UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS MÉDICAS

KARINA TAMY KASAWARA

EXERCÍCIOS FÍSICOS NA GESTAÇÃO DE ALTO RISCO – ESTUDOS TRANSLACIONAIS E MODELO EXPERIMENTAL

EXERCISES IN HIGH RISK PREGNANCY – TRANSLATIONAL STUDIES AND EXPERIMENTAL MODEL

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Tese apresentada ao Programa de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciências da Saúde na área de concentração Saúde Materna e Perinatal.

Thesis presented to the Graduate Program in Obstetrics and Gynecology, School of Medical Science from University of Campinas as part of the requirements for obtaining the title of Doctor in Health Science in the concentration area of Maternal and Perinatal Health.

ORIENTADOR: PROF. DR. JOÃO LUIZ DE CARVALHO PINTO E SILVA
COORIENTADORA: PROFA. DRA. FERNANDA GARANHANI DE CASTRO SURITA

ESTE EXEMPLAR CORRESPONDE À VERSÃO
FINAL DA TESE DEFENDIDA PELA
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PROF. DR. JOÃO LUIZ DE CARVALHO PINTO E SILVA.

CAMPINAS

2016

Agência(s) de fomento e nº(s) de processo(s): Não se aplica.

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Maristella Soares dos Santos - CRB 8/8402

K15e

Kasawara, Karina Tamy, 1986-
Exercícios físicos na gestação de alto risco - estudos translacionais e modelo experimental / Karina Tamy Kasawara. – Campinas, SP : [s.n.], 2016.

Orientador: João Luiz de Carvalho Pinto e Silva.
Coorientador: Fernanda Garanhani de Castro Surita.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Gravidez. 2. Exercício. I. Silva, João Luiz de Carvalho Pinto e,1944-. II. Surita, Fernanda Garanhani de Castro. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Exercises in high risk pregnancy - translational studies and experimental model
Palavras-chave em inglês:
Pregnancy
Exercise
Área de concentração: Saúde Materna e Perinatal
Titulação: Doutora em Ciências da Saúde
Banca examinadora:
João Luiz de Carvalho Pinto e Silva
José Carlos Peraçoli
Mary Angela Parpinelli
Egle Cristina Couto de Carvalho
Nelson Lourenço Maia Filho
Data de defesa: 19-02-2016
Programa de Pós-Graduação: Tocoginecologia

BANCA EXAMINADORA DA DEFESA DE DOUTORADO

KARINA TAMY KASAWARA

ORIENTADOR: PROF. DR. JOÃO LUIZ PINTO E SILVA

COORIENTADOR: PROFª. DRª. FERNANDA GARANHANI DE CASTRO SURITA

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Data: 19/02/2016

DEDICATÓRIA

Dedico este trabalho aos meus pais, Jum e Neusa Kasawara, em especial à minha mãe, que durante a trajetória do desenvolvimento desta tese, teve a oportunidade de ter uma vida nova. Nada seria se não fosse pelo amor incondicional que temos uns pelos outros.

Cláudia, à minha eterna gratidão.

AGRADECIMENTOS

Agradeço a Deus por todo amor concedido, por todas as oportunidades que tive em minha vida. Sou grata a todas as pessoas que estiveram em meu caminho durante esses anos e que contribuíram de maneira direta ou indireta para o desenvolvimento deste trabalho.

Aos meus pais Jum e Neusa Kasawara por todo apoio e incentivo durante essa jornada. Vocês foram essenciais para o desenvolvimento desta tese de Doutorado.

Ao Professor Doutor João Luiz Pinto e Silva por ter acreditado em mim, por ter aceitado ser meu mentor, por guiar meus passos científicos. Meu maior exemplo profissional, meu professor, me deu a honra de ser sua aluna durante minha qualificação profissional do mestrado e doutorado. Minha imensa admiração.

À Professora Doutora Fernanda Surita pela coorientação, por sua colaboração imensurável neste trabalho, contribuiu com sua visão científica e experiência.

Ao Professor Doutor Charles Graham por ter me recebido em seu laboratório, fazendo-me parte integrante de um grupo de excelência em pesquisa. Agradeço por receber-me como orientanda. Este agradecimento é estendido a todos os integrantes do laboratório, que me proporcionaram momentos agradáveis.

Ao Professor Doutor Chandrakant Tayade pela supervisão e apoio constante no desenvolvimento deste projeto de pesquisa.

À Professora Doutora Anne Croy por me receber no Canadá, por toda sua preocupação e cuidado durante a minha estada. Foi um prazer ter sua companhia em momentos especiais e eventos comemorativos, fez-me sentir acolhida.

Um agradecimento especial ao Professor Doutor Áureo Yamada, que abriu as portas do seu laboratório para treinar-me e preparar-me para o programa de

intercâmbio. Aprendi a superar meus medos e a enfrentar com sabedoria as dificuldades para alcançar meus objetivos. Serei eternamente grata por tudo o que aprendi no Instituto de Biologia da UNICAMP.

À Professora Doutora Maha Othman, pelo auxílio na análise da tromboelastografia. Sou grata por ter compartilhado comigo seu conhecimento e sua experiência na hematologia.

Ao Professor Terence Ozolinš, pela concessão de seus equipamentos para a realização deste estudo. Agradeço pelo tempo que passamos juntos discutindo pesquisa em saúde e o impacto do exercício físico em diferentes contextos.

À Tiziana Cotechini, por compartilhar seu conhecimento, pelo auxílio no desenvolvimento do projeto de pesquisa, por sua disposição e por estar presente em todos os momentos necessários. Seu auxílio foi de extrema importância para a conclusão deste estudo.

À Shannyn Macdonald-Goodfellow, Kevin Robb e Maggie Chasmar que me auxiliaram na coleta de dados, colaboraram nas discussões dos casos e me apoiaram em todos os momentos durante meu intercâmbio.

Ao Bruno Zavan, amigo-parceiro, sua disposição e seu auxílio foram essenciais para a coleta de dados deste estudo. Agradeço pela companhia, pela amizade e pelo cuidado durante esses anos de Canadá-Brasil.

À Amanda Carniato, pelo treinamento com os animais no Instituto de Biologia da Unicamp, obrigada pela paciência e cuidado ao me ensinar sobre pesquisa experimental.

Ao grupo de pesquisa em Saúde Reprodutiva e Hábitos Saudáveis (SARHAS) por compartilharem comigo os momentos de discussão sobre pesquisa científica, sempre muito proveitosos. O grupo está crescendo e agradeço a oportunidade de fazer parte de um núcleo de pesquisa tão comprometido.

Ao Programa de Pós-Graduação em Tocoginecologia, à Denise e Melissa, obrigada pelo auxílio durante todo o processo de intercâmbio, pelo cuidado durante todos esses anos e pelo auxílio na preparação para a defesa de tese.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa de estudos de Doutorado, pelo programa de intercâmbio e pelo acordo com a instituição Canadense, “Canadian Bureau for International Education”.

Ao Fundo de Apoio ao Ensino, à Pesquisa e Extensão (FAEPEX) pelo auxílio-viagem para a apresentação dos resultados deste estudo no 35th American Society for Reproductive Immunology, em Junho de 2015, em Kingston, Ontario, Canadá.

Ao Departamento de Tocoginecologia do Hospital da Mulher Prof. Dr.José Aristodemo Pinotti – CAISM, Margareth e Márcia, pela paciência e auxílio na logística de agendamento da defesa de tese.

Ao Pedro, meu parceiro, obrigada pelo apoio durante esses anos, sua força e seu cuidado me incentivaram a seguir em frente e a continuar buscando o melhor em mim. Agradeço pelo companheirismo presencial/virtual em todos os momentos que precisei, sem você minha permanência no Canadá não seria a mesma.

À minha família e meus amigos queridos, vocês me fazem sentir completa, vocês são essenciais em minha vida.

À tia-avó Toshiko Ito pela revisão ortográfica da tese. Obrigada por acompanhar minha formação ao longo desses anos.

A todos os animais que participaram desse estudo, que esses resultados possam guiar outros estudos translacionais e que suas vidas não tenham sido em vão para a pesquisa científica.

MUITO OBRIGADA, sem vocês nada disso seria possível.

RESUMO

Objetivo: avaliar os efeitos do exercício físico nas desordens hipertensivas da gestação através de revisão de literatura e do desenvolvimento de um modelo experimental. Métodos: revisão da literatura sobre os aspectos atuais do exercício físico nas pesquisas translacionais e os potencias modelos experimentais das síndromes hipertensivas na gestação. Além disso, foi desenvolvido um estudo experimental com modelo animal de ratos da raça Wistar, fêmeas, com três a quatro meses de idade. Os animais foram submetidos a exercício físico de intensidade moderada (65% da capacidade aeróbica máxima), realizado em esteira ergométrica, antes e durante a gestação. Os grupos experimentais foram constituídos por ratos que realizaram exercícios e receberam lipopolissacarídeo (LPS) durante a gestação (Ex+LPS), ratos que realizaram exercícios e receberam solução salina durante a gestação (Ex+Salina), ratos sedentários que não realizaram exercício e receberam LPS durante a gestação (Se+LPS) e ratos sedentários que não realizaram exercícios e receberam solução salina durante a gestação (Se+Salina). Para a indução da inflamação foram realizadas quatro injeções intraperitoneais de LPS nos dias de gestação 13,5 (10 µg/kg), 14,5 (40 µg/kg), 15,5 (40 µg/kg) e 16,5 (40 µg/kg). No dia de gestação 17,5 os ratos foram submetidos à eutanásia para análise do hemograma completo, peso dos fetos e tromboelastografia para análise de coagulação sanguínea. Considerou-se significativo p-valor<0,05. Resultados: conforme a revisão da literatura, existem diversos modelos animais para simular os efeitos deletérios das síndromes hipertensivas na gestação, dentre eles: animais knockout (transgênicos ou geneticamente modificados), cirurgias que induzem a redução do fluxo sanguíneo

útero-placentário, dieta enriquecida de sódio e modelos de inflamação que promovem alterações imunológicas. Neste sentido, os estudos sobre o efeito do exercício físico voluntário em modelos animais com complicações similares às síndromes hipertensivas apresentam efeito promissor no controle da pressão arterial, restrição de crescimento fetal e estresse oxidativo. Já os estudos clínicos translacionais apresentam evidência limitada com relação aos benefícios do exercício físico nas síndromes hipertensivas da gestação. No estudo experimental, a contagem de células brancas (monócitos, linfócitos e granulócitos) apresentou-se reduzida quando comparadas Ex+LPS versus Se+LPS. O grupo Ex+LPS não apresentou diferença significativa no aumento do peso fetal comparado ao grupo Se+LPS. Os parâmetros da tromboelastografia mostraram-se normalizados nos ratos que realizaram exercício Ex+LPS versus Se+Salina. Conclusões: os modelos animais são de extrema importância e utilidade para compreensão do impacto do exercício físico na gestação de alto risco, em especial nas desordens hipertensivas. Neste sentido, os estudos translacionais podem propor condutas utilizadas na prática clínica para adequada orientação da recomendação do exercício físico nas síndromes hipertensivas da gestação. No modelo experimental, o exercício preveniu o aumento na contagem de células brancas e nas coagulopatias relacionadas ao efeito do LPS na gestação. O exercício físico antes e durante a gestação pode promover benefícios a longo prazo como nos casos de coagulopatias induzidas pela inflamação relacionadas a desfechos maternos e fetais desfavoráveis.

Palavras-chave: exercício, gravidez de alto risco, inflamação, pré-eclâmpsia pesquisa médica translacional.

ABSTRACT

Objective: to evaluate the effects of exercise in hypertensive disorders of pregnancy through literature review and development of an experimental model. Methods: a literature review of the current aspects of physical exercise in translational research and the potential experimental models of hypertensive disorders in pregnancy. Furthermore, it was developed an experimental study on female Wistar rats, from three to four months old. The animals were submitted to physical exercise of moderate intensity (65% of maximal aerobic capacity), performed on a treadmill before and during pregnancy. The experimental groups consisted of rats who exercise and received lipopolysaccharide (LPS) during pregnancy (Ex+LPS), rats who underwent exercise and received saline during pregnancy (Ex+Saline), sedentary rats that performed no exercise and received LPS during pregnancy (Se+LPS) and sedentary rats that did not undergo any exercise and received saline during pregnancy (Se+Saline). For inflammation induction four intraperitoneal injections of LPS were performed on day 13.5 of gestation (10 µg/kg), 14.5 (40 µg/kg) 15.5 (40 µg/kg) and 16.5 (40 µg/kg). On the day 17.5 of gestation the rats were sacrificed for analysis of complete blood count, fetal weights and thromboelastography for blood coagulation analysis. It was considered significant p-value <0.05. Results: according to the literature review, there are several animal models for simulating the deleterious effects of hypertensive disorders in pregnancy, such as: knockout animals (transgenic or genetically modified), surgeries that induce a reduction in the utero-placental blood flow, enriched diet sodium and models of inflammation promoting immunological changes. In this sense, studies on the effect of voluntary exercise in animal models

with similar complications to hypertensive disorders have promising effect in controlling blood pressure, fetal growth restriction and oxidative stress. On the other hand, clinical translational studies provide limited evidence regarding the benefits of exercise in hypertensive disorders of pregnancy. In an experimental study, white blood cell count (monocytes, lymphocytes and granulocytes) was reduced when compared Ex+ LPS versus Se+LPS. The Ex+LPS group showed no significant difference in the increase of fetal weight compared to the group Se+LPS. The thromboelastography parameters were normalized in the mice that performed exercise Ex+LPS versus Se+Saline. Conclusions: animal models are of utmost importance and usefulness for understanding the impact of exercise on high-risk pregnancy, especially in hypertensive disorders. In this regard, translational studies can guide conducts used in clinical practice for proper orientation of the recommendation of exercise in hypertensive disorders of pregnancy. In the experimental model, the exercise prevented the increase in white blood cell count and coagulation disorders related to the effect of LPS in pregnancy. Physical exercise before and during pregnancy can promote the long-term benefits such as in cases of coagulopathy induced inflammation related to adverse maternal and fetal outcomes.

