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EDITORIAL

Developmental Origin of Health and Diseases (DOHaD) Concept and Brazil

Two decades ago sustained by epidemiological and experimental data emerge ideas that malnourishment during pregnancy has a link with low bird weight, which is associated to high risk to developing cardiometabolic diseases later in life. David Barker, an epidemiologist, and Charles Nicholas (Nick) Hales, a biochemist, gave the basis to build the developmental origins of health and disease (DOHaD) concept. Large prevalence of metabolic diseases worldwide must consider DOHaD as firing to justify, at least in part, the obesity pandemic, for example. Not only food restriction but also overfeeding as many other insults (smoke, pesticides, toxic substances, general urban pollution, among other stressful conditions) during pregnancy is inductor programming factors. Pregnancy is not the unique "window" to metabolic programming; other windows have been found, such as lactation, adolescence and young adulthood phases. Eventhough there are some experimental evidences that before the conception future father and mother should be target to programming the children/ adult phenotype. This programming is conduct by not genetic heritage but by epigenetic causes. Recently it was recorded that pancreatic beta-cell malfunctions in high-fat-diet-obese rat male were transmitted to offspring gestated in lean mother. Two major factors are crucial to understanding DOHaD concept: development phase of life and environmental. It has been shown that environmental aggression during neural brain connections onset can change functions of periphery tissues such as metabolism, which dysfunction persist later in life, showing obesity, type 2 diabetes, hypertension, stroke, and many other non-communicable diseases. Luckily, yet with few evidences it has also been shown that nutritional corrections can prevent and even treat these diseases caused by early programming. However, that evidences are not enough in number and quality to convincing our public health authorities and health personal to do intervention to reduce the metabolic syndrome pandemic. A big step to highlight and to explore DOHaD concept was made 25 years ago with creation of International Society for Developmental Origins of Health and Disease

Next November, from 16 to 20, in Singapore will be held the 8th Word Congress on the DOHaD, which will be discussed new data and efforts to put in evidence that considering DOHaD concept lives could be save and our life quality should be better improved.

Brazilian scientists, in clinic, epidemiology and experimental side, since two decades ago have gave considerable data contribution to test the DOHaD concept. Brazil is a continental country and has also continental big economic and health problem to manage diseases, including non-communicable ones. Like Japan, China and France, our country must to create DOHaD society section to bring attention over DOHaD concept to whom are responsible to do science and to plane our public health polices, as well as to whom take care of our lives.

To help a little bit in the next September 25-28, in Morretes, PR, will be held the 3rd International Symposium on Metabolic Programming and Stress (3ISMPS). Like the precedents, this meeting will received international and Brazilian epidemiologists, biochemists, biologists, physicians, nurses, pharmaceutics among others health personnel, as well as other professionals and students that produce scientific data and thinking about DOHaD concept as a window to save lives. The organization committee of 3ISMPS is hard working to develop the idea to create a Brazilian section of DOHaD

association. May be the Morretes´ meeting will be the first step. Whiling, with this taffy duty 3ISMPS invites you, readers of ***Endocrinologia & Diabetes Clínica e Experimental***, to come to nice touristic Morretes city to enjoy with nature and also enjoy to discussions about DOHaD concept. Please do not forget to spread the invitation to your colleagues and students, because will be a great pleasure to receive you in adorable scenario to advance our knowledge in endocrinology & diabetes clinics and experimental.

To further information about 3ISMPS go to http://www.dbc.uem.br/3Simp2013.htm

See you in September

Paulo Cezar de Freitas Mathias

Head of Obesity and Diabetes Studies Centre of Universidade Estadual de Maringá

Editor in Chief of Endocrinology and Experimental Diabetes

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Sumário

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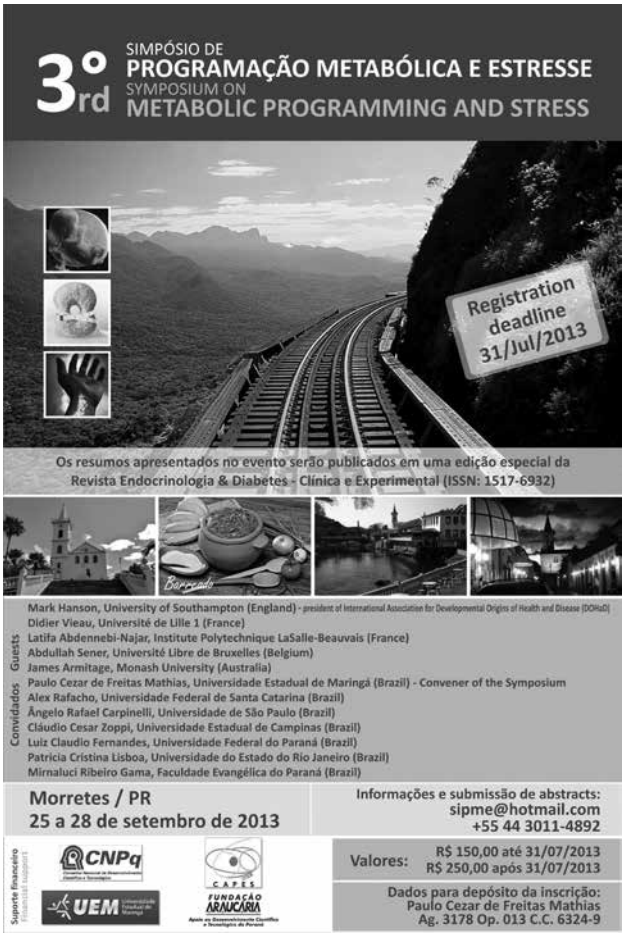

CONTRIBUIÇÃO ORIGINAL

ESTATINA: BENEFÍCIO X RISCO NA HOMEOSTASE DA GLICOSE

STATIN: BENEFIT X RISK IN GLUCOSE HOMEOSTASIS

JULIANA YUKA ARAI*

Descritores: Estatina, Diabetes mellitus, Colesterol
Keywords: Statins, Diabetes mellitus, Cholesterol

Resumo

As estatinas estão sendo cada vez mais usadas para a prevenção primária e secundária de eventos cardiovasculares em pacientes de alto risco. Contudo, muitos estudos apontam a associação do uso de estatinas com o desenvolvimento de diabetes. O presente estudo visa a comprovar o benefício sobre o risco com o uso dessa medicação. **Endocrinol diabetes clin exp 2013; 1571-1574.**

Abstract

Statins are being increasingly used for primary and secondary prevention of cardiovascular events in high risk patients. However, many studies indicate the association between the use of statins and development of diabetes. The objective of this report is to demonstrate the benefit over the risk of this medication. **Endocrinol diabetes clin exp 2013; 1571-1574.**

INTRODUÇÃO

As estatinas são medicamentos amplamente utilizados para a prevenção de infarto agudo do miocárdio (IAM), acidentes vasculares encefálicos (AVE) e mortes por causas cardiovasculares em pacientes com doença vascular, diabetes e dislipidemia (1,2,3). Entretanto, muitos estudos têm mostrado um elevado risco de desenvolvimento de diabetes com o uso dessa medicação. Com isso, muito se tem questionado quanto à possibilidade, ou não, dos danos potenciais do diabetes superarem os benefícios da terapia com a estatina.

A ASSOCIAÇÃO COM A PROTEÍNA C REATIVA (PCR) E O EFEITO CARDIOPROTETOR

O mecanismo de ação das estatinas está relacionado com a inibição da enzima HMG-CoA redutase que leva a um aumento na depuração das lipoproteínas de densidade baixa (LDL) através do aumento da síntese de receptores de LDL nas células (4).

O papel do LDL-colesterol na aterogênese já está bem documentado na literatura (5,6). Contudo, segundo Ross, a fisiopatologia da aterosclerose vai muito mais além do que o simples depósito de LDL- colesterol na camada subendotelial da parede arterial, envolvendo também mecanismos inflamatórios agravantes no dano endotelial (7). É nesse contexto que a dosagem da proteína C reativa (PCR) é considerada um biomarcador de inflamação e portanto de eventos cardiovasculares futuros, independentemente dos níveis de LDL-colesterol (8,9,10).

Estudos mostram que a estatina tem efeitos antiinflamatórios diretos denominados pleiotróficos. A redução dos níveis séricos de PCR está relacionada com a redução dos macrófagos dentro da placa aterosclerótica vulnerável (11), supressão da expressão das metaloproteinases envolvidas na dissolução capa fibrosa da placa aterosclerótica (12), aumento da biodisponibilidade do óxido nítrico o que promve a reendotelização, reduz o estresse oxidativo e diminui a inflamação. Além disso promove a inibição das moléculas de adesão que são essenciais para a ligação de monócitos na parede endotelial (13).

O estudo *PRINCE* randomizou 1702 pacientes sem doença cardiovascular prévia, sendo que 865 pacientes receberam pravastatina 40 mg/dia, e 867 receberam placebo por 24 semanas (14). O que se observou foi a redução média de 16,9% (p< 0,001) do nível de PCR no grupo com estatina, refletindo um decréscimo de 0,02 mg/dl, enquanto que no grupo placebo não houve alteração dos seus níveis. Além disso, o estudo mostrou que a redução dos níveis de PCR não guarda correlação com os níveis de LDL, o que já havia sido confirmado no estudo *CARE* (15). Contudo, o estudo *PRINCE* não avaliou o impacto da redução dos níveis de PCR sobre a incidência dos eventos cardiovasculares, mas concluiu que a Pravastatina exerce um efeito anti-inflamatório, reduzindo os níveis de PCR.

O estudo *JUPITER* foi um estudo randomizado, duplo-cego e prospectivo, que tem particular importância, uma vez que é o maior estudo de prevenção primária com estatinas até a presente data. Este tinha como um dos seus objetivos mostrar a benefício do uso da estatina em pessoas com níveis elevados de PCR e sem dislipidemia (16). Foram randomizados 17.802 pacientes saudáveis com LDL-colesterol <130 mg/dl e níveis de PCR ≥ 2 mg/dl, sendo 8.901 no grupo com rosuvastatina 20 mg/dia e 8.901 no grupo placebo. Estes pacientes foram acompanhados durante uma média de 1,9 anos (máximo de 5 anos) para avaliar a ocorrência de eventos cardiovasculares (como IAM, AVE, revascularização arterial e internamentos por angina instável) ou qualquer morte por causa cardiovascular.

O resultado do estudo mostrou uma redução dos níveis de LDL-colesterol em aproximadamente 50%, e dos níveis de PCR em 37% no grupo com rosuvastatina. Quanto ao benefício da estatina na prevenção primária de eventos cardiovasculares, foi observada a ocorrência de 142 primeiros eventos cardiovasculares no grupo com estatina, enquanto que o grupo placebo totalizou 251 eventos. Isso corresponde a 0,77 e 1,36 por 100 pessoas/ano no grupo com hipolipemiante e no grupo placebo respectivamente (OR para rosuvastatina, 0,56; intervalo de confiança (IC): 95%; p < 0,00001). Analisando separadamente os eventos cardiovasculares, observaram-se as seguintes taxas no grupo com rosuvastatina e placebo respectivamente: 0,17 e 0,37 para IAM (OR: 0,46; IC: 95%; p= 0,0002), 0,18 e 0,34 para AVE (OR: 0,52; IC: 95%; p=0,002), 0,41 e 0,77 para revascularização do miocárdio e angina instável (OR: 0.53; IC: 0,40; p< 0,00001) e 1,00 e 1,25 para mortes de qualquer causa (OR: 0,53; IC: 95%; p= 0,02).

Quanto à ocorrência de tromboembolismo venoso nesse mesmo estudo, foi observado em 94 participantes, sendo 34 no grupo com rosuvastatina e 60 no grupo placebo (17). As taxas para tromboembolismo pulmonar (TEP) foram de 0,09 no grupo controle e de 0,12 no grupo placebo (OR: 0,77; IC: 95%; p= 0,03), enquanto que para o tromboembolismo venoso profundo (TVP) foram de 0,09 e 0,20 respectivamente (OR: 0,45; IC: 95%; p= 0,004).

O estudo *JUPITER*, portanto, concluiu que o uso de estatina em pessoas saudáveis sem dislipidemia, mas com níveis elevados de PCR, reduziu significativamente o risco de eventos cardiovasculares maiores e a ocorrência de tromboembolismo venoso sintomático. Esses efeitos foram consistentes em todos os subgrupos analisados, incluindo aqueles com escore de *Framingham* (18) menor que 10%, aqueles com LDL <100 mg/

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dl, pacientes com PCR > 2mg/dl sem nenhum outro fator de risco identificado e pacientes sem síndrome metabólica (19).

Uma metanálise do grupo *Cholesterol Treatment Trialistis* (CTT) com 14 trials randomizados com estatina, por sua vez, mostrou a importância desse grupo de medicamento na redução dos níveis de LDL-colesterol e sua repercussão sobre o risco cardiovascular (20). *Baigent* e col observaram uma queda de 20% no risco cardiovascular para cada redução de 1 mmol/L (38,7 mg/dl) no nível de LDL-colesterol. O estudo também mostrou uma redução proporcional de 10% dos eventos cardiovasculares para cada redução de 1 mmol/L no primeiro ano de uso da estatina, enquanto que houve reduções maiores (20-30% por mmol/L) para cada ano de tratamento. Assim, concluiu-se que é necessário um tratamento prolongado com estatina em pacientes com elevado risco cardiovascular ou naqueles com doença cardiovascular estabelecida. Altas doses de estatinas também estão relacionadas com os mesmos efeitos cardioprotetores observados nos estudos mencionados (21,22). Os dados de *Preiss* e cols indicam uma redução de 16% (OR: 0,84; IC 95% 0,75- 0,94) de eventos cardiovasculares em pacientes tratados com dose intensiva, quando comparados com doses terapêuticas moderadas (23).

ESTATINA E DIABETES

Muitos estudos têm mostrado a elevação na incidência de diabetes nos pacientes em uso de estatinas. Entretanto, o mecanismo molecular que explica essa associação ainda é desconhecido.

Sabe-se que a insulina estimula a captação de glicose pelos adipócitos através da ativação da tirosina quinase dos receptores de insulina. Isso leva à um aumento da expressão dos transportadores de glicose 4 (GLUT4) portanto uma maior captação periférica da glicose (24). Baseado nisso, *Nakata* e cols observaram uma redução na expressão do GLUT4 em ratos tratados com altas doses de atorvastatina, o que levou à inibição da maturação dos adipócitos e à intolerância à glicose (25).

Narayanan e cols explicaram a associação da estatina com o diabetes observando a redução dos níveis de IGF1, IGF2 .O fator de crescimento insulina símile - insulin like growth factor - (IGF1), é um fator de crescimento celular *like* insulina e a redução de seus níveis plasmáticos está envolvida na fisiopatologia do diabetes. Evidências mostram que o IGF1 protege o endotélio impedindo o desenvolvimento e a fragilidade da placa aterosclerótica, além de aumentar a sensibilidade à insulina exercendo efeitos hipoglicêmicos (26).

Estudos têm mostrado que o IGF1 baixo em indivíduos com idade menor de 65 anos tem forte valor preditivo para diabetes, porém esta associação desaparece com a idade > 65 anos. A BP3 (binding protein) funciona como reservatório para o IGF1, portanto a IGDBP3 sequestra o IGF1 inibindo sua atividade, reduzindo a fração livre do IGF1. Esta atividade aumentada da IGFBP3 estaria elevada em diabéticos jovens. Esta é uma das explicações a respeito da implicação do IGF1 (25,26,27).

A ação hipoglicemiante do IGF1 é exercida através da ativação da PI3 (*phosphatidyl inositol* 3-kinase) que por sua vez aumenta a expressão da AKT e consequente formação do NO (óxido nítrico) o qual exerce ação benéfica no endotélio, aumenta os transportadores de glicose no músculo e adipócitos (26,27). A diminuição do IGF1 levaria a um desequilíbrio (*up regulation*) entre os receptores híbridos de insulina I/IGF1. Isto acontece porque estes dois hormônios podem ocupar receptores um do outro e a desestabilização criaria uma situação de resistência à insulina. Outra explicação para a resistência à insulina pela diminuição do IGF1 seria a falta da retroação negativa do IGF1 sobre o hipotálamo – hipófise com aumento da produção do hormônio do crescimento que se sabe ser fortemente antagônico à insulina.

Um estudo mostrou efeitos benéficos com o uso da fluvastatina em ratos alimentados com frutose e intolerantes à glicose. A fluvastatina aumentou o IGF1 que por sua vez aumentou a sensibilidade hepática e dos adipócitos à insulina (27).
No entanto a fluvastatina neste ensaio mostrou não ser redutora do GH e novas pesquisas deverão ser feitas para comprovar se esta ação maléfica ou neutra é inerente a todas as estatinas (27).

Apesar de ser necessário mais estudos para comprovar a fisiopatogenia das estatinas quanto à incidência de diabetes, o FDA (*Food and Drug Administration*) anunciou em fevereiro de 2012 importantes advertências de segurança sobre esse grupo de medicamentos, alertando para uma possível associação entre as estatinas e o aumento da hemoglobina glicada (HbA1c) e da glicemia em jejum (28). Com isso, muito se tem questionado sobre o uso indiscriminado dessa medicação.

O FDA baseou-se no estudo *JUPITER (Justification for the use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin*) (29) para justificar as mudanças nas bulas de segurança (28). Neste estudo, observou-se a incidência de 270 casos de diabetes no grupo em uso de rosuvastatina 20mg/dia, em comparação com 216 casos no grupo placebo (OR: 1,25; IC: 95% 1,05- 1,49, p= 0,01). Isso corresponde a um aumento de 26% no relato médico de diabetes de início recente em pacientes que receberam a medicação.

