JÉSSICA PRISCILA FRAGOSO DE MOURA

ATIVIDADE FÍSICA VOLUNTÁRIA E DIETA HIPOPROTEICA MATERNA: EFEITO SOBRE A ATIVIDADE LOCOMOTORA EM FILHOTES DE RATOS

RECIFE

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I Tessalonicenses 5:18

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"Para ter sucesso, é necessário

amar de verdade o que se faz".

Steve Jobs

RESUMO

O objetivo deste estudo foi avaliar o efeito da atividade física voluntária materna sobre alguns parâmetros da atividade locomotora em filhotes de ratas que receberam dieta hipoproteica nos períodos de gestação e lactação. Ratas da linhagem *Wistar* (*n*=29) foram alojadas individualmente em gaiolas de atividade física voluntária, contendo roda de corrida. Nessas gaiolas foram acoplados ciclocomputadores que permitiram o registro da distância percorrida, estimativa do gasto calórico e tempo de atividade. As ratas passaram por um período de adaptação (30 dias), recebendo neste período dieta AIN-93M. Posteriormente, foram classificadas de acordo com o nível diário de atividade física em: Inativas (*n*=15) e Muito Ativas (*n*=14). Um grupo de ratas (*n*=8) foi adicionado ao estudo no qual permaneceu durante todo o experimento em gaiola padrão de biotério, sem acesso a roda de corrida, sendo considerado nosso Grupo Controle. Após detecção da prenhez, metade de cada grupo recebeu dieta normoproteica (18% proteína) e a outra metade recebeu dieta hipoproteica (8% proteína) durante todo o período de gestação e lactação. No desmame (aos 22 dias de vida), foram escolhidos aleatoriamente 3-4 filhotes machos de cada grupo experimental para avaliação de alguns parâmetros da atividade locomotora. As avaliações foram realizadas no 23º, 45º e 60º dia de vida, num campo aberto, no qual os animais foram filmados por 5 minutos. Foram avaliados os seguintes parâmetros: Distância percorrida, deslocamento rotacional, velocidade média, potência média, energia total, tempo de imobilidade, número de paradas, relação entre tempo de imobilidade/número de paradas e tempo de permanência nas áreas do campo. Nossos resultados demonstraram que filhotes de mães submetidas à dieta hipoproteica durante os períodos de gestação e lactação, apresentaram alteração na trajetória de todos os parâmetros de atividade locomotora avaliados. Além disso, apresentaram maior distância percorrida e consequentemente menor tempo de imobilidade, aos 60 dias de vida. Filhotes de mães que realizaram atividade física voluntária antes e durante a gestação apresentaram aumento da distância percorrida e menor tempo de imobilidade. Contudo, filhotes de mães que receberam dieta hipoproteica e realizaram atividade física apresentaram menor distância percorrida em relação aos filhotes de mães muito ativas nutridas, normalizando este parâmetro. Dessa forma, a prática de atividade física materna foi capaz de atenuar os efeitos da dieta hipoproteica. Assim, podemos concluir que estímulos maternos, como a dieta e a atividade física, podem modular a atividade locomotora dos filhotes, devido ao fenômeno biológico "Plasticidade Fenotípica" que permite ao organismo a capacidade de adaptação em resposta ao meio.

Palavras-chave: Plasticidade; Gestação; Nutrição; Locomoção; Prole.

ABSTRACT

The aim of this study was to evaluate the effects of maternal voluntary physical activity on some parameters of locomotor activity in offspring of rats submitted low protein diet during periods of pregnancy and lactation. Female *Wistar* rats (*n*=29) were housed individually in voluntary physical activity cages, containing running wheel. In these cages were coupled ciclocomputadores that allowed the registration of the distance traveled, estimated caloric expenditure and time of activity. The rats passed for an adaptation period (30 days) and received AIN-93M diet. Then, were classified according to the level daily of physical activity: Inactive (*n*=15) and Very Active (*n*=14). A group of rats (*n*=8) was added in the study and remained throughout the experiment in standard cage, without access to running wheels, considered our control group. After detection of pregnancy, half of each group received normal protein diet (18% protein) and the other half received low protein diet (8% protein) during gestation and lactation periods. At weaning (at 22 days), were randomized 3-4 male offspring from each experimental group to evaluate some parameters of locomotor activity. The evaluations were performed at 23, 45 and 60 day of life, in an open field, in the animals were filmed for 5 minutes. The following parameters were evaluated: distance traveled, rotational displacement, average speed, average power, total energy, immobility time, number of stops, relationship between immobility time/number of stops and length of stay in the areas of the field. Our results showed that offspring of mothers when received low protein diet during periods of pregnancy and lactation, showed alterations in all parameters evaluated of locomotor activity. Furthermore, showed greater distance traveled and consequently shorter immobility, at 60 days of life. Pups from mothers that performed voluntary physical activity before and during pregnancy showed increased distance and shorter immobility. However, pups from mothers that received low protein diet and physical activity performed showed less distance compared to offspring of nourished very active mothers, normalizing this parameter. Thus, the practice of maternal voluntary physical activity was able to attenuate the effects of low protein diet. Thus, we can conclude that maternal stimuli such as diet and physical activity, can modulate the locomotor activity of pups due to biological phenomenon "Phenotypic Plasticity" that allows the organism ability to adapt in response to the environment.

Keywords: Plasticity; Pregnancy; Nutrition; Locomotion; Pups.

SUMÁRIO

1 APRESENTAÇÃO

Os períodos iniciais da vida são considerados críticos para o desenvolvimento dos diversos sistemas do organismo, devido à rápida proliferação e diferenciação celular (DOBBING, 1964; MORGANE *et al.*, 1993; MORGANE, MOKLER e GALLER, 2002). O desenvolvimento do sistema nervoso (SN), nos mamíferos, começa na embriogênese e continua durante o início da vida pós-natal (GUEDES, ROCHA-DE-MELO e TEODÓSIO, 2004). Em seres humanos, essa fase termina entre 2 e 4 anos de idade, enquanto que, nos ratos albinos, essa fase continua até o fim do período de lactação (21º dia de vida pós-natal) (GUEDES, ROCHA-DE-MELO e TEODÓSIO, 2004). Neste sentido, o desenvolvimento locomotor inicia no período gestacional, com o surgimento das estruturas nervosas e musculares e dos primeiros movimentos dos membros (MORGANE *et al.*, 1993; CLARAC *et al.*, 1998) e continua no período de aleitamento, onde ocorre a maturação das estruturas relacionadas à locomoção e a aquisição do comportamento (WESTERGA e GRAMSBERGEN, 1990; CLARAC *et al.*, 1998). Assim, nos períodos inicias da vida o são considerados críticos para o desenvolvimento da locomoção (WESTERGA e GRAMSBERGEN, 1990; CLARAC *et al.*, 1998).

A proteína é um nutriente fundamental para adequada formação e estruturação dos sistemas do organismo (MORGANE *et al.*, 1993; ZHANG *et al.*, 2010). Tem sido mostrado que o aporte inadequado de proteína no início da vida está relacionado com prejuízos em indicadores de crescimento somático, atraso no desenvolvimento do sistema nervoso e alteração no padrão de locomoção nos filhotes durante a lactação (BARROS *et al.*, 2006; FALCAO-TEBAS *et al.*, 2012). Além disso, restrição proteica durante os períodos de gestação e lactação resultou em aumento dos níveis de corticosterona e menor exploração da zona central do campo aberto em filhotes de ratos na idade adulta (REYES-CASTRO *et al.*, 2012). A relação entre insultos ambientais no início da vida e suas repercussões ao longo da vida pode ser explicada pelo fenômeno biológico chamado de plasticidade fenotípica (WEST-EBERHARD, 1989). Esse fenômeno permite à prole em desenvolvimento a capacidade de se adaptar em resposta a estímulos ambientais.

Além da nutrição, o estilo de vida materno ativo tem sido estudado para avaliar como o organismo se adapta ao meio, devido à plasticidade fenotípica (AMORIM *et al.*, 2009; FIDALGO; *et al.*, 2010; FALCAO-TEBAS *et al.*, 2012; FALCÃO-TEBAS; *et al.*, 2012). Estilo de vida materno ativo está relacionado com repercussões no desenvolvimento intra-uterino, mesmo em caso de aporte inadequado de nutrientes (AMORIM et al., 2009;

FIDALGO; et al., 2010; FALCAO-TEBAS et al., 2012; FALCÃO-TEBAS; et al., 2012). O mecanismo fisiológico parece está associado com o fato de que a atividade física materna proporcionaria maior fluxo sanguíneo placentário, aumentando assim, a disponibilidade de nutrientes e oxigênio para o feto (CLAPP, 2003).

Apesar de alguns estudos demonstrarem que o treinamento físico antes e durante a gestação causa efeitos para o desenvolvimento do feto, estes efeitos variam de acordo com o período em que esse estímulo é aplicado na gestação, intensidade e tipo de exercício (ROSA *et al.*, 2011). Além disso, o exercício forçado pode causar estresse nos animais, podendo ser um fator de confusão na interpretação dos resultados (CONTARTEZE *et al.*, 2008). Por isso, se faz necessário a utilização de modelos de atividade física que não cause estresse nos animais e que não afete de forma negativa o crescimento do feto (ROSA *et al.*, 2011). Recentemente, pesquisadores têm estudado o efeito da atividade física voluntária materna e suas repercussões na prole (CARTER *et al.*, 2012; SANTANA MUNIZ *et al.*, 2014). Foi demonstrado que a atividade física materna em roda de corrida está relacionada com aumento em indicadores de crescimento da prole durante a lactação (SANTANA MUNIZ *et al.*, 2014). Além disso, é capaz de melhorar a sensibilidade dos tecidos a insulina, aumentando a captação de glicose na prole (CARTER *et al.*, 2012). Desta forma, a atividade física materna pode atuar como mecanismo protetor para o aparecimento de doenças como diabetes tipo 2 (CARTER *et al.*, 2012).

Contudo, é escasso na literatura trabalhos que tratam de atividade física voluntária materna, especialmente em relação à plasticidade fenotípica, e suas consequências sobre a atividade locomotora da prole. Sendo assim, a pergunta condutora que norteou esta pesquisa foi: "Quais os efeitos da atividade física voluntária e da dieta hipoproteica materna sobre a atividade locomotora em filhotes de ratos no período pós-desmame?". O presente estudo teve como objetivo avaliar o efeito da atividade física voluntária materna sobre alguns parâmetros da atividade locomotora em filhotes de ratas que receberam dieta hipoproteica nos períodos de gestação e lactação. Nossa hipótese é que a atividade física voluntária materna atua como um estímulo capaz de atenuar os efeitos da dieta hipoproteica materna sobre a atividade locomotora da prole.

A pesquisa foi desenvolvida em colaboração com os laboratórios da Universidade Federal de Pernambuco (UFPE): Laboratório de Fisiologia da Nutrição Naíde Teodósio (LAFINNT) e Laboratório de Nutrição Experimental e Dietética (LNED), tendo como orientadoras a Profª Drª Carol Virginia Góis Leandro e a Profª Drª Raquel da Silva Aragão.

O estudo gerou 02 artigos científicos originais, no qual serão enviados para publicação após suas correções. Os dois artigos estão apresentados como resultado da pesquisa. O primeiro, intitulado: “Maternal voluntary physical activity alter some patterns of locomotor activity in offspring during development”, será enviado para Revista Physiology & Behavior. Esta revista é classificada com qualis A2 no comitê de Nutrição da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). E o segundo, intitulado: “Can maternal voluntary physical activity attenuate the effects of a perinatal low-protein diet on the patterns of locmotor activity of pups?”, será enviado para o European Journal of Nutrition, classificada com qualis A1 no comitê de Nutrição da CAPES.

2 REVISÃO DA LITERATURA

2.1 Ontogênese da locomoção

A locomoção é uma característica fundamental da vida animal, pois permite sua relação com o ambiente (BARROS, 2006; GARLAND *et al.*, 2011). O comportamento locomotor se constitui em elementos fundamentais da vida diária que estão relacionados à sobrevivência dos mamíferos como a busca de alimento, abrigo, interação com competidores e evitar predadores (GARLAND *et al.*, 2011). Esses comportamentos requerem coordenação fina e precisa da atividade simultânea das diversas vias motoras e da maturação e integração funcional de diversos sistemas, como o nervoso e o muscular (WESTERGA e GRAMSBERGEN, 1990; BARROS, 2006).

O desenvolvimento do sistema nervoso central (SNC), nos mamíferos, começa no período gestacional e continua durante o início da vida pós-natal (MORGANE *et al.*, 1993; GUEDES, ROCHA-DE-MELO e TEODÓSIO, 2004). No período gestacional, o processo de formação do tecido nervoso pode ser dividido em três fases principais: organogênese (processo de desenvolvimento do embrião que ocorre em cinco etapas: segmentação, mórula, blástula, gastrulação e neurulação); neurogênese e gliogênese (formação de neurônios e glias que são células constituintes do sistema nervoso) e diferenciação das células neurais imaturas (MORGANE *et al.*, 1993).

No período de lactação, ocorrem os eventos tardios da neurogênese e gliogênese seguindo de migração e diferenciação celular, formação de mielina e sinaptogênese, em ratos (MORGANE *et al.*, 1993). Ademais, é neste período que ocorre maior integração e maturação da comunicação entre o SNC e a periferia. No quinto dia de vida pós-natal, as primeiras fibras do trato corticoespinhal (composto principalmente de axônios motores) atingem os segmentos lombares e entre o 12º e 20º dia de vida pós-natal ocorre um rápido desenvolvimento do córtex sensório motor com aumento máximo na conectividade (WESTERGA e GRAMSBERGEN, 1990).

Da mesma forma, o desenvolvimento muscular, em ratos, tem início no período gestacional, no qual surgem as células progenitoras do músculo esquelético que se diferenciam nas fibras musculares (BIRESSI, MOLINARO e COSSU, 2007). No período de formação das fibras, inicia-se a inervação muscular e cada fibra é inervada por muitos axônios para posteriormente, serem eliminados restando apenas um para cada fibra muscular

(BIRESSI, MOLINARO e COSSU, 2007). Na ausência de inervação funcional, a formação de fibras musculares é prejudicada, levando a uma redução no número total de fibras (BIRESSI, MOLINARO e COSSU, 2007). Durante o período de lactação, ocorre a maturação do controle das ações de contração e relaxamento musculares permitindo a realização de movimentos coordenados (GRAMSBERGEN, 1998).

O desenvolvimento da locomoção parece seguir uma sequência de acontecimentos em diferentes espécies (MUIR, 2000). Em ratos, até o 10º dia de vida pós-natal, o rastejar é a forma predominante de locomoção do animal (WESTERGA e GRAMSBERGEN, 1990). A partir do 11º dia pós-natal, o animal consegue realizar uma caminhada com a superfície ventral do corpo afastada do chão (quadrupedia com caminhadas curtas) (WESTERGA e GRAMSBERGEN, 1990). Da metade para o fim da terceira semana, os animais chegam a atingir o padrão de locomoção semelhante ao adulto (WESTERGA e GRAMSBERGEN, 1990; CLARAC *et al.*, 1998).

Os períodos de gestação e lactação são críticos para o desenvolvimento da locomoção. A integração dos sistemas nervoso e muscular é fundamental para a construção do padrão locomotor adulto adequado. O sistema nervoso está relacionado com a coordenação e controle da ação motora, enquanto o sistema muscular, tem função de gerar força mecânica para permitir o deslocamento do corpo (BARROS, 2006).

2.2 Plasticidade fenotípica

Nos períodos iniciais da vida (como gestação e lactação), os órgãos e tecidos apresentam fases de rápida diferenciação celular, hiperplasia e hipertrofia e, por isso, são denominados de períodos críticos para o desenvolvimento (MORGANE, MOKLER e GALLER, 2002). Estímulo ambientais (como o fumo, álcool, estresse, nutrição e atividade física) podem atuar nos processos de plasticidade alterando o desenvolvimento do organismo (GLUCKMAN, 2005). A plasticidade fenotípica pode ser definida como um fenômeno biológico no qual um único genótipo pode dar origem a diversos fenótipos em resposta a diferentes condições ambientais (WEST-EBERHARD, 1989). Assim, a plasticidade permite ao organismo em formação modificar sua trajetória de crescimento e desenvolvimento através de processos adaptativos (WEST-EBERHARD, 1989).