Key words: exercise, high-risk pregnancy, inflammation, pre-eclampsia, translational medical research.

LISTA DE ABREVIATURAS E SIGLAS

% – Porcentagem

µg/kg – Micrograma(s) por kilograma(s)

µl – Microlitros

A + – Angiotensinogen

ACE2 – Angiotensin Converting Enzyme

ACOG – American College of Obstetrics and Gynecology

Ad – Adenovirus

AEM – Avaliação de exercício máximo

AT1R – Antagonist Angiotensin II receptors

AU – Umbilical Arteries

BP – Blood Pressure

CH – Chronic Hypertension

CI – Confidant Interval

CRP – C-reactive protein

DP – Desvio Padrão

FGR – Fetal growth restriction

GD – Gestational Day

HAC – Hipertensão arterial crônica

hAng – Transgenic mice with Angiotensinogen expression

HG – Hipertensão gestacional

HIF – Hypoxia-Inducible Factor

HLA-G – Human Leukocyte Antigen

HR – Heart Rate

hRN – Transgenic mice with Renin expression

IC – Intervalo de confiança

IL-10 – Interleucina dez

IL-6 – Interleucina seis

kg/m² - Kilograma(s) por metro(s) quadrado(s)

LPS – Lipopolissacarídeo

MasR – Mas receptor

MCA – Middle Cerebral Artery

MeSH – Medical Subject Headings

mmHg – Milímetros de Mercúrio

p – Nível de significância estatística

PE – Pré-eclâmpsia

PGF – Placental Growth Factor

PI – Pulsatility Index

R + – Renin

RCF – Restrição de crescimento fetal

RI – Resistance Index

RN – Recém-nascido

RUPP – Reduction of Uteroplacental perfusion

sFlt-1 – Soluble fms-like tyrosine kinase-1

SOD – Superoxide Dismutase

TEG – Thromboelastography

TNF-α – Fator de Necrose Tumoral Alpha/ Tumor Necrosis Factor Alpha

UNICAMP – Universidade Estadual de Campinas

VEGF – Vascular Endothelial Growth Factor

WHO – World Health Organization

SUMÁRIO

INTRODUÇÃO

As gestações de alto risco configuram uma ameaça à saúde do binômio mãe-feto, descritas ordinariamente como um fator potencialmente associado a desfechos gestacionais e/ou perinatais desfavoráveis. Foram descritas por Caldeyro-Barcia et al. (1973) (1) como “aquelas na qual a vida ou a saúde da mãe e/ou do feto e/ou do recém-nascido têm maiores chances de serem atingidas que as da média da população considerada”. Nesse sentido, deve-se compreender as múltiplas particularidades dos casos especiais de gestações de alto risco, para seu adequado seguimento durante o período gravídico-puerperal e minimizar os seus efeitos perigosos e adversos para a integridade da gravidez (2).

As complicações obstétricas associadas aos altos níveis de gravidade gestacional podem resultar em maiores taxas de morbidade e mortalidade maternas. A taxa de mortalidade materna foi estimada com 376.034 mortes no ano de 1990, sendo que houve uma redução dessa taxa comparados aos 292.982 casos estimados no ano 2013 em todo o mundo, cifras que continuam sendo muito grandes comparativamente às encontradas no mundo desenvolvido (3,4).

Neste ano de 2015, as Nações Unidas estão promovendo um plano de metas e estratégias, conhecido como “Millennium Development Goals”, com o objetivo de incentivar a sustentabilidade para o desenvolvimento por meio da erradicação da pobreza, melhorando a economia, protegendo a saúde e o ambiente, com promoção da paz em todas as comunidades e países do mundo.

Neste sentido, a saúde da mulher está presente no objetivo cinco que visa a igualdade de gênero por meio do fortalecimento das meninas e mulheres (5).

Nos últimos anos, a saúde da mulher tem sido foco de atenção em diversos países, com a adoção de medidas especiais de atenção a fim de reduzir a mortalidade e comorbidades relacionadas à gestação (3,6,7).

Analisando as diversas patologias que comprometem significativamente a saúde materna, as síndromes hemorrágicas encontram-se como primeira causa de morte materna em muitos países, sendo que na América Latina e no Caribe são responsáveis por aproximadamente 23,1% das mortes maternas, seguidas das síndromes hipertensivas (22,1%) (3,8). As síndromes hipertensivas configuram além da elevada mortalidade, a principal causa de morbidade materna grave, com repercussões a curto e a longo prazo na vida das mulheres e de seus filhos (9).

Fazem parte destas síndromes um amplo espectro de expressões de hipertensão em etiologia e grau de severidade como a hipertensão arterial crônica (HAC), a hipertensão gestacional (HG), a pré-eclâmpsia (PE) em suas múltiplas formas (PE e HAC sobrepostas, eclâmpsia, síndrome HELLP) situações patológicas com risco de morte materno e fetal aumentados, principalmente quando associadas à prematuridade (10,11).

A supervisão regular antenatal das gestantes é a maneira adequada de identificar aquelas que estão, ou poderão ser afetadas por estas complicações,

estimar os riscos envolvidos e propor agendas apropriadas de seguimento e tratamento (2).

A fisiopatologia da PE ainda permanece desconhecida. Sabe-se ser uma desordem caracterizada por uma placentação anormal com subsequente resposta materna de ordem inflamatória e vascular (12-14). O não reconhecimento destas alterações precocemente, determina um cuidado inadequado da gravidez e um aumento significativo de complicações e de mortes que poderiam ser evitadas ou minimizadas (15).

A maneira mais eficiente de otimizar a qualidade do manuseio pré-natal destas gestantes, seria desenvolver modelos de predição que pudessem identificar mulheres com alto risco de desenvolver a doença, e permitissem monitorar durante o período gestacional sua evolução e a tomada de atitudes profiláticas correspondentes (16).

Com o intuito de reproduzir em modelo animal as repercussões causadas nas gestações de alto riso, particularmente das síndromes hipertensivas, diversos estudos desenvolveram métodos de simulação das alterações observadas, particularmente aquelas associadas à PE e suas múltiplas variantes de evolução e gravidade (17-27). Um deles, propõe a inflamação induzida por lipopolissacarídeo (LPS), descrito como modelo experimental capaz de reproduzir manifestações clínicas da PE durante a gestação (26,27). O LPS é conhecido como uma endotoxina constituinte da membrana de bactéria gram negativa e é responsável por promover resposta imunológica inflamatória no hospedeiro (28).

O modelo de indução de inflamação com LPS administrado em baixas doses (10 – 40 µg/kg) durante a gestação de ratas da raça Wistar mostrou-se capaz de promover a restrição de crescimento fetal (RCF), distúrbios da coagulação materna, liberação de citocinas pró-inflamatórias, como o TNF-α, alterações encontradas nas gestações humanas acometidas de PE (26,27).

Dentre as possibilidades disponíveis de intervenção anti-inflamatória, a literatura sugere que os exercícios físicos encontram-se como aliados conhecidos para produzir este comportamento, por aumentar a imunidade do indivíduo, através da liberação de citocinas anti-inflamatórias (29,30). São sistematicamente recomendados para indivíduos afetados por doenças cardiovasculares como coadjuvantes de terapias medicamentosas ou cirúrgicas (31).

Os exercícios físicos durante o período gestacional configuram uma intervenção não invasiva, relacionado à mudança de estilo de vida da mulher, sendo recomendado por diversos colegiados (32,33) para serem utilizados nas gestações de baixo risco, para controle de peso (34), redução de desconfortos articulares e de coluna, com comprovada eficácia para o incremento da auto-estima e qualidade de vida (35,36).

No entanto, as recomendações da realização do exercício físico nas gestações de alto risco, particularmente naquelas associadas às síndromes hipertensivas, ainda apresentam evidência científica limitada sobre seu benefício e segurança, sendo sua prescrição discutida quanto ao tipo, frequência, duração e intensidade (32,37).

Recente revisão da Cochranne sugere que a dieta e os exercícios físicos associados durante a gestação poderiam reduzir o impacto da hipertensão arterial crônica (RR: 0,70, 95% CI 0,96-00:51), no entanto, sem influência na ocorrência de PE (RR: 0,95, 95% CI 0,77-1:16) (34).

Entretanto, ainda não existe suficiente clareza na literatura com relação à indicação e segurança do exercício físico em casos de alto risco gestacional , em especial nas síndromes hipertensivas e particulamente na PE (34,38). Os estudos translacionais são propostas contemporâneas de utilizar modelos experimentais em animais para simular e provocar o desenvolvimento de doenças que podem atingir a mulher grávida, com a possibilidade de reproduzir seus resultados que poderiam servir para a utilização clínica em segurança (39,40).

Diante do exposto, o modelo que se propõe neste estudo, busca compreender o real efeito dos exercícios físicos antes e durante a gestação relacionado aos desfechos desfavoráveis das gestações de alto risco, sobre a mãe e sobre o concepto a partir da observação dos resultados obtidos pela inflamação induzida por LPS em ratas prenhes.

OBJETIVOS

2.1. Objetivo geral

Conhecer os efeitos dos exercícios físicos nas gestações de alto risco por meio de revisão de literatura e do desenvolvimento de um modelo experimental.

2.2. Objetivo específico

Revisar na literatura alguns aspectos sobre o efeito dos exercícios físicos nas desordens hipertensivas associadas à gravidez, analisando perspectivas com base em estudos experimentais, estudos clínicos e as recomendações existentes para a sua realização na gestação de alto risco na prática clínica.

Avaliar os efeitos dos exercícios físicos antes e durante a gestação de alto risco, a partir de um estudo experimental com resposta anti-inflamatória induzida por lipopolissacarídeo (LPS) no índice de restrição de crescimento fetal, perfil de coagulação materna e resposta imunológica em ratos submetidos ao programa .

METODOLOGIA

3.1. Metodologia do artigo 1

3.1.1. Desenho do estudo

Foi realizada revisão da literatura sobre os aspectos atuais dos exercícios físicos nas pesquisas translacionais e os potencias modelos experimentais para estudo das síndromes hipertensivas na gestação.

3.1.2. Seleção dos estudos

Os estudos foram selecionados de acordo com a evidência científica nas ciências básicas e nos estudos clínicos utilizando os seguintes decritores Medical Subject Headings (MesH): "*Translational Medical Research", "Animal Models", "Motor Activity", "Exercise", "Animal Pregnancy", "High-Risk Pregnancy", "Pre-Eclampsia"* e *"Pregnancy-Induced Hypertension*".

As buscas foram realizadas nas bases de dados: MEDLINE (via PubMed), Lilacs, EMBASE, Scopus, SciELO and The Cochrane Library.

A seleção dos estudos foi realizada de acordo com os tópicos de interesse: 1) Recomendações do exercício físico na gestação de alto risco, 2) Modelos animais de hipertensão na gestação, 3) Exercício físico nos modelos animais relacionado às desordens hipertensivas, 4) Estudos translacionais do exercício físico na gestação de mulheres com desordens hipertensivas.

3.1.2.1. Critérios de inclusão

Foram incluídos estudos publicados em inglês, português e espanhol.

Não houve delimitação da data de publicação na sessão referente aos modelos animais para hipertensão na gestação, de modo a investigar todas as possibilidades de modelos que simulassem os sinais e sintomas clínicos desta patologia na gestação. Em todas as outras sessões foram consideradas publicações nos anos de 2000 a 2015.

Os estudos foram revisados por todos os autores antes da sua inclusão final.

3.1.2.2. Critérios de exclusão

Foram excluídos estudos que não contemplavam a temática proposta e que não se enquadravam nos tópicos de interesse delimitado para esta revisão da literatura.

3.2. Metodologia do artigo 2

3.2.1. Desenho do estudo

Foi realizado um estudo experimental com modelo animal de ratos da raça Wistar, submetidos a um protocolo de exercício físico antes e durante a gestação, com indução de inflamação por LPS (lipopolissacarídeo).

3.2.2. Variáveis

3.2.2.1. Variáveis independentes

3.2.2.1.1. Exercício físico

Exercício físico realizado em esteira especial para roedores (LE 8700 series, Panlab, Harvard Apparatus, Barcelona, Spain). A velocidade da esteira foi ajustada e personalizada para cada animal de acordo com o seu desempenho na avaliação de exercício máximo (AEM).

