blood was sampled every 20min during 2h. **Results:** Myo-inositol 5mg/kg produced a non-significant effect on plasma glucose. Otherwise, myo-inositol 15mg/kg reduced glycemia 19% ($p<0.05$). The coadministration of myo-inositol and Mn^{++} produced a hypoglycemic effect of 33% wich was not statistically different to that promoted solely by myo-inositol 15mg/kg ($p<0.05$). **Conclusion:** Myo-inositol was not favored by coadministration with Mn^{++}, indicating that Mn^{++} interacts specifically with DCI perhaps to form a chemical complex with greater activity.

05.002

EFFECT OF SWIMMING STRESS ON LIPOLYTIC RESPONSE TO CATECHOLAMINES.

Sampaio-Barros, M.M., Farias-Silva, E., Grassi-Kassisse, D.M., Spadari-Bratfisch, R.C. Depto de Fisiologia e Biofisica, UNICAMP, Campinas, SP, Brasil.

Goals: To evaluate the lipolytic response to adrenergic agonists in white epididymal adipocytes (WEA) from stressed rats. **Methods:** Wistar rats were submitted to one (1x), three (3x), or five (5x); 5, 15, 30, 45 and 60 min duration daily swimming sessions, in water at 35°C. Rats were sacrificed and WEA isolated. Dose response curves to isoprenaline (ISO), BRL 37344 (BRL), norepinephrine (NE), epinephrine (EPI) and d-butyryl-cAMP (d-BUT) were *in vitro* obtained. Cells glycerol release (µmol/60 min/100 mg total lipids) was taken as lipolysis index. **Results:** pD_2 values for control, 1x, 3x or 5x were respectivelly: BRL: 8.54 ± 0.18 ; 8.15 ± 0.56 ; 8.43 ± 0.52 ; 8.54 ± 0.59; ISO: 7.11 ± 0.08 ; 7.15 ± 0.21 ; 6.47± 0.22*; 6.84 ± 0.14; NE: 6.98 ± 0.13 ; 6.99 ± 0.19 ; 6.64 ± 0.19 ; 7.27 ± 0.36; EPI: 5.41 ± 0.13 ; 6.56 ± 0.05; 6.50 ± 0.50; 5.70 ± 0.19. **Conclusion:** This protocol of swimming stress did not induce any alteration in WEA sensitivity to β-adrenergic agonists as it does in cardiac tissue or likewise footshock stress (*Can. J. Physiol. Pharmacol.* 77: 1-6, 1999; *J. Lipid Res.*40: 1719-1727,1999). Financial Support: FAPESP.

FeSBE 2002

Certificamos que

o resumo número 16.021 intitulado EFEITO DA TEMPERATURA DA ÁGUA SOBRE INDICADORES METABÓLICOS. de autoria Sampaio-Barros, M. M.; Farias-Silva, E.; Souza, E. P. M.; Zoppi, R. C.; Grassi-Kassisse, D. M.; Spadari-Bratfisch, R. C. foi apresentado sob a forma de painel na

XVII Reunião Anual da Federação de Sociedades de Biologia Experimental - FeSBE, realizada no Centro de Convenções do Carlton Bahia Hotel, Salvador - Bahia, de 28 a 31 de agosto de 2002.

Comissão Organizadora

FeSBE2002 - Endocrinologia

16.021

EFEITO DA TEMPERATURA DA ÁGUA SOBRE INDICADORES METABÓLICOS. Sampaio-Barros, M. M.[*] ; Farias-Silva, E. ; Souza, E. P. M. ; Zoppi, R. C. ; Grassi-Kassisse, D. M. ; Spadari-Bratfisch, R. C. ; Fisiologia e Biofísica, UNICAMP; Fisiologia e Biofísica - IB, UNICAMP; Fisiologia e Biofísica/IB, UNICAMP; LABEEST - Departamento Fisiologia e Biofísica, UNICAMP .

Objetivo: Avaliar indicadores metabólicos em ratos submetidos a natação em água a 35°C ou 18°C.

Métodos e Resultados: Ratos Wistar, adultos foram submetidos a três sessões de natação a 35° C (A) ou 18°C (B), em dias consecutivos. Após a última sessão, foram sacrificados, sendo removidas amostras: fígado (GF) e músculo sóleo (GS) para dosagem de glicogênio (mg/100mg); sangue para glicose (mg/dL), lactato (mmol/L) e AGL (mmol/L) e tecido adiposo epididimal (TA) para liberação basal e estimulada (1 mM d-butiril-AMPc /d-but) de glicerol (mmol glicerol em 60 min/100 mg lipídeos totais).