Alguns autores mostram que o aporte inadequado de nutrientes nas fases iniciais da vida está relacionado com o aparecimento de doenças como hipertensão, diabetes tipo 2 e doenças cardiovasculares (HALES e BARKER, 1992; BARKER, 2007). Umas das justificativas para esta relação é a proposição que ficou conhecida como hipótese do fenótipo poupador "thrifty phenotype hypothesis". Segundo esta hipótese, o organismo em desenvolvimento se adaptaria às condições de baixo aporte nutricional, modificando seu metabolismo para o melhor aproveitamento energético e maior capacidade de estocagem de energia (HALES e BARKER, 1992). Porém, quando há aumento do aporte nutricional no período pós-natal, o organismo apresenta alterações metabólicas que podem levar ao aparecimento de doenças (HALES e BARKER, 1992).

Wells (2010) propôs um novo modelo para explicar a relação entre o ambiente perinatal, a trajetória de desenvolvimento do indivíduo e o aparecimento de doenças na vida adulta. Este modelo é constituído de dois componentes de fenótipo metabólico: "capacidade metabólica" que seria representada pelo peso ao nascer e "carga metabólica" que seria a trajetória de crescimento podendo ser representado pelo ganho de peso, estatura, massa gorda ou massa magra (WELLS, 2010). Assim, o risco da síndrome metabólica e doença cardiovascular podem ser atribuídos à razão de carga metabólica pela capacidade metabólica (WELLS, 2010). Um exemplo seriam crianças que nascem pequenas (baixa capacidade metabólica) e que tem crescimento acelerado (alta carga metabólica), ou seja, há grande carga sobre uma capacidade pequena e essa incompatibilidade estaria relacionada com maiores chances de aparecimento de doenças na vida adulta (WELLS, 2010). Tanto a hipótese do fenótipo poupador (thrifty phenotype hypothesis) como o modelo de capacidade-carga metabólica estão inseridas na "Plasticidade durante o desenvolvimento" ou, em termos mais amplos, na "Plasticidade Fenotípica".

Os processos envolvidos nesse fenômeno ainda não estão totalmente esclarecidos, mas parecem estar relacionados a mecanismos epigenéticos (WELLS, 2010; MARTIN-GRONERT e OZANNE, 2012). Os efeitos epigenéticos são gerados por meio de alterações no epigenoma com consequente efeito sobre a expressão de genes, tais como a metilação do DNA, modificações nas histonas e expressão de microRNAs, sem que ocorram alterações na sequência do DNA (WELLS, 2010). Este processo permite que a reprogramação do fenótipo materno influencie o perfil epigenético da prole, gerando efeitos em longo prazo sobre seu fenótipo (WELLS, 2010). As alterações fenotípicas ocorrem devido aos sinais que o ambiente envia a prole em desenvolvimento, como estratégia de prepará-lo as condições futuras previstas.

2.3 Dieta hipoproteica materna e plasticidade fenotípica

Dieta hipoproteica é um dos fatores ambientais mais bem estudados para avaliar como o organismo se adapta a tal condição ambiental, devido à plasticidade fenotípica. Estudos com humanos e ratos, mostram a relação entre restrição nutricional nos períodos críticos do desenvolvimento e suas repercussões ao longo da vida (HALES e BARKER, 1992; RAVELLI *et al.*, 1998; RAVELLI *et al.*, 1999; YZYDORCZYK *et al.*, 2006). Estudos com humanos têm mostrado que a restrição nutricional durante o inicio da vida está relacionada com obesidade, intolerância glicose e doenças cardiovasculares na vida adulta (HALES e BARKER, 1992; RAVELLI *et al.*, 1998; RAVELLI *et al.*, 1999).

Em ratos, dieta hipoproteíca durante os períodos de gestação e lactação provocou déficit em indicadores de crescimento somático, como peso e comprimento corporal, comprimento da cauda, eixo laterolateral do crânio e eixo anteroposterior da cabeça (FALCAO-TEBAS *et al.*, 2012). Além disso, retardou o aparecimento das características físicas (abertura do conduto auditivo e abertura dos olhos) e a ontogenia dos reflexos na prole durante a lactação (FALCAO-TEBAS *et al.*, 2012).

Pesquisadores têm investigado os efeitos da restrição proteica precoce sobre a musculatura esquelética e desempenho motor (BARROS *et al.*, 2006; TOSCANO, MANHAES-DE-CASTRO e CANON, 2008; MOURA-DOS-SANTOS *et al.*, 2013). Estudo recente demonstrou que crianças dos 7-10 anos, que nasceram com baixo peso (indicador de restrição nutricional materna) apresentam déficit permanente na força muscular e na performance na velocidade de corrida (MOURA-DOS-SANTOS *et al.*, 2013). Estudo com ratos mostrou que a dieta com baixo teor proteico durante a gestação provocou atrofia muscular e alterações no percentual de fibras (no músculo sóleo houve aumento no percentual de fibras do tipo IIa e no músculo extensor longo dos dedos houve aumento no percentual de fibras do tipo IIb). Houve também alterações nas propriedades mecânicas do músculo aos 25 e 90 dias de vida dos filhotes (TOSCANO, MANHAES-DE-CASTRO e CANON, 2008). Outro estudo utilizando dieta básica regional (7,87% proteína) durante o período de lactação demonstrou alteração no padrão de atividade locomotora em filhotes de ratos aos 21 dias de vida (BARROS *et al.*, 2006).

Diante disso, vê-se que insultos ambientais, tal como a dieta hipoproteica, nos períodos iniciais da vida está relacionada com alterações no crescimento e desenvolvimento da prole, além de predispor ao aparecimento de doenças crônicas na vida adulta.

2.4 Atividade física materna e plasticidade fenotípica

Atividade física é definida como qualquer movimento do músculo esquelético que demande gasto energético acima do metabolismo basal (LEANDRO *et al.*, 2009). Já o termo exercício físico refere-se a uma atividade física realizada sistematicamente e pode ser classificada de acordo com a intensidade de esforço em leve (20 a 50% do $VO_{2máx}$ e da $FC_{máx}$), moderada (50-80% do $VO_{2máx}$ e da $FC_{máx}$) e intensa (acima de 80% do $VO_{2máx}$ e da $FC_{máx}$) (LEANDRO *et al.*, 2009). Se o exercício físico é realizado regularmente e com um objetivo é denominado de treinamento físico (LEANDRO *et al.*, 2009).

Estudos têm suportado a ideia de que um estilo de vida materno ativo causa alterações no desenvolvimento intrauterino, mesmo em caso de aporte inadequado de proteína (AMORIM *et al.*, 2009; FIDALGO; *et al.*, 2010; FALCAO-TEBAS *et al.*, 2012; FALCÃO-TEBAS; *et al.*, 2012). Em animais, os filhotes de ratas treinadas em esteira com intensidade moderada (5 dias/semana e 60 min/dia, a 65% VO2máx) antes da gestação e intensidade leve (5 dias/semana e 20min/dia, a 40% $VO_{2máx}$) durante a gestação apresentaram aumento nos valores de indicadores de crescimento somático (taxa de crescimento, comprimento da cauda, eixo laterolateral do crânio e eixo anteroposterior da cabeça) e antecipação na maturação de alguns reflexos quando comparado com o grupo de filhotes provindos de mães que receberam dieta hipoproteica e que não realizaram treinamento físico (FALCAO-TEBAS *et al.*, 2012).

Utilizando esse mesmo desenho experimental, foi demonstrado que filhotes de mães que realizaram treinamento físico apresentaram diminuição nos níveis de colesterolemia, glicemia, valores de circunferência da cintura relativa ao peso corporal e menor percentual de ganho de peso quando comparado com o grupo de filhotes provindos de mães que receberam dieta hipoproteica e que não realizaram treinamento físico (AMORIM *et al.*, 2009; FIDALGO; *et al.*, 2010; FALCAO-TEBAS *et al.*, 2012; FALCÃO-TEBAS; *et al.*, 2012). O mecanismo fisiológico para essas repercussões positivas parece está associado com o fato de que a um maior fluxo sanguíneo placentário, aumentando assim, a disponibilidade de nutrientes e oxigênio para o feto (CLAPP, 2003).

Apesar de alguns estudos demonstrarem que o treinamento físico na gestação tem efeitos sobre o desenvolvimento do feto, esses efeitos podem variar de acordo com o período em que esse insulto é aplicado na gestação, intensidade (leve, moderado ou intenso) e tipo (anaeróbico, aerobico ou combinado) de exercício (ROSA *et al.*, 2011). Por exemplo, estudo realizado em população rural da Índia demonstrou que a atividade física intensa durante a gestação está associado com baixo peso ao nascer (RAO *et al.*, 2003). Por outro lado, atividade física de intensidade leve durante a gestação está relacionada com aumento do peso ao nascer (CLAPP, 2003). Além disso, o exercício forçado pode causar estresse nos animais, podendo ser um importante fator de confusão na interpretação dos resultados (CONTARTEZE *et al.*, 2008). Por isso, se faz necessário a utilização de modelos de atividade física que não cause estresse nos animais e que não afete de forma negativa o crescimento do feto (ROSA *et al.*, 2011).

Recentemente, pesquisadores têm estudado o efeito da atividade física voluntária materna e suas possíveis repercussões na prole (CARTER *et al.*, 2012; SANTANA MUNIZ *et al.*, 2014). Estudo com ratos Wistar mostrou que atividade física voluntária materna em roda de corrida antes e durante a gestação, repercutiu no aumento do comprimento corporal, comprimento da cauda e do eixo laterolateral da cabeça em filhotes durante o período de lactação (SANTANA MUNIZ *et al.*, 2014). Outro estudo, utilizando modelo animal, demonstrou que filhotes na idade adulta provindos de mães que realizaram atividade física voluntária em roda de corrida antes da gestação (2 semanas), durante a gestação e até o 14º dia de lactação, apresentaram maior captação de glicose em resposta a insulina no músculo esquelético e tecido adiposo em relação aos filhotes de mães sedentárias (CARTER *et al.*, 2012). Esse estudo mostra que a prática de atividade física pode diminuir o risco de aparecimento de doenças, como diabetes tipo 2, visto que o músculo esquelético e o tecido adiposo são os principais tecidos responsáveis pela captação de glicose em reposta a insulina (SHEPHERD e KAHN, 1999). Dessa forma, o estilo de vida materno ativo parece ser uma intervenção que pode melhorar a homeostase de glicose na prole (CARTER *et al.*, 2012).

Devido à capacidade de adaptação do organismo ao meio, estímulos ambientais maternos como a dieta, prática de atividade física ou os dois fatores aliados, podem ser utilizados para investigar diferentes estratégias de investimento na prole. Nesse sentido, é necessária a realização de estudos que visem investigar melhor os efeitos do estilo de vida materno e suas possíveis repercussões sobre os seus descendentes.

3 HIPÓTESE

A atividade física voluntária materna é capaz de atenuar os efeitos da dieta hipoproteica materna sobre a atividade locomotora da prole.

4 OBJETIVOS

4.1 Geral:

Avaliar o efeito da atividade física voluntária materna sobre alguns parâmetros da atividade locomotora em filhotes de ratas que receberam dieta hipoproteica nos períodos de gestação e lactação.

4.2 Específicos:

Ratas:

- Quantificar diariamente a distância percorrida, estimativa do gasto calórico e tempo de atividade durante os períodos de adaptação à atividade física, gestação e lactação;

- Avaliar o consumo alimentar e o ganho de peso corporal durante os períodos de adaptação à atividade física, gestação e lactação.

Filhotes:

- Avaliar o peso corporal, peso da ninhada e o número de filhotes no nascimento;
- Acompanhar o crescimento somático;
- Avaliar alguns parâmetros do padrão de atividade locomotora, sendo eles: Distância percorrida, deslocamento rotacional, velocidade média, potência média, energia total, tempo de imobilidade, número de paradas, relação entre tempo de imobilidade/número de paradas e tempo de permanência nas áreas do campo.

5 MÉTODOS

5.1 Animais e dietas experimentais

Foram utilizadas 37 ratas albinas da linhagem *Wistar* (peso corporal 220-260g, idade entre 85-95 dias) provenientes da colônia de criação do Departamento de Nutrição da Universidade Federal de Pernambuco. Os animais foram mantidos em biotério de experimentação, com temperatura de 22°C±1, num ciclo 12/12h [ciclo claro (20:00 às 08:00 h) e ciclo escuro (08:00 às 20:00 h)] com livre acesso à água e alimentação. As ratas nulíparas (*n*=29) foram alojadas individualmente em gaiolas de atividade física voluntária (GAFV) para um período de adaptação (30 dias), recebendo durante esse período dieta AIN-93M (tabela 1) (REEVES, 1997), para a fase de manutenção dos roedores. As outras ratas (*n*=8) permaneceram durante todo o experimento em gaiola padrão de biotério, sem acesso a roda de corrida, sendo consideras nosso Grupo Controle.

Após o período de adaptação, as ratas foram colocadas em gaiola padrão de biotério feita de polipropileno (33x40x17cm) para o acasalamento e após a presença de espermatozoide na cavidade vaginal (MARCONDES, BIANCHI e TANNO, 2002), as ratas foram recolocadas nas suas respectivas gaiolas de atividade física, onde metade das ratas de cada grupo recebeu dieta a base de caseína de acordo com a AIN-93G (18% de proteína, tabela 1) (REEVES, 1997), e a outra metade recebeu a mesma dieta, porém com menor quantidade de proteína (8% de proteína, tabela 1).

Após o parto, cada ninhada foi ajustada para oito filhotes (com o máximo de filhotes machos, sendo utilizadas as fêmeas apenas para completar a ninhada). Dos oito filhotes de cada ninhada, foram escolhidos aleatoriamente quatro machos para realização das avaliações no período pós-desmame. A partir do desmame (22º dia de vida) os filhotes passaram a receber dieta padrão de biotério (Presence-Brasil) até o fim do experimento.

O manejo e os cuidados com os animais seguiram as recomendações do Colégio Brasileiro de Experimentação Animal (COBEA) (BAYNE, 1996). O projeto foi aprovado pela Comissão de Ética no uso de Animal do Centro de Ciência Biológicas da UFPE (ANEXO A).

Tabela 1. Composição das dietas

Ingredientes	AIN-93M* g/1Kg	AIN-93G* g/1Kg	Hipoproteica g/1Kg
Amido de milho (87% carboidratos), g	465.692	397.486	476.686
Caseína (proteína ≥80%), g	140.0	200.0	94.1
Amido de milho dextrinizado (92% tetrasaccharides), g	155.0	132.0	158.7
Sacarose, g	100.0	100.0	100.0
Óleo de soja, g	40.0	70.0	70.0
Celulose, g	50.0	50.0	50.0
Mix de Mineral (AIN-93M-MX), g	35.0	-	-
Mix de Mineral (AIN-93G-MX), g	-	35.0	35.0
Mix de Vitaminas (AIN-93-VX), g	10.0	10.0	10.0
L-Metionina, g	1.8	3.0	3.0
Bitartarato de Colina (41.1% colina), g	2.5	2.5	2.5
Tert-butylhydroquinone (TBHQ), g	0.008	0.014	0.014
Macronutrientes			
Energia total (cal/g)	3.44	3.56	3.56
Proteínas	14%	18%	8%
Lipídios	11%	18%	18%
Carboidratos	75%	64%	74%

*(REEVES, 1997)

5.2 Gaiola de atividade física voluntária

Foi elaborada uma gaiola de atividade física voluntária (GAFV) de acrílico com as seguintes dimensões: 27 cm de largura, 34 cm de altura e 61 cm de comprimento (Figura 1 A e B). Em uma das extremidades foi posicionado um cicloergômetro (roda de corrida) com 27 cm de diâmetro, composto por acrílico e raios em aço inoxidável (Figura 2 A e B). Acoplado a gaiola e ao cicloergômetro há um sistema de monitoramento por sensor (ciclocomputador

Cataye, model CC-VL810, Osaka, Japan) que permitiu o registro de algumas grandezas físicas relacionadas à prática de atividade física, como: distância percorrida (km), estimativa do gasto calórico (Km.s^{-1}.dia^{-1}) e tempo de atividade (minutos) (Figura 3 A-D).