Protocolo de avaliação de exercício máximo

A AEM foi realizada individualmente para cada animal como publicado anteriormente por Jiao, et al. (2009). A inclinação da esteira foi posicionada em zero grau e a velocidade inicial ajustada em 30 cm/s, sendo que a cada 30 segundos foi reajustado o aumento de dois cm/s, até que o animal não conseguisse mais correr adequadamente, ou até que o rato repousasse na grade de choque (intensidade <1mA) mais de três vezes seguidas, caracterizando falha.

A velocidade final atingida pelo rato na esteira foi considerada como valor individual de AEM. A avaliação de AEM foi repetida três vezes para cada animal, com repouso de uma hora entre cada uma delas. A média de AEM foi calculada antes e depois do protocolo de exercício antes da gestação com o objetivo de avaliar a capacidade aeróbica antes de acasalamento e durante o período gestacional.

Protocolo de exercício pré-gestacional

O protocolo de exercício foi baseado na AEM de cada rato estabelecido antes de iniciar o programa, estabelecido por Amorim, et al. (2009) e modificado. Todos os ratos foram submetidos a quatro semanas de exercício pré-gestacional. As sessões aconteciam cinco vezes por semana, seguidos por dois dias de repouso entre as semanas. Cada sessão consistia em três estágios: (1) aquecimento; (2) treinamento e (3) desaquecimento. Durante o estágio (1) de aquecimento, os ratos corriam em 40% do AEM individual por cinco minutos. Durante o estágio (2) de treinamento os ratos iniciavam a sessão com 40% do AEM por 20 minutos por cada sessão durante uma semana, 50 minutos durante a segunda semana, 60 minutos durante a terceira semana e 60 minutos com 65% do AEM durante a quarta semana antes da gestação. Durante o estágio (3) de desaquecimento a velocidade da esteira foi reduzida gradualmente, dois a três cm/s a cada 30 segundos até a parada total da esteira.

Protocolo de exercício durante a gestação

Durante a gestação os ratos foram submetidos à intensidade moderada de exercício físico. Todos os ratos exercitavam-se a 65% do AEM por 50 minutos em cada sessão na primeira semana, 30 minutos por dia na segunda semana e 20 minutos por dia na terceira semana. Os animais exercitavam-se cinco vezes por semana com um dia de descanso entre cada semana, até o dia gestacional 17,5.

3.2.2.1.2. Indução da inflamação

O modelo utilizado para indução das repercussões associadas à inflamação, como a restrição de crescimento fetal, foi originado por doses diárias de LPS (Escherichia coli serotype 0111:B4; Sigma-Aldrich, Oakville, ON, Canada), seguindo o protocolo estabelecido por Cotechini et al. (2014) . Os ratos Wistar receberam diariamente baixa dose de LPS injetada intraperitonealmente conforme os dias gestacionais: 10µg/kg no dia gestacional 13,5, seguida por 40µg/kg nos dias de gestação 14,5, 15,5 e 16,5, sendo todos sacrificados no dia 17,5 .

3.2.2.1.3. Solução salina

O grupo controle recebeu solução salina (0.1 ml/100 g) nos mesmos dias gestacionais do grupo estudo (13,5, 14,5, 15,5 e 16,5), sendo que todos foram sacrificados igualmente, no dia 17,5 de gestação.

3.2.2.2. Variáveis dependentes

3.2.2.2.1. Contagem de células sanguíneas materna

A contagem de células sanguíneas materna foi realizada a partir do plasma sanguíneo coletado, via cardíaca, no dia de gestação 17,5, no momento da eutanásia. A seringa continha 0,1 ml de anticoagulante (ácido etilenodiamino tetra-acético, EDTA) e a amostra do sangue materno somado ao anticoagulante (1ml) foram centrifugados (2000g x 20 min) para a coleta do plasma sanguíneo.

A análise foi realizada para cada amostra (12 µl) por ABC Vet Animal Blood Counter (Scil Animal Care Company) nas instalações do biotério da Queen's University, Kingston, ON, Canada. A contagem de células sanguíneas materna foi realizada para os glóbulos vermelhos, glóbulos brancos, linfócitos, monócitos, granulócitos e plaquetas.

3.2.2.2.2. Peso fetal

O peso fetal foi avaliado no dia gestacional 17,5, medida em gramas, utilizando-se uma balança de precisão e calibrada previamente. O peso do feto foi normalizado pelo tamanho da ninhada, sendo que para cada feto foi realizado a proporção com relação aos outros fetos, para compreender a destruição e nutrição sanguínea materna em cada rato.

$$\text{Peso fetal normalizado} = \frac{\text{Peso fetal}}{\text{tamanho da ninhada}}$$

3.2.2.2.3. Tromboelastografia

Antes da eutanásia no dia gestacional 17,5, os ratos foram anestesiados com 40-50 mg/kg de Pentobarbital (CEVA Santé Animale, Rutherford, NJ, USA) intraperitoneal. O sangue materno foi coletado com a seringa preparada com 0,1ml de citrato de sódio, agulha de gauge 26', via cardíaca. O sangue materno somado ao citrato de sódio foi utilizado para análise da tromboelastografia (TEG), seguindo as recomendações do fabricante TEG® 5000 Haemostasis System e TEG® Haemostasis Analyzer software versão 4.2 (Haemoscope Corporation, Skokie, IL, USA).

Os dados foram coletados após 75 a 90 minutos e os parâmetros analisados foram: tempo de formação do coágulo (R), velocidade de propagação do coágulo (α), taxa de formação do coágulo (K), força e estabilidade do coágulo (MA), índice de coagulação (CI; esse valor é estabelecido a partir dos quatro componentes avaliados acima) e LY30 (porcentagem de dissolução do coaágulo em 30 minutos).

3.2.2.3. Variáveis de controle

3.2.2.3.1. Intensidade do exercício físico

A intensidade do exercício físico foi delimitada de acordo com a performance de cada rato, respeitando assim, a velocidade e individualidade de cada animal. Neste sentido, a AEM foi utilizada como estratégia para personalizar

a intensidade do exercício físico durante o protocolo, antes e durante a gestação, estimulando o exercício em intensidade moderada (65% da AEM).

3.2.3. Seleção da amostra

Foram estudados ratos da raça Wistar, fêmeas, com idade de três e quatro meses, fornecidos pelo laboratório Charles River (Montreal, QC, Canada), acomodados em uma sala com luz e umidade controladas nas facilidades da Queen's University (Kingston, ON, Canada).

3.2.3.1. Critérios de inclusão

Os ratos foram submetidos à aclimatização na esteira especial para roedores, durante 14 dias consecutivos, por no máximo 15 minutos, ou até três falhas consecutivas para lograrem a realização do exercício físico programado. Após cada sessão de aclimatização, os ratos receberam uma unidade de cereal à base de mel, recomendado pelo veterinário responsável pelo biotério. Os ratos que cumprissem os critérios estabelecidos no período de aclimatização foram considerados aptos para a inclusão no estudo experimental e iniciar o protocolo de exercício antes e durante a gestação.

3.2.3.2. Critérios de exclusão

Foram excluídos os ratos que não conseguiram realizar o exercício físico durante o período de aclimatização, ratos que apresentassem alguma limitação física em realizar o exercício físico e ratos que não engravidaram.

3.2.4. Coleta dos dados

Os desfechos maternos e fetais foram coletados no dia 17,5 de gestação. Todos animais foram anestesiados com 40–50 mg/kg Pentobarbital sódico (CEVA Santé Animale, Rutherford, NJ, USA) para a coleta de sangue e análise da contagem das células sanguíneas materna, tromboelastografia e avaliação do peso fetal.

A coleta de dados foi realizada durante o período de fevereiro a agosto de 2013 e de janeiro a agosto de 2014, no Laboratório de Ciências, Botterell Hall, Queen's University, Kingston, Canada.

3.2.5. Análise estatística

As análises estatísticas foram realizadas por meio do programa GraphPad Prism versão 6.0 (Graph-Pad, La Jolla, USA). Os dados foram apresentados em média ± desvio padrão (DP). O teste t de Student for utilizado para comparação dos dois grupos. Para avaliação do efeito do exercício físico nos resultados relacionados ao LPS, os dados foram analisados pelo teste ANOVA seguido por

uma comparação múltipla com o *post hoc* teste Bonferroni. Foi considerado estatisticamente significativo $p<0,05$.

3.2.6. Considerações éticas

Este estudo foi aprovado pelo comitê de ética da Queen's University (Anexo 1), e para isso a pesquisadora responsável realizou todos os cursos qualificatórios para a adequada manipulação de pequenos roedores (Anexo 2) oferecidos pela própria universidade.

RESULTADOS

Artigo 1

Carta de Submissão

Submission Confirmation

Thank you for your submission

Submitted to	Hypertension in Pregnancy
Manuscript ID	LHIP-2015-0105
Title	Translational studies for exercise in high-risk pregnancy: pre-eclampsia model
Authors	Kasawara, Karina Surita, Fernanda Pinto e Silva , João Luiz
Date Submitted	27-Oct-2015

Artigo 1

Review

Translational studies for exercise in high-risk pregnancy: pre-eclampsia model

Karina Kasawara, Fernanda Surita, João Luiz Pinto e Silva

Department of Obstetrics and Gynecology, University of Campinas (UNICAMP), Campinas, SP, Brazil.

Translational studies for exercise in high-risk pregnancy: pre-eclampsia model

Abstract

Objective: Reviewed literature regarding exercise effects on pregnancy related hypertensive disorders, analyzing basic science perspectives and clinical studies. ***Methods:*** Scientific databases were accessed by research strategy combining Medical Subject Headings terms. Studies published between 2000 and 2015, in English, Portuguese and Spanish language were considered. ***Results:*** Studies were classified in: Recommendations for exercise on high-risk pregnancy; Animals models for hypertension in pregnancy; Exercise on hypertensive disorders in animal models and pregnant women. ***Conclusion:*** There are several animal models to mimic hypertensive disorders in pregnancy, however, clinical studies are still needed for exercise recommendation in pregnant women with hypertensive disorders.

Keyword: Translational Medical Research, Motor Activity, Exercise, High-Risk Pregnancy, Pre-Eclampsia, Pregnancy-Induced Hypertension

1. Introduction

Hypertensive syndromes during pregnancy can pose significant health risks and are frequently associated with non-favorable maternal and fetal outcomes. Despite of all the scientific advances, maternal mortality rates related to hypertensive disorders during the gestational period are still high and considered the second most prevalent cause of death during pregnancy, responsible for approximately 14.0% (343 000, 11.1–17.4) of maternal deaths globally (1).

One of these syndromes, chronic hypertension (CH), is estimated to affect 1-5% of women during pregnancy (2); it has also been considered a particularly significant risk factor for developing severe complications when associated with pre-eclampsia (PE). This combination called superimposed PE, significantly compromises the maternal, neonatal, and fetal health (3-5).

Moreover, PE is a hypertensive disorder that occurs exclusively during the pregnancy and post-partum period, and it is characterized by a deficiency in spiral artery remodeling that leads to a dysfunction in intervillous space perfusion with oxidative stress. This hemodynamic stress causes the placenta to release pro-inflammatory and anti-angiogenic factors into the maternal circulatory system. Nevertheless, PE has been described as a pregnancy complication with severe perinatal consequences, though its etiology is still unknown (5,6).

Hypertensive syndromes during the pregnancy and post-partum period are classified by clinical manifestations that are periodically reviewed by many colleges

and associations. However, these organizations differ in their conceptualization and classification of hypertensive disorders (7-12). These differences in classification may lead to challenges for the interpretation of statistics and for the appropriate results comparison, considering these differences on classification of hypertension.

Severe cases of hypertensive syndrome are frequently associated with increased risks for non-favorable pregnancy outcomes, such as abruptio placentae, disseminated intravascular coagulopathy, eclampsia, acute renal failure, liver hemorrhage or failure, intracerebral hemorrhage, hypertensive encephalopathy, pulmonary edema, and death. Additionally, they may have a significant impact on women long-term health, increasing their risk for chronic diseases after the gestational period, including atherosclerosis, cardiovascular disease, end-stage renal disease, stroke, and retinopathy (13).

Although hypertension during pregnancy has a significant impact on short and long-term maternal health, there are also others fetal and neonatal complications those can do the same. The short-term complications may include severe fetal growth restriction (FGR), oligohydramnios, preterm delivery, hypoxia–acidosis, neurologic injury, and death; potential long-term complications might lead to cerebral palsy, fetal programming, cardiovascular disease, hypertension (13).

Up to date, there are no effective preventive measures with high quality of evidence indicated for avoid PE occurrence, and the only effective treatment known for the disease is the termination of pregnancy (14,15). Although there is no standard prevention for PE, some studies recommend the use of low dose

acetylsalicylic acid (aspirin, 75 mg) for women with risk factors (16). Furthermore, other studies suggest the use of calcium supplementation (at doses of 1.5–2.0g elemental calcium/day), especially for women with low basal calcium intake (less than 900 mg/day) (17). Either, acetylsalicylic acid or calcium supplementation are recommended during pregnancy by the World Health Organization (WHO), however with a moderate quality of scientific evidence (18).

In addition to studies targeting the prevention of PE, others are currently investigating factors related to genetic predisposition for and poor maternal immunologic adaptation in cases of PE. Associations between the circulatory and placentary protein human leukocyte antigen (HLA-G) and immune response modulation and vascular remodeling during the first trimester of pregnancy were done (19). Circulatory levels and placental expression of HLA-G are reduced in women who will develop PE (20). However, the relation between HLA-G levels, poor or deficiency on trophoblastic invasion and the impact on placental vascular organization is not well established.