Embora a incidência de diabetes tenha sido surpreendente no estudo *JUPITER*, algumas considerações devem ser feitas, o que limita a generalização do estudo (29). Em primeiro lugar, os indivíduos elegíveis eram descritos como “aparentemente saudáveis”, no entanto, 65% dos participantes apresentavam pelo menos um fator de risco maior para diabetes, como síndrome metabólica de base, glicemia de jejum alterada (glicemia de jejum entre 100 e 126 mg/dl) (30), índice de massa corporal (IMC) ≥ 30 kg/m2 ou hemoglobina glicada (HbA1c) ≥ 6%. Os demais participantes não apresentavam nenhum fator de risco para diabetes. Além disso, a HbA1c inicial média em ambos os braços deste ensaio clinico foi de 5,7% - nível que a Associação Americana de Diabetes (ADA) utiliza para classificar os indivíduos como pré-diabeticos (30). Assim, a população do estudo *JUPITER* apresentava um alto risco para desenvolver diabetes, independente de terem ou não recebido tratamento com estatina.

Em segundo lugar, deve-se levar em consideração também que não houve um protocolo padronizado para o diagnóstico de novos casos de diabetes, sendo válidos os casos relatados por médicos e aqueles confirmados laboratorialmente. Isso poderia explicar a diferença nas taxas de surgimento de diabetes nos dois braços do estudo (29).

Portanto, devido a essas limitações no desenho do estudo, os dados do *JUPITER* indicam a associação entre rosuvastatina e surgimento de diabetes, mas não demonstram causalidade.

Uma metanálise posterior de *Sattar* e cols (31), em 2010, procurou confirmar a associação entre o surgimento de diabetes e a terapia com estatina. Foram reunidos 13 ensaios clínicos randomizados, totalizando 91.140 pacientes. O que se observou foi um risco de 9% maior de desenvolver diabetes (OR: 1,09; IC: 95%; 1,02-1,17) no grupo tratado com estatinas, com pouca heterogeneidade entre os ensaios. Assim notaram que, para provocar um novo caso de diabetes, é necessário tratar 255 pacientes com estatina durante 4 anos.

Dos 13 ensaios clínicos randomizados, 6 deles (HPS, *JUPITER, CORONA, MEGA, AFCAPS/ TexCAPS, 4S)* basearam-se parcial ou totalmente em dados de relatos médicos não-padronizados como meio de determinação de diabetes incidental. Contudo, quando os investigadores analisaram apenas os estudos que utilizaram medidas pré-especificadas de glicemia de jejum para o diagnóstico de diabetes, o risco de desenvolvimento de diabetes foi atenuado e não atingiu significância estatística (OR: 1,07; IC: 95%, 0,97-1,17). Assim como no estudo *JUPITER*, a metanálise de *Sattar* e cols mos-

trou uma associação entre diabetes e o uso de estatina, mas não provou causalidade.

Questiona-se também a relação entre dose das estatinas com o risco de hiperglicemia. O estudo *PROVE-IT TIMI 22* (32) foi o primeiro estudo que avaliou a equivalência entre duas estatinas diferentes. Foram randomizados 4.612 indivíduos com síndrome coronariana aguda para o tratamento com dose moderada de estatina (pravastatina 40 mg/dia) ou dose intensiva (atorvastatina 80 mg/dia). O resultado foi um aumento de 0,37% nos valores de HbA1c em pacientes tratados com atorvastatina, quando comparados com um aumento de 0,18% naqueles que usaram a pravastatina. Os pacientes tratados com atorvastatina também tiveram um risco aumentado de desenvolver uma HbA1c > 6% em comparação com o grupo pravastatina (RR: 1,84; IC: 95% 1,52-2,22).

Posteriormente, uma metanálise com 5 ensaios clínicos randomizados também mostrou um risco de 12% maior (OR: 1,12; IC:95% 1,04-1,22) de surgimento de diabetes associado com doses maiores de estatina, em comparação com doses moderadas (23).

Em 2011, *Waters* e cols também reforçaram a associação entre doses elevadas de estatina e a incidência de diabetes através do estudo de 3 ensaios com altas doses de atorvastatina (33). Dois dos três ensaios, *TNT* (34) e *IDEAL* (35), compararam atorvastatina 80 mg/dia com doses terapêuticas moderadas (atorvastatina 10 mg/d e sinvastatina 20 mg/dia, respectivamente), enquanto que o terceiro estudo (*SPARCL*) comparou atorvastatina 80 mg/dia com placebo (36). Nos dois primeiros estudos, observou-se um aumento de diabetes de início recente associado com altas doses de estatina, em comparação com doses moderadas (para o *TNT*: OR: 1,10; IC: 95% 0,94-1,29; para o *IDEAL*: OR: 1,19; IC: 95% 0,98-1,43). O estudo *SPARCL* também mostrou um aumento significativo no surgimento de diabetes quando comparado com o placebo (OR: 1,37; IC: 95% 1,08-1,75).

O estudo de *Waters* e cols, portanto, apenas reiterou o que os demais estudos haviam concluído quanto à associação entre dose de estatina e a ocorrência de diabetes. No entanto, o que torna esse estudo especial foi a identificação de 4 preditores independentes para o surgimento de diabetes: glicemia de jejum alterada, obesidade, hipertrigliceridemia e hipertensão arterial.

Os pacientes que apresentaram um ou nenhum preditor, o risco de desenvolver diabetes foi relativamente baixo e não houve diferença estatística entre o grupo com altas doses de estatina e o grupo com moderadas doses. Já os pacientes com 3 ou mais desses fatores de risco apresentaram um aumento de casos de diabetes em ambos os grupos, sendo mais importante no grupo com elevadas doses de atorvastatina. Com isso, comprovou-se o papel da síndrome metabólica no desenvolvimento de diabetes (37).

Apesar de todos os estudos e metanálises ainda é desconhecida a relação entre estatina e desencadeamento do diabetes. A discussão sobre dose e tipo de estatinas lipofílicas ou hidrofílicas também não foi elucidado. Alguns estudos apontam efeito diabetogênico em estatinas lipofílicas como a sinvastatina e que este efeito seria atenuado se fosse usada estatina hidrofílica tipo pravastatina, fluvastatina ou rosuvastatina. E o JUPITER que fez uso da rosuvastatina? Portanto vê-se que este tipo de atividade inerentes à determinadas estatinas provavelmente também não é a culpada do efeito diabetogênico. Pequenos estudos têm mostrado que a associação do ezetemibe diminui a insulinemia e melhora a resistência à insulina através da medida do HOMAr (38).

Outro possível mecanismo mais recente de inibição da secreção da insulina pelas estatinas é a diminuição de produtos decorrentes do ciclo do mevalonato como isoprenoids farnesyl pyrophosphate (FPP) ou geranylgeranylpyrophosphate (GGPP). Estes isoprenóides estão envolvidos na regulação dos transportadores de glicose –GLUT4- na membrana celular dos adipócitos (39). Em adição a este mecanismo as estatinas por diminuírem o 3-hydroxy-3-methylglutaryl-coenzyme A (HMG--CoA) reductase reduziriam a atividade das pequenas proteínas ligadoras, as GTPs binding protein, grandes coadjuvantes da secreção de insulina (40). Outros estudos ainda implicam as estatinas lipofílicas vilãs pelo bloqueio dos canais de cálcio L-type Ca2+ responsáveis pela indução da secreção de insulina (41).

Em um estudo publicado recentemente foi mostrado que a rosuvastatina foi única estatina cujo risco diabetogênico aumentado é dose dependente. Neste estudo mostrou-se que a atorvastatina tinha um risco de 22%, a rosuvastatina de 18% a sinvastatina de 10%, fluvastatina de 5% e a lovastatina de 1% quando comparadas a 0% de risco da pravastatina no desencadeamento do diabetes (42).

O RISCO CARDIOVASCULAR DA DIABETES X O BENEFÍCIO DA ESTATINA

A essa altura deste artigo pergunta-se: será que os riscos cardiovasculares da diabetes superam os benefícios da estatina? Até o momento não parece haver um aumento da mortalidade ou de eventos cardiovasculares em pacientes que desenvolveram diabetes com o uso de estatina.

No estudo de *Waters* e cols (33), os autores mostraram que os eventos cardiovasculares maiores ocorreram em 11,3% dos pacientes que desenvolveram diabetes e em 10,8% daqueles que não desenvolveram esta comorbidade (OR: 1,02; IC: 95% 0,77- 1,35), ou seja, não houve diferença estatística significante. Assim como não houve diferença ao se comparar a incidência de eventos cardiovasculares entre os pacientes do grupo atorvastatina 80 mg/dia que desenvolveram diabetes (10,1%) com aqueles que não apresentaram diabetes (10,0%).

Além disso, *Waters* e cols observaram que aqueles pacientes que desenvolveram diabetes com o uso de estatina apresentaram uma menor taxa de eventos cardiovasculares em comparação com aqueles que já eram diabéticos no início do estudo (10,1% e 17,5%, respectivamente).

CONCLUSÃO

É inegável a associação entre o desenvolvimento de diabetes e o uso de estatinas. No entanto, não há provas que demonstram que o desenvolvimento de diabetes secundário ao uso de estatina resulte em risco aumentado de eventos cardiovasculares. Além disso, devemos questionar se o desenvolvimento da diabetes é uma progressão natural da disglicemia, uma vez que muitos pacientes já apresentam algum grau de intolerância à glicose, ou é decorrente de alguma propriedade da estatina ainda não conhecida.

O que está bem esclarecido, entretanto, é o benefício das estatinas na prevenção primária e secundária de eventos cardiovasculares em pacientes de alto risco e na redução da morbidade e da mortalidade (37). Assim, o benefício das estatinas ainda superam seus riscos.

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ARTIGO ORIGINAL

PREVALÊNCIA DA SÍNDROME DE *SJÖGREN* SECUNDÁRIA EM PACIENTES COM ESCLERODERMIA

PREVALENCE OF SECONDARY SJÖGREN SYNDROME IN PATIENTS WITH SCLERODERMA

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Descritores: Esclerodermia limitada, esclerodermia difusa, síndrome de *Sjögren*.
Key words: Scleroderma limited, scleroderma diffuse, Sjögren's syndrome.

Resumo

Justificativa: A ocorrência de Síndrome *Sjögren* secundária (SS) é bem estudada no contexto da artrite reumatóide e do lúpus eritematoso sistêmico. Todavia a sua associação com esclerodermia é menos conhecida e não existem dados brasileiros a respeito de sua prevalência. **Objetivos:** Observar a prevalência de SS em pacientes com esclerodermia na população local, avaliar possíveis diferenças no perfil de envolvimento de órgãos sistêmicos e de auto-anticorpos entre pacientes com esclerodermia associada ou não à SS. **Métodos:** Foi realizado um estudo transversal observacional no ambulatório de Reumatologia do HUEC entre todos os pacientes com esclerodermia cadastrados nesse serviço. Foram coletados dados demográficos, de envolvimento cutâneo e sistêmico secundários à esclerodermia, perfil de auto-anticorpos e presença de SS. **Resultados:** Foram incluídos 48 pacientes sendo 10.41% homens e 89,58% mulheres, com idade média de 47,6 ±13,21 anos e tempo médio de doença 8,29 ±6,56 anos. Em 20,83% a forma era difusa, 14,58% de esclerodermia tipo "overlap" e 64,58% da forma limitada. A prevalência de SS secundária foi de 10,66%. Houve relação significativa entre o tempo de evolução da esclerodermia e a presença de SS (p=0,0166). Não houve associação da presença dos anticorpos ou achados clínicos estudados com a presença de SS. **Conclusão:** A SS secundária aparece em 10.6% dos pacientes com esclerodermia, principalmente naqueles com maior tempo de doença. Não existem diferenças no envolvimento de órgãos sistêmicos ou de anticorpos naqueles com e sem SS secundária. **Endocrinol diabetes clin exp 2013; 1575-1578.**

Abstract

Background: The occurrence of secondary Sjögren's syndrome (SS) is well studied in the context of rheumatoid arthritis and systemic lupus erythematosus. However its association with scleroderma is less known and there are no Brazilian data about its prevalence. **Objectives:** To observe the prevalence of SS in patients with scleroderma of the local population; to assess possible differences in the profile of organ involvement and scleroderma auto antibodies between patients with and without SS. **Methods:** We conducted a cross-sectional observational study at the HUEC Rheumatology clinic among all patients with scleroderma enrolled in the clinic. It was collected demographic data, cutaneous involvement secondary to systemic scleroderma, autoantibody profile and presence of SS. **Results:** We included 48 patients: 10.41% men and 89.58% women with mean age of 47.6± 13.21 years and mean disease duration of 8.29±6.56 years. In 20.83% the form was diffuse, 14.58% of overlap type and 64.58% was limited. It was found that 43/38 had Raynaud, 27/48 had arthralgia, 27/48 had esophageal involvement and 19/48 had myalgias. The prevalence of secondary SS was 10.66%. No associations were found between SS presence and clinical or autoantibodies profile. **Conclusion:** The secondary SS appears in 10.6% of local patients with scleroderma, especially in those with longer disease duration. There are no differences in the involvement of organ systems or antibodies in those with and without secondary SS. **Endocrinol diabetes clin exp 2013; 1575-1578.**

INTRODUÇÃO

A esclerodermia sistêmica (ES) é um distúrbio sistêmico crônico de etiologia desconhecida que se caracteriza por espessamento da pele (esclerodermia) e comprometimento de múltiplos órgãos internos, principalmente os pulmões, trato gastrintestinal, coração e rins. (1) O grau de comprometimento sistêmico e de pele varia entre os pacientes. Esta é uma doença relativamente rara: 10 a 20 casos a cada um milhão de pessoas, sendo mais comum em mulheres do que homens, com relação 3:1, porém mais grave nos homens e em idosos. A idade média de incidência da doença é de 30-50 anos.(2)

A etiologia auto-imune é assumida de maneira indireta pela presença de auto-anticorpos e pela associação com outras doenças sabidamente autoimunes como lúpus eritematosos sistêmico (LES) e artrite reumatóide (AR). (3)

A ES pode manifestar-se de várias formas, desde uma forma localizada até uma síndrome onde ocorre fibrose generalizada na pele e em outros órgãos que contêm tecido conjuntivo. (1)

Pacientes com ES podem ter síndrome de *Sjögren* (SS) associada, principalmente naqueles com a forma limitada e com cirrose biliar primária (1).

A SS é uma doença autoimune crônica, lentamente progressiva, das glândulas exócrinas e caracterizada pela sua infiltração linfocitária. (4-9) A maioria dos pacientes tem sintomas relacionados com a diminuição de função das glândulas lacrimais e salivares, resultando em xerostomia e ceratoconjuntivite seca. A doença se apresenta de forma isolada (primária) ou em associação a outras doenças reumáticas auto-imunes (secundária), como pode acontecer com a esclerodermia, podendo evoluir para uma alteração proliferativa generalizada, pseudolinfoma e até neoplasias linfóides. Aproximadamente 60% dos pacientes com SS tem uma doença de base auto-imune como AR, LES e ES. (9)

O envolvimento ocular é devido às alterações fibróticas nas glândulas lacrimais (1,9). Já o principal sintoma oral é a xerostomia, a qual ocasiona queixas como dificuldade de deglutir alimentos secos, incapacidade de falar continuamente, sensação de queimação na boca, aumento de cáries e problemas de adaptação à próteses dentárias. (5) O envolvimento de outras glândulas exócrinas ocorre

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com menor frequência. (5) Manifestações sistêmicas são encontradas em 33% dos pacientes com SS. As queixas mais freqüentes são fenômeno de *Raynaud*, mialgias, artralgias, fadiga e febrícula. (1)

A ocorrência de SS associada com as outras colagenoses é bem estudada no contexto da AR e do LES. Todavia as suas associações com casos de ES são menos conhecidas e não existem dados brasileiros a respeito de sua prevalência.

O objetivo deste trabalho é observar a prevalência de SS em pacientes com ES na população local, avaliar possíveis diferenças no perfil de envolvimento de órgãos sistêmicos e analisar a diferença do perfil de autoanticorpos: FAN, anti-Scl70, anti-centrômero, anti-RNP, anti-Ro e anti-La entre pacientes com ES isolada e associada a SS.

METODOLOGIA

Este é um estudo transversal observacional realizado no ambulatório de Reumatologia do Hospital Universitário Evangélico de Curitiba ao longo dos anos de 2009 e 2010. Foram convidados a participar do estudo todos os pacientes com ES, cadastrados nesse ambulatório, os quais concordaram em participar dessa pesquisa assinando o Termo de Consentimento Livre e Esclarecido. Foram considerados elegíveis aqueles com diagnóstico de ES confirmado através dos critérios criados pelo Subcomitê de Critérios Diagnósticos do *American College of Reumatology* especificados no Quadro 1, sendo necessária a presença do critério maior ou dois menores. (1)

QUADRO 1 – **Critérios diagnósticos para Esclerodermia de acordo com o *Subcommitee for Scleroderma Criteria of American Rheumatism Association Diagnostic and Therapeutic Criteria Committee* (1).**

Critério Maior: Esclerodermia Proximal.

Critérios menores:

- Esclerodactilia;
- Cicatrizes estelares nas polpas digitais e perda do coxim da porção distal dos dedos;
- Fibrose pulmonar bibasilar observada ao Raio X.

Para diagnóstico da SS secundária utilizaram-se os Critérios Americanos- Europeus descritos no quadro 2.

QUADRO 2 – Critérios Americano- Europeus Diagnóstico da Síndrome de *Sjögren* Secundária (1)

Presença de uma colagenose de fundo (no caso esclerodermia)

+

um achado subjetivo e dois achados objetivos da lista abaixo:

- Sintomas oculares por 3 meses;
- Sintomas orais por 3 meses;
- Ceratoconjuntivite seca ao exame - teste de *Schirmer*, rosa-bengala ou tempo de ruptura lacrimal;
- Sialoadenite focal – biópsia de glândula salivar menor com pelo menos um grupo de 50 linfócitos em 4 mm²;
- Evidência de envolvimento de glândula salivar – fluxo salivar não estimulado menor que 1,5 mL em 15 minutos ou sialografia ou cintilografia de parótida.