Resultados:

Tabela 1. Marcadores metabólicos de ratos (média ± epm)

	Controle	A	B
GF	5,21±0,80[a](7)	3,32±0,40[b](7)	4,98±0,48[a](5)
GS	0,37±0,06[c](7)	0,24±0,04[d](7)	0,31±0,01[c](5)
Glicose	118,8±7,1[e](6)	159,9±8,8[f](7)	91,7±16,9[e](4)
Lactato	1,95±0,24[g](6)	2,88±0,26[h](8)	4,46±0,49[i](5)
AGL	0,62±0,07[j](5)	1,03±0,13[k](4)	1,68±0,13[l](4)
TA/Basal	0,55±0,13[m](5)	1,45±0,19[n](5)	1,72±0,32[n](5)
TA/d-but	0,79±0,13[o](7)	1,73±0,14[p](7)	1,49±0,17[p](5)

Conclusões: A natação em temperatura baixa bloqueou a mobilização de carboidratos e o metabolismo aeróbico de glicose que ocorrem quando a temperatura é de 35°C, causando aumento do lactato e AGL no plasma, além de aumentar a lipólise basal e a estimulada em adipócitos isolados.

Apoio Financeiro: FAPESP

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o resumo número 16.022 intitulado EFEITO DA DURAÇÃO E DA REPETIÇÃO DAS SESSÕES DE NATAÇÃO EM INDICADORES METABÓLICOS. de autoria Sampaio-Barros, M. M.; Farias-Silva, E.; Grassi-Kassisse, D. M.; Spadari-Bratfisch, R. C. foi apresentado sob a forma de painel na

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16.022

EFEITO DA DURAÇÃO E DA REPETIÇÃO DAS SESSÕES DE NATAÇÃO EM INDICADORES METABÓLICOS.

Sampaio-Barros, M. M.[*] ; Farias-Silva, E. ; Grassi-Kassisse, D. M. ; Spadari-Bratfisch, R. C. ; Fisiologia e Biofísica - IB, UNICAMP; Fisiologia e Biofísica/IB, UNICAMP; LABEEST - Departamento Fisiologia e Biofísica, UNICAMP .

Objetivo: Avaliar indicadores metabólicos em ratos submetidos a 50-60 minutos de natação, de acordo com três protocolos experimentais.

Métodos e Resultados: Ratos Wistar, adultos foram submetidos a uma (A), três (B) ou cinco (C) sessões de natação a 35°C, com durações de 50; 5,15,30,45 e 60 minutos de duração, em dias consecutivos. Após a última sessão, foram sacrificados, sendo removidas amostras: fígado (GF) e músculo sóleo (GS) para dosagem de glicogênio (mg/100mg); sangue para glicose (mg/dL), lactato (mmol/L) e AGL (mmol/L) e tecido adiposo epididimal (TA) para liberação basal e resposta a 1 mM d-butiril-AMPc (d-but) de glicerol (µmol glicerol em 60 min/100 mg lipídeos totais). No TA foram obtidas curvas dose-resposta para noradrenalina (NA) e adrenalina (ADR).

Resultados:

Tabela 1. Marcadores metabólicos em ratos (média ± epm)

	Controle	A	B	C
GF	5,21±0,80^{a}(7)	2,69±0,68^{b}(7)	3,32±0,40^{b}(7)	3,51±0,24^{b}(7)
GS	0,37±0,06^{c}(7)	0,16±0,05^{d}(7)	0,24±0,04^{d}(7)	0,25±0,03^{d}(7)
Glicose	118,8±7,1^{e}(6)	127,2±6,7^{e}(7)	159,9±8,8^{f}(7)	114,9±5,4^{e}(7)
Lactato	1,95±0,24^{g}(6)	2,93±0,35^{h}(4)	2,88±0,26^{h}(8)	2,40±0,26^{g}(5)
AGL	0,62±0,07^{i}(5)	1,10±0,06^{j}(4)	1,03±0,13^{j}(4)	1,20±0,11^{j}(4)
TA-Basal	0,55±0,13^{k}(5)	0,44±0,08^{k}(12)	1,45±0,19^{l}(5)	0,78±0,23^{k}(12)
TA-d-but	0,79±0,13^{o}(7)	1,38±0,17^{p}(11)	1,73±0,14^{p}(7)	0,86±0,06^{o}(14)

Conclusões: Alterações em marcadores metabólicos induzidas por natação dependem da duração da sessão. A repetição das sessões (grupo C) resultou em menor aumento dos níveis plasmáticos de lactato e da resposta ao d- but do que no grupo A, sem alterações nos outros parâmetros.