Consequently, exercise has emerged as an alternative to promote maternal circulation, improving maternal-fetal vascularity — including an additional anti-inflammatory effect — and boosting the immune system of women at high risk to develop PE (21). In fact, several observational studies suggest the benefits of exercise in the prevention of PE development (22-24). The role of exercise in the prevention of cardiovascular diseases is well established in the literature (25,26), and in a low-risk pregnancy it is considered safe and recommended for its maternal health benefits, including control of weight gain during pregnancy, muscle and

posture adaptation, stress relief and the reduction of back and lumbar-pelvic pain (27-29).

Following the recommendations of the Centers for Disease Control and Prevention and American College of Sports Medicine (30, 31), The American College of Obstetrics and Gynecology (ACOG) (32) suggests that women exercise during pregnancy with moderate intensity, 30 minutes or more, in cases without medical and obstetrics complications. However, there is no clear recommendation regarding exercise characteristics (type of exercise, frequency, duration, and intensity) for maternal-fetal safety in pregnancies at risk for PE development.

Experimental studies represent an interesting possibility to explore the real effect of exercise on animal subjects that exhibit pre-eclampsia-like symptoms, and translational research may enable the transfer of these results in a different clinical degree, considering different variables during high risk pregnancy to PE development.

Therefore, the present article reviews the literature regarding the effects of exercise on pregnancy-related hypertensive disorders, analyzing the different perspectives of basic science and clinical science studies, as well as presenting actual guidelines with exercise recommendations for high-risk pregnancies in clinical practice.

2. Methods

This review evaluated the literature regarding exercise effect on high risk pregnancy. In order to develop an evidence based context in relation to different perspectives of basic science and clinical science studies the following Medical Subject Headings (MeSH) terms were used for research: “Translational Medical Research”, “Animal Models”, “Motor Activity”, “Exercise”, “Animal Pregnancy”, “High-Risk Pregnancy”, “Pre-Eclampsia” and “Pregnancy-Induced Hypertension”.

Different scientific databases were analyzed: MEDLINE (via PubMed), Lilacs, EMBASE, Scopus, SciELO and The Cochrane Library. Studies were selected by the interest topic: 1) Recommendations for exercise on high-risk pregnancy, 2) Animals models for hypertension in pregnancy, 3) exercise on hypertensive disorders in animal models, 4) Translational research for exercise in pregnant women with hypertensive disorders.

Studies published in English, Portuguese and Spanish language were considered for inclusion criteria. There was not date limited on session regarding animal models for hypertension in pregnancy in order to review all the experimental models available in the literature to mimic this disease during pregnancy. In all other sessions studies published between 2000 and 2015 were reviewed, however, the most recent publication were considered for inclusion in this review.

Studies were reviewed by the authors before inclusion on this review.

3. Results

Recommendations for and positive effects of exercise on high-risk pregnancy

In cases of higher gestational risk exercises are recommended with some restrictions, or there are no clear recommendations for safe performance. However, the lack of scientific evidence to support a recommendation of exercise in high-risk pregnancy has led some to consider it a threat for maternal and fetal safety.

Some special morbid medical conditions comprise a set of restrictions and are classified as relative or absolute contraindications to performing physical exercise during pregnancy: chronic hypertension, fetal growth restriction, anemia with hemoglobin <10 mg/dL, cardiac arrhythmias, bronchitis, uncontrolled diabetes, epilepsy or thyroid disease, extreme obesity, malnutrition or eating disorders, excessive smoking, and a sedentary lifestyle (32,33). Absolute contraindications for exercise during pregnancy include: PE or any type of uncontrolled hypertension or heart disease, restrictive lung disease, cervical incompetence, a multiple pregnancy (after 30 weeks), bleeding during pregnancy, placenta previa, preterm labor, and premature rupture of membranes (32,33).

Physical exercise is recognized as an alternative to reduce the risk of developing hypertensive disorders, as well as reducing associated comorbidities, such as obesity. Some studies support that exercise is associated with reduced gestational weight gain in overweight and obese pregnant women (28,34,35). As

physical exercise increases energy expenditure (kcal), it may leads to a reduction on body fat storage, improving the positive effects on maternal and fetal health when women become more active during their pregnancies (36,37).

Seneviratne et al. (2014) (38) presented recommendations for exercise programs in pregnancies complicated by obesity. According to their proposal, for women who live sedentary lifestyles, physical activity should only begin in the second trimester with low-impact exercises, always avoiding vigorous activities. Further, exercise intensity should be evaluated in accordance with the variation of heart rate in the overweight and obese, with a 30 minute exercise period that includes a warm up and cool-down, three to a maximum of four sessions per week. According to this protocol, obese women may benefit from an adequate program of physical activity when prescribed and properly carried out.

Regarding pregnancy complications related to hypertensive disorders, there are some hypotheses about the possible positive effects of exercise in prevention of PE. Specifically, physical activity would stimulate vascularization and placental growth, reduce oxidative substances, reverse endothelial dysfunction found in pathology, and minimize inflammatory effects (5,39,40) (Figure 1). The abnormal development of the placenta is associated with PE, and it is a condition associated with fetal growth restriction (FGR). The inadequate trophoblastic invasion of the spiral arteries could lead to loss of sensitivity in the utero-placental blood vessels to vasoconstriction, causing intermittent hypoxia and placental ischemia with potentially harmful obstetric and fetal consequences (40,41).

Exercise could promote the development of the placenta, increase organization of the immune system, activate antioxidant enzymes such as superoxide dismutase (SOD), and limit cell damage caused by oxidative stress. Long-term exercise leads to the improvement of endothelial functions in cases of diabetes mellitus type 2, heart failure, and dysfunction caused by an aging mother. Therefore, women at risk for developing PE could benefit from the positive effects of exercise on endothelial dysfunction (40-42).

The anti-inflammatory effects of exercise outside of pregnancy on conditions such as high blood pressure and coronary heart disease are well established in the literature. It has also been shown to reduce the risk of stroke, mellitus type 2 diabetes, and dementia, and to stimulate the immune system in cancer patients. Furthermore, exercise is associated with a reduction in C-reactive protein (CRP); cytokines of the interleukin-1, interleukin-6, and interferon-ɣ; the decreased expression of Toll-like receptors in monocytes and macrophages; and increased anti-inflammatory cytokines, such as interleukin-10. Considering these benefits, exercise could have similar anti-inflammatory effects on pregnant women, and so prevent and mitigate the systemic inflammation caused by PE (40,43,44).

To date, there is no definitive consensus on the indication for rest in pregnant women with normal blood pressure who are seeking to prevent PE and its complications. Additionally, there is insufficient evidence for recommending exercise or other activities for the prevention of PE, so according to Cochranne Library, the benefits related to exercise or rest during pregnancy (as they relate to PE prevention) are inconclusive (45-47). It is worth mentioning that once

diagnosed with PE, the most prevalent indication is hospitalization for appropriate intervention and clinical frame control (18).

Pregnancy hypertension and pre-eclampsia models in animals

There are several models of induction of hypertensive disorders, including PE, which are used in experimental studies on animal subjects. The mechanism of action used to promote clinical manifestations related to hypertensive disorders varies from one model to another. Among the experimental models known as knockout animals (48,49), some of the resources used to mimic the condition of PE include: transgenic or genetic modification (50-52), placental surgery to reduce blood flow to the uterus (53,54), administration of a high-sodium diet (55), and induction of inflammation to promote immunological changes (56-58).

Some genetically modified animals reproduce the deficiencies in angiogenesis factors as placental growth factor (PGF) knockout mice (PGF - / -) (48). This is a protein of the same family as vascular endothelial growth factor (VEGF), which is responsible for vascular endothelial differentiation during the peaking of embryogenesis at 30 weeks gestation in humans, as well as its decrease towards the end of pregnancy. Maternal PGF deficiency is recognized in early pregnancy and is associated with clinical cases that may lead to PE development (49).

In addition to these animals models of PE and those genetically modified animals, it was verified that the mating of a female expressing angiotensinogen (A

+) and a male expressing renin (R +) results in increased blood pressure and proteinuria in the female. However, after changing the combination of mating so that the female expresses R + and the male expresses A +, the female presents no physiological hypertension (50,51). In this context, there is another transgenic line: the combination of R + and A + that is produced by the mating of heterozygous mice with human renin expression and heterozygous mice with human angiotensinogen expression. During pregnancy, these females demonstrated exacerbated expression of angiotensinogen and human renin (R + and A +), thereby developing hypertension before and during pregnancy (52).

To accomplish the pharmacological induction of anti-angiogenic models, healthy animals are subjected to intervention with the goal of reversing the placental angiogenic action of VEGF and PGF. The soluble fms-like tyrosine kinase-1 (sFlt-1) is considered an anti-angiogenesis protein and is presented systemically and in placenta in cases of PE. The SFlt-1 is administered through an adenovirus (Ad)-mediated gene transfer; the sFlt-1 promotes increased blood pressure, proteinuria, and gromelurar endotheliosis associated with reduced concentrations of VEGF and PGF in the maternal blood plasma of mice (59).

The induction of PE by immunological changes in experimental studies is linked to the exacerbation of the inflammatory response. In this model, the inflammatory cytokine tumor necrosis factor α (TNF-α) is administered for induction of hypertension during pregnancy in mice (56). Other studies have linked the induction of inflammation by low doses of lipopolysaccharide (LPS) in pregnant rats with an increased TNF-α concentration, hypoxia-inducible factor (HIF), with

consequent high blood pressure, proteinuria, maternal coagulopathy, increased spiral artery resistance index and FGR changes very similar to those that appear in pregnant women with PE (57,58).

Another model is the surgery for reduction of uteroplacental perfusion (Rupp), was described in 1940 by Ogden et al. (53) as being performed in dogs by partial occlusion of the infrarenal abdominal aortic. Since then, the technique has been modified and tested in various animals, in order to reduce the uteroplacental blood flow by 40% and simulate the development of PE. Experimental studies in male Sprague-Dawley rats revealed blood pressure (BP) increases—as well as reductions in VEGF levels and antioxidative capacities of placentas—by placing a clip into the lower abdominal aorta above the iliac bifurcation and right and left ovarian arteries, when compared to control mice undergoing placebo, or sham, surgeries (54).

While not very common model of PE complications induction, changes in sodium diets were proposed in 2010 by Burke et al.(55) and administered with the intention of assessing the sensitivity of BL6, BalbC, and Rag mice. In this experiment, the mice received different concentrations of sodium doses: one that was considered normal (0.67%), another that was considered to be low dose (0.43%), and one that was considered to contain a high concentration of sodium (8.5%). All animals showed sensitivity to the amount of sodium administered, presenting variations of the BP with the change of diet, regardless of the strain of mouse.

These experimental models mimic the clinical implications of hypertensive disorders, but PE is a multifactorial condition of unknown etiology, thus making it a difficult animal model to reproduce. In addition, the pregnancy itself is considered a stress factor for the animals, which need to adapt to the various physiological changes associated with it in just a few days. In this case, surgery, excessive manipulations, and dietary changes could be considered additional stress factors during pregnancy, and thus variables that must be considered carefully when selecting the animal model to be used for the research.

The effect of exercise on hypertensive disorders in animal models

Some experimental studies have focused on the ability of exercise to prevent hypertensive disorders and comorbidities during pregnancy. Among those carried out on the relationship between exercise and gestational hypertensive disorders, the most described model in the literature is the voluntary exercise model, in which the animal has free access to the exercise instrument for the realization of daily physical activity before and during pregnancy.

In one study involving transgenic female mice (hAng) that demonstrate increased angiotensinogen expression when mated with transgenic male mice with renin expression (hRN), the subjects presented with hypertension and proteinuria during pregnancy. The animals had access to a special racing wheel for rodents for one month before and during pregnancy, and the group that performed exercise showed normal protein secretion compared to the sedentary group (60). In

addition, this study demonstrated other benefits associated with the effect of exercise on hAng females, such as a reduction of placental necrosis, normal placenta development accompanied by the necessary changes in the expression of VEGF, and increased fetal and placental weight (60).

Another study utilized a different animal model in which Sprague-Dawley rats underwent reduced uterine perfusion pressure (RUPP) surgery and researchers evaluated the effect of voluntary exercise before and during pregnancy on placental ischemia associated with hypertension and angiogenesis. Mice with RUPP that submitted to exercise exhibited reduced blood pressure levels compared to control groups, as well as increased VEGF circulation and antioxidative capacity in their placentas and kidneys. However, the exercise had no effect on the weight of the fetus and placenta in these cases, and did not reverse the harm caused by reduced uteroplacental blood flow (54).