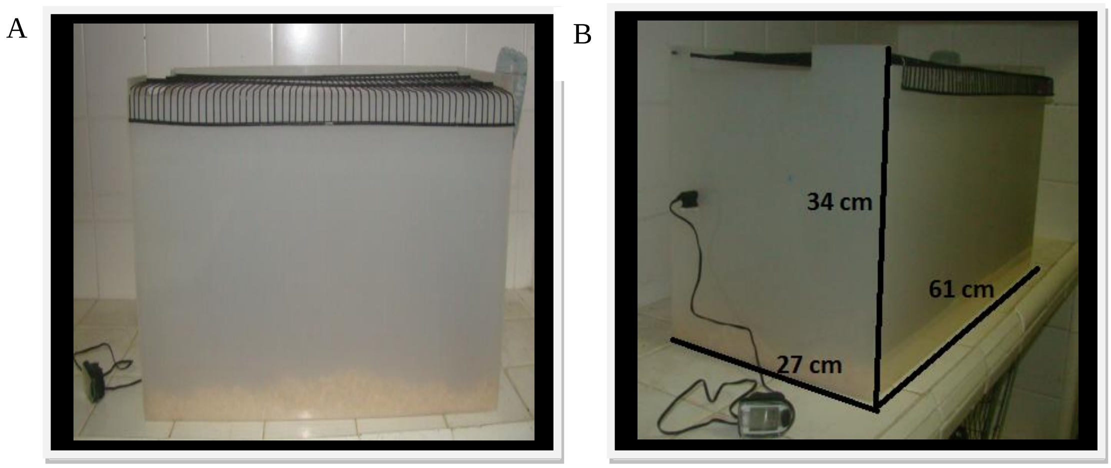

Figura 1. Gaiola de atividade física voluntária (A) e dimensões (B).

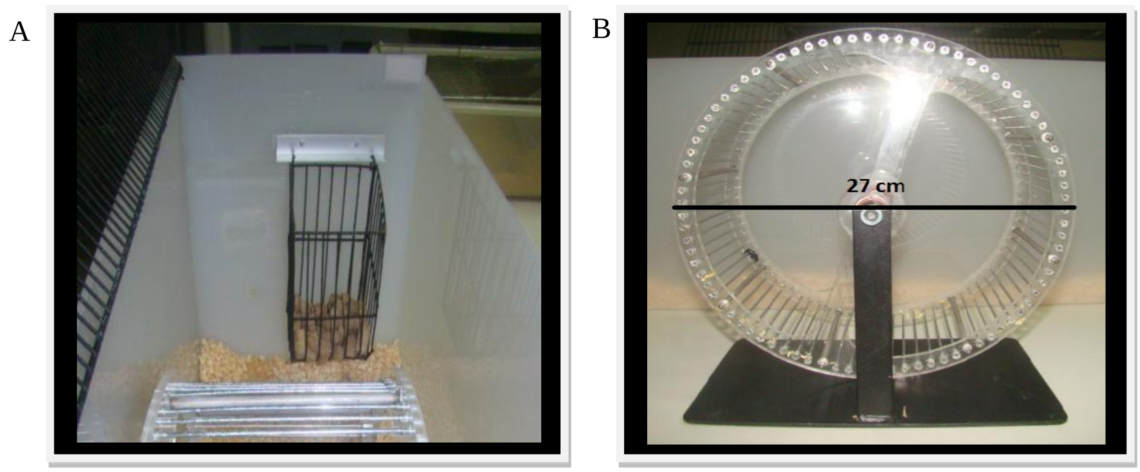

Figura 2. Gaiola de atividade física voluntária com cicloergômetro e comedouro (A) e cicloergômetro fora da Gaiola de atividade física voluntária (B).

5.3 Protocolo de atividade física voluntária

As ratas (*n*=29) aos 85-95 dias de vida foram colocadas individualmente nas GAFV para um período de adaptação. A atividade física das ratas foi avaliada pela movimentação do cicloergômetro e quantificada através dos sensores acoplados na gaiola. Foram registrados diariamente: Distância percorrida (km), estimativa do gasto calórico (km.s^{-1}. dia^{-1}) e o tempo de atividade (mim), para classificar as ratas de acordo com o nível diário de atividade física em: Inativas ou Muito Ativas, seguindo classificação sugerida por estudo anterior (SANTANA MUNIZ *et al.*, 2014) (Tabela 2).

Após detectado o estado de prenhez, através da técnica de esfregaço vaginal, as ratas foram recolocadas nas suas respectivas GAFV permanecendo até o final da lactação. No 14º dia pós-parto o cicloergômetro foi travado para impedir a utilização pelos filhotes, pois estes já apresentam a abertura dos olhos. Nesta data foi finalizado o período de atividade física voluntária das ratas.

Figura 3. Esquema do funcionamento do ciclocomputador com os sensores [Cataye, model CC-VL810, Osaka, Japan] (A); Posicionamento de um sensor na porção externa da GAFV, acoplado ao ciclocomputador (B); visão interna dos sensores, um aclopado ao cicloergômetro e outro na GAFV (C); Rata realizando atividade física (D).

Tabela 2. Classificação dos grupos experimentais de acordo com o nível diário de atividade física.

Grupos experimentais	***N***	**Distância percorrida (km.dia^{-1})**	**Estimativa do gasto calórico (km.s^{-1}.dia^{-1})**	**Tempo de atividade (min.dia^{-1})**
Inativo	*15*	< 1.0	< 10.0	< 20.0
Muito Ativo	*14*	>5.0	>40.0	>120.0

(SANTANA MUNIZ *et al.*, 2014)

5.4 Desenho experimental

Após o período de adaptação, foram formados os seguintes grupos experimentais: Controle (C, *n*=8), Inativo (I, *n*=15) e Muito Ativo (VA, *n*=14). Após acasalamento, foi realizada a manipulação nutricional onde metade de cada grupo experimental recebeu dieta normoproteica e a outra metade recebeu dieta hipoproteica, formando assim os seguintes grupos: Controle Normoproteico (C-NP, *n*=4), Controle Hipoproteico (C-LP, *n*=4), Inativo Normoproteico (I-NP, *n*=8), Inativo Hipoproteico (I-LP, *n*=7), Muito Ativo Normoproteico (VA-NP, *n*=8) e Muito Ativo Hipoproteico (VA-LP, *n*=6).

No desmame, cada ninhada (composta por 3-4 filhotes), representou uma amostra a partir do total de *n* avaliado: Ninhada de mães do grupo Controle Normoproteico ($C\text{-}NP_L$, *n*=4), Ninhada de mães do grupo Controle Hipoproteico ($C\text{-}LP_L$, *n*=4), Ninhada de mães do grupo Inativo Normoproteico ($I\text{-}NP_L$, *n*=8), Ninhada de mães do grupo Inativo Hipoproteico ($I\text{-}LP_L$, *n*=7), Ninhada de mães do grupo Muito Ativo Normoproteico ($VA\text{-}NP_L$, *n*=8) e Ninhada de mães do grupo Muito Ativo Hipoproteico ($VA\text{-}LP_L$, *n*=6).

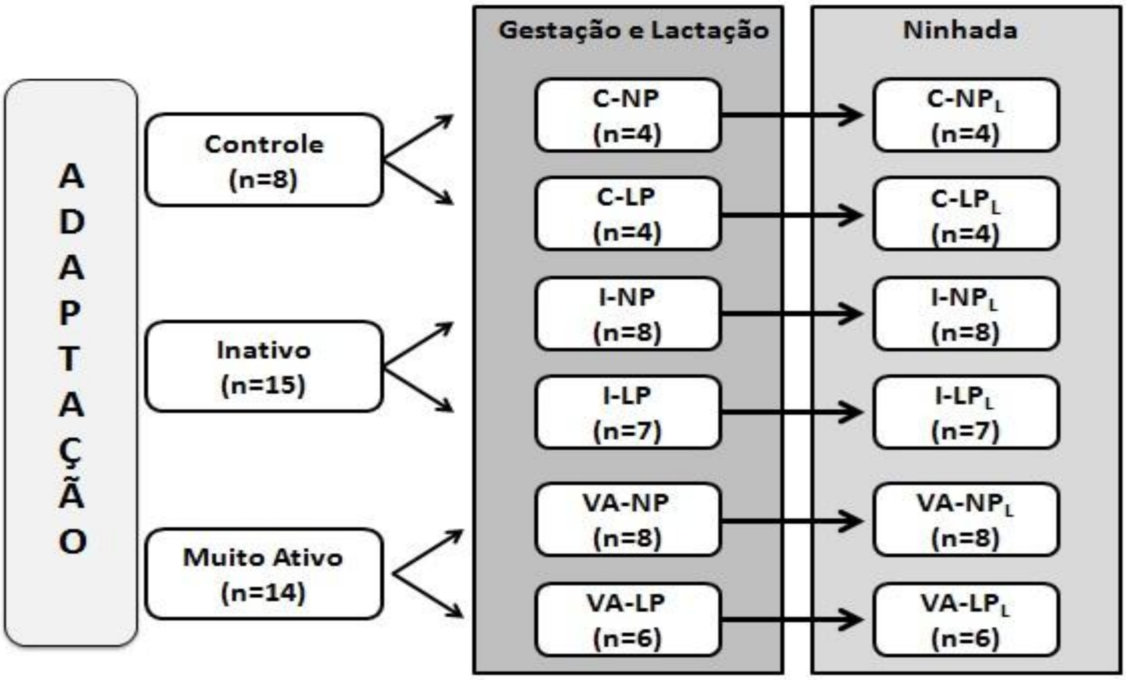

Figura 4. Esquema de formação dos grupos experimentais.

5.5 Consumo alimentar das ratas

O consumo alimentar (g) das ratas foi avaliado a cada três dias durante os períodos de adaptação, gestação e lactação, através da subtração do peso da ração ofertada e o peso da ração restante, de acordo com a seguinte fórmula:

Consumo alimentar = Peso da ração ofertada (g) – Peso da ração restante (g)

(LOPES DE SOUZA *et al.*, 2008)

5.6 Avaliações somáticas

5.6.1 Avaliação do peso corporal e do ganho de peso das ratas

O peso corporal das ratas foi avaliado a cada três dias durante os períodos de adaptação, gestação e lactação. Foi utilizada uma balança eletrônica digital – Marte, modelo S-1000, com capacidade máxima de 1000g e sensibilidade de 0,01g. O percentual de ganho de peso corporal foi calculado tendo como base o peso do 1° dia de avaliação, segundo a fórmula:

% ganho de peso = [Peso do dia (g) x 100/ Peso do 1° dia (g)] – 100

(BAYOL *et al.*, 2004)

5.6.2 Avaliação do peso corporal dos filhotes

O peso corporal dos filhotes foi avaliado a cada três dias durante o período de lactação. Após desmame, o peso corporal foi avaliado no 30º, 40º, 50º e 60º dia de vida, através de uma balança eletrônica digital – Marte, modelo S-1000, com capacidade máxima de 1000g e sensibilidade de 0,01g.

5.7 Avaliação da atividade locomotora

5.7.1 Procedimentos

Para avaliar os efeitos da atividade física e da dieta materna sobre a atividade locomotora, os filhotes foram avaliados no 23º, 45º e 60º dia de vida pós-natal durante a fase escura do ciclo circadiano (entre 10h e 12h). Os procedimentos foram realizados conforme descrito em estudo prévio (ARAGAO RDA *et al.*, 2011), levemente modificado. Cada animal foi colocado individualmente no centro do campo aberto (1 m de diâmetro) e filmados durante 5 minutos, enquanto se locomoveram livremente. Nenhuma iluminação foi utilizada. Na troca dos animais, o campo foi limpo com solução de água e hipoclorito, e o etil vinil acetato (EVA) trocado, para eliminar odores que pudessem interferir no comportamento do animal seguinte. Os vídeos foram gravados diretamente em formato digital e analisados *offline* (ARAGAO RDA *et al.*, 2011). A Figura 5A-D mostra o campo aberto e o sistema de monitoramento utilizado na avaliação da atividade locomotora.

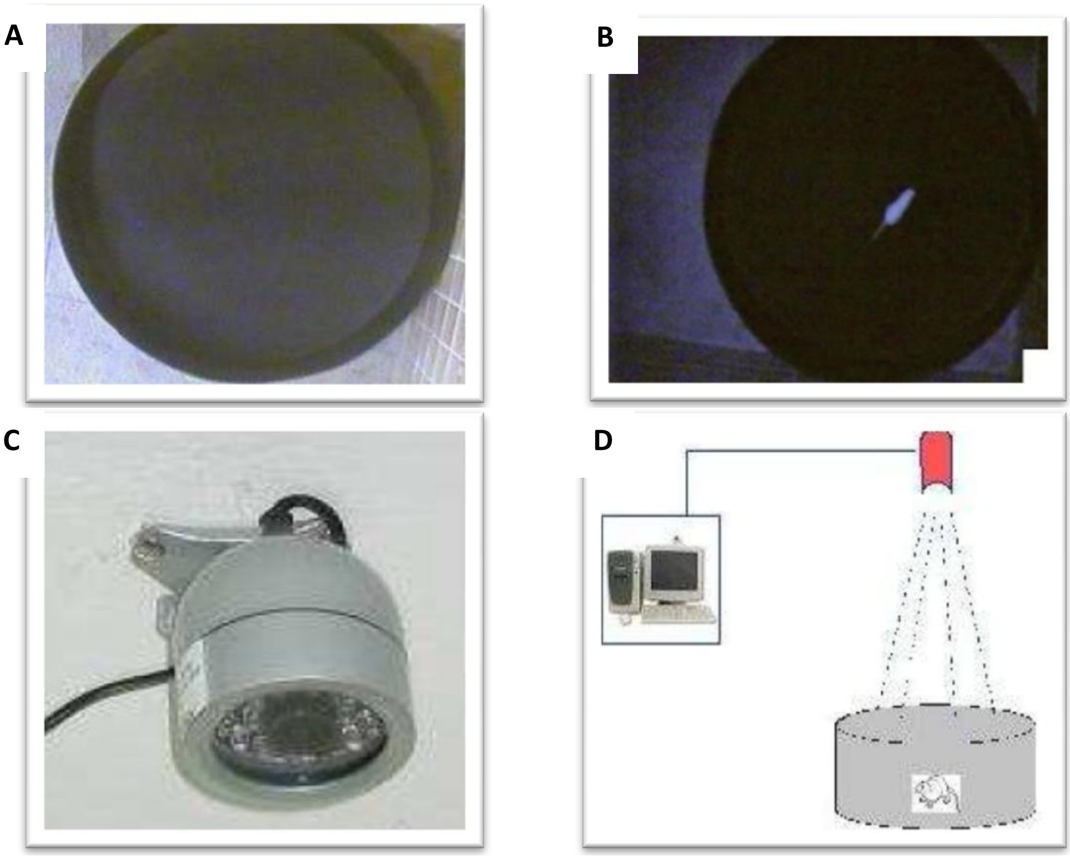

Figura 5. Vista superior do campo aberto em ambiente claro (A); Imagem do rato no campo (B); Câmera de captura das imagens dos animais (C); Representação esquemática do sistema de monitoramento no campo aberto (D).

5.7.2 Sistema de análise

O mesmo sistema de análise descrito em estudo anterior (ARAGAO RDA *et al.*, 2011) foi utilizado e alguns novos parâmetros foram incluídos. Foi possível reconstituir a trajetória dos animais e estabelecer os seguintes parâmetros:

- **Distância percorrida (m):** soma de todo o percurso realizado pelo animal que foi capaz de deslocar o seu centro de massa ao longo do comprimento do seu raio.
- **Deslocamento rotacional (m):** soma de todos os pequenos deslocamentos realizados pelo animal que não foi maior que o comprimento do seu raio. Esta análise foi incluída para serem considerados pequenos movimentos da cabeça e das patas.
- **Velocidade média (m/s):** taxa do deslocamento total pelo tempo que o animal permaneceu em movimento.
- **Potência média (mW):** potência produzida durante o período de deslocamento.
- **Energia total (kcal):** gasto energético total durante o período de deslocamento.
- **Tempo imóvel (s):** tempo total que o animal permaneceu parado no campo aberto.
- **Número de paradas:** número total de paradas realizadas no campo.
- **Tempo imóvel/número de paradas (s):** relação entre o tempo de imobilidade e o número total de paradas.
- **Tempo de permanência na área (s):** o campo aberto foi dividido virtualmente em três áreas (central, intermediária e periférica). Sendo dado o tempo total dos animais nestas áreas.

5.8 Análise estatística

O teste Kolmogorov–Smirnov foi realizado para determinar se os dados apresentam distribuição normal. Para as mães, avaliações da distância percorrida, estimativa do gasto calórico, tempo de atividade, consumo alimentar e peso corporal foram analisados por ANOVA two-way seguido do pós-teste de Bonferroni. Para análise da atividade locomotora da prole, cada ninhada de três a quatro filhotes foi considerada uma amostra, e as análises estatísticas foram realizadas por ANOVA two-way para medidas repetidas, seguida pelo pós-teste de Tukey. Todos os dados são apresentados como médias ± S.E.M. Significância foi estabelecida em $p < 0{,}05$. A análise dos dados foi realizada utilizando o programa estatístico GraphPad Prism 5® (GraphPad Software Inc., La Jolla, CA, EUA).