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ABSTRACTS

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EFFECT OF SWIMMING SESSION DURATION AND REPETITION ON METABOLIC TRACERS IN RATS

Sampaio-Barros MM, Farias-Silva E, Grassi-Kassisse DM, Spadari-Bratfisch RC*. LABEEST, State University of Campinas, São Paulo, Brazil

Aim: To evaluate metabolic tracers in rats submitted to three swimming protocols. **Methods**: Wistar rats were submitted to swimming sessions at 35°C, as follows: Group A: 1 session of 50 min; Group B: 1 session daily for 3 days of 5, 15 then 30 min (3 day total of 50 min); Group C: 1 session daily for 5 days of 5, 15, 30, 45 then 60 min on day 5. After the last session, animals were sacrificed and tissues removed: liver and soleus muscle for glycogen content (Glyc, mg/100 mg); blood for glucose (mg/dL), lactate (mmol/L) and free fatty acids (FFA-mmol/L) serum levels; and epididymal adipose tissue (AT) to determine adipocyte glycerol release, both basal and stimulated by 1 mM d-butyryl cAMP (dbut; mmol glycerol 60 min/100 mg total lipids), norepinephrine or epinephrine. **Results** are shown in the table:

	Liver Glyc	Soleus Glyc	Glucose	Lactate	FFA	AT basal	AT dbut
control	5.21±0.80	0.37±0.06	118.8±7.1	1.95±0.24	0.62±0.07	0.55±0.13	1.46±0.34
A	2.69±0.68*	0.16±0.05*	127.2±6.7	2.93±0.35*	1.10±0.06*	0.44±0.08	1.38±0.17
B	3.32±0.40*	0.24±0.04*	159.9±8.8*	2.88±0.26*	1.03±0.13*	1.45±0.19*	1.73±0.14
C	3.51±0.24*	0.25±0.03*	114.9±5.4	2.40±0.26	1.20±0.11*	0.78±0.23	0.86±0.06*

* significantly different from control ($p<0.05$; ANOVA plus Tukey test)

The norepinephrine response was not different between groups. Epinephrine pD2** values were higher in groups A (6.75±0.14) and B (6.50±0.33) than in control (5.56±0.26) and group C (6.12±0.27). **Conclusions**: After five sessions of swimming the metabolic tracer alterations were lower than after one session, even though the duration of each session was similar.

**pD2: negative log molar EC50 concentration

Keywords: metabolic tracers, stress, swimming

WATER TEMPERATURE EFFECT ON METABOLIC TRACERS OF RATS SUBMITTED TO SWIMMING

Sampaio-Barros MM[1,2], Farias-Silva E[1], Souza EPM[1], Zoppi RC[1], Grassi-Kassisse DM[1], Spadari-Bratfisch RC* [1,2]. [1]LABEEST, State University of Campinas (UNICAMP); [2]Paulista University (UNIP), Campinas, São Paulo, Brazil

Aim: To compare metabolite mobilisation in rats submitted to swim stress at two water temperatures: 35°C or 18°C. **Methods**: Adult Wistar male rats were submitted to three daily swimming sessions (5, 15 and 30 min) at 35°C or 18°C and sacrificed after the last session. Glycogen content (mg/100 mg) was determined in liver (LG) and soleus muscle (SG); glucose (mg/dL), lactate (mmol/L) and free fatty acids (FFA-mmol/L) were measured in the serum, and epididymal adipocyte glycerol release was evaluated, both basal and stimulated by 1 mM d-butyryl cAMP (dbut; mmol glycerol in 60 min/100 mg total lipids). **Results**: In the rats swimming at 35°C, LG (3.32 ± 0.40 vs 5.21 ± 0.80) and SG (0.24 ± 0.04 vs 0.37 ± 0.06) were significantly lower than control, whereas blood levels of glucose (159.9 ± 8.8 vs 118.8 ± 7.1), lactate (2.88 ± 0.26 vs 1.95 ± 0.24) and FFA (1.03 ± 0.13 vs 0.62 ± 0.07) were higher. In the rats swimming at 18°C, LG (4.98 ± 0.48) and SG (0.31 ± 0.01) were not depleted, glycaemia (91.7 ± 16.9) was not different from control but blood lactate (4.46 ± 0.49) and FFA (1.68 ± 0.13) were much higher. Adipocyte basal glycerol release was increased in both groups (35°C: 1.45 ± 0.19; 18°C: 1.72 ± 0.32; control: 0.55 ± 0.13); dbut-stimulated release was, respectively 35°C: 1.73 ± 0.14; 18°C: 1.48 ± 0.17; control: 1.46 ± 0.34. **Conclusions**: Swimming at 35°C causes substrate mobilisation and metabolism. However, swimming at low temperature impaired carbohydrate mobilisation and glucose aerobic metabolism, whereas basal lipolysis was increased, then causing increased serum lactate and FFA.

Keywords: cold water, metabolic tracers, stress, swimming