Foram coletados dados demográficos, grau de envolvimento cutâneo [medido pelo índice de *Rodnan* (10)], presença de envolvimento pulmonar, músculo-esquelético, gastrintestinal e renal secundários à esclerodermia e perfil de autoanticorpos (anti-Ro, anti-La, anticentrômero, anti Scl-70 e anti-RNP) para fins de análise comparativa na população esclerodérmica com e sem SS secundária.

Foram considerados como portadores de envolvimento pulmonar aqueles pacientes com capacidade de difusão de monóxido de carbono inferior ou igual a 70% do valor esperado, com volume expiratório forçado, capacidade vital forçada ou capacidade pulmonar total 75% inferior ou igual ao valor de referência, ou ainda achados radiológicos de doença intersticial crônica (10).

O envolvimento renal foi considerado nos pacientes em que a concentração da creatinina sérica era igual ou maior que o limite normal de 1,4 mg/dL ou *clearance* de creatinina inferior a 70 ml/min. (10)

O envolvimento muscular foi considerado presente quando a concentração da creatina-quinase estava superior a 200% do limite normal ou a força muscular diminuída em 4 graus de 5 nos músculos proximais. Já o envolvimento articular, foi positivo se presença de dor em mais de uma articulação. (10)

Os portadores de envolvimento cardíaco foram definidos a partir da história de insuficiência cardíaca congestiva, arritmia cardíaca em uso de medicamento, pericardite, derrame pericárdico, cardiomegalia como achado radiológico ou um índice cardiotorácico maior que 0,5 na radiografia de tórax, não relacionado a outras causas (10).

Os dados foram agrupados em tabelas de freqüência e de contingência, sendo usados para estudo de associação de variáveis nominais o teste de *Fisher* e o de qui-quadrado e para as variáveis numéricas o de *Mann Whitney* com auxilio do software *Graph Pad prism*, versão 4.0. Significância adotada de 5%.

RESULTADOS

Foram estudados 48 pacientes com ES sendo 5/48 (10,41%) homens e 43/48 (89,58%) mulheres, com idade de 24 a 78 anos (média de 47,6 ±13,21) e tempo de doença entre 1 e 31 anos (media de 8,29±6,56). Destas, 10 (20,83%) tinham a forma difusa, 7 (14,58%) esclerodermia do tipo "*overlap*" e 31 (64,58%) a forma limitada.

Os achados mais comuns foram a presença do fenômeno de *Raynaud* em 43/38 (89,5%), artralgia em 27/48 (56,2%), envolvimento esofágico em 27/48 (56,2%) e mialgia em 19/48 (39,5%). O *Rodnan* variou de 2 a 38 (média de 17,02± 8,79). A freqüência do aparecimento de envolvimento dos órgãos está registrada na tabela 1.

TABELA 1- Prevalência de achados clínicos na amostra de 48 pacientes com esclerodermia

Dados Clinicos	Número da amostra	%
Artrite	14/48	29,1
Artralgia	27/48	56,2
Miosite	14/48	29,1
Mialgia	19/48	39,5
Esôfago	27/48	56,2
Hipertensão pulmonar	4/48	8,3
Fibrose pulmonar	11/48	22,9
Raynaud 43/48	89,5	
Cicatrizes estelares	14/48	29,1
Úlceras digitais	13/48	27,08
Leucomelanodermia	12/48	25
Telangiectasia	13/48	27,0
Microstomia	8/48	16,6
Olho seco 5/48	10,4	
Boca seca	9/45	20

A prevalência de SS secundária foi de 10,66% (5/48).

No que se refere à presença de auto-anticorpos, na amostra estudada, os dados estão resumidos na tabela 2.

TABELA 2 - Perfil de autoanticorpos na população de 48 pacientes com esclerodermia

Anticorpo	Positividade	%
FAN	37/46	80,4
Anti Scl 70	7/35	20,0
Anticentrômero	11/37	29,7
Anti RNP	7/40	17,5
Anti Ro	6/40	15,0
Anti La	3/29	10,3

Dos 46 pacientes com diagnóstico de ES e sem queixas de xeroftalmia e xerostomia, 5 (10,86%) apresentaram a SS associada, sendo 4 mulheres e 1 homem (p= 0.437).

Comparando-se a população de pacientes de ES com e sem SS secundária, houve significância entre a associação da SS e o tempo de evolução da doença (p=0,016), conforme pode ser visto no gráfico da figura 1. Os pacientes com ES e SS tinham um tempo de doença com uma média de 14.6±.29 anos e os pacientes sem a associação apresentaram uma média de 7.55±5.88 anos.

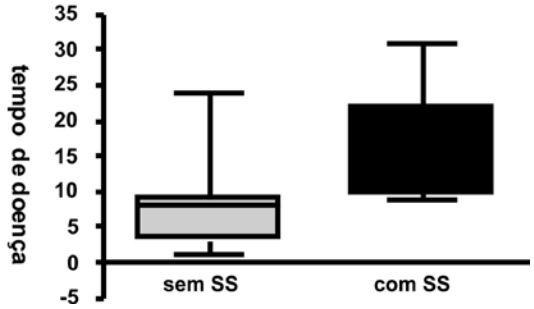

Figura 1- tempo de duração da esclerodermia em pacientes com e sem síndrome de *Sjogren* secundária (p=0,0166)

SS= Síndrome de Sjögren

Comparando-se as formas de ES naqueles com e sem SS secundária observou-se que entre aqueles com esta síndrome associada existiam 4 ES com forma limitada e 1 ES com a forma generalizada. Nos pacientes sem SS: 27 ES com a forma limitada, 9 ES com a forma difusa e 7 ES com a forma de "*overlap*". As porcentagens desta prevalência podem ser observadas na figura 2.

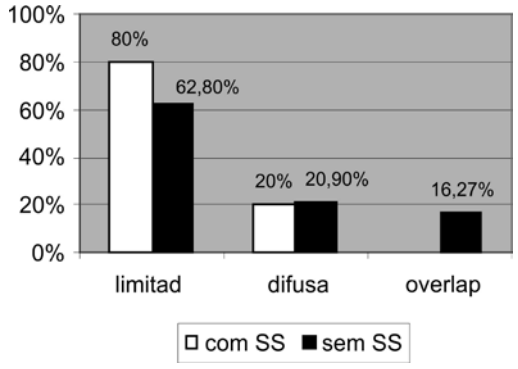

FIGURA 2 - Prevalência das diferentes formas de esclerodermia em pacientes com e sem síndrome de Sjogren (p=0,6001)

OBS - SS= Síndrome de Sjögren

A comparação dos dados clínicos entre pacientes com e sem SS pode ser avaliada na tabela 3.

TABELA 3 - Prevalência de manifestações clínicas na amostra de esclerodermia com e sem síndrome de *Sjogren* secundária (n=48)

Manifestação Clínica	Com SS (n=5)	Sem SS (n=43)	P
Artrite	1/5 - 20%	13/43 30,2%	1,000
Artralgia	3/5 - 60%	24/43 55,8 %	1,0000
Miosite	1/5 - 20%	13/43 30,2%	1,0000
Mialgia	1/5 – 20%	18/43 35,2%	0,3558
Esôfago	3/5 – 60%	24/43 55,8%	1,0000
Hipertensão pulmonar	0/5 - 0	4/43 9,3%	1,0000
Fibrose pulmonar	2/5 - 40%	9/43 20,9%	0,3212
Raynaud	4/5 - 80%	39/43 90,6%	0,4378
Cicatrizes estelares	1/5 - 20%	15/43 34,8%	1,0000
Úlceras digitais	1/5 -20%	12/43 27,8%	1,0000
Leucomelanodermia	0/5 0	12/43 27,9%	0,1464
Telangiectasia	0/5 0	13/43 30,2%	0,3043
Microstomia	2/5 40%	6/43 13,9%	0,1887
Rodnam	15,0±7,07	17,23±8,99	0,6386

SS= Síndrome de Sjögren
n= número da amostra

Não houve associação entre os anticorpos estudados (FAN, anti-Scl70, anticentrômero, anti-RNP e anti-Ro) e a presença de SS secundário. O anticorpo anti-La não foi estudado devido ao pequeno número de pacientes com esta informação. Estes dados podem ser observados na tabela 4.

TABELA 4 - Estudo comparativo dos autoanticorpos em pacientes portadores de esclerodermia com e sem síndrome de *Sjögren* secundária

	Com SS n=5	Sem SS n=43	p
FAN	4/5 – 80%	33/41 80.4%	1,0000
Anti Scl70	0/4 -0	7/31 22.5%	0,5620
Anticentrômero	1/4 -25%	10/33 30.3%	1,0000
Anti RNP	1/5 - 20%	6/35 17.1%	1,0000
Anti Ro	1/5 - 20%	5/35 14.2%	1,0000

SS= Síndrome de Sjögren
n= número da amostra

DISCUSSÃO

Uma doença autoimune está freqüentemente associada à outra. A etiologia da maioria das doenças autoimunes ainda permanece desconhecida, porém a alta incidência dessas associações sugere uma origem comum. (3,8,10) Dentre os principais fatores conhecidos que contribuem para o desenvolvimento de auto-imunidade estão a susceptibilidade genética e os desencadeantes ambientais.(8)

A SS pode aparecer antes ou depois do diagnóstico de outra doença auto-imune como a AR, o LES ou ES.(1) Alguns autores descreveram que a presença de SS associada exerce uma certa influência no grau de gravidade da doença subjacente. No caso do LES associado à SS, este parece se tornar menos agressivo, com menor risco de doença renal grave e com menos complicações de sistema nervoso central. (1,2,13) Já a associação com a AR parece ser um fator agravante, com maior ocorrência de fadiga, trombocitopenia, linfadenopatias e, principalmente, linfomas. (1,2)

Existem autores que defendem a idéia de que a maneira correta para se tratar a coexistência da SS e da ES seria considerar que uma doença acompanha a outra, e não que é secundária à ela. (2,7)

Não há estudos recentes e nem estudos com pacientes de nossa área geográfica que demonstrem a prevalência da

SS em pacientes com ES, o que torna relevante o propósito da nossa pesquisa.

A sintomatologia da SS é comum em pacientes com ES. (1,7) A presença de xerostomia ocasionada por sialoadenite linfocítica, característica da SS, confunde-se com aquela causada por fibrose das glândulas salivares provocada pela ES. (2,11) A própria clínica da ES dificulta a diferenciação entre as doenças. A microstomia, presente na ES, prejudica a higiene bucal dos pacientes e pode ter, como consequência, problemas dentários e gengivais. Essa dificuldade de higiene pode se confundir com resultados da escassez de saliva provocada pela SS, resultando nas mesmas complicações. Por isso, o diagnóstico de SS, neste contexto, deve ser feito através de busca ativa.

Não existem critérios oficiais para a classificação da SS. Neste estudo foram utilizados os Critérios Americano-Europeus, que incluem a presença de sintomas subjetivos, exame oftalmológico para pesquisa de xeroftalmia, biópsia de glândula salivar e análise dos anticorpos. A falta de critérios homogêneos para a identificação da SS dificulta a análise comparativa de achados entre os diferentes estudos.

O anticorpo anticentrômero pode estar positivo na SS e sabe-se que sua presença pode estar associada também com a ocorrência do fenômeno de *Raynaud*. (7) Esse anticorpo apresenta maior relação com a forma limitada da ES e parece estar associado à baixa freqüência de fibrose pulmonar nesses pacientes. (6,7) A maior prevalência do anticorpo anticentrômero poderia guardar maior associação da SS com a forma limitada da ES, mas isso não foi provado no presente estudo. (6)

Há uma variabilidade de resultados na literatura no que concerne à presença do anticorpo anti-Ro na ES. (7) Neste estudo, o anticorpo anti-Ro foi encontrado em 14,5% dos pacientes que apresentam apenas a ES e em 20% dos pacientes com SS associada. Na literatura os valores encontrados são de 12% e 40-70%, respectivamente. (7) Essa variação valida o fato de que, nos novos critérios para o diagnóstico da SS, a presença do anticorpo anti-Ro é apenas um dos ítens a serem considerados para sua classificação, enquanto esta presença era considerada fundamental nos antigos critérios diagnósticos. Outro anticorpo analisado para diagnóstico da SS é o anti-La. Não foi possível o estudo da associação com este anticorpo pois foi realizado em poucos pacientes da amostra.

O FAN apresentou-se positivo em cerca de 80% dos pacientes com a SS, não sendo possível estabelecer sua associação com a presença da SS secundária a ES.

A SS primária e a SS associada à ES apresentam as mesmas características segundo a literatura. (2) A associação dessas duas doenças não modifica a gravidade da SS (envolvimento extraglandular, presença de marcadores sorológicos e fatores prognósticos diferentes). (1,2,6)

Neste estudo, a forma limitada da esclerodermia representou 80% dos casos associados a SS, achado este compatível com o encontrado na literatura, que vai de 68 a 81%. (2,6) Também o envolvimento sistêmico da ES não foi diferente quando associada à SS na análise atual, embora seja descrito, pela literatura, maior possibilidade de complicações por neuropatia periférica ou ainda a presença de uma terceira doença auto-imune.(1)

A presença da SS secundária mostrou-se relacionada com o tempo de evolução da ES. O estudo demonstra que o maior tempo de evolução da doença, aumenta a chance do paciente desenvolver a SS, uma vez que foi encontrado um tempo de doença médio de 14.6 anos naqueles com SS secundária, e 7.55 anos nos pacientes sem a SS. Isso não exclui que, com o decorrer dos anos, pacientes que atualmente não preencham os critérios para diagnóstico de SS venham a desenvolver a doença, aumentando assim a sua prevalência.

CONCLUSÃO

A SS secundária à ES tem uma prevalência de 10,6% (5/48) na população local estudada. Encontrou-se associação entre maior tempo de evolução da ES e a presença da SS. Não foram encontradas diferenças no envolvimento de órgãos sistêmicos ou do perfil de auto-anticorpos nos pacientes que apresentaram apenas ES e os que apresentaram ES com SS secundária.

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ARTIGO DE REVISÃO

ALIMENTOS FUNCIONAIS: AS ISOFLAVONAS DA SOJA E A REGULAÇÃO DO METABOLISMO

FUNCTIONAL FOOD, SOYA ISOFLAVONES AND METABOLISM REGULATION

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Descritores: Isoflavona, Obesidade, Soja, Genisteína, Lipídios, Glicemia
Keywords: Isoflavone, Obesity, Soy, Genistein, Lipidis, Glycemia

Resumo

Uma dieta equilibrada e o consumo de alimentos saudáveis são muito importantes para a manutenção da saúde. A classe dos flavonóides, principalmente as isoflavonas, originada da soja, desperta interesse terapêutico devido suas amplas atividades biológicas. As isoflavonas são consideradas como fitoestrógeno por possuir semelhança estrutural com os estrogênios fisiológicos, e consequentemente provocar efeitos estrogênicos fracos no organismo. Adicionalmente também atuam na melhora da adipogênese, na homeostase glicêmica e no sistema nervoso central. Esta breve revisão discute as propriedades biológicas das isoflavonas da soja como um alimento funcional que pode ajudar no controle do metabolismo. **Endocrinol diabetes clin exp 2013; 1579-1584.**

Abstract

A balanced diet and healthy food consumption are very important for the maintenance of health. The class of flavonoids, especially isoflavonas from soya beens, therapeutic arouses interest because of their broad biological activities. Isoflavones are considered a phytoestrogen for having structural similarity to estrogens and consequently cause weak estrogenic effect in the body. Additionally also affect the improvement of adipogenesis, glucose homeostasis and has positive effect on the central nervous system.It has been demonstrated that isoflavones may lead to many positive effects in the body. The current mini review discusses the biological properties of soya isoflavonas as a functional food that can help the metabolic regulation. **Endocrinol diabetes clin exp 2013; 1579-1584.**

INTRODUÇÃO

É indiscutível a importância de uma dieta equilibrada na manutenção da saúde e na prevenção/redução do risco de desenvolver doenças (1). Não é de hoje que o ser humano busca nos alimentos, principalmente nos vegetais, a fonte de saúde e longevidade. Há 2.500 anos, Hipócrates já dizia: "Que o seu alimento seja seu medicamento, e seu medicamento seja seu alimento" (2). Esses alimentos que trazem benefícios ao organismo são ditos como "Funcionais".

Alimento Funcional é aquele que apresenta em sua composição uma ou mais substâncias que comprovadamente, além de nutrir tenham alguma função benéfica ao organismo (1). A ANVISA regulamentou esses alimentos em 2005 com a resolução RDC 278/2005, que estabelece: Os alimentos que apresentarem em seus dizeres de rotulagem e ou material publicitário as alegações aprovadas pela ANVISA devem ser registrados nas categorias de "Alimentos com Alegações de Propriedade Funcional e ou de Saúde" ou de "Substâncias Bioativas e Probióticos Isolados com Alegação de Propriedades Funcional e ou de Saúde" (3).

O interesse por esses alimentos não é novidade. Os flavonóides, em especial as isoflavonas, despertam interesse terapêutico á anos devido às suas diversas propriedades biológicas. Atualmente as isoflavonas são utilizadas principalmente como agente terapêutico para regulação hormonal em mulheres pós-menopausadas. Além disso, alguns estudos apontam que a isoflavona também atua sobre o metabolismo energético, em especial sobre a homeostase da glicose e lipídios (4,5).

ISOFLAVONA E SUA COMPOSIÇÃO

As isoflavonas são compostos polifenólicos encontradas principalmente na soja e nos seus derivados. São denominadas fitoestrógenos por apresentarem semelhanças estruturais e funcionais com o hormônio estrogênio (6). As isoflavonas podem ser encontradas na sua forma livre, onde são chamados de agliconas (Daidzeína e Genisteína), ou na forma conjugadas a uma ou mais moléculas glicídicas, onde são consideradas glicosiladas (Daidzina e Genistina) (7).