6 RESULTADOS – ARTIGOS ORIGINAIS

<u>Artigo 1</u> - Title: Maternal voluntary physical activity alter some patterns of locomotor activity in offspring during development

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Abstract

We evaluated the effects of maternal voluntary physical activity on some parameters of locomotor activity in offspring during development. Virgin female Wistar rats (*n*=16) were housed in voluntary physical activity cages (containing running wheel). It was recorded the distance traveled, estimated caloric expenditure and time of activity. The rats were submitted to a period of adaptation (30 days) subsequently classified according to the level of daily physical activity: Inactive (*n*=8) and Very Active (*n*=8). A group of rats (*n*=4) was added in the study in standard cages for control of the experiment. After confirmed the pregnancy, all rats remained to the special cages to the 14th day of lactation. At weaning, rats were randomized 3-4 male offspring from each experimental group to evaluate some parameters of locomotor activity. The evaluations were performed at 23rd, 45th and 60th days of postnatal life, in an open field. Pups from very active mothers traveled more distance with consequent reduction in immobility time, and increase the average power over other groups. Thus, we demonstrated that some parameters related to locomotor activity of pups were modulated due to maternal voluntary physical activity in running wheel before mating and during gestation.

Keywords: Plasticity during development, pregnancy, running wheel, locomotor development, pups.

Introduction

Locomotor activity is a behavior shared among animals and includes walking, running, hopping or jumping, and crawling or slithering (LIPPKE e ZIEGELMANN, 2006). Animals can store elastic potential energy (tendons) and contractile energy (muscle fiber) to beginning a movement (LIPPKE e ZIEGELMANN, 2006). The interaction between skeletal and muscular systems is required in each step of the development of locomotor activity (GARLAND *et al.*, 2011). In addition, balance, motor coordination, speed and power are required to establish the correct movements and help to exploratory activity (GARLAND *et al.*, 2011). During development, the primitive reflexes allow fetus to elaborate the first movements, then the maturation of superior center of the nervous system is responsible for the control of coordination and mature locomotor behavior (FOX, 1965; GRAMSBERGEN, 1998).

During development, the locomotor activity of offspring can be modulated by environmental stimuli, for example, maternal diet and physical activity (BARROS *et al.*, 2006; FALCAO-TEBAS *et al.*, 2012). Previous study showed that pups (21 d old) from malnourished (7.87% protein) mothers during lactation presented a reduced time of exploration in an open field (BARROS *et al.*, 2006). Moreover, adult pups from dams submitted to a high fat palatable diet during the period of lactation presented a reduced distance traveled in the open field (WRIGHT, LANGLEY-EVANS e VOIGT, 2011). In terms of maternal physical exercise, less is known about the interaction between perinatal stimuli and the repercussion on pups during development.

Maternal physical exercise has been studied and the type, intensity and duration are determinant for the short and long-last effects (CLAPP, 2003; 2006; AMORIM *et al.*, 2009; SANTANA MUNIZ *et al.*, 2014). Maternal exercise guidelines preconizes that at least 30 min of moderate-intensity exercise a day on most, if not all, days of the week is satisfactory for health (ARTAL e O'TOOLE, 2003). Following these recommendations, regular exercise during pregnancy increases the rate of placental bed blood flow at rest, so more glucose and oxygen delivery to the placental site may be expected (CLAPP, 2003). These physiological responses to exercise occur according time point in the pregnancy when it is carried out (CLAPP *et al.*, 2002; HOPKINS e CUTFIELD, 2011). Controlled prospective studies have demonstrated that moderate pre-gestational exercise (approximately 50% to 75% of VO_{2max}) is useful to increase metabolic rate (reduction of body weight), and improve cardiorespiratory fitness and maternal-fetal physiological reserve (WOLFE e WEISSGERBER, 2003). Recently, our previous study published a protocol to analyse the voluntary maternal physical activity in running wheel for female Wistar rats during pre-gestational, gestational and lactation periods (SANTANA MUNIZ *et al.*, 2014). In this study, we developed three categories of physical activity for rats: inactive, active and very active according to the distance travelled, estimated calorie burned and time spent in the running wheel (SANTANA MUNIZ *et al.*, 2014).

In our previous study, we evaluated the effects of maternal voluntary physical activity in pups during the reflexes acquisition (SANTANA MUNIZ *et al.*, 2014). However, there is no data about the repercussion of this maternal stimulus on some patterns of locomotor activity of the offspring. In the present study, we analyzed the repercussion of maternal physical activity on some biomechanical parameters of locomotor activity such as: distance traveled, rotational displacement, average speed and potency. We also analyzed some behavioral parameters: time of immobility, number of stops and the time spent in the different areas of the open field. In our previous study, we developed a protocol to analyze some patterns of locomotor activity for rats (ARAGAO RDA *et al.,* 2011). Thus, the main goal of the present study is to analyze the effects if maternal voluntary physical activity on the patterns of locomotor physical activity in offspring at different ages. Our hypothesis is that the maternal phenotype is passed to offspring and patterns of locomotor activity is more pronounced in pups from very active mothers.

Material and methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Center (protocol nº 23076.047664/2013-87), Federal University of Pernambuco, Brazil, and followed the Guidelines for the Care and Use of Laboratory Animals (BAYNE, 1996).

Animals

Twenty virgin female albino Wistar rats (*Rattus norvegicus*) aged 85-95 days de life, were obtained from the Department of Nutrition, Federal University of Pernambuco, Brazil. Animals were maintained at a room temperature of 22 ± 1°C with a controlled light–dark cycle (dark 08.00 am – 8.00 pm). Standard laboratory chow and water were given *ad libitum* throughout the experiment: period of adaptation (carbohydrates: 75%, lipids: 11% and protein: 14%) and period of gestation/lactation (carbohydrates: 64%, lipids: 18% and protein: 18%) (REEVES, 1997). Special cages were used with a stainless steel wheel running and dams were allowed to run for a period of four weeks. After this period, females were placed into a standard cage and mated (1 female for 1 male) for a period of 1–5 days. Females had no access to the running wheel during mating. The day on which spermatozoa were present in a vaginal smear was designated as the day of conception, day 0 of pregnancy. Pregnant rats were then transferred to their original cages with free access to the running wheel throughout pregnancy, and up to postnatal day 14. Wheels were locked on postnatal day 14 to prevent the pups from running and/or being injured. On postnatal day 1, litters were reduced to 8 pups per mother, ensuring only males per litter when possible. Eventually, litters were completed to 8 pups with 2–3 females when necessary. Of each litter, 3-4 males were randomly chosen for the evaluation of parameters of locomotor activity performed after suckling period. Each litter, composed for 3-4 offspring, represents one sample from total *n* evaluated: control (C, n=4); inactive (I, n=8) and very active (VA, n=8). At weaning (22 days), the offspring received a standard diet Presence-Brazil throughout the experiment.

Voluntary physical activity measurements

Female Wistar rats were singly housed into an acrylic cage (cage size: 34 cm height, 27 cm width and 61 cm length). A stainless steel wheel (27 cm diameter) was placed into the cage for running physical activity with food and water *ad libitum*. A wireless cyclocomputer (Cataye, model CC-AT200W, Colorado, USA) was attached in the wheel to calculate and display trip information, such as trip time, distance traveled and estimated calorie burned. Distance was determined by counting the number of rotations, which was translated into the number of wheel circumferences passed. The protocol to classify rats according to their physical activity followed previous study (SANTANA MUNIZ *et al.*, 2014). Briefly, daily distance traveled, time and estimated calorie burned were used to classify rats into different groups according to voluntary physical activity: Inactive [Distance traveled (Km/day): ≤1.0; Estimated calorie burned (Km/s/day): ≤10.0 and Time (min/day): ≤20.0] and Very Active [Distance traveled (Km/day): >5.0; Estimated calorie burned (Km/s/day): >40.0 and Time (min/day): >120.0] (SANTANA MUNIZ *et al.*, 2014). In this study there was no active group [Distance traveled (Km/day): >1.0≤5.0; Estimated calorie burned (Km/s/day): >10.0≤40.0 and Time (min/day): >20.0≤120.0], as the mothers who choose to perform or not physical activity on the wheel. A control group (n=4) with similar age and body weight was incorporated in the study and individually housed in a standard dimension cage without running wheel apparatus.

Mother's body weight and food intake

Mother's body weight and food consumption were recorded each three days throughout the experiment. Body and food weights were recorded each three days throughout the experiment, using a Marte Scale (AS-1000) with a 0.01-g accuracy.

Blood glucose measurements

Twelve hour fasting glycaemia levels were evaluated in the last day of adaptation and weekly during gestation using blood samples from the tail vein of the rats, using a glucometer (Accu Check Advantage and Accutrend GCT) and the glucose oxidase method. The animals were fasted overnight.

Offspring body weight

Body weight of the pups was measured at birth and every three days during the lactation period. After weaning, body weight was assessed on the 30th, 40th, 50th and 60th day of life. Body weight was recorded using a Marte Scale (AS-1000) with 0.01-g accuracy.

Assess of locomotor activity

Procedures

To evaluate the effects of the maternal physical activity on locomotor activity of the offspring, the animals were evaluated on the 23rd, 45th and 60th days of postnatal life during the dark phase of the circadian cycle (between 10.00am and 12.00pm). The procedures were performed as described in previous study (ARAGAO RDA *et al.*, 2011) slightly modified. Each animal was placed individually in the center of the open field (1 m diameter) and recorded for 5 minutes while it moved freely. No additional illumination was used. When the animals were exchanged, the field was cleaned with sodium hypochlorite and water, and the ethyl vinyl acetate (EVA) was changed to eliminate odors that could affect the behavior of the next animal. The videos were recorded directly at digital format and analyzed *offline* (ARAGAO RDA *et al.*, 2011).

Analysis System

The same analysis system described in previous study (ARAGAO RDA *et al.*, 2011) was used and some new parameters were included. It was possible to reconstruct the animal trajectory and establish the following parameters:

• Distance traveled (m): the sum of all displacements performed by the animal that was able to displaced its mass center over the length of its radius.

• Rotational displacement (m): the sum of all small displacements performed by the animal that was not over the length of its radius. This analysis was included to take into account small movements from the head and limbs.

• Average speed (m/s): the ratio of total displacement by the time the animal remained in motion.

• Average potency (mW): potency produced during the period of displacement.

• Total energy (kcal): total energy spent during the period of displacement.

• Time immobile (s): total time the animal remained standing in the open field.

• Number of stops: total number of stops made in the field.

• Time immobile/number of stops (s): relationship between the time immobile and the total number of stops.

• Length of stay in the area (s): the open field was divided virtually into three (central, intermediate and peripheral) areas. Being given the total time of the animals in these areas.

Statistical analyses

The Kolmogorov–Smirnov test was performed to determine if the data were normally distributed. For the mothers, measurements of distance traveled, estimated calorie burned e time of activity, were analyzed by ANOVA two-way and for body weight, food intake and blood glucose were analyzed by ANOVA one-way. For the analysis of offspring locomotor activity, each litter of four pups was considered one sample, and statistical analyses were performed by two-way ANOVA for repeated measures followed by the Tukey test. All data are presented as means ± S.E.M. Significance was set at $p<0.05$. Data analysis was performed using the statistical program GraphPad Prism 5® (GraphPad Software Inc., La Jolla, CA, USA).

Results

Very active mothers showed a progressive increment of the distance traveled, estimated calorie burned and time of activity during the adaptation period when compared to inactive group (Figure 1A, B and C). At pregnancy, the very active mothers reduced the distance traveled becoming inactive from of the second week of gestation until the 14^{th} day of lactation (day on which the running wheel was locked) (Figure 1A, B and C).

Very active mothers showed a high food intake during adaptation and gestation while body weight was high during gestation when compared to control. Food intake during gestation was higher in very active mothers than inactive. During lactation, very active dams showed an increase of the initial and final body weight when compared to control group. Groups did not alter fasting glycaemia (Table 1).

The number of pups born from very active and inactive mothers was higher than control mothers (Table 1). Very active mothers also had a higher number of pups than inactive mothers (Table 1). However, offspring's birth weight did not differ among groups (C = 6.4, SEM: 0.5; I = 5.6, SEM: 0.1; VA = 5.7, SEM: 0.2 $P>0.05$). Similarly, body weight did not differ among groups during lactation, 30^{th}, 40^{th}, 50^{th} and 60^{th} days old [data not shown].

Locomotor activity was evaluated at 23, 45 and 60 d old (Figure 2). All groups showed a reduction in the distance traveled throughout ages. Rotational displacement, average speed, average potency, total energy, immobility time, number of stops, and relative immobility time/number of stops were progressively increasing at different ages (Figure 2A-H). In relation to the time spent in each area of the field, with increasing age there is less time spent in the central and intermediate area and increased time spent in the peripheral area.

Difference inter-groups were evaluated in each age. At 23 d old, there were no differences among groups. At 45 d and 60 d old, pups from VA mothers showed high values of distance traveled and consequently less time immobile when compared with pups from mothers I and

C (Figures 2A and F). At 45 d old, the average potency and total energy were higher in pups from VA mothers than pups from control mothers (Figures 2D and E). At 60 d old, there was an increase in the average speed, total energy and number of stops in pups from VA mothers when compared to pups from control mothers (Figures 2C, E and G). In addition, the average potency was increased in pups from VA dams when compared to both control and inactive (Figure 2D). Relative time immobility/number of stops was lower in pups from inactive mothers than control while there were a reduction in pups from VA mothers when compared to both control and inactive (Figure 2H). There were no differences among groups in relation to the time spent in each area of the open field for each age studied.