Genest et al. (2013) (52) evaluated the effect of voluntary exercise in transgenic mice models with exacerbated expressions of renin and angiotensinogen (R + and A +) and exercise mechanisms of action before and during pregnancy with superimposed PE. These animals had a history of hypertension and increased BP associated with proteinuria during pregnancy. Exercise was associated with benefits such as BP control and proteinuria expression during pregnancy in mice that trained on the exercise wheel compared with those that were sedentary. To evaluate the possible role of exercise on the renin-angiotensin system, some proteins were studied in aorta, and the sedentary animals exhibited an 85% increase in antagonist angiotensin II receptors (AT1R), a

24% reduction of the Mas receptor (MasR), and a 32% decrease of the angiotensin converting enzyme (ACE2). Contrasted, however, with the results found in trained rats (R + and A +), where the MasR was found in its highest concentration (50%), associated with the maintenance of BP values. In this study, exercise promoted improvement in endothelial functions during pregnancy by reducing blood glucose levels and promoting increased angiogenesis, thus changing the components of the renin-angiotensin system.

In yet another study on the effects of exercise on the pregnancies of mice, Rocha et al. (2014) (61) examined a strain of mice that develop spontaneous hypertension, dividing the animals into three groups and submitting them to swimming exercises. The first was a control group, the second group began the exercise on day zero of pregnancy, and the third began on the seventh gestational day. The exercise duration was progressive until the rats reached an hour's daily exercise in late pregnancy (gestational day 20). Both groups that performed the exercise (gestational day zero and 7) presented with lower BP levels than did the control group. Furthermore, there was a reduction on fetal growth in the group that initiated the physical exercise on gestational day 7. This study differs from others by presenting an exercise protocol induced with control of variable intensity intervention.

The model of voluntary exercise among animals becomes, however, a complex situation to reproduce in humans. This represents the inability to understand the comparative intensity of activity, especially in voluntary exercise, although enrich literature with experimental background on the physiology of

exercise during animals high risk pregnancy. Despite the positive results in maternal and fetal outcomes shown in the studies cited above, it is important to understand the implementation of these physiological effects in women who are at high risk for gestational hypertensive disorders.

Translational research for exercise in pregnant women with hypertensive disorders

Clinical studies are considered the optimal method by which to assess cause and effect of a determinate intervention when an independent variable—such as treatment performed with physical exercise during high-risk pregnancy—may be associated with maternal and neonatal outcomes that should present the intervention-related effects. However, the effects of exercise on cardiovascular conditions of pregnancies accompanied by hypertensive disorders should be taken into account in order to guarantee maternal and fetal safety.

In order to evaluate the hemodynamic effects of exercise in women who had experienced PE in a previous pregnancy, Krabbendam et al. (2009) (62) studied nine non-pregnant women in the postpartum period for at least six months, during which they performed two to three sessions per week for four weeks with stationary exercise bicycles at levels of moderate intensity (60 to 70% of the heart rate). Hemodynamic assessments carried out before and after the training program found that the resting heart rate decreased with the activity, and there was an increase in plasma volume by 8%, as well as an 18% increase in venous

compliance. They concluded that although this training protocol was completed by mothers in a post-partum period, it demonstrated improvement in vascular function following the reduction in sympathetic activity, suggesting that these results can be used in early pregnancy for women with an increased risk for recurrence of gestational hypertension.

Additionally, in the same year, Medeiros et al. (2009) (63) evaluated the effects of aerobic exercise on a treadmill performed for 30 minutes by pregnant women with CH and the impact it had on their heart rate (HR), BP, the resistance index (RI) and pulsatility index (PI) of the umbilical arteries (AU), and fetal middle cerebral (MCA), which was evaluated by Doppler velocimetry before and after the day of physical activity. They examined 12 pregnant women who exhibited an increase in heart rate and systolic blood pressure shortly after the completion of the exercise, however, no significant changes were observed in other parameters. Physical exercise from walking did not change the utero-placental blood flow in high-risk pregnant women when assessed before and after the activity.

However, there are some evidence on the reduction of fetal HR while the mother is performing exercise during pregnancy. Transient fetal bradycardia is associated with maternal fitness and is considered as a mild adverse event with an approximately occurrence rate of 35% (64).

A randomized controlled trial evaluated the effect of the walking exercise compared to the effect of stretching in women who were sedentary before pregnancy and had a history of PE; researchers observed higher rates of PE in the walking group at 14.6% versus 2.6% in the stretching group. However, there were

fewer incidents of gestational hypertension in women who underwent walking as an exercise (22%, 95% CI 8.7-35.2) than in women who stretched (40% 95% CI 23.2 to 55.8). The walking group also presented lower transferrin levels during labor, which is a glycoprotein found in blood plasma that transports iron (65). A secondary analysis of the data showed that stretching group had lower resting HR compared to walking group, suggesting the benefits of stretching may improve autonomic response in reducing the risk for developing PE (66).

Nevertheless, Kasawara et al. (2013) (67) demonstrated that supervised exercise once a week during pregnancy in women with CH or previous PE was not linked to adverse maternal and neonatal outcomes. Supervised exercise programs involving a stationary bicycle did not increase the incidence of PE or any other maternal morbidity, nor did it make maternal hospitalization in the Intensive Care Unit more likely. With regards to potential neonatal repercussions, such as fetal weight, appropriate weight for gestational age, prematurity rate, Apgar score at birth, and neonatal morbidity, none were observed to be significant when compared with the results of exercise group and the control group. In this context, supervised exercise once a week was considered safe for these high-risk pregnancy populations.

A systematic review of Cochrane Library suggests that dietary choices and exercise during pregnancy can reduce maternal hypertension (average RR 0.70, 95% CI 0.96 to 00:51), but with no impact on the incidence of PE (RR 0.95, 95% CI 0.77 to 1:16) (47).

However, translational studies are needed in order to better understand the mechanisms of action and the effects of physical exercise observed in experimental models, as well as to transport them to women at high risk for gestational hypertensive disorders. These clinical trials are not sufficient and definitive enough to change clinical practice, nor to establish systematic exercise of recommendations with complete safety during pregnancy with the goal of preventing hypertensive disorders.

4. Conclusions

Animal models are extremely important and useful in order to understand the impact of exercise on high-risk human pregnancy, specifically with hypertensive disorders, because of the potential risk involved in clinical studies in this situation. However, although their findings represent a fundamental contribution to understanding hypertensive disorders during pregnancy, exercise during high risk pregnancy, especially for hypertensive syndromes still requires high-quality randomized controlled trials with high-risk pregnant women in order to comprehend all the variables involved in this important proposed lifestyle change during pregnancy.

Furthermore, experimental studies have to be interpreted carefully, and when reproduced in humans, must have a rigorous control of results evaluation according to the same hypothesis presented in the animal models. The results of future studies on this topic must be evidence based and coordinated with clinical

practices. This is critical, as they could form the basis of recommendations for exercise in pregnant women with hypertensive disorders.

Hopefully the future will feature sufficient scientific literature in order to support women in high-risk pregnancies; in this future, those with conditions such as hypertension will be offered a specific recommendation based on robust evidence regarding the benefits—or real risks—of exercise in the prevention of PE and its complications.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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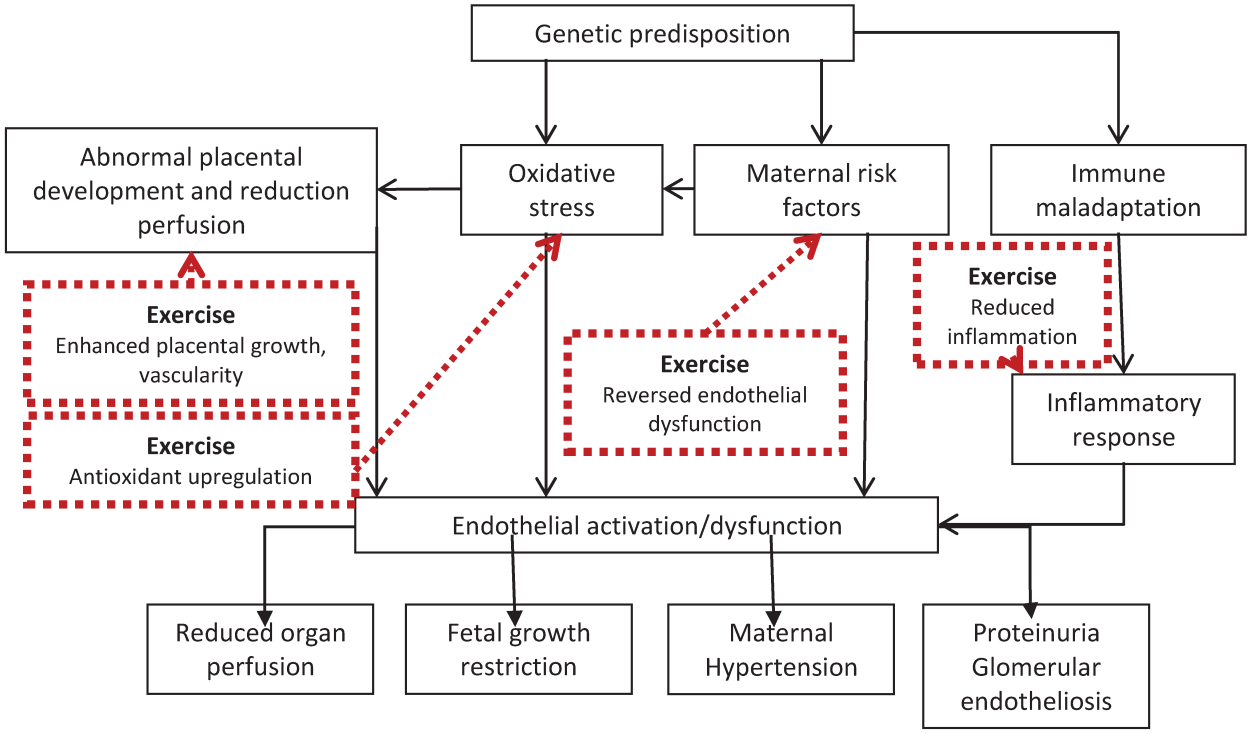
Figure 1: Flow chart of the etiology, interaction, and clinical morbidity associated with pre-eclampsia and the possible positive effects of exercise.



Solid lines indicate the effects of pre-eclampsia, while dotted lines suggest the effects of physical exercise as they relate to pathology (Weissgerber, et al, 2006; Sircar, 2015 modified).

Artigo 2

Carta de Submissão

Submission Confirmation

Thank you for your submission

Submitted to	Reproductive Sciences
Manuscript ID	RSCI-15-702
Title	Moderate Exercise Attenuates LPS-Induced Inflammation and Associated Maternal and Fetal Morbidities in Pregnant Rats
Authors	Kasawara, Karina Cotechini, Tiziana Macdonald-Goodfellow, Shannyn Surita, Fernanda Pinto e Silva, João Tayade, Chandra Othman, Maha Ozolinš, Terence Graham, Charles
Date Submitted	10-Dec-2015

Artigo 2

Experimental study

Moderate Exercise Attenuates LPS-Induced Inflammation and Associated Maternal and Fetal Morbidities in Pregnant Rats

Karina T. Kasawara, PT, MSc[1,2]; Tiziana Cotechini, PhD[1,3]; Shannyn K. Macdonald-Goodfellow, BSc[1], Fernanda G. Surita, MD, PhD[2]; João Luiz Pinto e Silva, MD, PhD[2]; Chandrakant Tayade, PhD[1]; Maha Othman, MD, PhD[1]; Terence R.S. Ozolinš, PhD[1]; Charles H. Graham, PhD[1]

[1]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada
[2]Department of Obstetrics and Gynaecology, University of Campinas, Campinas, SP, Brazil
[3]Current address: Oregon Health & Science University, Portland, Oregon, USA

Karina T. Kasawara, e-mail: karina.tamy@gmail.com

Tiziana Cotechini, e-mail: tiziana.cotechini@gmail.com

Shannyn K. Macdonald-Goodfellow, e-mail: sm23@queensu.ca

Fernanda G. Surita, e-mail: surita@unicamp.br

João Luiz Pinto e Silva, e-mail: jlcpes@uol.com.br

Chandrakant Tayade, e-mail: tayadec@queensu.ca

Maha Othman, e-mail: othman@queensu.ca

Terence R.S. Ozolinš, e-mail: ozolinst@queensu.ca

Charles H. Graham, Corresponding author: Telephone: +1 (613) 533-2852; e-mail: grahamc@queensu.ca, Mailing address: 18 Stuart Street, Room 859. Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada. K7L 3N6

Running title: Exercise and inflammation in rat pregnancy

K.T. Kasawara received scholarships from the Canadian Bureau for International Education – Department of Foreign Affairs and International Trade (CBIE/DFAIT) and Coordination for the Improvement of Higher Education Personnel –The Ministry of Education of Brazil (CAPES/MEC) and Canada-Brazil Awards – Joint Research Projects (2011-2012) and from FAEPEX (University of Campinas). This research was supported by a grant from the Canadian Institutes of Health Research (CIHR) awarded to C.H.G. Graham. The TEG studies were supported by a grant from the Canadian Haemophilia Society awarded to M. Othman.