Na natureza a isoflavona encontra-se na forma glicosilada que é biologicamente inativa e precisa ser hidrolisada para desencadear seus efeitos (8). As isoflavonas ingeridas nos alimentos são hidrolisadas no intestino delgado pelas enzimas intestinais β-glicosidases, as quais liberam as agliconas (Genisteína e Daidzeína). Assim as isoflavonas são absorvidas na forma biologicamente ativa e transportadas para o fígado, onde são removidas da circulação sanguínea através da veia porta. Elas retornam ao intestino pela via biliar e podem ser excretas pelas fezes. Uma porcentagem, porém consegue escapar do sangue portal sem passar pelo fígado e entra na circulação periférica, alcançando os tecidos. Neste caso as isoflavonas são eliminadas pelos rins, de maneira similar aos estrogênios endógenos (9).

A estrutura química das isoflavonas é muito variada. Somente a soja contém três tipos de isoflavonas com quatro formas isoméricas, totalizando doze diferentes tipos desse composto. Dentre os principais destacam-se daidzina, genistina, glicitina, malonidadzina, malonigenistina, maloniglicitina, acetildaidzina, acetilgenistina, acetilglicitina, equol (10). As formas que têm recebido maior atenção, apresentando maior atividade em humanos, são a daidzeína, genisteína (11) **Figura 1 próxima página.**

ATIVIDADE ESTROGÊNICA

As isoflavonas de soja são compostos não esteroidais, estruturalmente similares ao 17-□ estradiol, que apresenta um anel fenólico, com um radical hidroxila no carbono três. Esta estrutura confere ao 17-□ estradiol a capacidade de ligação seletiva e de alta afinidade aos receptores estrogênicos (12).

Os estrogênios exercem seus efeitos através de dois tipos de receptores: REα e REβ, que apresentam vasta distribuição nos tecidos (8). O estradiol tem afinidade por ambos os recep-

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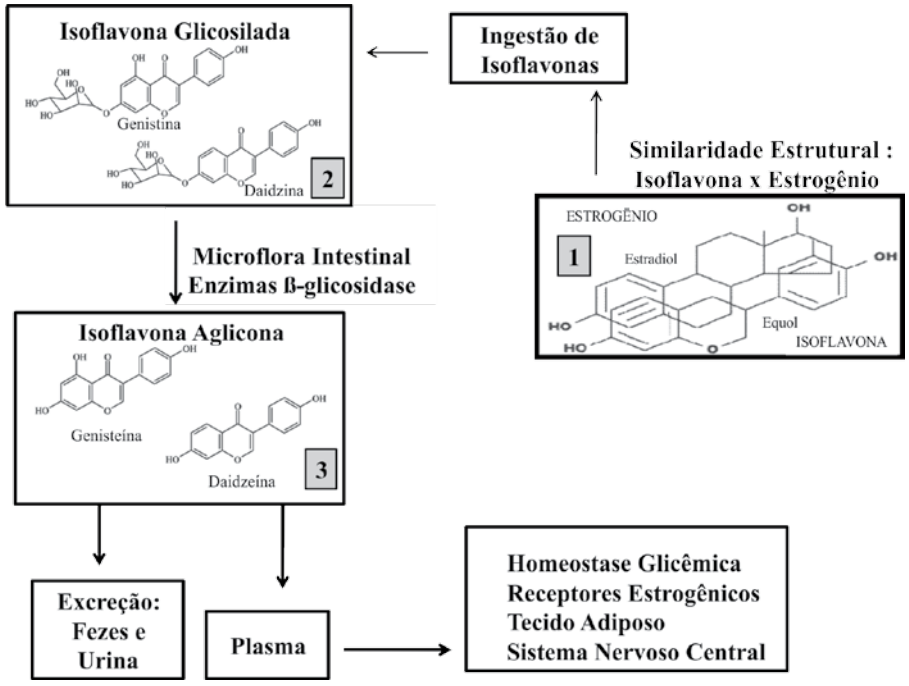

Figura 1: Desenho esquemático do metabolismo da isoflavona. 1) Apresenta similaridade estrutural da isoflavona (equol) com o estrogênio (Modificado a partir de *Knight & Éden*, 1997). 2 e 3) Apresenta etapas da metabolização da isoflavona ingerida na dieta. Isoflavonas ingeridas na sua forma glicosilada, Genistina e Daidzina (Modificado *Bhathena & Velasquez*, 2002), são hidrolisadas pelas enzimas β-glicosidases da microflora intestinal, as quais liberam as agliconas, Genisteína e Daidzeína (*Guidoni et al*.,2007). Uma porcentagem é excretada pelas fezes, outra parte vai para o plasma alcançando os tecidos.

tores, já as isoflavonas são mais seletivas para os receptores β (13), apresentando potente ação agonista β e fraca ação mediada por receptores α. Isto permite classificá-las como agonista/antagonista naturais seletivos do receptor de estrogênio (8), ação que será melhor descrita em seguida.

A similaridade estrutural da isoflavona com o estrogênio permite que ela se ligue aos receptores estrogênicos em vários tecidos, atuando como agonista quando a produção do hormônio está diminuída, como na menopausa. Devido sua "fraca ação estrogênica" as isoflavonas também podem agir como antagonistas competitivos quando a concentração de hormônio circulante está normal (14). A propriedade estrogênica ou antiestrogênica depende, portanto, da concentração de isoflavona, concentração de hormônios esteróides endógenos e do órgão alvo específico envolvido na interação com os receptores de estrogênio (12).

Em mulheres na pré-menopausa, quando a concentração de hormônios estrogênicos circulantes é alta, os receptores de estrógeno estão ocupados e as isoflavonas competem por estes receptores, resultando em uma fraca ação estrogênica ou antiestrogênica. Na menopausa, quando a concentração do estrogênio diminui em torno de 60%, os receptores ficam mais disponíveis, favorecendo a fraca ação estrogênica das isoflavonas (15). Nestas condições as isoflavonas agem coadjuvando na redução dos sintomas da menopausa (16), na prevenção da osteoporose (17) do câncer de mama (19) e das doenças cardiovasculares (20).

Quando se trata de efeito estrogênico, estudos usando isoflavonas em humano, no geral são mais focados em mulheres, com pouco interesse em homens. Evidências epidemiológicas sugerem uma associação inversa entre concentrações séricas de andrógenos fisiológicas e consumo de soja em homens (21). Pesquisas em homens que usaram isoflavona relataram sintomas de feminilização tal como ginecomastia (22), hipogonadismo e disfunção erétil (23). Portanto as isoflavonas podem ter efeitos diferentes no sexo masculino e feminino. No entanto, mais estudos epidemiológicos, ensaios clínicos e experimentais são necessários para determinar as ações do consumo de isoflavona na feminilização de homens.

Investigações em animais, principalmente roedores, mostram que as isoflavonas têm efeitos estrogênicos relevantes. Estas ações podem ser mediadas pelo mecanismo de ação similar a humanos, com implicação de receptores estrogênicos. Portanto, podem ter consequências semelhante ao hormônio feminino no que diz respeito ao desenvolvimento de saúde e de doença (24).

EFEITOS SOBRE A ADIPOGÊNESE

A obesidade pode ser caracterizada como o acúmulo excessivo de gordura corporal com etiologia multifatorial pautada em fatores genéticos, comportamentais e ambientais (25,26,27). Adicionalmente, pode levar a instalação de outras doenças como: hipertensão arterial, cardiopatias, dislipidemias, resistência à insulina, diabetes do tipo 2, distúrbios respiratórios, problemas articulares, aumento da incidência de câncer, além de distúrbios psicossociais (28). Tendo em vista o aumento da obesidade na população mundial e das doenças relacionadas à síndrome metabólica, os efeitos benéficos das isoflavonas sobre a regulação da adipogênese despertou o interesse de muitos pesquisadores em investigar suas ações sobre as doenças cardiometabólicas (29).

Estudos em animais mostram que ratos *Zucker* geneticamente obesos e ratas ovariectomizadas, ambos tratados com isoflavona, apresentaram redução do peso corporal, diminuição da lipogênese em tecido adiposo branco e do metabolismo dos lipídios (30,31,32,33). Parte destes efeitos pode ser mediado pela apoptose de adipócitos induzida pela isoflavona (34).

Há relatos em roedores de que as isoflavonas, sobretudo a genisteína, aumentaram a lipólise de adipócitos isolados (35), e inibiram a captação de glicose pelos adipócitos (36,37). Similarmente a incubação de adipócitos com genisteína resultou em inibição da conversão da glicose em lipídios na ausência

ou presença de insulina (38). Estes estudos indicam uma ação específica da genisteína sobre os adipócitos no sentido de reduzir o armazenamento de energia na forma de lipídios.

O potencial das proteínas de soja em modificar o metabolismo lipídico deve-se a diferentes mecanismos. Parte destes são devido ao fato das isoflavonas apresentarem similaridade estrutural com o hormônio feminino. Evidências experimentais mostram que o estrogênio exerce importante papel na regulação do tecido adiposo branco (39), que expressa ambos receptores estrogênicos α e β (40). O receptor α seria o responsável pela modulação da lipogênese no tecido adiposo (41). Através da ligação com seus receptores o estrogênio inibe a lipogênese principalmente através da diminuição da atividade da lipoproteína lipase (LPL), uma enzima que regula o metabolismo dos lipídios nos adipócitos (41).

Vários estudos demonstraram que a genisteína liga-se a receptores ativados pelo proliferador de peroxissomo (PPAR) e induz atividades transcricionais envolvidos na regulação do tecido adiposo, gerando vários efeitos em adipócitos numa maneira dose-dependente (42). Os PPARs são um grupo de proteínas receptoras nucleares que funcionam como fatores de transcrição adipogênico. Existem em três isoformas PPARα, PPARβ/δ, PPARγ, todas estão presentes em adipócitos, sendo que o PPARγ é considerado o maior regulador da adipogênese. Juntos, os PPARs são alvo da genisteína, que pode ativar sua fosforilação (43,44), conduzindo a um aumento da β-oxidação dos ácidos graxos (45).

A ingestão de isoflavona, em especial a genisteína, tem mostrado efeito sobre os níveis séricos de leptina (46). Este é o hormônio importante derivado do tecido adiposo, desempenhando um papel chave na regulação do consumo calórico via inibição da ingestão alimentar entre outros (47). Estudos sugerem que a genisteína inibe a secreção de leptina em adipócitos e melhora o estado inflamatório em indivíduos obesos, o que poderia restaurar a ação deste hormônio em condições de resistência observada na obesidade (48).

EFEITOS SOBRE A HOMEOSTASE GLICÊMICA

O mecanismo de ação das isoflavonas pode variar de acordo com o contexto celular, e a dose administrada. Assim fica evidente que a isoflavona não age por um único mecanismo. A homeostasia da glicose também é afetada beneficamente pelo consumo deste fitoestrógenos. Ratas ovariectomizadas tratadas com isoflavona apresentaram uma queda significativa na glicemia durante o estado de alimentação (33). Adicionalmente, observou-se que a glicemia de jejum também está diminuída em ratos tratados com isoflavonas (49). *Lavigne* e colaboradores demonstraram que ratos alimentados com soja, apresentam menor área sob a curva para a glicose durante o teste de tolerância à glicose intravenosa (50). Adicionalmente, *Park* e colaboradores mostraram em camundongos diabéticos db/db tratados com genisteína (0,02%) um aumento na razão insulina/glucagon, associado a uma hiperatividade da glicoquinase hepática e baixa atividade da glicose-6-fosfato, fosfoenolpiruvato carboxiquinase e ácido graxo sintase (51). Este estudo sugere que a genisteína pode exercer um efeito antidiabético via modulação da glicose hepática e metabolismo de lipídios. Estudos relataram que a genisteína pode atuar diretamente sobre as células betas das ilhotas pancreáticas, por outro mecanismo que não seja pelo receptor de estrogênio (52). Esta age como um fator de crescimento para células beta "in vivo", levando, por consequência, a um aumento da secreção de insulina (53). Ratos tratados com aloxano (um análogo tóxico da glicose, que seletivamente destroem as células β pancreática) e posteriormente tratados com genisteína, obtiveram uma redução da hiperglicemia e aumento dos níveis de insulina. Este resultado demonstra que a genisteína é capaz de reduzir a perda de células das ilhotas induzida pelo aloxano, facilitação da restauração do número de células e estimulação da secreção de insulina (52).

Akiyama e colaboradores mostraram que a genisteína é um inibidor altamente específico para a proteína tirosina quinase (PTK) (54). Estudos da década de 90 sugeriram que o efeito insulinotrópico da genisteína depende de seu efeito inibitório sobre a PTK (55). É bem estabelecido que receptores de insulina e de fatores de crescimento semelhantes à insulina (IGF-I) estão presentes nas células secretoras de insulina e possivelmente estes receptores medeiam o mecanismo de retrocontrole negativo de secreção de insulina. *Verspohl* e colaboradores mostraram que o efeito inibitório da insulina e do IGF-I sobre a secreção de insulina foi abolido pela genisteína, mas não por outra isoflavona sem ação sobre a PTK, a daidzeína (56). Este estudo sugere que que o aumento de insulina observado em animais tratados com isoflavona pode ser dependente da inibição de mecanismos de retrocontrole negativo dependente da PTK. Estudos mais recentes desacordam desta hipótese e sugerem que genisteína potencializa a secreção de insulina estimulada pela glicose (GSIS) tanto em linhagens de células secretoras de insulina, como em ilhotas pancreáticas de rato. *Liu* e colaboradores demonstraram que o efeito insulinotrópico da genisteína é independente de sua ação inibitória sobre a PTK, mas depende do acúmulo intracelular de adenosina 3',5'-monofosfato cíclico (AMPc), que posteriormente ativa proteína quinase A (PKA). Este mecanismo de secreção de insulina dependente da elevação no nível de AMPc/PKA é bem estabelecido (57).

Pode-se, portanto, sugerir que as isoflavonas, principalmente a genisteína, tem um potencial antidiabético devido seu efeito redutor sobre os níveis de glicose sanguínea e sua ação positiva sobre a proliferação das células-beta e redução da apoptose, preservando assim a função das células beta (58).

EFEITO CENTRAL DA ISOFLAVONA

As primeiras evidências de que isoflavonas administradas perifericamente são encontradas no sistema nervoso central (SNC) datam do fim da década de 60 (59). Contudo o interesse pela ação central das isoflavonas ganhou destaque apenas mais recentemente, a partir da década de 90. Estudos em ratos tratados com dieta rica em isoflavonas mostram que as concentrações de agliconas estão muito elevadas (de 8 a 50 vezes) em regiões do SNC ricas em receptores estrogênicos, como hipotálamo basomedial e a região cortical frontal (60). Adicionalmente, estudos sugerem que fitoestrógenos modulam a regulação central do balanço energético no hipotálamo (61).

Estudos sugerem que animais tratados com isoflavonas apresentaram alterações nos níveis de neuropeptídios moduladores da ingestão alimentar e atividade locomotora. *Cederroth* e colaboradores mostraram que camundongos tratados com soja apresentaram hiperfagia associado a um fenótipo magro, com aumento da taxa metabólica, utilização de lipídios e atividade locomotora (62). Eles sugerem que este perfil foi modulado centralmente pelas isoflavonas, uma vez que os animais tratados apresentaram alterações em neuropeptídios hipotalâmicos implicados na regulação do metabolismo energético, com redução na expressão de RNA mensageiro (mRNA) do peptídeo A*goutie* (AgRP), aumento na expressão de mRNA da orexina A e do hormônio melanocortina (63). Os autores argumentam que o perfil destes animais, com fenótipo magro e aumento da taxa metabólica, foi similar ao encontrado em camundongos que não apresentam o gene AgRP (64). Também ressaltam que a orexina A estimula a ingestão alimentar e a atividade física (65). Estes estudos sugerem que um desbalanço dos neuropeptídios implicados na modulação central do equilíbrio energético (ingestão/gasto) seria um dos fatores desencadeantes do fenótipo magro associado a hiperfagia. Um estudo semelhante mostrou que ratos expostos a uma dieta a base de soja apresentaram, nos núcleos paraventriculares e arqueado, aproximadamente 40% de aumento na expressão

de neuropeptídio Y, que também é um neuropeptídio orexígeno conhecido por estimular a ingestão alimentar (66). Outro estudo confirma o aumento da atividade locomotora em roedores expostos a soja na dieta (67). Juntos, estes estudos sugerem uma regulação central das isoflavonas sobre o balanço energético modulado pelo hipotálamo. Uma meta-análise realizada com estudos em mulheres não asiáticas na pós-menopausa suplementadas com isoflavona aponta para os benefícios deste fitoestrógeno na redução do peso corporal (68). O aumento do gasto energético modulado centralmente, como sugerido por estudos *in vitro* e em animais, é potencialmente um fator preponderante para a redução do peso corporal observada em estudos em humanos.

Além de seu efeito sobre a regulação central do balanço energético, as isoflavonas também têm apresentado potencial neuroprotetor em situações de acidente vascular cerebral e esclerose cerebral amiotrófica (69).

EFEITOS ADVERSOS DO USO DE ISOFLAVONAS

A categoria dos flavonóides contém mais de 5000 moléculas (70), o que indica a diversidade destes compostos. Nos alimentos observa-se uma composição variada de isoflavonas, a qual pode ser alterada por vários fatores como: época do ano em que é plantada, localização geográfica do plantio, nutrientes e fertilizantes utilizados no solo, duração do dia, cultivo em estufa, temperatura, umidade do ar, processamento, aquecimento, tratamento químico, transporte e armazenamento (71). Assim, não é fácil garantir e reproduzir a mistura perfeita e mais benéfica para tratamento em seres humanos.

Dados referentes aos efeitos destas moléculas ainda são inconsistentes. Faltam estudos adequados para definir os efeitos das isoflavonas em mulheres, e referendar sua indicação como tratamento alternativo. A principal preocupação é quanto ao uso prolongado e os efeitos adversos, questão pouco conhecida devido à escassez de estudos que visem à suplementação em longo prazo (72).

Quando testadas individualmente isoflavonas, em doses elevadas, colocam um ponto de interrogação sobre a sua segurança (73) visto que apresentam toxicidade relacionada à inibição de enzimas digestivas, a toxicidade para o fígado e o rim. Além de alguns efeitos carcinogênicos, que parecem ser dependentes das doses ingeridas (74). Dados epidemiológicos considerando o efeito das isoflavonas na prevenção de doenças cardiovasculares e câncer não são consistentes, pois, depende da população “alvo” testada (66,69).