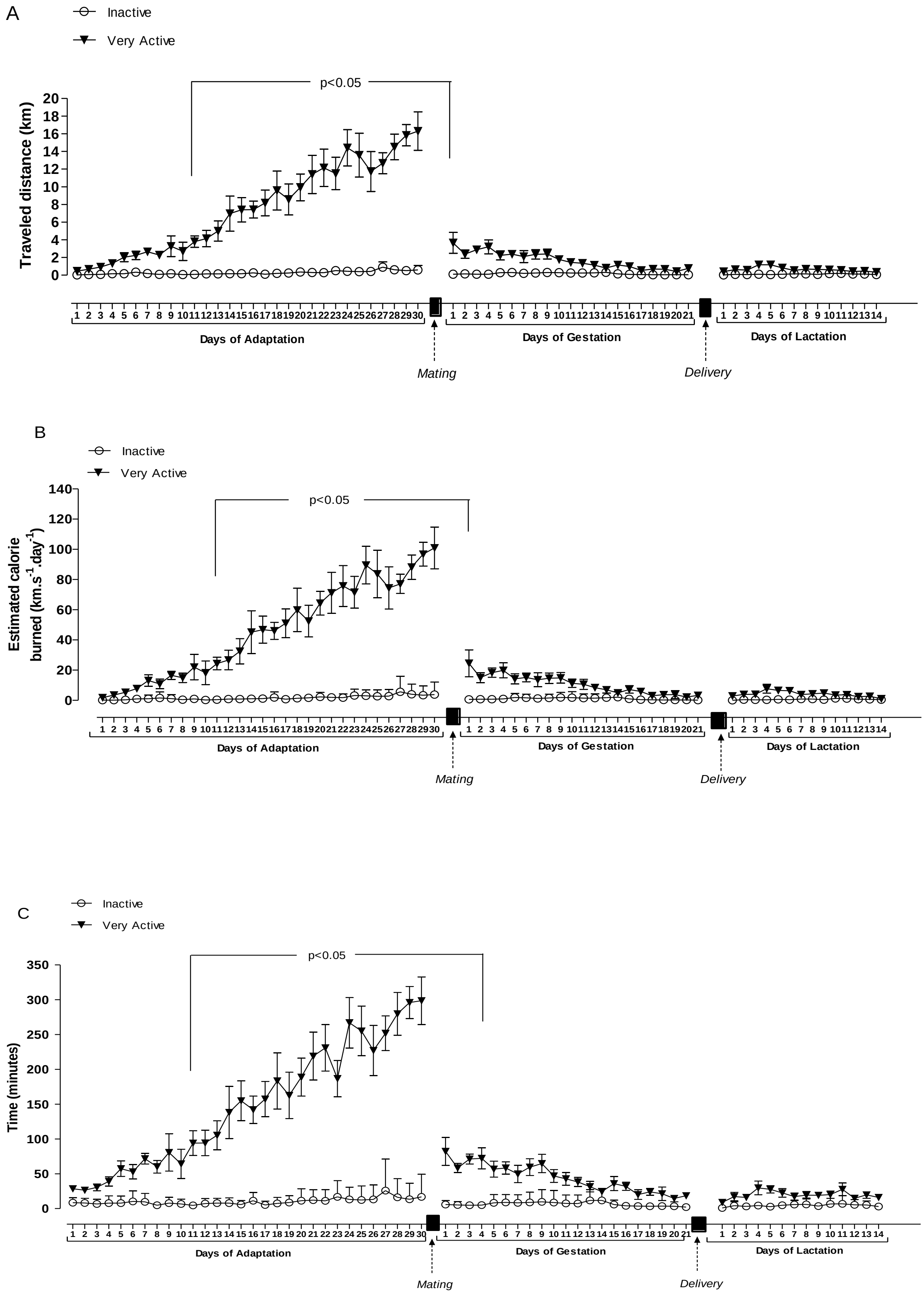
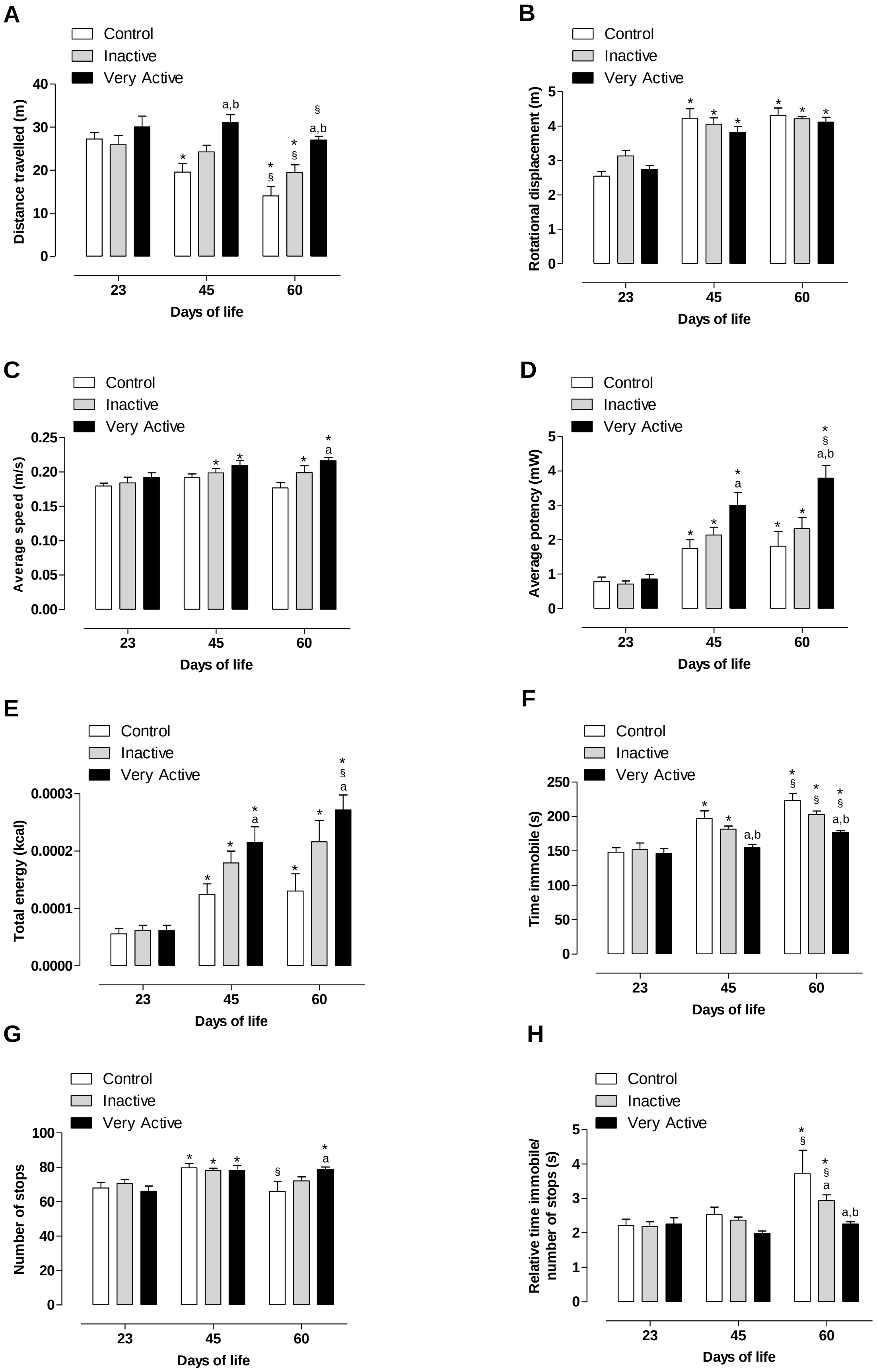


Figure 1. Parameters of voluntary physical activity. Daily distance traveled (A), estimated calorie burned (B) and time (C) were recorded during periods of adaptation, gestation and lactation. Values are presented as mean ± S.E.M. *$p<0.05$ vs. Inactive using two way ANOVA with Bonferroni post-hoc test.

Table 1. Data of mothers on periods of pre-conception, gestation and lactation. Values expressed as Mean ± E.P.M

	Initial BW (g)		**Final BW (g)**		**Δ% BW**		**Food consumption (g/day)**		**Glycaemia (mg/dL)**		**Number of pups at delivery**	
Pre-conception												
Control	218.7	14.2	226.5	6.2	4.3	4.4	12.0	0.5	109.3	4.3		
Inactive	233.2	3.9	238.6	4.9	2.4	2.1	13.6	0.9	108.8	2.8		
Very Active	234.2	4.7	237.5	5.8	1.6	2.9	15.4[a]	0.6	108.8	3.3		
Gestation												
Control	234.0	7.4	325.4	11.2	39.0	1.2	14.6	0.6	74.2	3.7	9.2	0.75
Inactive	257.0	6.2	351.3	6.4	37.1	3.6	16.1	0.5	76.8	2.7	11.7[a]	0.45
Very Active	254.6	7.4	363.9[a]	9.0	43.9	5.9	20.7[a,b]	0.7	69.1	3.4	14.0[a,b]	0.53
Lactation												
Control	252.0	6.3	249.9	9.8	-0.8	2.9	37.1	3.4	-	-		
Inactive	275.5	4.5	276.7	3.8	0.5	1.3	40.5	1.5	-	-		
Very Active	284.9[a]	7.2	282.8[a]	9.4	-0.6	2.7	42.4	2.3	-	-		

Control (n=4); Inactive (n=8) and Very Active (n=8), [a] $p<0.05$ vs Control and [b]$p<0.05$ vs Inactive using one way ANOVA with Tukey's post-hoc.


A
Control
Inactive
Very Active
Distance travelled (m)
0
10
20
30
40
a,b
*
§
a,b
*
§
*
§
23
45
60
Days of life
B
Control
Inactive
Very Active
Rotational displacement (m)
0
1
2
3
4
5
23
45
60
Days of life
C
Control
Inactive
Very Active
Average speed (m/s)
0.00
0.05
0.10
0.15
0.20
0.25
*
a
23
45
60
Days of life
D
Control
Inactive
Very Active
Average potency (mW)
0
1
2
3
4
5
*
a
*
§
a,b
23
45
60
Days of life
E
Control
Inactive
Very Active
Total energy (kcal)
0.0000
0.0001
0.0002
0.0003
*
a
*
§
a
23
45
60
Days of life
F
Control
Inactive
Very Active
Time immobile (s)
0
50
100
150
200
250
a,b
*
§
a,b
23
45
60
Days of life
G
Control
Inactive
Very Active
Number of stops
0
20
40
60
80
100
§
*
a
23
45
60
Days of life
H
Control
Inactive
Very Active
Relative time immobile/
number of stops (s)
0
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2
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*
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a
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23
45
60
Days of life

I

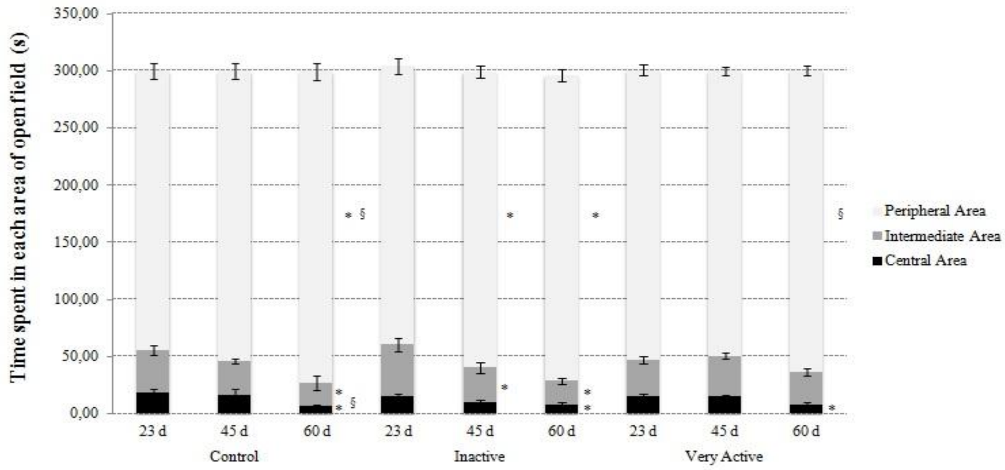

Figure 2 – Parameters of locomotor activity of offspring at 23, 45 and 60 days of life. Litters were classified according to maternal voluntary physical activity during the adaptation period (30 days before mating), being constituted by groups: Control (n=4), Inactive (n=8) and Very Active (n=8). A, Distance traveled (m); B, Rotational displacement (m); C, Average speed (m/s); D, Average pontency (mW); E, Total energy (kcal); F, Time of immobility (s); G, Number of stops; H, relative time of immobility/number of stops (s); I, Time spent in each area of open field (s). Values are presented as mean ± S.E.M. *p<0.05 vs. 23 days; §p<0.05 vs. 45 days; ^{a}p<0.05 vs. Control and ^{b}p<0.05 vs. Inactive using two way ANOVA with Tukey's post-hoc test.

Discussion

There are few and divergent data to infer a real benefit or detriment to offspring associated with physical activity during pregnancy. In addition, due to the lack in categorization of physical activity or exercise in some studies, it becomes difficult to understand its effects. In the present study, we used a protocol of maternal voluntary physical activity in order to establish the active phenotype during pre-gestational period. Dams were classified either inactive or very active according to a previous study (SANTANA MUNIZ *et al.*, 2014). During gestation, there was reduction in the distance traveled and rats from all experimental groups became inactive since the second week of gestation until the 14th day of lactation. Our results are aligned with our previous study that identified a more preservative behavior from mothers in order to save energy for their fetus (SANTANA MUNIZ *et al.*, 2014). A meta-analyse reported no association between regular physical activity (unspecified) and birth weight and preterm birth (KRAMER e MCDONALD, 2006).

Very active mothers presented an increase in the body weight gain probable due the high number of pups during gestation. In spite of the number of pups was high for very active mother, we do not believe that this, somehow, is related with active maternal phenotype. There is no physiological reason to consider that physical activity can affect the number of pups in rats. The number of pups born from VA mothers was higher than the other groups, but there was no difference in the pups birth weight possibly due a high food consumption during the period of physical activity in order to balance the energy intake and waste (DIXON, ACKERT e ECKEL, 2003; CARTER *et al.*, 2012; NOVAK, BURGHARDT e LEVINE, 2012). Although a clinical study demonstrated that maternal moderate-intensity physical exercise throughout gestation (55-60% of the preconception maximum aerobic capacity, for 20 minutes, 3-5 times/week) increased offspring birth weight and body lean mass (CLAPP *et al.*, 2000), this increased birth weight (100-250g) was maintained within normal range (3000g to 3999g).

Pups from very active mothers showed a high distance traveled and a reduced time of immobilization when compared to other groups. Our data confirmed the hypothesis of maternal active phenotype can induce changes in the phenotype of the offspring in terms of locomotor activity. In pups from VA mothers, the trajectory of some patterns (distance traveled, rotational displacement, average speed, average potency, total energy, time of immobility, number of stops and time to spend in the areas of the open field) of locomotor activity was not altered at different ages (23, 45 and 60 d old). Our results are aligned with previous study (ARAGAO RDA *et al.*, 2011). In addition, pups from VA mothers did not reduce the distance traveled when compared with other groups. The mechanism underlying these effects can be related to the interaction between muscular and nervous system (BARROS *et al.*, 2006) that is very responsible to the maternal exercise (CLAPP, 2003). Maternal physical activity (treadmill running, beginning on the E15, 30 min/day at a low-intensity) during pregnancy promoted hippocampal neurogenesis and hippocampal brain-derived neurotrophic factor mRNA expression of the rat offspring on the 29 days old (KIM *et al.*, 2007). In addition, pups from VA mothers showed a high average speed and potency that are parameters that express the mechanical capacity of the skeletal muscle during movement (ARAGAO RDA *et al.*, 2011).

Another mechanism underlying the long-last effects of maternal physical activity is related to the high concentrations of insulin-like growth factor (IGF-1), adjustment of the hypothalamus-pituitary-adrenal axis (HPA axis) and brain-derived neurotrophic factor (BDNF) in some areas of the brain of mother and pups (NEEPER *et al.*, 1996; OLIFF *et al.*, 1998; CLAPP, 2006; SASSE *et al.*, 2008; M *et al.*, 2013). The IGF-1 and their associated binding proteins are thought to be an important factor which may modulate fetal response to early environment. Treadmill exercise (20 m/min, 20 min/day, during 19 days) results in an increase in plasma concentration of growth hormone (GH), IGF-I and insulin-like growth factor binding protein-3 (IGFBP-3) in the late period of pregnancy (TURGUT *et al.*, 2006).

In addition, the placental bed blood flow is also an important factor based on the compensatory mechanisms to protect both mother and developing fetus. It has been suggested that placental blood flow is higher at 20^{th} and 40^{th} week of gestation in exercising women (60% of VO_{2max}) when compared to non-exercising women (THOMAS, CLAPP e SHERNCE, 2008). At rest (after exercise), the rate of placental bed blood flow increases and more glucose and oxygen delivery to placental site are observed in women submitted to a physical activity (CLAPP, 2003). Furthermore, it was showed that mothers that began a regular exercise program in early pregnancy had increased mid-trimester placental growth rates and vascularization compared to control sedentary mothers (CLAPP *et al.*, 2000).

Conclusion

In the present study, we demonstrated that some parameters of the pattern of locomotor activity in the offspring were modulated by maternal physical activity. However, there was no alteration in the ordinary development of those patterns at different ages. This study considers that active maternal phenotype is established before pregnancy and can influence the development of locomotor activity in the offspring. Thus, maternal lifestyle can be considered an important strategy to intervene in the lifestyle development of the offspring.

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Artigo 2 - Title: Can maternal voluntary physical activity attenuate the effects of a perinatal low-protein diet on the patterns of locmotor activity of pups?

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Abstract

The aim of this study was to evaluate the effect of maternal voluntary physical activity on some parameters of locomotor activity in offspring of rats who received low protein diet during periods of pregnancy and lactation. Wistar rats (*n*=29) were housed in voluntary physical activity cages (containing running wheel) in which it was done record the distance traveled, estimate of energy expenditure and time of activity. The rats passed a period of adaptation (30 days) subsequently classified according to the level of daily physical activity: Inactive (*n*=15) and Very Active (*n*=14). A group of rats (*n*=8) was added to the study in which he remained throughout the experiment in standard cage, without access to running wheels, and is considered our control group. Following detection of pregnancy, half of each group received normal protein diet (18% protein) and the other half low protein diet (8% protein) during the period of gestation and lactation. At weaning, were randomized 3-4 male offspring from each experimental group to evaluated of some parameters of locomotor activity. Our results showed that pups, at 60 days of life, born to mothers provided with the low protein diet during gestation and lactation showed increased distance. However, this change was attenuated in offspring of mothers who received low protein diet and physical activity performed. In conclusion, the practice of maternal voluntary physical activity is able to modify some parameters of locomotor activity of offspring due to the organism ability to respond to the environment.

Keywords: Plasticity during development, pregnancy and lactation, nutrition, locomotor development, pups.