Abstract

Background: Fetal growth restriction (FGR) and coagulopathies are often associated with aberrant maternal inflammation. Moderate-intensity exercise during pregnancy has been shown to increase utero-placental blood flow and to enhance fetal nutrition as well as fetal and placental growth. Furthermore, exercise is known to reduce inflammation. **Objective:** To evaluate the effect of moderate-intensity exercise on inflammation associated with the development of maternal coagulopathies and FGR in pregnant rats. **Study Design:** Wistar rats were subjected to an exercise regime before and during pregnancy. To model inflammation-induced FGR, pregnant rats were given daily intraperitoneal injections of *E. coli* lipopolysaccharide (LPS) on gestational day (GD) 13.5 – 16.5 and sacrificed at GD17.5. Control rats were injected with saline. Maternal hemostasis was assessed by thromboelastography. **Results:** Moderate-intensity exercise prevented LPS-induced increases in white blood cell (WBC) counts measured on GD 17.5 and improved maternal hemostasis profiles. Importantly, our data reveal that exercise prevented LPS-induced FGR. **Conclusion:** Moderate exercise initiated before and sustained during pregnancy may decrease the severity of certain complications of pregnancy associated with abnormal inflammation.

Key words: Thromboelastography, Exercise, Inflammation, Fetal Growth Restriction, Leukocyte Count.

Introduction

Fetal growth restriction (FGR) affects 5 – 10% of clinically recognized pregnancies[1-3] and is often associated with aberrant maternal inflammation. Though normal pregnancy is considered to be a state of low-grade inflammation,[4,5] it has been postulated that adverse pregnancy complications, including FGR, are linked to aberrant maternal inflammation.[4,6,7]

Normal pregnancy is associated with a shift in maternal hemostasis towards a pro-thrombotic state,[8,9] and pregnancy-associated coagulopathies have been implicated in the pathophysiology of complications including pre-eclampsia (PE), fetal loss and FGR.[10-13] Whether disruptions in maternal hemostasis are causally linked to the deficient utero-placental perfusion that characterises these pregnancy disorders remains unknown. However, there is recent evidence that antenatal anti-thrombotic therapy reduces the risk of poor pregnancy outcomes, including FGR, in women at risk of placental dysfunction.[14] Moreover, thrombophilia is a common cause of fetal demise when utero-placental insufficiency is present.[15]

There is substantial cross talk between inflammatory and hemostasis pathways, and dysregulation of both systems is implicated in the pathophysiology of pregnancy complications.[13] Previous work from our laboratory revealed that inflammation-induced coagulopathies in a rat model are associated with altered utero-placental hemodynamics and fetal death.[11,12,16] Importantly, our data revealed that inhibition of tumour necrosis factor-alpha (TNF) successfully prevented inflammation-induced hemostatic alterations, normalized utero-placental perfusion and prevented fetal loss.[11,16]

Exercise has immunomodulatory effects. Specifically, exercise reduces the release pro-inflammatory cytokines, promotes the release of anti-inflammatory cytokines, decreases visceral fat mass and reduces the expression of Toll-Like Receptor 4 (TLR-4) on monocytes and macrophages.[17,18] The latter is a cell surface protein that plays a fundamental role in the release of pro-inflammatory cytokines and activation of innate immunity. Moderate exercise also regulates hemostasis by decreasing platelet reactivity and fibrin formation.[19,20]

Prospective studies have revealed the benefits of exercise on achieving optimal vascular function.[21] In particular, moderate-intensity exercise has been shown to promote beneficial vascular adaptations and reduce cardiovascular risks.[22] Additionally, moderate-intensity exercise during pregnancy increases utero-placental blood flow, enhances fetal nutrition and improves fetal and placental growth.[23,24] In the present study we tested the hypothesis that exercise attenuates fetal and maternal morbidities associated with aberrant inflammation. To the best of our knowledge this is the first study that examines the efficacy of an exercise protocol initiated before and during pregnancy in the attenuation of maternal inflammation, FGR and systemic coagulopathies.

Materials and Methods

Animals

All procedures for animal experimentation were undertaken in accordance with the principles and guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee. Virgin female Wistar rats (3 – 4 months old; Charles River Laboratories, St-Constant, QC, Canada) were housed in a light- and humidity-controlled facility.

Exercise protocol

Exercise under regulated conditions (*i.e.* constant time and intensity) was achieved using a rodent treadmill (LE 8700 series, Panlab, Harvard Apparatus, Barcelona, Spain). Belt speed was adjusted and individualized according to the maximal exercise ability (MEA; described below) determined for each rat during a training phase (see Figure 1 for timeline).

Evaluation of maximal exercise ability

The evaluation of maximal exercise ability (MEA) for each rat was performed as previously published by Jiao *et al.*[25] Briefly, belt inclination was set at 0˚ and belt speed was set to 30 cm/sec. Every 30 sec, belt speed was increased 2 cm/sec until rats were unable to run regularly, or until they rested on the shock grid (receiving a shock of <1mA) more than three times. The final belt speed achieved by each animal was set as the MEA for that subject. This MEA evaluation was repeated three times for each rat (with one-hour rests between trials) and the average MEA over the three trials was determined. Overall, mean MEA was calculated before and after the pre-pregnancy exercise protocol (described

below) was completed in order to evaluate the change in each rat's aerobic capacity prior to mating.

Pre-pregnancy exercise protocol

The protocol for exercise training was based on the MEA for each rat and was established from an exercise training protocol for pregnant rats published by Amorim, *et al*. [26] and modified by us. Briefly, all rats were subjected to four weeks of pre-pregnancy exercise training. Each training week consisted of five consecutive days of training followed by two days of rest. The daily pre-pregnancy exercise training program was divided in three stages consisting of warm-up, training, and cool-down. During the warm-up stage, belt speed intensity was set to 40% of the subject's MEA. During the training phase, belt intensity and duration of exercise were modulated based upon the week of training. Specifically, rats ran at 40% of their MEA for 20 min during week one, 50 min during week two, 60 min during week three and 60 min at 65% of their MEA during week four. During the cool-down period, belt speed was slowly decreased 2-3 cm/sec every 30 sec until the treadmill was stopped.

Mating

Following four weeks of pre-pregnancy exercise training, virgin female rats were co-housed overnight with a male rat (at a 2:1 ratio). The detection of sperm in the vaginal lavage the following morning represented gestational day (GD) 0.5. Pregnant rats were then subjected to the pregnancy exercise protocol (described below) the same day.

Pregnancy exercise protocol

Throughout pregnancy, rats were subjected to a moderate-intensity exercise program. Pregnant rats ran at 65% of their MEA for various durations over gestation (week one = 50 min/day); week two = 30 min/day; and week three = 20 min/day). Animals ran five days/week with one day of rest between each week until the study endpoint on GD 17.5.

Complete blood cell count analysis

Complete blood cell (CBC) count analysis was performed on maternal plasma samples collected on GD 17.5 at the time of euthanasia. Briefly, whole maternal blood was taken via cardiac puncture using a syringe pre-filled with EDTA; samples were centrifuged (2,000 g x 20 min) to collect plasma. Analysis was performed on each sample (12 µl) using the ABC Vet Animal Blood Counter (Scil Animal Care Company, Gurnee, IL, USA) according to the manufacturer's instructions. Red blood cell, white blood cell, lymphocyte, monocyte, granulocyte and platelet populations were assessed.

Inflammation-induced rat model of FGR

We used our previously established model of LPS-induced FGR.[7] Briefly, pregnant Wistar rats received daily intraperitoneal (i.p.) injections of low-dose lipopolysaccharide (LPS; 10 µg/kg on GD 13.5 followed by 40 µg/kg on GD 14.5, 15.5 and 16.5) or saline (0.1 ml/100 g) during the second half of gestation, and were euthanized on GD 17.5.[7] To control for the effects of exercise, we utilized our previously published data collected from *sedentary* rats treated with saline (Se + saline) or LPS (Se + LPS).[7,12] In that study, FGR was defined as a fetal weight falling below the 10th percentile for gestational age. The threshold of FGR was determined by evaluating the distribution of all fetal weights from the saline-treated control

cohort (n = 22 dams; n = 305 fetuses; mean fetal weight = 0.9244 ± 0.007 g); fetuses with weights below 0.8071 g (lower 10th percentile) were designated as FGR.[7] Treatment of sedentary rats with LPS in our previous study resulted in a mean fetal weight = 0.8421 ± 0.006 g (n = 28 dams; n = 258 fetuses).

To evaluate the effect of exercise on LPS-induced FGR, exercised rats (as described above) received daily i.p. injections of saline or LPS (*Escherichia coli* serotype 0111:B4; Sigma-Aldrich, Oakville, ON, Canada) according to the same protocol established for the sedentary group.[7] Fetal weights were measured on GD 17.5 and were normalized to litter size (fetal weight/number of fetuses in litter) to account for alterations in fetal weight due to litter size.[27]

Thromboelastography (TEG)

Prior to euthanasia, pregnant rats were anaesthetized on GD 17.5 using 40-50 mg/kg sodium pentobarbital (CEVA Santé Animale, Rutherford, NJ, USA). Maternal whole blood was collected via cardiac puncture, using a 26'-gauge needle, and placed into a tube pre-filled with trisodium citrate. Thromboelastography (TEG) was performed on this citrated blood as previously described[11,12,16] using a TEG® 5000 Haemostasis System and TEG® Haemostasis Analyzer software Version 4.2 (Haemoscope Corporation, Skokie, IL, USA). Prior to each analysis, an electronic quality test was performed on the TEG® 5000 Haemostasis System by a trained operator. Blood (340 µl) was re-calcified by adding 20 µl of 0.2 M calcium chloride and loaded into a disposable plastic cuvette for analysis. Data were collected for 75-90 min and the following parameters were evaluated: time to clot formation (R), speed of clot propagation (α), rate of clot formation (K), strength/stability of

clot (MA), clotting index (CI; a value that is based on the four parameters above,) and LY30 (percent clot dissolution in 30 min). As stated above, previously published data collected from the sedentary cohort of saline- and LPS-treated rats (n = 9 and 13, respectively)[12] were used as the reference range and controls. Exercised rats from the current study were considered to exhibit hemostatic alterations if two or more parameters fell beyond reference ranges previously established during normal pregnancy in saline-treated rats.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 6.0 Software (GraphPad Software Inc., La Jolla, CA, USA). Data are presented as mean ± standard deviation (SD). Student's t-test was used to compare means between two groups. To evaluate whether exercise was able to ameliorate LPS-induced outcomes, data were analysed using two-way ANOVA and the Bonferroni correction was applied to determine significant differences between comparison groups. Differences between groups were considered significant when $p < 0.05$ for the null hypothesis.

Results

Exercise training increased the aerobic capacity of rats prior to pregnancy

Five rats were unable to complete the pre-pregnancy training phase due to persistent failure to run on the treadmill for unknown reasons; an additional rat was diagnosed with hip displacement. These six rats were excluded from the study. All the trained rats (n = 10) increased their aerobic capacity following four weeks of pre-pregnancy exercise. Specifically, MEA significantly increased from 63.7 cm/sec ± 8.6 cm/sec prior to training, to 79.1 cm/sec ± 6.4 cm/sec following training. Since all rats achieved a similar final belt speed following the training phase, animals were randomly assigned to the saline (Ex+Saline, n = 5) or LPS (Ex+LPS, n = 5) group.

Exercise prevented LPS-induced increases in white blood cell counts

Though not significant, exercise trended ($p = 0.12$) towards promoting increased red blood cell counts in LPS-treated animals (Figure 2A). Whereas our previous data revealed that white blood cell (WBC) counts measured in blood from sedentary, LPS-treated rats were significant increased compared with sedentary controls (saline-treated), WBC counts measured from Ex+LPS-treated animals were significantly reduced compared with WBC counts measured from Se+LPS-treated rats (Figure 2B). Differential WBC analysis revealed that exercise significantly prevented LPS-induced increases in the number of circulating monocytes, granulocytes and lymphocytes (Figure 2C, D, E).

Exercise attenuated inflammation-induced FGR

Our previously published data revealed that administration of LPS to sedentary pregnant rats induced FGR[7] (Figure 3A, B). This LPS-induced reduction in fetal weights observed in

sedentary rats was not observed in exercised rats (Figure 3A, B). Moreover, weights of fetuses from Ex+LPS dams trended ($p = 0.07$) towards being significantly increased compared with weights of fetuses from Se+LPS dams (Figure 3A). There were no differences in total litter size (all implantation sites) or fetal viability (number of live pups in a litter) when all treatment groups were compared (data not shown).

Exercise normalized coagulation parameters measured by TEG

TEG coagulation parameters obtained from exercised rats were compared with our previously published coagulation parameters obtained from sedentary rats[12] (Table 1). Results showed that exercise normalized coagulation parameters as described below (Figure 4). Of the four rats in the Ex+Saline group, three had normal TEG parameters when compared with Se+Saline rats (Figure 4A, B). One rat (Rat 9) had TEG parameters indicative of a hypocoagulable state with more than two parameters beyond the reference ranges for normal pregnant rats, including increased K and decreased alpha angle and MA (Table 1).

The TEG parameters assessed from blood collected from four of the five rats from the Ex+LPS group were not significantly different from TEG parameters assessed from blood samples collected from the Se+Saline group (Table 1; Figure 4A, C). For the remaining animal (Rat 3; Table 1), an increased K combined with reduced α angle, MA and CI was indicative of a hypocoagulable state. Overall, exercise normalized LPS-induced coagulation parameters as evaluated by TEG.