Estudos em animais têm sugerido que a utilização de uma única proteína ou de alguns componentes isolados das isoflavonas parece ser uma abordagem ideal. Entretanto é freqüentemente impraticável reproduzir esses resultados, utilizando, em humanos, uma dieta rica em soja (75).

Pesquisa realizada com chineses de meia-idade e idosos, com uma maior ingestão de isoflavona, mostrou uma tendência à redução dos riscos associado à síndrome metabólica em mulheres, mas um risco aumentado em homens (76). Os sintomas de hipogonadismo, disfunção erétil, diminuição da porcentagem livre de testosterona e aumento dos níveis de desidroepiandrosterona (DHEA) também foram relacionados com pacientes do sexo masculino que ingeriram 360 mg de isoflavonas totais por dia, ao longo de um ano (77). *Sherrill* e colaboradores (2010) constataram que a isoflavona afeta a produção de testosterona e consequentemente conduz a uma toxicidade reprodutiva (78). Esses resultados sugerem que as isoflavonas, têm um mecanismo de ação diferenciado em mulheres e homens (79), modulando diretamente concentrações circulantes de hormônios sexuais em uma forma sexo-específica. Na verdade, evidências epidemiológicas sugerem uma associação inversa entre concentrações séricas de andrógenos e consumo de soja em homens (80,81).

Consequentemente, isoflavonas podem ter efeitos adversos no sexo masculino, enquanto que produzem efeitos favoráveis no sexo feminino. No entanto, mais pesquisas nesta área precisam ser feitas para determinar se a ingestão de soja e os hormônios sexuais estão realmente correlacionados.

CONCLUSÃO

Evidências a partir de experimentos em animais e humanos sugerem que as isoflavonas possuem efeitos benéficos em doenças como diabetes e obesidade. Em estudos *in vitro* e com animais, as isoflavonas são eficazes em diminuir a hiperglicemia, aumentar os níveis de insulina, reduzir o peso corporal e tecido adiposo. Contudo, pouco se sabe sobre os mecanismos que medeiam os efeitos benéficos das isoflavonas.

Embora as tendências sociais e demográficas favoreçam o desenvolvimento e a popularização de alimentos funcionais, as investigações nesta área são escassas e dispersas. Isto se deve ao alto custo e complexidade para o desenvolvimento destes novos produtos. Adicionalmente, os alimentos funcionais têm grande ligação à saúde pública, a qual tem sido cada vez mais regulamentada. Cada vez mais são exigidas provas científicas para a autorização da comercialização de um alimento dito como funcional, colocando-se a exigência ao nível da qualificação de evidência científica.

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TÓPICOS EM CLÍNICA MÉDICA
DESCRIÇÃO DE CASO

HEPATITE C COMO CAUSA DE UVEITE BILATERAL
HEPATITIS C AS A CAUSE OF BILATERAL UVEITIS

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Descritores: Virus C, Hepatite, Uveite, Autoimunidade
Key words: C vírus, Hepatitis, Uveitis, Autoimmunity

Resumo

Descreve-se o caso de uma paciente com uveíte intermediária, secundária à hepatite C crônica cujo diagnóstico da infecção viral foi feito a partir das manifestações oculares. A paciente foi submetida à terapia combinada com interferon alfa e ribavirina durante 24 semanas associada à corticoterapia para o controle das manifestações oculares. Este caso é descrito para ilustrar a importância da investigação de uma doença sistêmica a partir do diagnóstico de uveíte. **Endocrinol diabetes clin exp 2013; 1585-1586.**

Abstract

We describe a patient with intermediate uveitis secondary to chronic hepatitis C infection. The diagnosis of the viral infection was done by the ophtamologic manifestations. The patient received combined treatment with alpha interferon and ribavirin for 24 weeks associated to glucocorticoids to control ocular manifestations. This case is described to illustrate the importance of investigation of a systemic disease when the diagnosis of uveitis is done. **Endocrinol diabetes clin exp 2012; 1585-1586.**

INTRODUÇÃO

O diagnóstico etiológico de uma uveíte exige sempre um cuidadoso exercício de diagnóstico diferencial. Embora quase 50% sejam atribuídos a uma doença ocular primária, doenças como infecções e de autoimunidade necessitam ser excluídas (1). A identificação de uma possível doença sistêmica associada à uveíte é fundamental para o tratamento correto do paciente. Naturalmente, o fenótipo apresentado da manifestação ocular auxilia na identificação de determinadas etiologias: uveítes anteriores agudas e unilaterais são comuns em pacientes HLA B27 positivos (1); já as uveítes posteriores podem ter etiologia infecciosa (1,2). Dentre as doenças infecciosas associadas encontram-se a toxoplasmose, tuberculose, sífilis, infecção pelo vírus da síndrome da imunodeficiência adquirida, herpes vírus etc (1,2).

A hepatite C é uma infecção causada por um pequeno vírus RNA classificado no gênero hepacivirus, família *Flaviridae* (2) que cursa com muitos fenômenos autoimunes associados, dada a capacidade de invasão de linfócitos B pelo vírus C (3). Embora a uveíte conste como uma das manifestações clínicas extra-hepáticas dessa doença (3), sua pesquisa não faz parte da investigação rotineira de casos de uveíte.

Descreve-se aqui um caso de uveíte associado à infecção pelo vírus C para alertar acerca da necessidade de incluir esta infecção na investigação etiológica nessa situação.

DESCRIÇÃO DE CASO

Paciente feminina, branca, 58 anos, do lar, procurou atendimento oftalmológico referindo baixa da acuidade visual para longe e perto progressiva há 4 meses e dor ocular em ambos os olhos. Referia ainda cefaléia bitemporal, mialgias e fadiga. Apresentava história de cirurgia de pterígio em ambos os olhos há 15 anos. Nos antecedentes existia história de hipertensão arterial, tireoidopatia, depressão, fibromialgia, artrose e cistite crônica. Havia feito uma cirurgia para correção de cistocele há 11 anos necessitando de transfusão sanguínea.

O exame oftalmológico constatou acuidade visual de 20/50 OD e 20/40 OE (J2 para perto) com melhor correção. Na biomicroscopia observavam-se precipitados ceráticos finos difusos e catarata subcapsular posterior +/++++ AO. Tonometria: 10 mmHg AO. Fundoscopia: vitreíte intensa e *snowbanks* AO.

Optou-se, então, pela realização de corticóide subconjuntival AO (dose de 0,3 ml em 2 aplicações com intervalo de 30 dias) associado a corticóide tópico 6/6h e VO, prednisona na dose de 20 mg/dia .

Investigação laboratorial mostrou: hemograma, função renal e tireoidiana normais, Fator reumatoide positivo (64 UI), FAN negativo; radiografias de tórax, mãos e pés e sacroilíacas normais; VHS 57 mm; HLA B27 negativo. As transaminases estavam alteradas com TGO=115 UI/ml (N até 52UI); TGP=136 UI/ml (N até 48UI/ml). VDRL, FTA-ABs IgM e IgG, HBsAg, anti-HBs e HIV eram não reagentes; Herpes simples IgM não reagente e IgG reagente; anti-HCV reagente. RNA quantitativo de hepatite C (VHC) mostrou 8.580.000 cópias/mL. Uma ecografia abdome total mostrou lesão hepática infiltrativa crônica.

Verificou-se melhora da sintomatologia ocular e da acuidade visual para 20/25 AO com correção após 60 dias do início da corticoterapia, além da remissão completa da vitreíte e diminuição dos precipitados ceráticos. O uso dos corticosteróides foi regredido lentamente até sua retirada completa e mantido apenas lubrificante ocular.

Após o diagnóstico de hepatite C, a paciente foi encaminhada para Infectologia onde foi confirmado o diagnóstico de hepatite C crônica genótipo 2, sendo iniciado tratamento com interferon peguilado e ribavirina durante 6 meses. Desde então, a paciente encontra-se estável, sem queixas oculares ou sistêmicas.

DISCUSSÃO

Estima-se que 1,8 milhões de pessoas estejam infectadas pelo vírus C no Brasil e esta infecção tem sido considerada a principal causa de transplante hepático em todo o mundo (4). A infecção pelo vírus C causa, peculiarmente, vários fenômenos autoimunes como artrites, glomerulonefrites, vasculites, presença de autoanticorpos como fator reumatóide, FAN, anti RO, anti LA e crioglobulinas (4), além dos achados da infecção em si.

Manifestações oculares associadas com a exposição crôni-

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ca ao vírus da hepatite C, já descritas, são a ceratoconjuntivite seca (5,6), retinopatias isquêmicas, hemorragias retinianas e úlcera de *Mooren* (6). Muitas destas correlacionam-se com deposição de complexos imunes em tecidos alvo (6). A ocorrência de uveítes, embora citada, não tem sido muito valorizada (5,6).

Vários dos efeitos oculares observados em casos de hepatite C têm sido atribuídos ao uso do tratamento com interferon (7); no caso descrito isso não ocorreu uma vez que a doença ocular apareceu antes do uso de tais medicamentos. RNA do vírus C tem sido detectado na lágrima de portadores da doença sendo considerado como fonte potencial de contaminação e mostrando a presença dos mesmos nos tecidos oculares durante a infecção (8). *Pazienza* chama a atenção para o fato de que, estudos experimentais *in vitro* demonstram que a infecção viral no olho induz alterações de genes envolvidos em doenças oculares, desregulando níveis de auto antígenos e enfatizando, assim, a possibilidade de que a própria infecção, e não seu tratamento, seja responsável pelos achados oftalmológicos (9). Tem sido verificado que, com a infecção pelo vírus C, existe aumento de vários auto antígenos dentro do olho, incluindo-se nisso auto antígenos uveais como o UACA (*uveal autoantigen with coiled coil domains and ankyrin repeats*) (10). Níveis significantes de anti-UACA têm sido encontrados em pacientes com panuveíte quando comparado com controles saudáveis mostrando que a autoimunidade contra UACA é um fenômeno existente nesse tipo de doença (10).

CONCLUSÃO

É importante o reconhecimento desta infecção como causa de uveíte e de que existe a possibilidade de diagnóstico de hepatite C pelo oftalmologista. Isto pode representar, para o paciente, a possibilidade de tratamento precoce de uma doença potencialmente grave.

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HOSPITAL UNIVERSITÁRIO EVANGÉLICO DE CURITIBA

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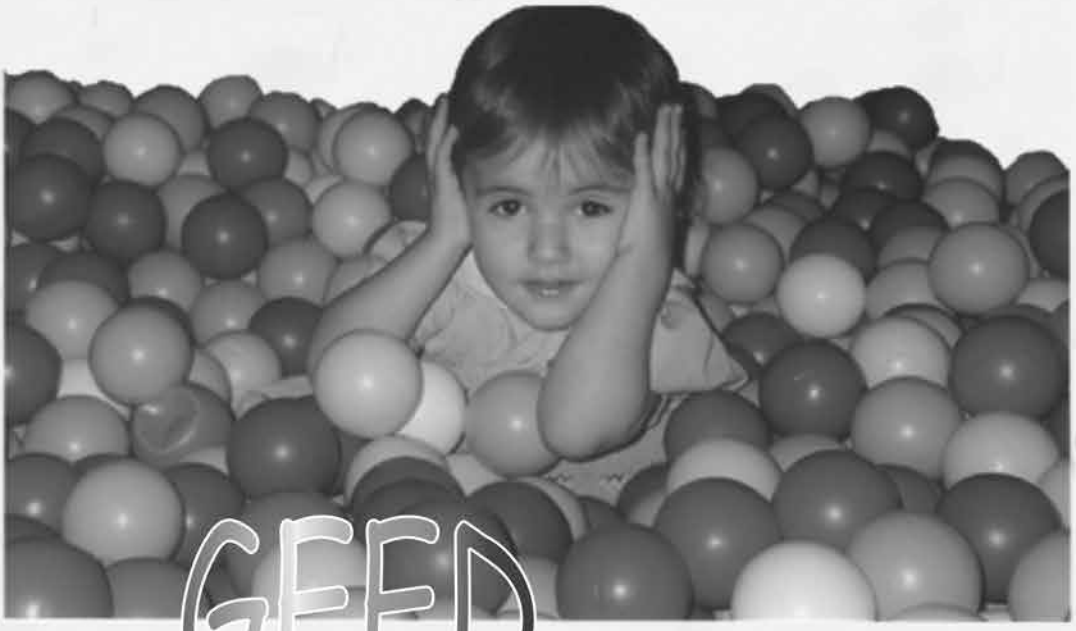

GEED

GRUPO DE ESTUDOS EM ENDOCRINOLOGIA E DIABETES

THE 3RD INTERNATIONAL SYMPOSIUM ON METABOLIC PROGRAMMING AND STRESS (3ISMPS) - SEPTEMBER 25-28

LECTURES

Full Communications – (FC)

FC001

Revisiting the fetal origin of adult diseases (FOAD) hypothesis: recent findings and future directions

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Studies indicate that intrauterine growth retardation (IUGR) and macrosomia sensitises to the development of chronic adult diseases, suggesting that alterations of the growth velocity of the fetus has long-term consequences. These observations have led to the fetal programming hypothesis. However, it has been reported that modification of the growth velocity of the neonate has also programming effects indicating that the sensitive periods are not strictly limited to gestation. The pathophysiological mechanisms involved in the so-called "Developmental Origins of Health and Diseases (DOHaD)" are largely unknown and depend on the type of alteration (nutritional, psychological, among others), its intensity and duration, species, sex and the time during which it is applied. Among the mechanisms, organs and tissues susceptible to be involved, it has been reported that perinatal stress, via disturbances of both hypothalamo-pituitary-adrenal (HPA) axis and sympatho-adrenal-system (SAS), as well as brain-adipose axis and pancreas alterations could play a crucial role. Recently, studies opened exciting perspectives and important questions need to be addressed: 1) do other pathologies such as neurodegenerative disorders or cancer may also have a developmental origin?; 2) could specific molecules exert long-term programming effects?; 3) could we identify early predictive markers of "programmed" diseases?; 5) are the mechanisms different in males and females offspring?; 6) could paternal environmental alterations also program the offspring?; 7) could perinatal alterations exert transgenerational effects?; 8) is it possible to "deprogram" diseases or "program" health in people at risk? Interestingly, the answer to all these questions seems to be yes, suggesting that it might be possible to perinatally program health and to improve to well-being during the aging process.

Keywords: perinatal stress, transgenerational effects, epigenetic, placental markers.

FC002

Involvement of pancreatic α-cells in the glucocorticoids-induced glucose dyshomeostasis

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Besides their important role in the regulation of glucose homeostasis and nutrient metabolism, glucocorticoids (GCs), when in excess, likely in GC-based therapies produce several side effects that include insulin resistance (IR), glucose intolerance, hyperglycemia and overt diabetes in susceptible individuals. Thus, understanding of mechanisms behind these metabolic derangements could improve the management of glucose homeostasis in patients under chronic GC treatment or excessive acute exposure to GCs. Male adult Wistar rats were treated with daily injection of dexamethasone (1 mg/kg, b.w., i.p.) for 5 consecutive days (DEX), whereas control rats received saline. Glycemia, insulinemia and glucagonemia were determined. Insulin and glucagon secretion protocols, pancreas immunohistochemistry, and western blot for determination of islet proteins were performed. DEX rats developed IR, hyperglycemia, hyperinsulinemia and hyperglucagonemia in both metabolic states ($p<0.05$). The expected shift towards increase for insulin to glucagon ratio from fasted to fed condition was absent in DEX rats. The elevation in blood glucose was abrogated by treatment of DEX rats with glucagon receptor antagonist ($p<0.05$). Insulin secretion in the presence of glucagon and the glucagon receptor content were significantly augmented in islets from DEX rats ($p<0.05$). The inhibitory effect of high glucose on α-cells was impaired in islets from GC-treated rats and the absolute α-cell mass, normalized by the body weight, was higher ($p=0.07$) when compared with CTL rats. The 11βHSD-1, pre-receptor of GC metabolism, contributes for the insulin hypersecretion in basal glucose conditions in DEX rats ($p<0.05$). Altogether, high dose GC treatment induces hyperglucagonemia that contributes for the glucose homeostasis imbalance. The impairment of α-cell response to the inhibitory glucose signals seems to be the cause for hyperglucagonemia that, in parallel, may contribute for the compensatory insulin hypersecretion. Blockage of the glucagon receptor is effective in prevent the GC-induced hyperglycemia.

Keywords: Insulin resistance, pancreatic α-cells, glucocorticoids, glucagon

FC003

Early life protein malnutrition metabolic programming of insulin resistance in diet-induced obesity (DIO)

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Increased fatty acids beta-oxidation by products such as ceramides, acylcarnitine diacilglycerol as well as reactive oxygen species are associated with insulin resistance and type 2 diabetes development. Early life protein malnutrition and obesity separately lead to mitochondrial impairment. However, little is known regarding the impact of DIO after early life protein malnutrition. Thus, we aimed to investigate if protein malnutrition would worsen insulin resistance in DIO mice as well as the effects of some treatments to restore insulin signaling. After weaning, C57BL-6 mice were fed during 45 days with a protein-restricted diet (6% of protein). After that, they received high fat diet (HFD) for 60 days. Mice were also treated with taurine (TAU, 5% in the drinking water) or were submitted to an endurance exercise training protocol. Liver and skeletal muscle samples were assessed in order to establish metabolic alterations and insulin signaling. Protein-restricted mice experienced a higher catch-up growth after DIO showing similar levels of body weight, fat content and insulin resistance reported in normoprotein-fed mice. Although metabolic alterations as well as insulin signaling impairment were, generally, similar in normo- and low-protein-fed mice, protein malnourished group was less prone to reestablish insulin signaling in response to TAU supplementation and exercise training. Although protein malnutrition did not improve DIO mice insulin resistance, it impaired insulin sensitivity reestablishment.