Introduction

The locomotor behavior is related to the animal survival such as, seeking for food, shelter, interaction with competitors, mating and avoids predators (Garland, Schutz et al. 2011). This behavior requires a fine coordination with simultaneous activity of different motor pathways and maturation and functional integration of the nervous and muscular systems (Westerga and Gramsbergen 1990). Pregnancy and lactation are considered the critical period of the development in which environmental stimuli can impact on morphological, physiological and/or behavioral development (Dobbing 1964; Morgane, Austin-LaFrance et al. 1993).

It has been shown that maternal malnutrition can change the behavioral phenotype of offspring during development (Barros, Manhaes-De-Castro et al. 2006; Reyes-Castro, Rodriguez et al. 2012). Previous study demonstrated that pups from mothers submitted to a regional basic diet (7.87% protein) during lactation had a reduction in the time of exploration in a open field at 21 days of life (Barros, Manhaes-De-Castro et al. 2006). Another study showed that protein restriction during gestation and lactation induced an increase in the plasma corticosterone concentration and a reduction in the exploration of the central zone of the open field, with no change in total distance (Reyes-Castro, Rodriguez et al. 2012).

Recently, another stimulus that has been well described in previous studies refers to maternal life style (Amorim, dos Santos et al. 2009; Falcao-Tebas, Bento-Santos et al. 2012; Leandro, Fidalgo et al. 2012; Fidalgo, Falcao-Tebas et al. 2013; Santana Muniz, Beserra et al. 2014). Maternal physical activity has been also used as a model to study investment strategies and the short and long-last impacts on the offspring during development (Carter, Lewis et al.

2012; Santana Muniz, Beserra et al. 2014). Our previous study showed that physical activity before and during pregnancy was related to the increased somatic growth indicators in the offspring at different ages (Santana Muniz, Beserra et al. 2014). Another study, using an animal model showed that adult pups from active mothers (running wheel during 2 weeks before pregnancy until the 14^{th} day of lactation) had an increased in the glucose uptake and the insulin response in the skeletal muscle and adipose tissue (Carter, Lewis et al. 2012).

Studies have supported the hypothesis that an active maternal lifestyle can attenuate changes in the offspring in response to an inadequate nutrient intake (Falcao-Tebas, Bento-Santos et al. 2012; Falcão-Tebas;, Tobias; et al. 2012). In rats, maternal physical training during pregnancy showed an increase in some somatic growth indicators (growth rate, tail length, laterolateral axis of the skull and anteroposterior axis of the head) and anticipation in the maturation of some reflexes when compared with pups from mothers who received diet low protein (Falcao-Tebas, Bento-Santos et al. 2012). In addition, it was shown that maternal physical training reduced blood cholesterol levels, serum glucose and waist circumference values in adult offspring (Falcão-Tebas;, Tobias; et al. 2012). Although some studies have shown that maternal physical training is related to benefits to mothers and their offspring during development, maternal voluntary physical activity seems to be more interesting in terms of the active phenotype establishment (Contarteze, Manchado Fde et al. 2008).

Therefore, the main goal of the present study was to evaluate the effect of maternal voluntary physical activity on some parameters of locomotor activity in offspring of rats submitted to a maternal low protein diet during pregnancy and lactation. Our hypothesis is that maternal voluntary physical activity is able to attenuate the effects of maternal low protein diet on locomotor activity of offspring due to the adaptive capacity of the organism to respond to environmental changes.

Material and methods

The experimental protocol was approved by the Ethical Committee of the Biological Sciences Center (protocol nº 23076.047664/2013-87), Federal University of Pernambuco, Brazil, and followed the Guidelines for the Care and Use of Laboratory Animals (Bayne 1996).

Animals and experimental diets

Thirty seven virgin female albino Wistar rats (*Rattus norvegicus*) aged 85-95 were obtained from the Department of Nutrition, Federal University of Pernambuco, Brazil. Animals were maintained at a room temperature of 22 ± 1°C with a controlled light–dark cycle (dark 08.00 am – 8.00 pm). Special cages were used with a stainless steel wheel running and dams were allowed to run for a period of four weeks, in this period the rats received water and were fed AIN-93M diet (Table 1) *ad libitum* (Reeves 1997). After this period, females were placed into a standard cage and mated (1 female for 1 male) for a period of 1–5 days. Females had no access to the running wheel during mating. The day on which spermatozoa were present in a vaginal smear was designated as the day of conception, day 0 of pregnancy.

Pregnant rats were then transferred to their original cages, where half of the rats of each experimental group received a diet based on casein according to the AIN-93G diet (18% protein, Table 1) (Reeves 1997) and the other half received the same diet, but with less amount of protein (8% protein, Table 1). Rats had free access to the running wheel throughout pregnancy, and up to postnatal day 14. Wheels were locked on postnatal day 14 to prevent the pups from running and/or being injured. On postnatal day 1, litters were reduced to 8 pups per mother, ensuring only males per litter when possible. Eventually, litters were completed to 8 pups with 2–3 females when necessary. Of each litter, 3-4 males were randomly chosen for the evaluation of parameters of locomotor activity performed after suckling period. Each litter, composed for 3-4 offspring, represents one sample from total *n* evaluated: Control

Normo Protein (C-NP_L, n=4); Control Low Protein (C-LP_L, n=4), Inactive Normo Protein (I-NP_L, n=8), Inactive Low Protein (I-LP_L, n=7), Very Active Normo Protein (VA-NP_L, n=8) and Very Active Low Protein (VA-LP_L, n=6). At weaning (22 days), the offspring received a standard diet Presence-Brazil throughout the experiment.

Table 1. Composition of the diet

Ingredients	**AIN-93M* g/1Kg**	**AIN-93G* g/1Kg**	**LOW PROTEIN g/1Kg**
Corn starch (87% carbohydrates), g	465.692	397.486	476.686
Casein (protein ≥80%), g	140.0	200.0	94.1
Dextrinized starch (92% tetrasaccharides), g	155.0	132.0	158.7
Sucrose, g	100.0	100.0	100.0
Soya oil, g	40.0	70.0	70.0
Cellulose, g	50.0	50.0	50.0
Mineral mixture (AIN-93M-MX), g	35.0	-	-
Mineral mixture (AIN-93G-MX), g	-	35.0	35.0
Vitamin mix (AIN-93-VX), g	10.0	10.0	10.0
L-Methionine, g	1.8	3.0	3.0
Choline bitartrate (41.1% choline), g	2.5	2.5	2.5
Tert-butylhydroquinone (TBHQ), g	0.008	0.014	0.014
Macronutrients			
Total energy (cal/g)	3.44	3.56	3.56
Protein	14%	18%	8%
Lipids	11%	18%	18%
Carbohydrates	75%	64%	74%

*(Reeves 1997)

Voluntary physical activity measurements

Female Wistar rats were singly housed into an acrylic cage (cage size: 34 cm height, 27 cm width and 61 cm length). A stainless steel wheel (27 cm diameter) was placed into the cage for running physical activity with food and water *ad libitum*. A wireless cyclocomputer (Cataye, model CC-AT200W, Colorado, USA) was attached in the wheel to calculate and display trip information, such as trip time, distance traveled and estimated calorie burned. Distance was determined by counting the number of rotations, which was translated into the number of wheel circumferences passed. The protocol to classify rats according to their physical activity followed previous study (Santana Muniz, Beserra et al. 2014). Briefly, daily distance traveled, time and estimated calorie burned were used to classify rats into different groups according to voluntary physical activity: Inactive [Distance traveled (km/day): ≤1.0; Estimated calorie burned (Km/s/day): ≤10.0 and Time (min/day): ≤20.0] and Very Active [Distance traveled (Km/day): >5.0; Estimated calorie burned (km/s/day): >40.0 and Time (min/day): >120.0] (Santana Muniz, Beserra et al. 2014). In this study there was no active group [Distance traveled (km/day): >1.0≤5.0; Estimated calorie burned (km/s/day): >10.0≤40.0 and Time (min/day): >20.0≤120.0], as the mothers who choose to perform or not physical activity on the wheel. A control group (n=8) with similar age was incorporated in the study and individually housed in a standard dimension cage without running wheel apparatus.

Mother's body weight and food intake

Mother's body weight and food consumption were recorded each three days throughout the adaptation, gestation and lactation periods. Body and food weights were recorded using a Marte Scale (AS-1000) with a 0.01-g accuracy.

Offspring body weight

Body weight of the pups was measured at birth and every three days during the lactation period. After weaning, body weight was assessed on the 30th, 40th, 50th and 60th day of life. Body weight was recorded using a Marte Scale (AS-1000) with 0.01-g accuracy.

Assess of locomotor activity

Procedures

To evaluate the effects of the maternal physical activity and diet on locomotor activity of the offspring, the animals were evaluated on the 23^{rd}, 45^{th} and 60^{th} days of postnatal life during the dark phase of the circadian cycle (between 10.00am and 12.00pm). The procedures were performed as described in previous study (Aragao Rda, Rodrigues et al. 2011) slightly modified. Each animal was placed individually in the center of the an open field (1 m diameter) and recorded for 5 minutes while it moved freely. No additional illumination was used. When the animals were exchanged, the field was cleaned with sodium hypochlorite and water, and the ethyl vinyl acetate (EVA) was changed to eliminate odors that could affect the behavior of the next animal. The videos were recorded directly at digital format and analyzed *offline* (Aragao Rda, Rodrigues et al. 2011).

Analysis System

The same analysis system described in previous study (Aragao Rda, Rodrigues et al. 2011) was used and some new parameters were included. It was possible to reconstruct the animal trajectory and establish the following parameters:

• Distance traveled (m): the sum of all displacements performed by the animal that was able to displaced its mass center over the length of its radius.

• Rotational displacement (m): the sum of all small displacements performed by the animal that was not over the length of its radius. This analysis was included to take into account small movements from the head and limbs.

• Average speed (m/s): the ratio of total displacement by the time the animal remained in motion.

• Average potency (mW): potency produced during the period of displacement.

• Total energy (kcal): total energy spent during the period of displacement.

• Time immobile (s): total time the animal remained standing in the open field.

• Number of stops: total number of stops made in the field.

• Time immobile/number of stops (s): relationship between the time immobile and the total number of stops.

Statistical analyses

The Kolmogorov–Smirnov test was performed to determine if the data were normally distributed. For the mothers, measurements of distance traveled, estimated calorie burned e time of activity, food intake and body weight were analyzed by ANOVA two-way followed by the Bonferroni post-hoc test. For the analysis of offspring locomotor activity, each litter of three-four pups was considered one sample, and statistical analyses were performed by two-way ANOVA for repeated measures followed by the Bonferroni or Tukey post-hoc test. All data are presented as means ± S.E.M. Significance was set at $p<0.05$. Data analysis was performed using the statistical program GraphPad Prism 5® (GraphPad Software Inc., La Jolla, CA, USA).

Results

Very active mothers were different in terms of distance traveled when compared to inactive mothers from the second week of adaptation period (Figure 1A, B and C). During this period, very active mothers decreased body weight gain percentage at the beginning of adaptation when compared to control and inactive mothers and increased food consumption in the final period of adaptation (Figure 2A and B).

Dams submitted to a low protein diet did not change the level of physical activity during pregnancy, with a progressive reduction of the level of physical activity (Figure 3A, B and C). There were no differences among groups in terms of food consumption during gestation (Figure 4A). At the 21st day of gestation, I-LP mothers presented a reduced percentage of body weight gain when compared to I-NP mothers (Figure 4B). Mothers submitted to a low-protein diet presented lower food consumption from the 12th to 21st day of lactation than their respective controls (Figure 4A), while body weight gain did not change (Figure 4A and B).

The number of pups and the litter weight at birth was higher for the VA-NP group when compared with C-NP and I-NP groups (Table 2). However, there was no difference between groups in terms of birth weight (Table 2). Pups from LP mothers showed a lower body weight from of the 12th to 21st day of lactation (Figure 5A). At weaning, I-LP pups had lower body weight at 30th and 40th days old than I-NP pups and VA-LP pups had lower body weight at 30th to 60th days old than VA-NP pups (Figure 5B).

The trajectory of locomotor activity at 23, 45 and 60 d old is shown in the figure 6 (A – H). Pups from I-NP mothers presented a similar trajectory of locomotor activity of the pups from C-NP mothers. All parameters of locomotor activity were changed in pups from I-LP mothers when compared to pups of C-NP mothers. Maternal physical activity also changed the trajectory of all parameters of locomotor activity in pups, except for the rotational displacement. Pups from VA-LP mothers showed no differences in terms of the trajectory of locomotor activity, as average power, total energy and immobility time when compared to pups from C-NP mothers.

Difference inter-groups were evaluated in each age (Table 3). At 23 d old, pups from I-LP mothers showed a reduced rotational displacement when compared to pups from I-NP mothers. At 45 d old, pups from VA-NP mothers showed a higher traveled distance and reduced time of immobilization when compared to pups from I-NP mothers. In addition, pups from VA-LP mothers showed reduced average potency when compared to pups from VA-NP mothers. Most visible changes were seen in pups at 60 d old in relation to low protein diet and physical activity. Pups from I-LP mothers showed a high traveled distance and reduced time of immobilization and relation time of immobilization/number of stops when compared to pups from I-NP mothers. Similarly, pups from VA-NP mothers also showed a high traveled distance and reduced time of immobilization and relation time of immobilization/number of stops when compared to pups from I-NP mothers. In addition, pups from VA-LP mothers showed reduced traveled distance when compared to pups from VA-NP mothers e reduced average potency and total energy when compared to pups from I-LP and VA-NP mothers.

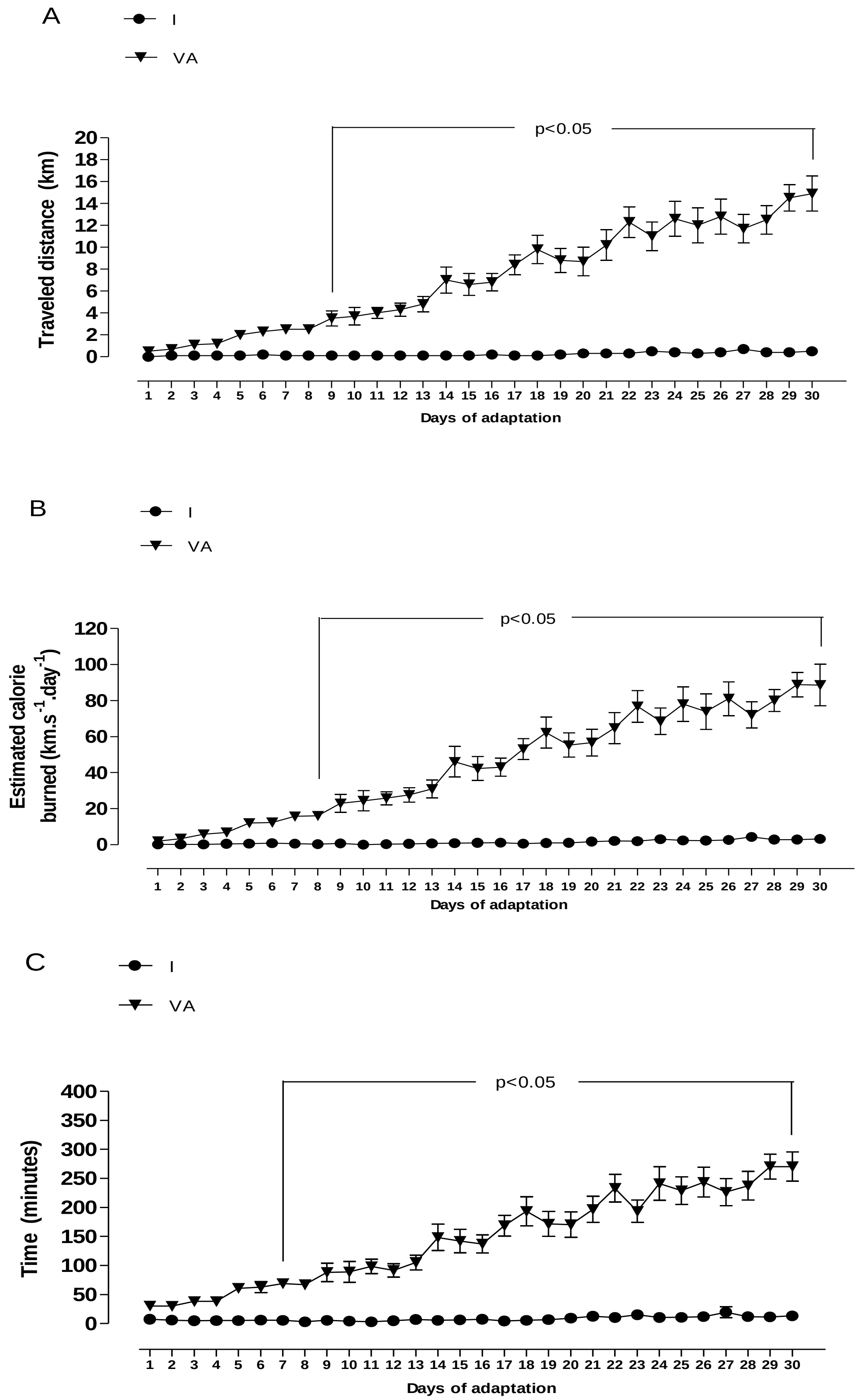
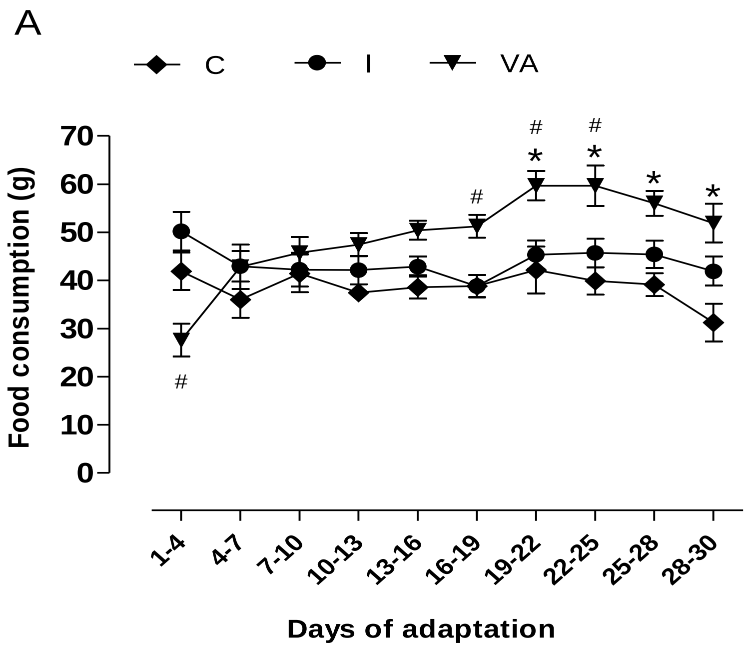