Discussion

Here we describe the effect of moderate-intensity maternal exercise before and during pregnancy, on the prevention of LPS-induced FGR and its associated hemostatic alterations. The results of this study are in agreement with similar data reported in mouse studies in which voluntary exercise performed before and during pregnancy revealed positive effects on maternal outcomes. In those studies, voluntary maternal exercise was associated with better control of blood pressure during pregnancy, reduced proteinuria (albumin/creatinine ratio), decreased oxidative stress increasing placental antioxidant capacity, and reduced maternal and placental circulating sFlt-1 levels.[28-30] Despite the beneficial effects of exercise reported, voluntary exercise can result in intervention bias as the intensity of exercise experienced by each animal may differ. There is evidence that animals voluntarily reduce their physical activity later in gestation and that this reduction in activity varies between individuals.[28,29] Though the reason for this variance is not well described, it is possible that physiological and biomechanical changes during pregnancy (*i.e.* weight gain and cardiovascular adaptations) play a role. In consideration of such inter-animal variability, we designed our study so that all rats were exposed to the same exercise regime throughout the study.

In our study, seven weeks of moderate-intensity exercise (including the pre-pregnancy training phase and exercise during gestation) prevented LPS-induced increases in white blood cell counts, normalized coagulation parameters and attenuated the development of LPS-induced coagulopathies. In addition, we observed a beneficial effect of maternal exercise on fetal outcomes such that exercise prevented LPS-induced FGR. Our data align with previous studies revealing that voluntary exercise ameliorates poor fetal growth.[28,29]

However, work from Rocha and colleagues revealed that maternal exercise (swimming) in a rodent model did not increase fetal weight in spontaneously hypertensive (SHR) rats.[31] In contrast to our model, in that study SHR rats began exercise on GD 7 (20 min of initial daily exercise increased gradually to one hour per day until GD 20). Overall, swimming promoted produced greater FGR than in sedentary SHR control rats.[31] It is possible that the gestational period during which maternal exercise is initiated is important to the prevention of FGR. Whereas the data from our study suggest that exercise initiated prior to pregnancy and sustained throughout gestation may be beneficial, exercise initiated during an established pregnancy may be detrimental as a result of increased maternal physiological demands and cardiovascular overload.[29]

Exercise promotes positive physiological adaptations during pregnancy.[28,29] However, forced exercise (as in our study) could also induce physiological stress.[32] In our study, we chose a regimented exercise protocol in order to control exercise intensity and duration. We chose to model moderate-intensity exercise (65% of maximum exercise ability) because this level of exercise has been associated with maternal benefits and, importantly, because it has been recommended for pregnant women by the American College of Obstetrics and Gynecology.[26,33] In our study, though moderate-intensity exercise was advantageous, it did not completely prevent the LPS-induced maternal and fetal alterations. Therefore, it is possible that exercise at a different level of intensity (*i.e.* high or low) may be a more effective option with broader outcomes. Indeed, there is evidence that differing exercise intensities during pregnancy have variable effects on maternal and neonatal health outcomes.[34,35]

To the best of our knowledge, this study provides the first evidence that moderate-intensity maternal exercise before and during pregnancy prevents inflammation and its associated hemostatic alterations in a rat model. TEG is an effective tool for the evaluation of global hemostatic changes associated with both normal pregnancy[36] and adverse pregnancy outcomes.[37] Moreover, we previously demonstrated that inflammation-induced maternal hemostatic alterations, detected systemically using TEG, are comparable to hemostatic alterations detected locally at the utero-placental interface in a model of inflammation-induced fetal demise.[11] Though some studies have described a pro-thrombotic effect of high intensity exercise (>75% of VO_{2max}),[38,39] there is evidence that exercise prevents thrombosis during hospitalization and prolonged bedtime periods in non-pregnant individuals, and that exercise improves the hemostatic profile.[40,41] In the present study, exercise normalized LPS-induced alterations in TEG parameters such that the values were no different from sedentary, saline-treated, control rats. The use of TEG has been an important tool for the evaluation of the coagulation index in rats[11,12] and in this study TEG was used to assess individual hemodynamic changes in response to LPS and exercise. Moreover, our data align with the literature, which proposes a beneficial effect of exercise on coagulation parameters and an overall enhanced fibrinolytic state.[40,42]

Exercise is known to reduce the release of pro-inflammatory cytokines, stimulate the release of anti-inflammatory cytokines and diminish the physiological consequences of exaggerated inflammation. In light of accumulating evidence for a role of abnormal inflammation in the development of pregnancy complications,[43] including FGR[7] and spontaneous pregnancy loss,[11,12,16] our study points to the need to assess the potential

benefits of exercise in clinical studies involving women with clearly identified complications (*e.g.* recurrent pregnancy loss) associated with aberrant inflammation.

Acknowledgements

We wish to thank the following individuals for technical help: Bruno Zavan, Maggie Chasmar and Kevin Robb for assistance with animal care.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

K.T. Kasawara received scholarships from the Canadian Bureau for International Education – Department of Foreign Affairs and International Trade (CBIE/DFAIT) and Coordination for the Improvement of Higher Education Personnel –The Ministry of Education of Brazil (CAPES/MEC) and Canada-Brazil Awards – Joint Research Projects (2011-2012) and from FAEPEX (University of Campinas). This research was supported by a grant from the Canadian Institutes of Health Research (CIHR) awarded to C.H.G. Graham. The TEG studies were supported by a grant from the Canadian Haemophilia Society awarded to M. Othman.

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Figure

Figure 1. Timeline of exercise protocol. Rats were subjected to an exercise protocol before and during pregnancy. Maximal exercise ability (MEA) was determined to adjust the exercise intensity before pregnancy (four weeks, five days/week with two-day rest) and rats were subjected to the pregnancy exercise protocol with 65% of their MEA during pregnancy (three weeks, five days/week with one-day rest).

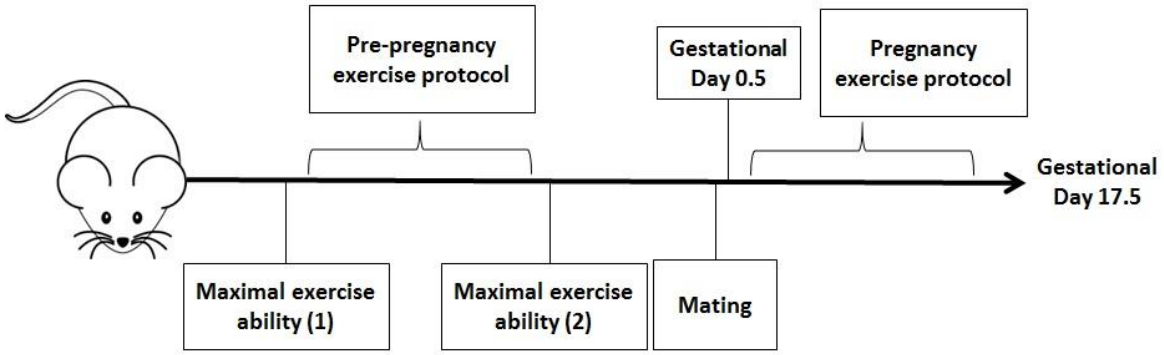

Figure 2: Exercise prevented LPS-induced increases in white blood cell counts measured on gestational day 17.5.

Exercise did not alter red blood cell counts (A), but prevented LPS-induced increases in white blood cell counts measured in blood samples collected on GD 17.5 (B). Exercise significantly abrogated LPS-induced increases in the number of circulating granulocytes (C), lymphocytes (D) and monocytes (E). *p < 0.05; WBC, white blood cell; RBC, red blood cell; gra, granulocyte; lym, lymphocyte; mon. monocyte; Ex+LPS, n = 5; Ex+Saline, n = 5; Se+LPS, n = 11; Se+Saline, n = 10.

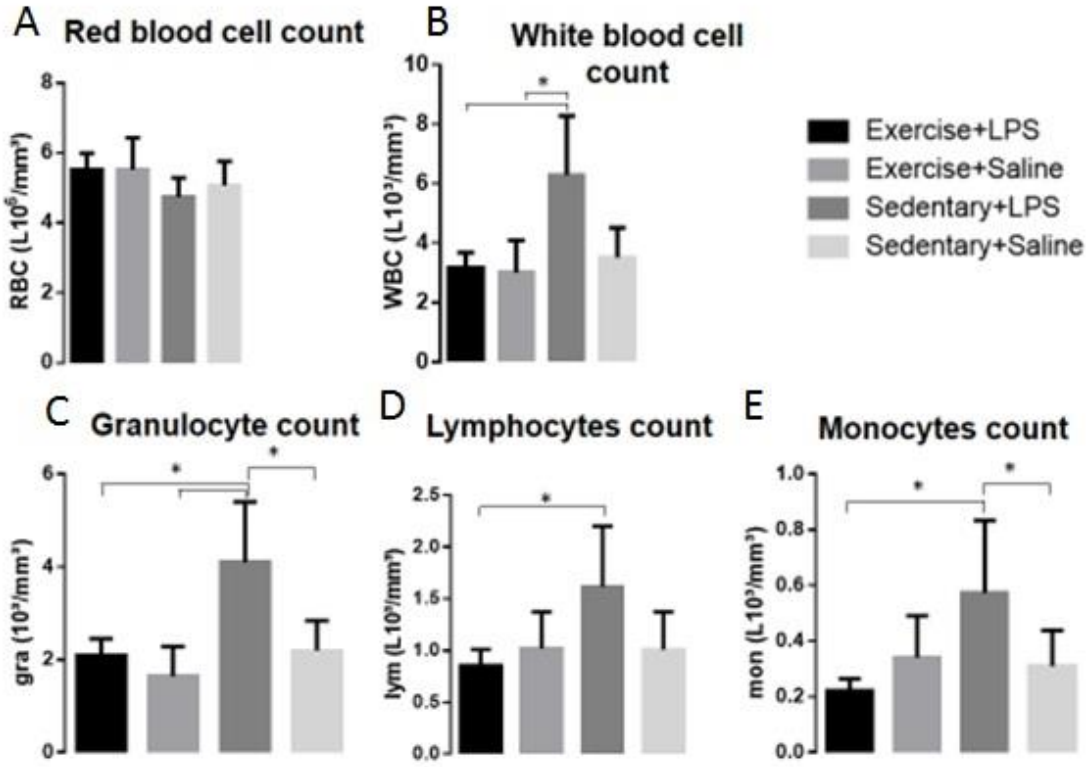

Figure 3. Exercise prevented LPS-induced fetal growth restriction.

LPS significantly reduced fetal weights/litter size in sedentary rats (Se+LPS), whereas fetal weights in exercised rats treated with LPS (Ex+LPS) trended ($p = 0.07$) towards being significantly higher than in the Se+LPS cohort (A). Although the proportion of fetuses that were growth restricted was higher in Se+LPS rats than in Se+Saline rats (Cotechini et al. 2014)[7], exercise did not significantly alter this proportion in the present study (B). FGR: fetal growth restriction. *$p < 0.05$. Ex+LPS (number of pups = 41, litter size varied 5 to 11), Ex+Saline (number of pups = 63; range in litter size = 4 – 19), Se+LPS (number of pups = 258), Se+Saline (number of pups = 305) (A); Ex+LPS (n = 5), Ex+Saline (n = 5), Se+LPS (n = 28), Se+Saline (n = 22) (B).

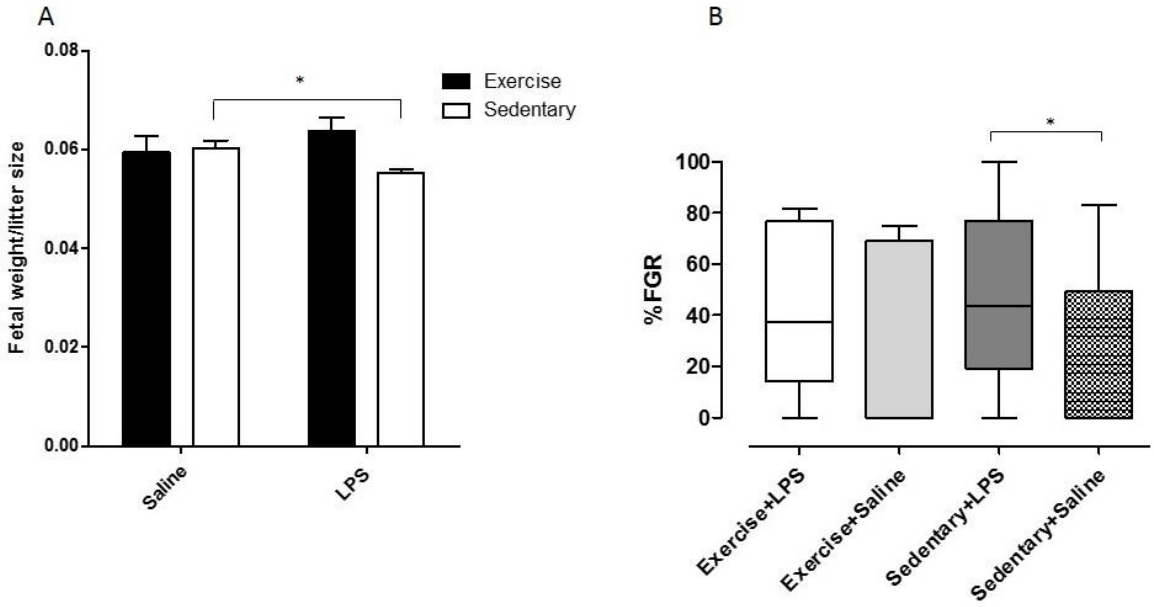

Figure 4. Exercise normalized thromboelastography parameters and tracings.