Keywords: protein malnutrition, metabolic programming, obesity, insulin resistance

FC004

Cardiovascular, renal and metabolic sequelae in offspring exposed in utero to a maternal diet high in saturated fat.

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The prevalence of cardiovascular renal and metabolic disease has risen sharply in the past 3 decades across the globe. Although adult lifestyle factors have been identified as playing a role in the aetiology of the metabolic syndrome, it is also appreciated that the diet consumed by a pregnant mother can program obesity, hypertension and metabolic disease in her offspring. Here we describe the consequences for offspring health in rat dams of consuming a diet rich in saturated fat during pregnancy and suckling. Sprague-Dawley rats were fed either a control diet (7% fat w/w) or a diet rich in saturated fat (23% w/w) for 3 weeks prior to mating, during pregnancy and in the suckling period. Pups were weaned at 21 days of age to a control (7% fat w/w) diet. At 6 and 12 months of age, the offspring were examined. Cardiovascular and renal function were assessed by indwelling telemetry radiolabelled tracer clearance respectively. Metabolic parameters (blood glucose, insulin, leptin) were assessed and body composition determined by DEXA. Next generation sequencing was used to determine transcriptomic profiles in skeletal muscle and Gene Set Enrichment Analysis to detect coordinated changes in transcription. Even though mothers consuming the high fat diet did not develop overt obesity their offspring were programmed to develop hypertension, altered renal function and accelerated glomerulosclerosis. The offspring of saturated fat fed dams demonstrate insulin resistance and a transcription profile of inflammation, cytokine activation and mitochondrial dysfunction in skeletal muscle. Consumption of a high fat diet during pregnancy and suckling can result in permanent alterations in offspring health even though neither mother nor offspring develop frank obesity. These data highlight the importance of advising women to moderate the intake of saturated fat during pregnancy.

Keywords: developmental programming, maternal diet, metabolic syndrome, hypertension, gene set enrichment.

FC005

Leptin: new insights into IUGR pathology

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Intra-uterine growth retardation (IUGR) often experience adverse perinatal outcomes. Their general developmental delay affects the growth and functional properties of various organs. In rodents, the temporal windows of development for many organs differ from those of humans, making it difficult to directly extrapolate results to humans. Pigs are an advantageous model for the timing of development and maturation of its organs. In pigs, IUGR occurs naturally and IUGR results in similar long-term pathological consequences as in humans. We previously showed that IUGR postnatal leptin piglets enhanced their ponderal index and linear growth and was associated with an improvement in the growth of several organs. The purpose of this presentation is to underlie some of the physiological processes that occur at the cellular level of the immune, gastrointestinal, and reproductive systems after leptin neonatal supply. IUGR piglets were injected from day 0 to day 5 with either 0.5 mg/kg/d leptin (IUGRLep) or saline (IUGRSal), organs were collected and sampled at day 21. Leptin induced an increase of the relative weights of the liver, spleen, pancreas, kidneys, and small intestine. Notable structural and functional changes occurred in the ovaries, pancreas, and secondary lymphoid organs. The ovaries of IUGRLep piglets contained less oogonia but more oocytes enclosed in primordial and growing follicles than the ovaries of IUGRSal piglets, and FOXO3A staining grade was higher in the germ cells of IUGRLep piglets. Within the exocrine parenchyma of the pancreas, IUGRLep piglets presented a high rate of apoptotic cells associated with a higher trypsin activity. In the spleen and the Peyer's patches, B lymphocyte follicles were much larger in IUGRLep piglets than in IUGRSal piglets. Moreover, IUGRLep piglets showed numerous CD79+cells in well-differentiated follicle structures. This study highlights a new role for leptin in general developmental of the IUGR.

Keywords: leptin, IUGR, pig, organ maturation, ovaries.

FC006

Neonatal environmental stress on neuronal changes and behavior in rats

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Early life stress has been related to neuroendocrine and behavioral disorders later in life. The understanding of the mechanisms that have altered in the infants, as a consequence of the environmental intervention, seems to be crucial to unravel the development of the subject. Neonatal handling has been used experimentally with rodents in order to analyze the long--lasting effects of environmental intervention on mother-infant relationship disruption. Using this procedure, it has been shown that the repeated handling procedure reduces stress responses, probably due to the elevated expression of corticotropin releasing hormone (CRH) receptors in the hippocampus, which increases effectiveness of the stress negative feed-back. This observation shows that the handling procedure may produce a morphological change that is a stable signature in the central nervous. In female rats, we demonstrated a reduction in reproductive parameters both in female and male rats, as well, decreased sexual behavior. Neonatal handling reduces plasma levels of luteinizing (LH), follicle stimulating hormones and prolactin in the afternoon of the proestrus. The LH-releasing hormone content in the medial preoptic area (MPOA) was significantly higher than in the non-handled group. A stable reduction in the number of cells and in the size of the cell soma, which were lower in handled females than in non-handled ones, was shown. The repeated mother–infant disruption imposed by the handling procedure "lesioned" the MPOA. In the locus coeruleus, there was also a reduction in the number of cells. Moreover, related to the reduction in social behaviors, handling decreases the number of oxytocin-positive parvocellular cells in the paraventricular nucleus (PVN) in adult females, but not in the magnocellular, PVN or in the supraoptic nucleus. Although the responsiveness of the hypothalamic–pituitary–adrenal axis to stressors is reduced in the neonatal period, environmental interventions may impact behavioral and biochemical mechanisms relevant to the animal at that early age.

Keywords: neonatal, stress, early life programming.
Funding: FAPERGS, FAPESP, CNPq & CAPES.

FC007

Metabolic flexibility in adipose tissue and skeletal muscle of MGS-obesity model is positively modified by chronic aerobic exercise

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Metabolic flexibility represents the body efficiency to use different energy sources, in accordance to their availability. This physiological aptness is dependent on the oxidative capacity and insulin sensitivity in peripheral tissues, being impaired in numerous pathophysiological conditions. Here we investigated the effect of monosodium L-glutamate (MSG)-induced obesity on metabolic flexibility in adipose tissue (AT) and skeletal muscle (SM), and also the chronic effect of aerobic exercise on it. Newborn males Wistar rats were injected with a MSG solution (4 mg/g bw). Exercise protocol consisted of swimming, during one hour, five days a week, for 8 weeks. The metabolic flexibility in AT and SM was evaluated by in vitro analysis of diverse metabolic pathways, protein expression of immunometabolic regulators, and infiltration of immune cells in the adipose tissue. At 150 days after birth, the MSG-treated animals had hyper adiposity, hypertrophy of adipocytes, insulin resistance, hyperinsulinemia, dyslipidemia and ectopic triacylglycerol (IMTG) accumulation. AT oxidative capacity was reduced, and also unable to raise insulin-stimulated glucose oxidation, characterizing a metabolic inflexibility state. There was an inability to raise lactate production and glycogen synthesis by insulin stimulation, along with a lower expression of AMPKα1 and PGC-1α. Macrophage and CD8+ T-cells infiltration were increased. Oxidative capacity was not impaired in SM of MSG-treated animals. Exercised MSG-treated animals had lower adiposity, no insulin resistance, dyslipidemia or IMTG accumulation. Exercise improved the oxidative capacity of fatty acids in the AT and SM, as well as insulin stimulated glucose oxidation in both of them. Exercise partially preserved AMPKα1 expression and prevented AT infiltration. Our results show in MSG-obesity model impairment of metabolic flexibility in AT and SM. Chronic aerobic exercise prevents these features, constrains the adiposity progression, and modulates immunometabolic pathways.

Keywords: metabolic flexibility, insulin resistance, obesity, L-monosodium glutamate, aerobic exercise, adipose tissue, skeletal muscle.

FC008

Evolutional characterization of nonalcoholic steatohepatitis in monosodium L-glutamate-induced obese mice

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This study aimed to characterize the evolution of metabolical and histopathological patterns of nonalcoholic steatohepatitis (NASH) in monosodium L-glutamate (MSG)--induced obese mice. Male offspring of Mus *musculus* litters were injected with MSG (4 mg/g/day, sc, n=21) or 0.9% NaCl (0.01ml/g/day, sc, n=22, CTR group) in alternate days during the first 10 days of life. At the ages of 60, 120 and 180 days, the animals were euthanized for blood and liver samples collection. Serum and liver lipid profile, as well as, hepatic histopathological analysis were undertaken. All protocols were approved by UFMA's CEUA (process #5845/2012-41). Results were expressed as mean ±SEM and analyzed by Student's t test or ANOVA for $p<0.05$. MSG mice showed higher serum triglyceride levels at all ages (60, 50.9±10.7; 120, 132.9±26.9;

180, 119.0±7.5mg/dl) as compared to CTR (60, 30.4±2.7; 120, 65.9±19.8; 180, 54.9±4.5mg/dl, p<0.05). Hepatic total fat content of MSG group was increased in a non-age-dependent manner, contributing to triglyceride accumulation (MSG: 60, 49.8±7.1; 120, 42.7±5.3; 180, 126.6±15.7mg/g of liver, CTR: 60, 10.2±1.4; 120, 19.7±1.1; 180, 31.06±5.532mg/g of liver), p<0.05. At the age of 60, 83.3% of MSG mice presented nonalcoholic fatty liver, which evolved to 57.1% in those of 120 days of life. Notwithstanding, 28.6% of MSG120 and 100% of MSG180 presented NASH. On the other hand, CTR mice presented NASH only at the age of 180 days of life (20%). Real-time PCR protocols for NASH and ER stress markers are under analysis. Taken together, these results show that MSG-induced obesity cause nonalcoholic fatty liver disease in young animals, which promptly evolves to NASH. However, there is still a gap concerning the main molecular targets involved in this pathophysiological mechanism.

Keywords: non-alcoholic steatohepatits, hypertriglyceridemia, L-monosodium glutamate, obesity and rodent.

Funding: FAPEMA, CNPq & UFMA.

FC009

Diet and exercise interventions before and in pregnancy prevent adverse programming offspring outcomes of obese mothers

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Human and animal studies indicate that maternal obesity predisposes offspring to later-life chronic diseases. There is a need for effective maternal interventions that prevent these outcomes. Hypothesis: interventions involving a change in maternal diet or level of exercise may act to improve maternal and offspring outcomes. From weaning through pregnancy and lactation rats ate chow (C) or high energy obesogenic diet (MO). A third part of MO wheel-ran 30 min, 5 times/ week from day 90 to delivery (MOEx), another third of MO group was switched to control diet from day 90 to delivery (dietary intervention group, DINT). All mothers were bred at day 120 and continued their diet. Offspring were weaned on to control diet. At 110 days, offspring from different litters were euthanized by decapitation, and fat depots weighed, body weight, serum insulin, glucose, leptin, TG and cholesterol were measured. At the end of lactation, MO mothers were 16% heavier than C, which did not change with exercise but was recuperated with DINT. MO increased all offspring parameters of fat metabolism: body and fat weight, serum insulin, leptin and triglycerides without changes in cholesterol and glucose. DINT and exercise prevents offspring insulin and triglycerides increased, DINT partially prevents higher levels of fat weight and exercise prevents leptin rise by MO. Maternal obesity increased offspring metabolic parameters. Maternal diet and exercise interventions prevent some of the outcomes. Other potential interventions remain to be investigated to permit evidence-based changes in clinical management. These include supplemented diets with polyunsaturated fatty acids or anti-oxidants. Different interventions to improve outcomes may act through different mechanisms. If so, a combination of approaches may lead to even better results for the mother and the offspring.

Keywords: maternal obesity, exercise, dietary intervention.

FC010

Effects of early adrenaldemedullation on fat accumulation in lean and obese trained rats

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Obesity is a disease of multifactorial etiology, which is associated to metabolic, hormonal and inflammatory dysfunctions. Several studies have suggested that physical exercise is able to prevent or attenuate factors linked to obesity. Additionally, it is known that adrenal catecholamine plays an important role on lipolysis. Nevertheless, the influence of catecholamine on obesity development and on physical training is still controversial. The goals of the present work were 1) evaluate whether adrenodemedullation increases fat accretion in sedentary lean and diet-induced obese rats; 2) investigate whether the lack of adrenal catecholamine impairs fat reduction on lean and obese trained rats. All protocols were performed in accordance with Brazilian College of Animal Experimentation. Male Wistar rats were distributed in 8 groups: Sham sedentary control (SSedC), Sham exercised control (SExC), adrenodemedullated sedentary control (AdSedC), adrenodemedullated exercised control (AdExC), Sham sedentary obese (SSedOb), Sham exercised obese (SExOb), adrenodemedullated sedentary obese (AdSedOb) and adrenodemedullated exercised obese (AdExOb). Animals were adrenodemedulated at 21-day-old, and at 22-day-old, obese-induced groups received high-sugar diet. Physical training started at 35-day-old, and consisted on treadmill running 5 times a week during 8 weeks. Animals were euthanized at 90-day-old. Retroperitoneal fat pad was removed and weighted. Total catecholamine content was measured by fluorimetric method. High-sugar diet increased the retroperitoneal fat mass by 143% compared to SSedC (p<0.001). Despite AdSedC and AdSedOb presented high fat accretion, the values were not statistic significantly. SExC and SExOb presented a reduction of 43% and 37% in retroperitoneal fat pad (p<0.01); however, AdExOb rats had only 27% reduction compared to AdSedOb (p<0.05). Exercise training restored SExOb fat mass compared to SSedC. Total catecholamine content was not statistically different in all experimental groups. Exercise training is less effective on fat reduction when associated to a high-sugar diet. Sympatoadrenal axis is required to fat mobilization stimulated by exercise.

Keywords: Obesity, high-sugar diet, catecholamines, exercise training.

FC011

Maternal separation and feeding behavior in adult life

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The periodic separation between mothers and infants has been used as a perinatal stress model. Rats submitted to this stressful model show high responsiveness in adult age to stress and anxiety, deficits in learning and memory, and changes in the behaviors patterns. The present study aimed to investigate in adult rats the effects of periodic maternal separation during lactation in different light cycles on parameters of the feeding behavior control. The maternal separation for six hours during periods of light or dark cycle was performed from the 1st to 14th day of life. At 150 days of life, we evaluated the behavioral satiety sequence, macronutrients preference, reaction to palatable diet and fasting, and corticosterone levels. Related to food intake, we observed that females submitted to maternal separation during light cycle shows increase compared to control. In the test of food preference, females submitted to maternal separation shows increase in protein intake, decrease in fat intake, no change in carbohydrate intake and increase in intake of palatable diet compared to their controls. We observed in male rats an increase in corticosterone levels only for those separated during the dark cycle. For females, it was observed higher corticosterone due to maternal separation independent of the light cycle. The study indicates possible phenotypic adjustments in the structure of feeding behavior promoted by maternal separation, especially with separating in dark cycle. This dissociation between mother's presence and milk intake probably acts as factors that induce adjustments in adulthood feeding behavior.
Keywords: stress, maternal separation, feeding behavior, satiety.

FC012
Differences in behavioral outcomes at adulthood between models of early weaning in rats

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Exclusive and prolonged breastfeeding has been associated with protection against long-term chronic diseases, such as obesity and diabetes. However, women are currently becoming more active in the labor market and, for many new mothers, the breastfeeding period has been shortening progressively. In fact, no more than 35% of infants worldwide are exclusively breastfed during the first four months of life. Epidemiological and experimental studies have shown that nutritional alterations during early periods of life can imprint epigenetic changes, which lead to disorders such as obesity, diabetes, hypertension and vulnerability to psychopathological disorders such as anxiety and depression at adulthood. Experimental models that address the immediate, early and late-emerging repercussions of shortened lactation may be useful to assess its long-lasting effects on the nutritional, metabolic, endocrine and behavioral status of the progeny. In this sense, three different models of early weaning (EW) in Wistar rats have been frequently used: 1) Maternal separation – progenitors are separated from the offspring three to five days before the usual weaning day (at postnatal day 21); 2) Pharmacological weaning – lactation is interrupted during the last three days of the lactation period through maternal treatment with bromocriptine, a prolactin inhibitor; 3) Physical barrier weaning – a breast bandage is placed around the dams preventing pups from suckling during the last three days of the lactation period. The offspring of the three aforementioned EW models have been shown to present significant metabolic and endocrine alterations at adulthood. For example, the pharmacological EW was shown to result in obesity, insulin and leptin resistance, lower levels of serum TSH, T3 and T4, hypoadiponectinemia, hypoprolactinemia, hypercorticosteronemia and higher total adrenal catecholamine content. The physical barrier EW was shown to result in obesity, hyperphagia, hypoadiponectinemia, hyperglycemia, leptin and insulin resistance, hypoprolactinemia and higher total adrenal catecholamine content. Curiously, the behavioral outcomes vary considerably between EW models: While maternal separation results in higher anxiety, higher motor activity and reduced cognitive performance, and pharmacological weaning was shown to result in higher anxiety and poorer memory/learning performance, no significant behavioral effects were observed regarding the adult offspring of the physical barrier weaning model. These results indicate that aspects other than early weaning are interfering with the development of the nervous system, which puts into question the validity of the different EW models as a means to study the repercussions of a shortened period of lactation. Here we will discuss the methodological procedures associated with each of the three models that may help explain the differences in behavioral outcomes.

Keywords: early weaning, developmental plasticity, anxiety, novelty-seeking, memory, learning, motor activity.
Funding: FAPERJ, CNPq & CAPES.