Figure 1. Parameters of maternal voluntary physical activity in the adaptation period. Groups constituted by: Inactive (I, n=15) and Very Active (VA, n=14). Daily traveled distance (A), estimated calorie burned (B) and time (C). Values are presented as mean ± S.E.M. *$p<0.05$ VA vs I, using two way ANOVA with Bonferroni post-hoc test.



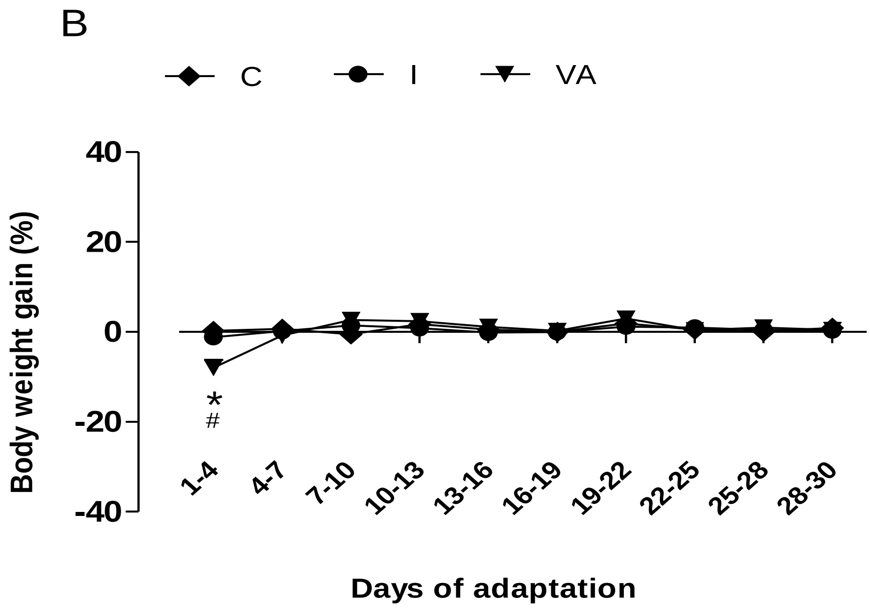


Figure 2. Food consumption in grams (A) and Body weight gain in percentage (B) of mothers in the adaptation period. Groups constituted by: Control (C, n=8), Inactive (I, n=15) and Very Active (VA, n=14). Values are presented as mean ± S.E.M. *$p<0.05$ VA vs C and $^{\#}p<0.05$ VA vs I, using two way ANOVA with Bonferroni post-hoc test.

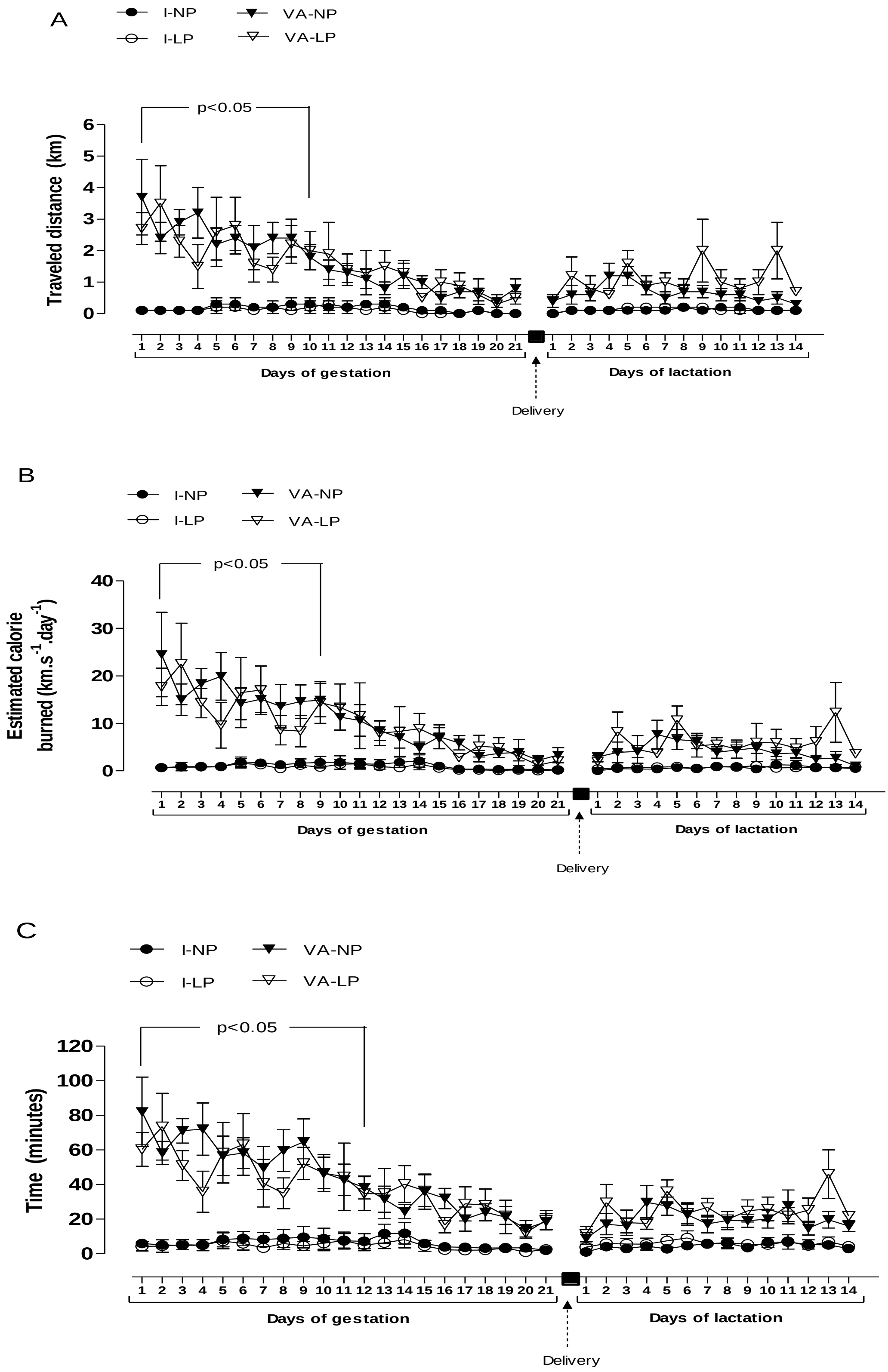


Figure 3. Parameters of maternal voluntary physical activity in the gestation and lactation periods. Groups constituted by: Inactive Normo Protein (I–NP, n=8), Inactive Low Protein (I-LP, n=7), Very Active Normo Protein (VA-NP, n=8) and Very Active Low Protein (VA-LP, n=6). Daily traveled distance (A), estimated calorie burned (B) and time (C). Values are presented as mean ± S.E.M. *$p<0.05$ VA-NP vs I-NP and VA-LP vs I-LP, using two way ANOVA with Bonferroni post-hoc test.

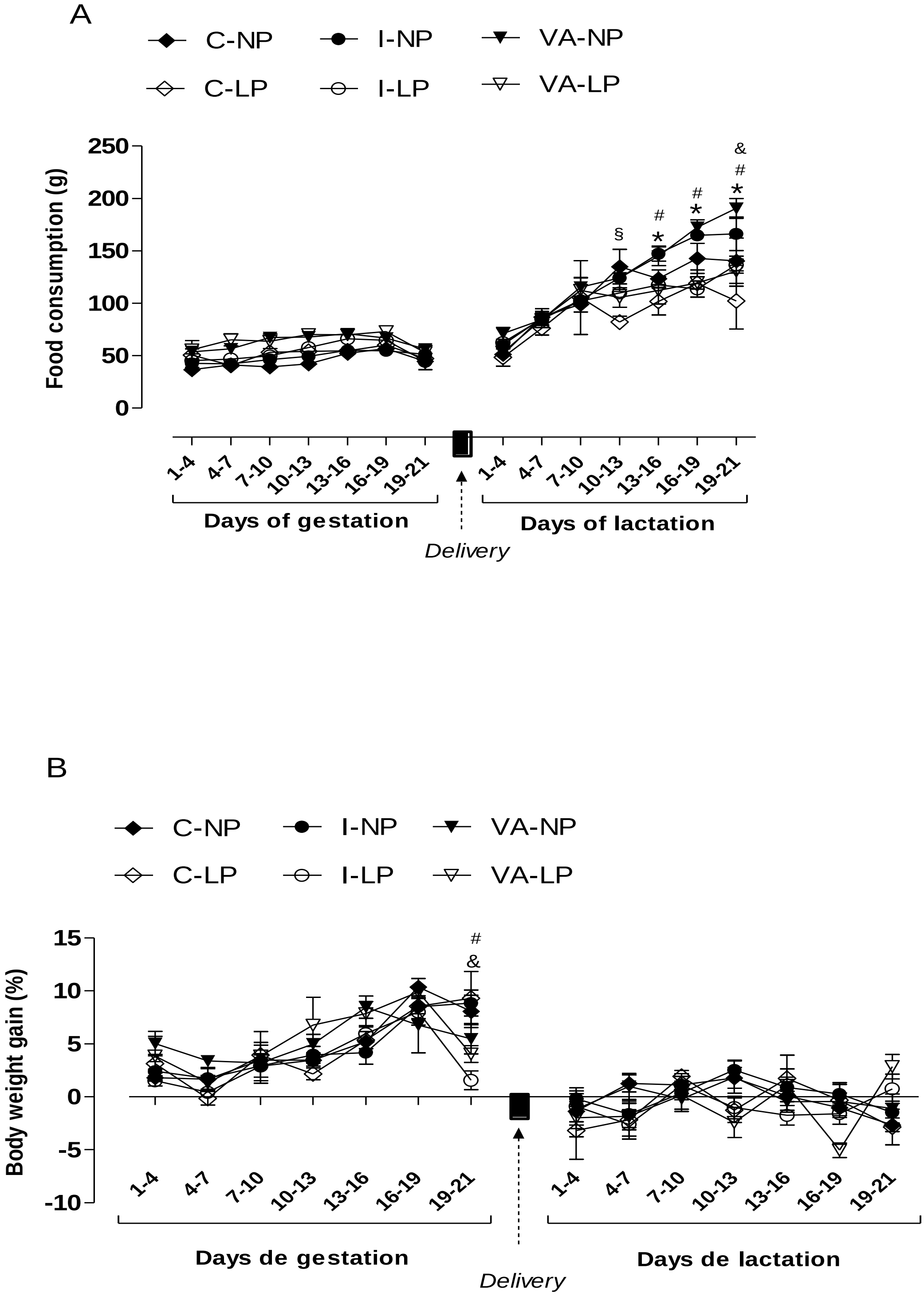


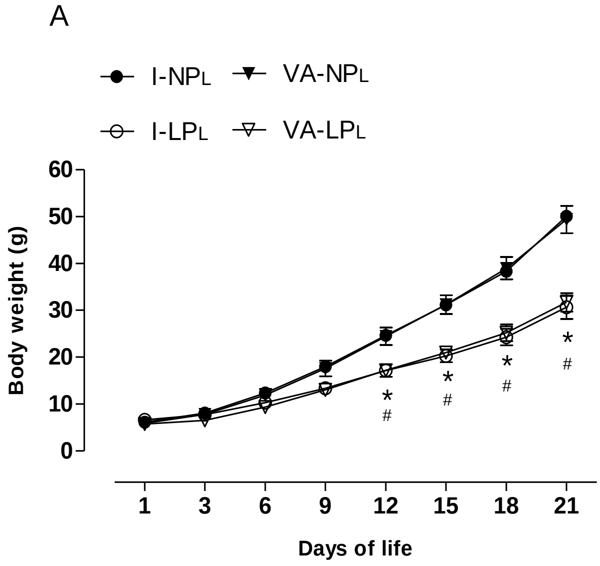
Figure 4. Food consumption in grams (A) and Body weight gain in percentage (B) of mothers in the gestation and lactation periods. Groups constituted by: Control Normo Protein (C-NP, n=4), Control Low Protein (C-LP, n=4), Inactive Normo Protein (I–NP, n=8), Inactive Low Protein (I-LP, n=7), Very Active Normo Protein (VA-NP, n=8) and Very Active Low Protein (VA-LP, n=6). Values are presented as mean ± S.E.M. *$p<0.05$ VA-LP vs VA-NP, #$p<0.05$ I-LP vs I-NP, §$p<0.05$ C-LP vs C-NP and &$p<0.05$ I-LP vs C-LP, using two way ANOVA with Bonferroni post-hoc test.

Table 2. Data of the pups at birth. Values expressed as Mean ± E.P.M

Groups	**Number of pups at delivery**		**Weight of litter**		**Average weight of pups at birth**	
Control NP	9.2	0.7	59.7	6.5	6.4	0.6
Control LP	7.7	1.2	49.9	6.7	6.5	0.4
Inactive NP	11.7	0.4	66.8	2.6	5.6	0.1
Inactive LP	10.1	0.9	61.8	2.4	6.3	0.5
Very Active NP	14.0 [a,b]	0.5	80.9 [a,b]	5.5	5.7	0.3
Very Active LP	11.6 [c]	0.9	62.3 [d]	4.5	5.3	0.2

Control NP (n=4); Control LP (n=4); Inactive NP (n=8); Inactive LP (n=7); Very Active NP (n=8) and Very Active LP (n=6), [a] $p<0.05$ vs Control NP, [b] $p<0.05$ vs Inactive NP, [c] $p<0.05$ vs Control LP and [d] $p<0.05$ vs Very Active NP, using two way ANOVA with Bonferroni post-hoc test.