Representative TEG trace revealing TEG parameters measured from blood collected from a Se+Saline control dam on GD 17.5 (A). TEG tracing from an Ex+Saline dam on GD 17.5 (B). TEG tracing from an Ex+LPS dam on GD 17.5 (C). R, time to clot formation; α, speed of clot propagation; K, rate of clot formation; MA, strength/stability of clot; CI, clotting index; LY30, percent clot dissolution in 30 min (%).

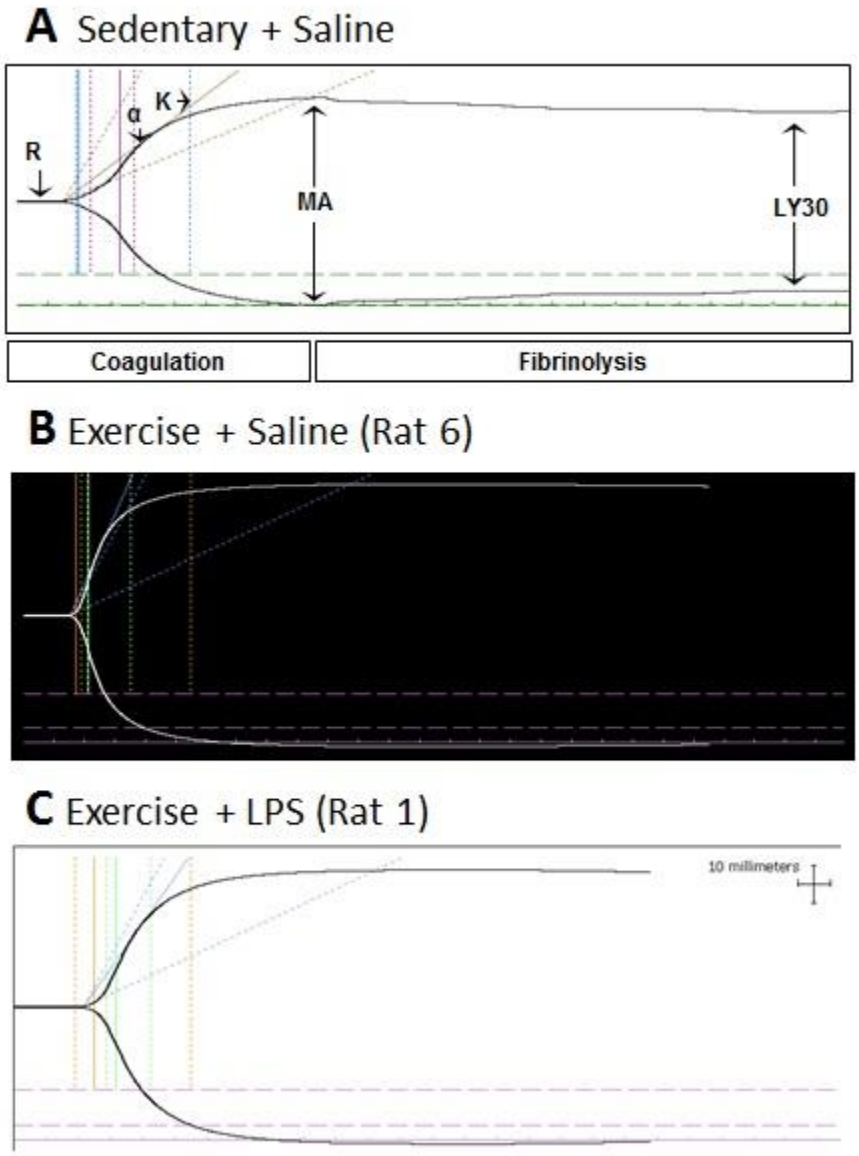

Table 1: Thromboelastography parameters assessed on GD 17.5.

	R	K	α angle	MA	CI	LY30	Coagulopathy[#]
Exercise + LPS	10.78	3.68	50.86	62.7	1.54	0.34	
(n = 5)	(7.5-13.8)	(2.3-7.2)	(31.8-61.5)	(48.1-71.9)	(-1.1-3.1)	(0-1.7)	
Rat 1	10.4	2.8	53.7	71.9	3.1	0.0	Normal
Rat 2	9.9	2.6	56.6	59.0	1.0	0.0	Normal
Rat 3	13.8	7.2	31.8	48.1	-1.1	1.7	Hypocoagulable
Rat 4	12.3	3.5	50.7	71.4	2.6	0.0	Normal
Rat 5	7.5	2.3	61.5	63.1	2.1	0.0	Hypercoagulable
Exercise + Saline	9.8	3.98	46.28	59.55	1.5	1.15	
(n = 4)	(8.6-10.9)	(2.1-8.1)	(29.2-62.9)	(52.8-71.8)	(0.8-3.3)	(0-2.6)	
Rat 6	8.6	2.1	62.9	71.8	3.3	0.0	Normal
Rat 7	10.9	2.8	36.6	56.1	0.8	2.6	Normal
Rat 8	10.8	4.4	42.1	57.5	0.9	2.0	Normal
Rat 9	8.9	8.1	29.2	52.8	1.0	0.0	Hypocoagulable
Sedentary + LPS[a,12]	8.9	2.5	60.6	67.4	3	5.4	
(n = 13)	(2.4-21.5)	(0.8-7.4)	(25.2-78.4)	(36.9-82.3)	(-3.3-5.8)	(0-47.5)	
Sedentary + Saline[a,12]	11.6	3.6	51.6	62.5	1.2	5.1	
(n = 9)	(7.9-18.2)	(1.8-5.9)	(33.2-65)	(56.7-71.5)	(-0.9-3.4)	(0-13.1)	

[a] Historical data from previoulsy published work [12]. All values presented in mean (range of minimum and maximum); R: time for clot formation, α: speed of clot propagation, K: rate of clot formation, MA: strength/Stability of clot, CI: clotting index, LY30: percent of clot dissolution in 30 min (%). [#]Coagulopathy was compared with Sedentary + Saline group.

DISCUSSÃO GERAL

A gestação é um momento especial na vida da mulher, em que são realizadas importantes mudanças no estilo de vida e em geral é recomendada a adoção de hábitos saudáveis que possam proteger e/ou beneficiar o binômio materno-fetal. Quase que invariavelmente se propõe a todas as gestantes, independentes do risco qualificado, uma alimentação equilibrada e a prática regular de exercícios físicos orientados e preferencialmente supervisionados. Admite-se que, a continuidade de hábitos de vida saudável durante a gravidez e no período pós-parto poderia promover benefícios a longo prazo, como controle do peso, prevenção de doenças crônicas, como hipertensão ou diabetes mellitus e o benefício suplementar de incorporação de atitudes de interesse para a saúde como um todo para toda a vida.

Neste sentido, os períodos gestacionais e puerperal configuram-se num contexto essencial para a saúde pública, por oportunizar em momento de alta motivação pessoal, a adoção ou o reforço de atitudes, práticas e comportamentos, que podem concorrer para reduzir comorbidades que podem estar associadas à desfechos desfavoráveis durante a gestação, como ocorrem frequentemente com as síndromes hipertensivas.

Dentre os diversos guidelines de exercícios físicos durante a gestação e período puerperal, que visam orientar as recomendações seguras e apropriadas para serem adotadas, as síndromes hipertensivas específicas ou crônicas encontram-se como contraindicação relativa para a indicação do mesmo no

período gravídico. Há uma escassez importante de evidência científica para embasamento com relação ao tipo, duração, frequência e intensidade a ser recomendado de exercício físico para essa população.

Desta maneira, os estudos translacionais configuram a possibilidade de compreender os mecanismos de ação do exercício físico na gestação de alto risco associado às repercussões maternas e fetais desfavoráveis. Estes estudos, descritos e frequentemente realizados pela ciência básica e a pesquisa clínica, são capazes de direcionar conduta e gerar grau de recomendação para diretrizes/guidelines utilizados na prática clínica.

Neste estudo observamos os benefícios do exercício físico na gestação de ratas induzidas à inflamação, sendo que o exercício promoveu incremento imunológico avaliado pela contagem de células brancas e normalização no padrão de coagulação materna. No entanto, a curto prazo o exercício foi incapaz de reverter uma inflamação generalizada induzida que favoreceu a restrição de crescimento fetal, divergindo dos resultados observados em outros estudos por administração de substâncias anti-inflamatórias.

O exercício físico vem sendo recomendado como um poderoso aliado na prevenção de doenças inflamatórias, sendo capaz de promover, a longo prazo, respostas imunológicas favoráveis, principalmente para a proteção de doenças crônicas como o câncer.

Entretanto, vale ressaltar que de acordo com a literatura revisada, os ensaios clínicos com gestantes de alto risco para as síndromes hipertensivas são

limitados e ainda inconclusivos. Desta maneira, o exercício físico e a mudança de estilo de vida devem ser estimulados com prudência visando benefícios a longo prazo, de modo a prevenir doenças inflamatórias, estimulando a resposta imunológica e beneficiando a saúde geral dessas mulheres.

O período gestacional é caracterizado como um período ideal para mudanças positivas no estilo de vida das mulheres, no entanto, essas devem ser incentivadas à adoção de hábitos de vida saudável ainda no momento pré-gestacional, reduzindo assim riscos relacionados ao ciclo gravídico-puerperal. Neste sentido, políticas de saúde pública devem agregar os exercícios físicos como fator de prevenção de doenças inflamatórias e crônicas, principalmente em mulheres em idade reprodutiva.

CONCLUSÕES

Modelos animais são importantes e úteis para a compreensão do impacto do exercício físico na gestação de alto risco, em especial naquelas associadas às desordens hipertensivas. Estudos experimentais devem ser interpretados com cautela e quando reproduzidos em humanos, devem ter controle rigoroso. A conexão entre as evidências da ciência básica com pesquisas clínicas deve estar de acordo com a prática clínica baseada em evidência, sendo esses os principais fatores a se considerar quando da indicação do exercício físico na gestação com alto risco.

O exercício físico antes e durante a gestação de alto risco no estudo experimental com inflamação induzida pelo LPS mostrou redução na contagem de células brancas, não alterou o peso fetal e reverteu alterações laboratoriais da coagulação induzidas pela inflamação na amostra avaliada.

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ANEXOS

9.1. ANEXO 1 – Carta de Aprovação do Comitê de Ética em Pesquisa

From: <uacc@queensu.ca>

Subject: Approval Notice for Amendment Graham-2010-012-R3-A8

Date: 17 January, 2014 11:07:46 AM EST

To: <sm23@queensu.ca>, <grahamc@queensu.ca>, <uvet.compliance@queensu.ca>, <uacc@queensu.ca>, <johnstns@queensu.ca>, <Kathy.Williams@queensu.ca>, <joan.tremblay@queensu.ca>

Your Animal Care Protocol Amendment Graham-2010-012-R3-A8

Title: The effect of an activated immune response on pregnancy complications in the rat

has now been approved by the Queen's University Animal Care Committee.

Expiry Date: 3/15/2014

Authorized Amount:
Conventional (Rat) #1
B Category: 24
C Category: 22
D Category: 241

You may now continue your study. If you have any questions please contact the UACC Coordinator.

Thank You

Office of University Animal Care Committee
Natalie Kolomeitz-Douglas X 78805
uacc@queensu.ca
http://www.queensu.ca/uvet

9.2. ANEXO 2 – Certificação de manipulação de pequenos animais

Office of the University Veterinarian
251 Richardson Hall
74 University Avenue
Kingston, Ontario
K7L 3N6
Tel (613) 533-6000 ext. 79013
Fax (613)-533-3278
uvet.compliance@queensu.ca

March 14th, 2013

Dr. Charles Graham
Professor, Department of Biomedical and Molecular Sciences
Botterell Hall 859
Queen's University
Kingston, Ontario K7L 3N6

Dear Dr. Graham:

Re: Karina Kasawara Practical Workshop Training

I would like to confirm Karina Kasawara attended Workshop #1 (Small Animal Handling and Routine Procedures) and Workshop #2 (Small Animal Technical Procedures). The dates were March 12th and 14th, 2013. As a follow up to hosting these sessions, I am responsible for providing feedback to the Principal Investigator.

Karina was a pleasure to work with – she was punctual, listened attentively and was eager to learn.

We had a brief overview of the regulations and requirements regarding animal research in Canada, and the role of the UACC. We then discussed Topaz, Granite, the UACC SOP's, and the importance of familiarizing oneself with the protocol(s) under which you are associated. Skills we covered included a basic health assessment, restraint, subcutaneous injections, intraperitoneal injections, blood collection – cardiac puncture, tail vein and saphenous, substance administration – iv, anesthesia, monitoring vitals, euthanasia – overdose, bilateral pneumothorax and exsanguination.

I look forward to seeing Karina in the near future at the post-training review (once she starts her animal work), and will advise ACS that she can proceed with setting up a facility tour through Joan Tremblay (joan.tremblay@queensu.ca). This will allow access to the animal quarters.

Please let me know if you have any questions or concerns.

Sincerely,

Erin Burnett
Quality Assurance and Training Coordinator