FC013
Undernutrition and stress

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Undernutrition is caused by poverty and disease, associated with inadequate food consumption during growth. It is the most powerful condition of physiologic stress, and over time, this can lead to weakening of the body and even death. Studies shows that undernourished children have an increase in sympathetic tone and higher blood cortisol concentrations. It is known that cortisol generates a redirect energy flow favoring fat accumulation mostly in the central region of the body, low fat oxidation low resting and postprandial energy expenditure, insulin resistance in adulthood, hypertension, dyslipidemia and a reduced capacity for manual work. Stress at the beginning of life results in an epigenetic effect, altered neurogenesis, cerebral plasticity, cognitive function and appetite control. We selected children by anthropometric surveys performed in schools next to slums in São Paulo city. Those selected were subjected to screening to determining pubertal stage, discard infections and endocrine disorders. Only those who had normal biochemical parameters were included in the study and divided into four groups: recovered from undernutrition (*n*=30; before treatment: HAZ and/or BAZ <-2.0; after treatment HAZ and BAZ >-2.0);

Stunting (*n*=24; HAZ <-2.0); Underweight (*n*=24; BAZ <-2.0) and Control (*n*=47; HAZ and BAZ >-2.0). At the protocol day, children were subjected to a stressor stimulus that consisted of immersion of the hand in cold water (2ºC – 5ºC) for one minute (10h) and a relaxing stimulus that consisted of watching a video with pictures of nature for 5min (14h). We collected 13 saliva samples of each child throughout the day. The stunting group had high mean values of salivary cortisol. The underweight group showed a decrease in mean cortisol after waking and a slow decrease throughout the day. The recovered group showed similar mean values compared to control. Nutritional recovery may have a positive impact in the stress response.

Keywords: Undernutrition, stunting, nutritional recovery, stress, cortisol.

FC014

Insulin and insulin-like growth factor 1 signaling as a fine control on insulin synthesis, and their secretion from different animal models.

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Insulin and insulin-like growth factor 1 (IGF-1) are involved with many metabolic process. These proteins as well as their receptors (insulin receptor – IR; insulin-like growth factor 1 - IGF-1R) can be detected in pancreatic beta cells where they seem to play an important role in insulin secretion and beta cell survival. However, at unbalance physiological conditions such as diabetes, caloric restriction, and obesity, showed a profound impact on insulin syntheses and their secretion. The present work show a brief review about the effects of IR/IGF-1R signaling, as well as proteins downstream in pancreatic islets from animals submitted to a divergent conditions as well diet, temperature, and environment.

Keywords: IR/IGF-1R signaling, pancreatic islet, environment adaptations.

Funding: FAPITEC/SE.

LECTURES

Short Communications – (SC)

SC001

Perinatal high-fat programming induces parasympathetic nervous system hyperactivity and affects pancreatic beta cell function in weanling rats

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High-fat diets have been associated to the development of obesity, insulin resistance and metabolic complications. Fetus and offspring development are highly sensitive to environment changes, and nutrition is an important factor affecting both growth and maturation, and this has been linked to the development of metabolic diseases later in life. We aimed to investigate the weanling rat offspring (at 22-day-old) from dams maintained on a standard (NF) or a high-fat diet (HF) throughout the lactation period. The HF diet (35% of fat) was offered to dams from the delivery day until the end of offspring nursing (day 21). At 22-day--old, the overnight fasted pups underwent different experimental assessments. Data were analyzed by Student t-test. HF pups gained more weight during the lactation period and they became heavier than the NF pups at 21-day-old (final body weight: NF, 50.9±0.9g vs HF, 55.8±0.6g, $p<0.05$). Both retroperitoneal and periepididymal fat pads were increased in HF rats (56% and 47%, respectively). HF rats had no differences in the fasting glycemia comparing with NF rats. The fasting insulinemia was 6-fold lower in HF rats ($p<0.05$). Regarding glucose-stimulated insulin secretion, HF rats secreted more insulin in all glucose concentrations ($p<0.05$), but not in 5.6mM. Interestingly, the recordings of the vagus nerve electrical activity in the HF rats showed 41% higher activity than NF pups ($p<0.05$). No changes were observed in the protein expression of islets from HF group regarding the cholinergic muscarinic receptor type M2 (M2mAChR), whereas the M3mAChR expression was 2.5-fold higher ($p<0.05$) in the HF rats compared with the NF group. HF diet may induce changes on milk quality of rat mothers, which may be transferring nutrients and biomolecules inducing obesity in offspring. We suggest that high parasympathetic activity and changes in the M3mAChR expression associated to impaired metabolism is a hallmark of an expression of malprogramming, already shown by these young weanling rats, which could result in the development of metabolic diseases when they become adults.

Keywords: high-fat programming, lactation period, autonomic nervous system, beta-cell function, muscarinic protein expression.

Funding: CNPq, CAPES & Fundação Araucária.

SC002

Changes in muscarinic receptor subtypes composition in pancreatic islets from obese rats

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Impaired pancreatic β-cell function and/or insulin secretion/action is a link between obesity and type 2 diabetes, a worldwide public health burden. Regarding the obese humans and animals, it has been showed increased parasympathetic activity and hyperinsulinemia associated with insulin oversecretion, which can suggest the idea that the composition of muscarinic acetylcholine receptors (mAChR) in pancreatic islets may be compromised. Thus, the use of an experimental model that presents these features is essential for the characterization of mAChR in pancreatic β-cells of obese individuals. We aimed to characterize the M1–M_4mAChR subtypes, in pancreatic islets from pre-diabetic obese rats, neonatally treated with monosodium L-glutamate (MSG). At 90-day-old, rats of both groups were used to biometric and biochemical evaluation; anti-muscarinic drugs were used to study the mAChR function, either in vivo or *in vitro*. Isolated pancreatic islets were used to mAChR characterization. Atropine treatment reduced the insulin secretion in MSG and control group; while the M_2mAChR selective antagonist increased this effect. Moreover, insulinostatic effect of M_3mAChR selective antagonist was significantly high in MSG group. The M_1mAChR and M3mAChR expression were increased in MSG-obese group by 55% and 73%, respectively. Inversely, the expression of M2mAChR decreased by 25% in MSG group, while it was unchanged to M_4mAChR. Immunofluorescence staining confirmed the changes observed by western blotting. The functional changes as well as altered composition of the mAChR (M_1–M_4) subtypes are pivotal on to demand high pancreatic β-cell insulin secretion in MSG-obese rats; which can be directly associated to vagal hyperactivity, and peripheral insulin resistance.

Keywords: MSG rats, muscarinic receptors subtypes, pancreatic islets.

Funding: CNPq, CAPES & Fundação Araucária.

SC003

Stressed pattern by maternal low-protein diet programs pancreatic β-cell function to resist dexamethasone disturbances in adult rat offspring

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Nutritional insults early in life have been linked with the metabolic diseases expression in adulthood. Low-protein diet, as well as maneuvers that increase blood glucocorticoids levels may induces metabolic syndrome. We aimed to study the synthetic glucocorticoids (dexamethasone, Dex) exposition on the glucose--insulin homeostasis and β-cell function of the adult rat offspring from dams fed a low-protein diet during lactation. Wistar rats were nursed by dams fed a low-protein diet (4%, LP group) during the first two weeks of lactation; while control were fed a normal--protein diet (23%, NP group). Adult rat offspring, were injected with Dex (1mg/kg bw) or saline solution by five consecutive days. After that, rats were submitted to intravenous glucose tolerance test (1g/kg bw). Insulin secretion from isolated pancreatic islets, under the action of glucose (mmol/L: 5.6, 8.3, 11.1, 16.7, 20.0 and 24.0) or Dex (μmol/L: 1, 2, 4, 8 and 16) were evaluated. Data were submitted to one-way ANOVA or Student t-test. At 90-day--old, LP rats showed a lean phenotype, hypoinsulinemia, high corticosteronemia, and high peripheral insulin sensitivity ($p<0.05$). Glucose-induced insulin secretion was lower in islets from LP than NP rats ($p<0.01$). Dex inhibited insulin secretion in islets from both groups; however, it was 44% less prominent in islets from LP than NP rats. In relation to rats treated with saline, chronic Dex induced fasting hyperglycemia, hyperinsulinemia, and insulin resistance; as well as glucose intolerance in both groups ($p<0.01$); however the magnitude of these effects was bigger in NP than LP rats ($p<0.01$). Maternal protein-malnourishment early in life induced a stressed pattern in adult rat offspring, which may confer resistance against the stressful action of dexamethasone on the LP rat's metabolism including pancreatic β-cell function.

Keywords: metabolic programming, low-protein diet, insulin resistance, dexamethasone.

Funding: CNPq, CAPES & Fundação Araucária.

SC004

Hypothalamus-pituitary-adrenal axis disruption changes pancreatic islets muscarinic receptors composition in programming-obese rats

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High levels of glucocorticoids are an important clinical signal in type 2 diabetes. The unbalanced action of hypothalamus--pituitary-adrenal (HPA) axis in the obese pre-diabetic rat model induced by neonatal administration of monosodium L-glutamate (MSG) has been observed; likewise, impaired autonomous nervous system (ANS) activity, together with impaired cholinergic response in pancreatic beta-cells may be a key factor for the high insulin secretion, imprinted in these MSG-programmed rats. A link between HPA axis and cholinergic parasympathetic pathways are not totally understood. The goal of the present work was to study the cholinergic insulinotropic effect in hypercorticosteronemic MSG-obese rats, focusing on the subtype M3 of the cholinergic muscarinic receptor (mAChR) in pancreatic islets. During the first five days of life, male pups received subcutaneous injections of MSG [4g/kg body weight (bw)/day; MSG group]; while, control rats received saline solution (CON group). At 80-day-old, MSG-rats underwent vagotomy (MSG-VAG), adrenalectomy (MGS-ADX) or the both surgery (MSG-VAG-ADX). At the same time, either CON or MSG rats were sham operated (CON-Sham and MSG--Sham). Fasting insulinemia, glycemia and corticosteronemia were evaluated. Pancreatic islets were isolated and M3mAChR expression was analyzed at 90-day-old by western blotting. Rats from CON-Sham, MSG-Sham groups were hyperinsulinemic and hypercorticosteronemic ($p<0.01$). Meanwhile, fasting glycemia, insulinemia, and corticosteronemia were not changed by vagotomy. The MSG-ADX and MSG-ADX-VAG groups did not present detectable corticosterone plasma levels, confirming the adrenalectomy surgery; however, glycemia and insulinemia were reduced ($p<0.001$). The M3mACh expression was higher in islets from MSG-Sham and MSG-VAG groups when compared to the CON-Sham; by the other hand, MSG-ADX and MSG-ADX--VAG presented a reduction of 24% and 34% respectively in the M3mACh expression in relation to MSG-Sham ($p<0.001$). HPA axis malfunction is involved in the hyperinsulinemia of programmed MSG-obese rats, rather than their high vagus tonus. The pancreatic islets M3mAChR overexpressed may be due to the high levels of corticosterone.

Keywords: hypothalamus, islets, muscarinic receptor, obesity.

Funding: CAPES, CNPq & Fundação Araucária.

SC005

The glucocorticoids and their effects on clock genes expressions from pineal glands.

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We evaluated isolated pineal from rats treated with dexamethasone and their effects on genes involved with clock genes. Rats with 170-190g body/weight were maintained at 23±2ºC under 12-h light/dark cycle, with water and standard rodent chow ad libitum. Two groups of animals were utilized, control group (received saline solution 0.9%) and Dexa group (received dexamethasone solution 2mg/g body wt), after 10 days of treatment, all animals were submitted to experimental protocols. Pineal glands were isolated from both group at zeitgeber time (ZT) zt17, zt21 and zt22, after that, were sonicated, and gene expressions (Brain and muscle ARNT-like – BMAL1, Period gene 1 – PER1, Period gene 2 – PER2, Cryptochromes 1 - CRY1, and Cryptochromes 2 – CRY2) by q-PCR. Gene expression of BMAL1 were enhanced in pineal glands from Dexa group by +15% (at zt21, and zt22), respectively when comparing to Control group ($p<0.05$). The Genes expressions of PER1 and PER2 were enhanced by +25% (at zt17, zt21, and zt22) in pineal glands from Dexa group when comparing

to Control one (p<0.05). The Genes expressions of CRY1 and CRY22 were enhanced by +20% at zt2, and zt21, respectively in pineal glands from Dexa group (p<0.05). The present work shows that treatment with dexamethasone impairs clock gene expressions from pineal gland from dexamethasone treated rats. These results suggested a possible role of these genes on pineal function.

Keywords: pineal gland, clock genes, dexamethasone treated-rats.

Funding: FAPITEC/SE

SC006

Omega fatty acid mixture n-6/n-3 (1.2:1.0) diet supplementation during perinatal life attenuates mice obesity programming

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Barker hypothesis indicates that stressful environmental factors during perinatal life, particularly nutrition, 'program' the babies for an increased risk of metabolic diseases in adulthood. However, functional nutrition containing vitamins and/or antioxidants, among other substances are used to treat metabolic dysfunction and even they can mitigate malprogramming. Evidences support that food containing omega fatty acids, mostly n-3 and n-6, helps to prevent metabolic diseases. It has been observed that diet with ratio of n-6/n-3 close to 1:1 offer during pregnancy and lactation induced beneficial early brain development of mice offspring. The initial week of lactation is crutial to brain development of rodents. Brain hypothalamus has important role for metabolic homeostasis regulation. High doses of monosodium L-glutamate (MSG) administrated in new born rodents causes partial destruction of hypothalamus and induces obesity onset. The goal of the current work was to know whether omega fatty acid n-6/n-3 with rate 1.2:1.0 in a supplemented diet offered during perinatal life can attenuate the MSG-induced obesity in mice. Pregnant mice received diet, containing omega n-6 fatty acid from sunflower oil, supplemented of flaxseed powder as source of omega n-3 fatty acid to reach a rate n-6/n-3 of 1.2:1.0. Diet treatment was extended to all lactation (Flax-diet). Another mice pregnant group received commercial diet (Com-diet). New born mice from both groups were treated neonatally with MSG. They were divided in two groups, from mothers that received Flax-diet (MSG-Flax) and from mothers Flax-diet untreated (MSG-Com). A batch of MSG-untreated mice offspring, from mother Flax--diet treated (Control-Flax) and untreated (Control-Com) were also studied. After weaning (21-day-old), pups from all groups ate commercial diet. Body weight (BW) and chow intake were taken each two days during 59 days. Intraperitoneal glucose tolerance test (ipGTT) and intraperitoneal insulin tolerance test (ITT) were used to observe the glucose homeostasis. Periepididymal fat pad was removed and weighted from killed 90-day-old mice by thiopental over anaesthesia. From another batch of mice pancreatic islets were isolated and exposed to different glucose concentrations to study insulin secretion. Data were submitted to two-way ANOVA by the *GraphPad Prism* version 6.01. As expected MSG-mice developed obesity with impaired glucose homeostasis, showing fasting hyperglycaemia and hyperinsulinaemia, glucose intolerance, insulin tissue resistance, dysfunction of pancreatic islets and huge fat tissue accretion. However, flaxseed supplementation during maternal phase was able to improve metabolism malfunction, including decrease of fat tissue accumulation and increase of glucose--induced insulin secretion in pancreatic islets from MSG-mice. Polyunsaturated fatty acids n-6 and n-3 with a rate of 1.2:1.0 in diet offered to mothers during perinatal life attenuate the metabolism malfunction in adult MSG-programed offspring mice.

Keywords: MSG-obese mice, insulin resistance, omega fatty acid-supplemented diet.

SC007

Short-term exercise prevents autonomic dysfunction related to obesity, but not cardiovascular changes induced by high-fat diet in rats

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Obesity is strongly associated with increased blood pressure (BP). It was observed that high-fat diet (HFD) provokes autonomic dysfunction related to metabolic and cardiovascular diseases. Exercise has been considered a non-pharmacological treatment strategy to combat obesity and its related diseases. Though, little is known about the long-lasting protective effect of exercise on BP. In the present study, we examined if previous exercise is able to prevent cardiovascular changes induced by a HFD. Young adult Wistar rats (60-day-old) were submitted to an aerobic exercise program with evolution from 10min (16cm/s) to 60min (26cm/s), three times a week, during 30 days. Just after, animals were exposed to a HFD (30% of lard) during 30 days. Control animals had access to normal-fat diet (commercial chow). Analyses were performed in 120-day-old rats. Animals were anaesthetised with thiopental (2.5ml/100g body weight) for measure of mean BP (MBP), heart rate and activity of vagal and major splanchnic nerve. Fat pad from retroperitoneal and visceral tissues were weighted. Data were submitted to two--way ANOVA (factors: diet and exercise) with *GraphPad Prism* software version 6.01. HFD increased MBP and heart rate. Furthermore, the lasting reduction of MBP induced by exercise was not observed in animals exposed later to HFD (Pdiet x exercise<0.01). Parasympathetic nerve activity was increase, while sympathetic splanchnic nerve activity was reduced by HFD. Though, previous exercise attenuated vagal changes induced by HFD (Pdiet x exercise<0,01). HFD increases retroperitoneal and visceral fat pads, but previous exercise attenuated fat deposition in both groups. Previous exercise prevents obesity induced by HFD, but is not able to avoid BP and heart rate increase induced by HFD. These results suggest that short-term exercise performed previously to a high-fat dietary insult may prevent autonomic dysfunction related to metabolism, but has little protective effects on cardiovascular system.

Keywords: obesity, exercise training, blood pressure.