NP: Normo Protein; LP: Low Protein



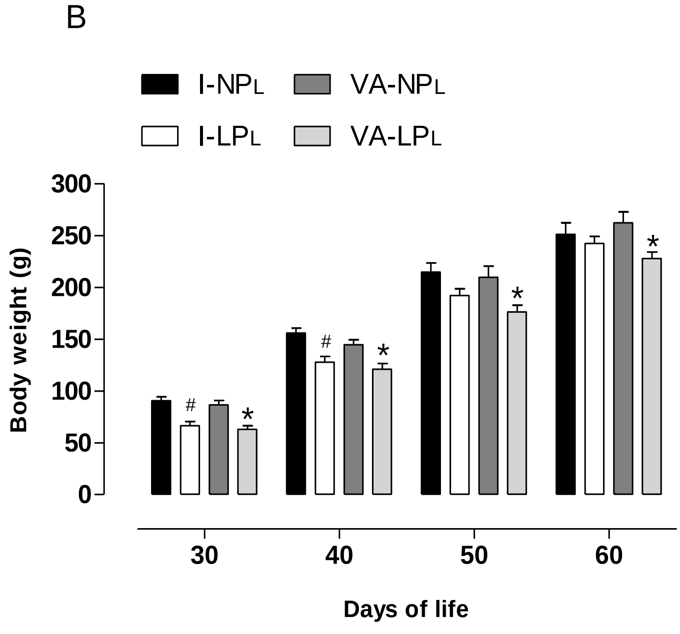


Figure 5. Body weight in grams in the lactation period (A) and Body weight in grams in the post weaning period (B) of the offspring. Groups constituted by: Inactive Normo Protein (I–NP_L, n=8), Inactive Low Protein (I-LP_L, n=7), Very Active Normo Protein (VA-NP_L, n=8) and Very Active Low Protein (VA-LP_L, n=6). Values are presented as mean ± S.E.M. *$p<0.05$ VA-LP_L vs VA-NP_L and $^{\#}p<0.05$ I-LP_L vs I-NP_L, using two way ANOVA with Tukey post-hoc test.

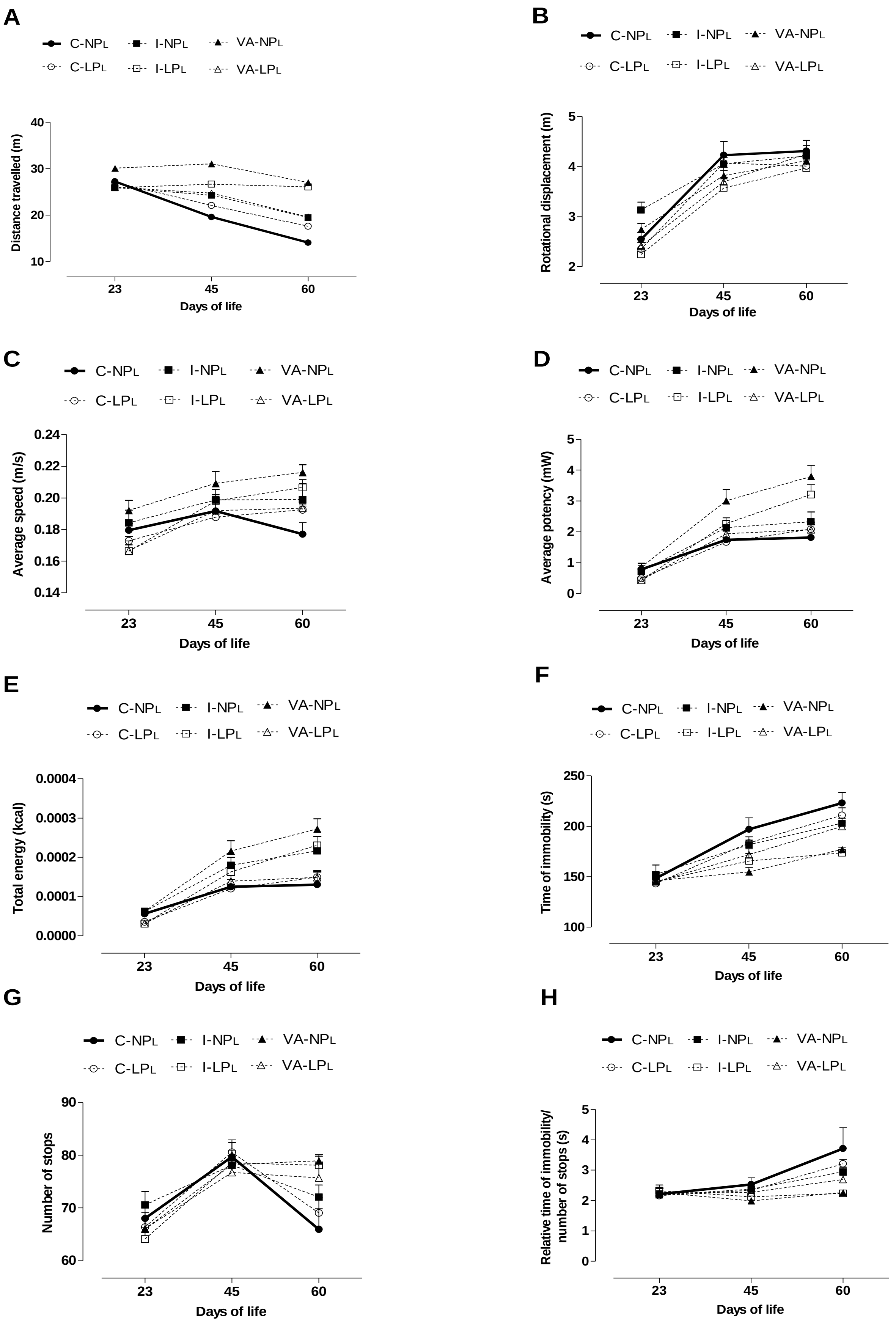

A
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Distance travelled (m)
40
30
20
10
23 45 60
Days of life
B
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Rotational displacement (m)
5
4
3
2
23 45 60
Days of life
C
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Average speed (m/s)
0.24
0.22
0.20
0.18
0.16
0.14
23 45 60
Days of life
D
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Average potency (mW)
5
4
3
2
1
0
23 45 60
Days of life
E
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Total energy (kcal)
0.0004
0.0003
0.0002
0.0001
0.0000
23 45 60
Days of life
F
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Time of immobility (s)
250
200
150
100
23 45 60
Days of life
G
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Number of stops
90
80
70
60
23 45 60
Days of life
H
C-NPL I-NPL VA-NPL
C-LPL I-LPL VA-LPL
Relative time of immobility/ number of stops (s)
5
4
3
2
1
0
23 45 60
Days of life

Figure 6. Trajectory of parameters of locomotor activity of offspring at 23, 45 and 60 days of life. Groups constituted by: Control Normo Protein (C-NP_L, n=4), Control Low Protein (C-LP_L, n=4), Inactive Normo Protein (I–NP_L, n=8), Inactive Low Protein (I-LP_L, n=7), Very Active Normo Protein (VA-NP_L, n=8) and Very Active Low Protein (VA-LP_L, n=6). A, Distance travelled (m); B, Rotational displacement (m); C, Average speed (m/s); D, Average pontency (mW); E, Total energy (kcal); F, Time of immobility (s); G, Number of stops; H, relative time of immobility/number of stops (s). Values are presented as mean ± S.E.M, using two way ANOVA with Tukey's post-hoc test.

Table 3. Data of Parameters of locomotor activity of offspring at 23, 45 and 60 days of life. Values expressed as Mean ± E.P.M

	Distance travelled (m)		**Rotational displacement (m)**		**Average speed (m/s)**		**Average potency (mW)**		**Total energy (kcal x 10^{-6})**		**Time of immobility (s)**		**Number of stops**		**Relative time of immobility/ number of stops (s)**	
23 days of life																
Control NP	27.2	1.5	2.5	0.1	0.17	0.004	0.78	0.12	5.58	0.93	148.4	6.0	68.0	3.2	2.1	0.25
Control LP	27.0	1.3	2.3	0.1	0.17	0.002	0.49	0.04	3.53	0.33	143.1	8.6	66.3	1.5	2.1	0.14
Inactive NP	25.9	2.1	3.1	0.1	0.18	0.008	0.71	0.09	6.12	0.91	152.2	9.2	70.6	2.5	2.1	0.13
Inactive LP	25.8	1.8	**2.2 [b]**	0.1	0.16	0.002	0.42	0.03	3.06	0.26	145.1	8.9	64.1	2.1	2.3	0.19
Very Active NP	30.1	2.5	2.7	0.1	0.19	0.006	0.85	0.12	6.16	0.88	145.9	8.0	66.0	3.1	2.2	0.17
Very Active LP	25.9	1.8	2.4	0.1	**0.16 [c]**	0.006	0.42	0.05	3.08	0.39	145.0	8.0	63.9	0.8	2.2	0.15
45 days of life																
Control NP	19.5	2.0	4.2	0.3	0.19	0.005	1.74	0.25	12.45	1.83	197.1	11.2	79.7	2.7	2.3	0.24
Control LP	22.0	1.5	4.0	0.1	0.18	0.003	1.66	0.12	11.98	0.87	183.0	6.0	80.5	2.4	2.3	0.13
Inactive NP	24.2	1.6	4.0	0.2	0.19	0.006	2.13	0.23	17.92	2.06	181.6	4.5	78.1	1.3	2.3	0.08
Inactive LP	26.6	1.7	3.5	0.2	0.19	0.003	2.16	0.19	16.23	1.39	165.5	7.3	70.5	1.3	2.1	0.08
Very Active NP	**31.0 [a]**	1.9	3.8	0.1	0.20	0.007	3.00	0.37	21.53	2.67	**154.6 [a]**	4.8	78.2	2.7	1.9	0.06
Very Active LP	24.7	1.7	3.6	0.2	0.19	0.005	**1.93 [c]**	0.19	13.88	1.36	171.8	6.0	76.7	2.2	2.2	0.09
60 days of life																
Control NP	14.0	2.2	4.3	0.2	0.17	0.007	1.81	0.41	13.03	2.98	223.2	10.2	65.9	6.0	3.0	0.02
Control LP	17.6	1.7	4.0	0.2	0.19	0.002	2.08	0.23	14.93	1.70	210.8	7.4	69.0	0.8	3.2	0.15
Inactive NP	19.4	1.8	4.2	0.07	0.19	0.010	2.32	0.32	21.62	3.72	202.9	5.0	72.0	2.3	2.9	0.15
Inactive LP	**26.0 [b]**	1.3	3.9	0.2	0.20	0.004	3.21	0.31	23.00	2.27	**173.6 [b]**	5.7	78.0	1.8	**2.2 [b]**	0.08
Very Active NP	**27.0 [a]**	0.9	4.1	0.1	**0.21 [a]**	0.004	**3.79 [a]**	0.36	27.16	2.62	**177.0 [a]**	2.0	78.9	1.2	**2.2 [a]**	0.05
Very Active LP	**20.5 [c]**	0.5	4.2	0.2	0.19	0.002	**2.06 [c,d]**	0.21	**14.83 [c,d]**	1.57	**199.9 [d]**	4.3	75.7	2.6	2.6	0.14

Control NP (n=4); Control LP (n=4); Inactive NP (n=8); Inactive LP (n=7); Very Active NP (n=8) and Very Active LP (n=6), [a] $p<0.05$ Very Active NP vs Inactive NP, [b] $p<0.05$Inactive LP vs Inactive NP, [c] $p<0.05$ Very Active LP vs Very Active NP and [d] $p<0.05$ Very Active LP vs Inactive LP, using two way ANOVA with Tukey's post-hoc test.

NP: Normo Protein; LP: Low Protein

Discussion

Maternal nutrition plays a key role in establishing the behavioral phenotype of offspring (Reyes-Castro, Rodriguez et al. 2012). In this study, was observed in a deficit in body weight in offspring of mothers who received inadequate supply of protein, from the 12th day of lactation until 60 days of life, corroborating previous studies (Barros, Manhaes-De-Castro et al. 2006; Falcão-Tebas;, Tobias; et al. 2012). It has been seen that maternal low protein diet altered the trajectory of all parameters of locomotor activity of the offspring. In a previous study, pups of mothers of who received low-calorie diet during late pregnancy and lactation all, no change in the trajectory of locomotor activity of the 8th until the 60th day of life (de Santana Muniz, da Silva et al. 2013). Therefore, the protein appears to be the most critical nutrient that can interfere in the locomotor development.

Regarding the differences between the groups, pups of mothers who received low protein diet showed an increase in distance traveled and consequently shorter immobility time at 60 days of life. Our results do not support with the previous study did not observe difference in distance traveled in pups, at 90 days of life, mothers who received protein restriction (10% casein) during pregnancy and lactation (Reyes-Castro, Rodriguez et al. 2012). The difference in the model used to low protein diet and age may explain the differences in the results. Similarly, pups from very active mothers also showed increased distance traveled and lower immobility time. Greater distance may represent further exploration of the environment by increasing the supply motor (Barros, Manhaes-De-Castro et al. 2006). Furthermore, studies have shown that greater distance in the peripheral area of the open field can represent anxious behavior and stress due to increased corticosterone (Prut and Belzung 2003; Reyes-Castro, Rodriguez et al. 2012; Reyes-Castro, Rodriguez et al. 2012). When physical activity was combined with low protein diet, pups showed a decrease in distance traveled in order to match the offspring of nourished inactive mothers. According to Wells (2014), the impact of a determined stimulus on the developing fetus can vary according to the capacity of the maternal organism to respond to such aggression. That is, mothers who performed physical activity may respond differently to protein restriction in relation to mothers who did not performed physical activity (Wells 2014).

A previous study shows that pups of mothers who have gone through protein restriction at the end of pregnancy and during lactation showed an increase of rotational movements during lactation (Smart and Dobbing 1971). The increase in these movements can damage the organization of proper movement (Barros, Manhaes-De-Castro et al. 2006). Our data showed that the low protein diet and maternal physical activity were not able to change some parameters of locomotor activity of the pups as the rotational displacement, average speed, total energy and the number of stops. However, reflected in the decrease in relative immobility time/number of stop. Thus, most evident damage in some parameters of locomotor activity may occur during lactation, it is during this period that the pups form the pattern of locomotion similar to adult (Westerga and Gramsbergen 1990; Gramsbergen 1998).

In addition, pups from very active mothers and who received low protein diet had lower total energy and average power in relation to pups from very active mothers and pups who received low protein diet mothers. This finding demonstrates that some environmental factors in isolation cannot have influence on the given parameter evaluated. But, when allies can pass on adaptations that modify certain behavior, corroborating previous study (Falcao-Tebas, Bento-Santos et al. 2012). In previous study it was seen that the maternal physical training was not able to change the reflex ontogeny in offspring during lactation, but when physical activity was combined with low protein diet was seen changes in some reflexes such as vibrissa placing and negative geotaxis (Falcao-Tebas, Bento-Santos et al. 2012).

In conclusion, in this study we demonstrated that maternal environmental stimuli, such as low protein diet and physical activity, were able to modulate some parameters of locomotor activity of the offspring in the post weaning period, especially at 60 days of life. We have seen that the practice of maternal physical activity was able to attenuate the effect of low protein diet on distance traveled. This investment strategy is due to the adaptive capacity of the organism is modular in response to the tax environment.

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7 CONSIDERAÇÕES FINAIS

No presente estudo, demonstramos que o modelo utilizado de atividade física voluntária é reprodutível, podendo ajudar na elaboração e comparação de futuros estudos. A prática de atividade física foi capaz de alterar alguns parâmetros da atividade locomotora da prole e ainda atenuou efeitos advindos da dieta hipoproteica. Dessa forma, fornecemos resultados importantes acerca da adaptação do organismo frente ao ambiente e suas repercussões sobre a atividade locomotora de filhotes no período pós-desmame. Assim, este trabalho abre um cenário para o melhor entendimento dos mecanismos relacionados com alterações encontradas tanto nas mães como nos filhotes.

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ANEXO

ANEXO A – Parecer do Comitê de Ética em Pesquisa

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Recife, 28 de novembro de 2013.

Ofício nº 655/13

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Profª. Carol Virginia Góis Leandro**
Universidade Federal de Pernambuco
Centro Acadêmico de Vitória
Processo nº 23076.047664/2013-87

Os membros da Comissão de Ética no Uso de Animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEUA-UFPE) avaliaram seu projeto de pesquisa intitulado, **"Efeitos da atividade física voluntária e da desnutrição perinatal materna sobre a atividade locomotora em filhotes de ratos".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEUA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 11.794 de 08 de outubro de 2008, que trata da questão do uso de animais para fins científicos e didáticos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais a serem realizados.

Origem dos animais: Biotério de criação do Departamento de Nutrição; Animal: Rato heterogênico; Linhagem: wistar; Idade: 90 dias; Peso: 220-260g; Sexo: machos e fêmeas; Nº Total de Animais: 120.

Atenciosamente

Profª Marcia Vasconcelos
Vice-Presidente da CEUA/CCB-UFPE
SIAPE 2176635

CCB: Integrar para desenvolver