SC008

Insulin secretion and action are altered by in vitro amino-acid restriction: possible role of sirtuins

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Protein malnutrition during early life leads to poor¬-cell function and may underlie some forms of diabetes in adulthood. Protein acetylation has emerged as a relevant process to the fine-tuning of metabolism. Sirtuins (SIRTs) are NAD+-dependent protein deacetylases and/or ADP-ribosyltranferases known to regulate insulin secretion and glucose homeostasis. Here, we evaluated insulin secretion and the expression of SIRTs in pancreatic islets cultured in an amino-acid (aa) deficient medium. We also assessed the insulin signaling and metabolic activity in HepG2 cells subjected to the same culture protocol. Pancreatic islets were isolated from weaned C57/Bl6 mice and cultured for 48h in aa-free RPMI medium adding back 1, 0.5 or 0.25x of its initial content. Glucose and KCL-induced insulin secretion as well as SIRT1 and 4 protein expression were determined. HepG2 cells were subjected to the same restriction protocol in DMEM medium. Insulin signaling (p-Akt) and a MTS assay were performed. Statistics: one-way ANOVA and Student's t-test where indicated. Statistical significance was set at $p<0.05$. Cultured islets exposed to 0.25x aa secreted less insulin at 22.2mM compared to 1x ($p<0.01$; 1x = 1.56±0.3; 0.5x = 0.86±0.2; 0.25x = 0.32±0.05ng/islet). KCL-induced insulin secretion was also impaired in 0.25x islets ($p<0.05$; 1x = 1.80±0.3; 0.5x = 1.13±0.2; 0.25x = 0.85±0.2ng/islet). SIRT4 protein expression was significantly increased in 0.25x islets ($p<0.05$; 1x = 0.62±0.2; 0.25x = 2.68±0.1 fold over 1x). SIRT1 expression was unaltered by aa restriction. In HepG2 cells, insulin-stimulated Akt phosphorylation over its basal values was significantly higher in 0.25x cells ($p<0.05$; 1x = 1.17±0.2; 0.5x = 1.12±0.07; 0.25x = 1.689±0.1 fold over basal) in comparison to 1x. Finally, metabolic activity assessed by the MTS assay was reduced in 0.25x cells in comparison to 1x ($p<0.05$; 1x = 213.9±3.4; 0.5x = 218.5±4.2; 0.25x = 180.7±5.2Abs490. min). In vitro aa restriction impaired glucose and KCl stimulated insulin secretion and increased SIRT4 expression in islets. In the same context, HepG2 cells showed improved insulin signaling and reduced cellular metabolism.

Keywords: amino-acid restriction, insulin secretion, sirtuin, insulin signaling.

Funding: FAPESP & CNPq

SC009

A role for chronic exercise on glucose metabolism of skeletal muscle from undernourished obese mice

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Obesity and protein malnutrition lead to insulin resistance. However, the changes induced by these two treatments together, and the effects of exercise in this model are unknown. Our aim was to investigate metabolic alterations associated with insulin resistance within skeletal muscle of malnourished obese mice subjected to chronic exercise. After weaning, 21-day-old male C57BL-6 mice were randomly assigned into the control group (C), which received a normoprotein diet (14%, protein) during 105 days; the control high-fat diet (HFD) (CH), which received a normoprotein diet for 45 days, and after that were fed a HFD (60%, fat) for 60 days; the control HFD exercised (CHE), which received a normoprotein diet for 45 days followed by HFD for 60 days, and at the same time, accomplished to an endurance training protocol. The protein restricted R, RH and RHE groups were fed with a low protein diet (6%, protein), receiving the same HFD and exercise treatments. Total body, retroperitoneal and epididymal fat pads were measured. Glucose and insulin tolerance were assessed by ipGTT and ipITT. Protein content from phospho AKT (p-AKT), phosphofructokinase 1 (PFK1), pyruvate dehydrogenase (PDH), citrate lyase (CL), and uncoupling protein 3 (UCP3) was assessed. HFD increased fat pads mass and decreased glucose tolerance in CH and RH, effects reverted in CHE and RHE. Insulin sensitivity was increased only in CHE group. p-AKT, PFK1 and ATP-CL were higher in R compared with C (94%, 73% and 134%, respectively). HFD decreased near 50% expression of CL, p-AKT in C and R, although expression of PFK1 decreased only in RHxR. Finally, the physical training increase expression of CL, p-AKT and PFK1 in C group, and reduced CL in R. Our data showed that glucose metabolism could be specifically modulated by HFD, low protein diet. Physical training can attenuate and change metabolic pathways that are altered in pathological conditions.

Keywords: exercise, skeletal muscle and metabolism.
Funding: Fapesp & CNPq.

SC010

Characterization of MSG-induced obesity as a new model for polycystic ovary syndrome

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We propose the classical L-monosodium glutamate (MSG)-induced obesity rat as a new model for polycystic ovary syndrome. Wistar female rats received MSG (4.0g/kg s.c., n=9) or equimolar saline (0.1mL/10.0g s.c., *n*=9) from 2nd to 10th day after birth. At day 60, Lee Index was calculated and estrous cycle was assessed by vaginal smear for at least 5 consecutive days. Blood samples from overnight fasted rats were collected for serum biochemical profile (glucose, triglycerides and TyG index calculation). When in estrus, animals were sacrificed for serum estradiol (E2), luteinizing hormone (LH) and testosterone (T) measurement. Retroperitoneal fat pads, ovaries and fallopian tubes were also collected. Oocytes were counted under light microscopy. Ovarian histology was performed to verify the number and quality of follicles and cysts. Results were expressed as mean ±SEM by the Student t-test considering $p<0.05$ as statistical significance. MSG rats showed higher Lee index (343±5g/cm3 vs 317±5g/cm3), peritoneal fat (2.5±0.5g/100g vs 0.6±0.1g/100g) serum glucose (134±11mg/dL vs 105±3mg/dL), triglycerides (92±13mg/dL vs 36±3mg/dL) and TyG index (8.8±0.2 vs 7.8±0.1), as compared to lean rats. MSG showed

oligoovulation with predominant diestrus phase (60%) over estrus phase (10%), whereas lean showed regular cycling (28% diestrus and 22% estrus). Sexual hormones were elevated in MSG rats (LH:1.1±0.2mUI/mL vs 0.4±0.2mUI/mL, T:1.9±0.1ng/mL vs 0.8±0.1ng/mL and E2:1.9±0.1pg/mL vs 1.4±0.1pg/mL), which had also fewer oocytes (5.6±0.2 vs 11.6±0.4) and lighter ovaries (0.007±0.0g/100g vs 0.01±0.0g/100g). Histological analysis showed increased number of pre-cysts in MSG (6.2±1.0 vs 1.4±0.4) though the total number of mature cysts did not differ (1.2±0.5 vs 1.0±0.5). MSG had also more atretic follicles (85% vs 43%). SG-obese female rat arises as a promising and low-cost model for PCOS studies, since it plenty fulfills the pathophysiological criteria for this syndrome, e.g., irregular cycle, oligoovulation, follicular atresia, hyperandrogenism, obesity, insulin resistance and dyslipidemia.

Keywords: polycystic ovary syndrome, hyperandrogenism, L-monosodium glutamate, obesity and rodent.

Funding: CNPq, FAPEMA & UFMA.

SC011

Metabolic programming by small litters impairs the VO2max performance of rats during the exercise program

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Obesity is a public health problem related with excessive food intake, sedentary lifestyle and low oxygen consumption (VO_{2max}), that may leads to insulin resistance in skeletal muscles and type 2 diabetes. We investigate whether metabolic programming by small litter size impairs the VO2max of rats exercised from 21- to 90-day-old. Three days after birth, litter size was standardized in 3 pups (small litter, SL) or 9 pups (normal litter, NL). At 21-day-old rats, were weanling and divided into: (sedentary-NL (NL) or sedentary-SL (SL) rats, and exercised-NL (NL-ex) or exercised-SL (SL-ex). At 30, 45, 60, 75 and 90-days-old rats from both groups, underwent an effort test. The test was performed by a gas analyzer in individual treadmill for rodents with initial velocity of 10cm/s and increment of 5cm/s every 3 minutes. VO_{2max} was defined as the largest value of the last 15 seconds, from the rats ran 6 sessions under 55, 58, 60, 60, 63 and 65% of VO2max throughout 2 weeks (3 times/week for 40min/day). At 90-day-old, the overnight fasted animals were sacrificed and blood collected to further glycemia measurement, and retroperitoneal fat pad was removed and weighted. Results were analyzed by one-way ANOVA, stipulating $p<0.05$. Exercise training was able on reducing body weight, retroperitoneal fat and fasting glycemia only in SL-ex (SL, 386±6.0g vs SL-ex, 380±7.5g; $p<0.05$), (SL, 1.62±0.17g/100g vs SL-ex, 0.79±0.15g/100g; $p<0.001$) and (SL-ex, 91.3±3.6mg/dl vs SL, 116.2±4.0mg/dl; $p<0.001$). VO_{2max}, at 30 and 45 days-old, was similar between all groups, at 60-day-old sedentary groups showed a drastic drop with average close of 20.3±1.7ml/min/kg, while the exercise remained on average 29.0±1.1ml/min/kg at 75 and 90-days-old, animals SL-exe (22.9±0.9ml/min/kg) were decreased of 12% compared to NL-exe (26.1±1.0ml/min/kg) and sedentary remained at 60-day-old. Programmed rats by SL display a weak capacity on VO_{2max}, even when exercised, which may be associated to fasting hyperglycemia and high body weight. Further investigation should be performed to better explaining the mechanisms behind of these features.

Keywords: VO_{2max}, hyperglycemia, small litter.

Funding: CAPES.

SC012

Duodenal-jejunal bypass improves insulin secretion stimulated by Cch and PMA in islets from obese rats

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Here we investigated the effect of duodenal-jejunal bypass (DJB) on insulin secretion in pancreatic islets from cafeteria diet-induced obese rats. Male Wistar rats with 60 days of life received chow-rodent (CTL group) or cafeteria diet (CAF group). After 10 weeks of diet, CAF group were divided in sham operated (CAF-SHAM) and CAF-DJB, submitted to bariatric surgery. After 8 weeks, we evaluated fasting and fed glucose and insulin. Static insulin secretion in presence of glucose, carbachol (Cch) and phorbol-ester (PMA) were analyzed. CAF-SHAM group showed increase of 11% and 16% in fasting and fed glycemia, respectively, when compared to CTL group. DDJ normalized these parameters. Fasting and feeding insulinemia were about 4- and 2-fold higher, respectively, in CAF-SHAM than CTL rats. DJB did not change these parameters. In the presence of 5.6 and 11.1mM glucose, insulin secretion was similar between the groups. In the presence of 11.1mM of glucose plus Cch and PMA, CAF-SHAM islets secreted 47% and 83% respectively, more insulin, when compared to CTL. DJB normalized the insulin secretion in presence of these secretagogues. DJB surgery was efficient to reduce glycemia and insulin secretion stimulated by 11.1mM glucose in the presence of Cch and PMA.

Keywords: obesity, Insulin secretion, duodenal-jejunal-bypass.

SC013

Neonatal serotonin reuptake inhibition promotes reduces metabolic indicators in adult rats

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Serotonin (5-HT) is the key neurotransmitter involved in nervous system ontogenesis and is important for energy balance. We aimed to investigate the effects of pharmacological inhibition of neonatal serotonin reuptake on biochemical indicators in adult rats exposed to a hypercaloric diet. Wistar rat pups received daily single subcutaneous (s.c.) injections of sterile saline 0.9% [Control (C), 1µl/g body weight (BW), n=10] or fluoxetine (F; 10mg/kg, 1µl/g BW, s.c., n=10) from postnatal day (PND) 1 to 21. The animals remained on standard diet ad libitum up to 180 days. Another set of animals (8 for each group, C and F) was subjected to a hypercaloric diet for 5 weeks (Cd group, n=8 and Fd group n=8). We analyzed abdominal white tissue deposition; glucose tolerance; and plasma triglycerides and cholesterol before and after the hypercaloric diet. Following the hypercaloric diet, Fd accumulated 64.32% of abdominal white adipose tissue in relation to F, and Cd accumulated 100.96% in relation to C. Two-way ANOVA of the GTT measurements identified interaction among the groups C and F vs Cd vs Fd at 15 and 30 minutes after the glucose injection [F(3.192) = 20.12]. SSRI-treated animals had lower fasting glucose than those in the C group (C = 93.0±4.10ng/dl, n=10 vs F = 85.7±2.63ng/dl, n=10, p<0.001). With the hypercaloric diet, fasting glucose increased more in Cd than in Fd group. There were changes in the amount of plasma cholesterol and relative abdominal adipose tissue after 5 weeks of the hypercaloric diet. Triglyceride levels did not differ between groups. One-way ANOVA showed interaction between the hypercaloric diet and plasma cholesterol levels [F(3.65) = 12.72]. The neonatal inhibition of serotonin reuptake was associated with best regard to abdominal deposition, blood glucose and cholesterol indicating that this experimental model could promote phenotypic adaptation in adulthood to an environment with higher energy consumption.

Keywords: SSRI, phenotypic plasticity, metabolic syndrome.
Funding: CNPq, CAPES-COFECUB & FACEPE.

SC014

Effects of maternal separation on eating behavior and stress reactivity in rats

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The present study investigated the effects of maternal separation on adult eating behavior. Wistar albino rats were separated from their mothers for six hours each day over postnatal day 1 to 14, from 18:00–00:00h (light phase) or from 06:00–12:00h (dark phase), forming the following groups: maternal separation during the light male (SLM) and female (SLF), maternal separation during the dark male (SDM) and female (SDF). Control group male (CM) and female (CF) remained with their mothers. At 120 days of age, 30g of palatable diet was offered in a container that did not allow animal feeding, only visual and olfactory information, during 15 minutes after four hours of fasting. Next, animals had access to the palatable diet during 1 hour. Only females separated during the dark period decreased food intake compared to controls (CF = 15.41±0.43 vs SDF = 14.13±0.24, n=10, p<0.001). There were also differences between animals separated in the light period [F=(3.36)=6.78 p<0.05] or in the dark period [F=(3.36)=19.58, p<0.05]. At 180 days, after euthanasia and plasma obtainment, the corticosterone concentration was determined by ELISA test. Males and females separated in the dark period increased plasma levels of corticosterone (CM = 112.70±7.00 vs SDM = 173.90±12.70, p<0.001; CF = 74.6±8 vs SDF = 153.50±8.90, p<0.001) when compared to their controls. In the light period, only females increased plasma levels of the hormone when compared to controls (CF = 74.6±8, n=8 vs SLF = 142.7±12, n=8, p<0.001). Then, it was possible to observe that maternal separation, depending on the period it occurs, can alter the phenotypic expression of eating behavior and the stress reactivity in adult animals.

Keywords: maternal separation, eating behavior, stress.

SC015

Endocrine and metabolic function are altered in a model of anxiety disorder

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Anxiety disorders are one of the most prevalent mental disorders widespread around the world. One of the features of this disease is an increase on glucocorticoids serum levels. The chronic activation of hypothalamus-pituitary-adrenal axis is associated with metabolic disturbances such as metabolic syndrome. Carioca High-Freezing rats (CHF) are animals selected by their high freezing response in contextual fear conditioning that mimic anxiety disorder. The aim of this study is to characterize CHF animals regarding their endocrine and metabolic backgrounds. Our results show an increase in serum corticosterone (CTRL: 96.7 ± 21.65 vs CHF 292.0 ± 40.71ng/mL) and leptin (CTRL: 9.4 ± 1.51 vs CHF 19.2 ± 4.31ng/mL) in CHF animals. Serum testosterone (CTRL: 3.3 ± 0.29 vs CHF: 2.0 ± 0.28ng/mL) and T3 (CTRL: 52.3 ± 2.73 vs CHF: 42.7 ± 2.93ng/dL) were low in CHF group, as well as brown adipose tissue (BAT) type 2 iodothyironine deiodinase activity (CTRL: 0.7 ± 0.17 vs CHF: 0.3 ± 0.04fmoles T4/min.mg ptn); however serum insulin, TSH and T4 were not affected. Body weight and food intake were unchanged in CHF, nevertheless retroperitoneal fat (CTRL: 2.1 ± 0.23 vs CHF: 4.7 ± 0.63g) and epididymal fat (CTRL: 2.6 ± 0.19 vs CHF: 4.7 ± 0.37g) depots were around 2-fold high in CHF group. BAT weight was similar in both groups. Serum triglycerides (CTRL: 41.4 ± 6.03 vs CHF: 83.2 ± 17.09mg/dL) and total cholesterol levels (CTRL: 181.6 ± 5.61 vs CHF: 226.4 ± 13.04mg/dL) were high in CHF group. Fasting glycemia (CTRL: 68.7 ± 3.04 vs CHF: 82.3 ± 2.99mg/dL) was also high in CHF group; however glucose tolerance test response was similar among groups. Oxygen consumption was lower in CHF group (CTRL: 10.5 ± 0.40 vs CHF: 7.9 ± 0.58 VO2 ml/min/kg^0.75). Our data show that anxiety could impair endocrine and metabolic function and may contribute to the development of metabolic diseases.

Keywords: anxiety, anxiety disorders, corticosterone, thyroid function, metabolism, fat depots.

SC016

Lactational serotonin reuptake inhibition increased hypothalamic expression of 5-HT2C receptor in adulthood rats

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The neurotransmitter serotonin (5-HT) influences the expression of various genes and is an important factor for both developmental and plastic changes in living organisms. Preliminary evidence suggests that the metabolic syndrome may be associated with decreased serotonin function. We aimed to investigate the effects of pharmacological inhibition of neonatal serotonin reuptake on body weight (BW) and food intake control in adult rats exposed to a hypercaloric diet. Wistar rat pups received daily single subcutaneous (s.c.) injections of sterile saline 0.9% [Control (C), 1µl/g BW, s.c., n=10] or Fluoxetine (F; 10mg/kg, 1µl/g BW, s.c., n=10) from postnatal day (PND) 1 to 21. The animals remained on standard diet *ad libitum* up to 180 days. From PND 180, animals from both groups were euthanized for analysis of baseline gene expression and phenotypic characteristics. Another set of animals (8 for each group, C and F) was subjected to a hypercaloric diet for 5 weeks (Cd group, n=8 and Fd group n=8). We analyzed BW; food intake; mRNA expression of hypothalamic 5-HT1B, 5-HT_{2C}, NPY, and POMC before and after the hypercaloric diet. In adulthood, animals treated with selective serotonin reuptake inhibitor (SSRI) during the neonatal period had lower BW and food intake than controls. Following the hypercaloric diet, animals in Cd and Fd showed equal food consumption and weight gain. Hypothalamic 5-HT2C receptor expression was lower in SSRI-treated rats (C = 1.08±0.02 vs F = 0.8±0.04, n=4, $p<0.001$). After energy overload, 5-HT2C receptor mRNA expression was higher in Fd rats fed a hypercaloric diet compared with the Cd group (Cd = 1.06±0.03 vs Fd = 1.23±0.01, n=4, $p<0.01$) and F group (F = 0.8±0.04 vs Fd = 1.23±0.01, n=4, $p<0.0001$). After energy overload, neonatally treated rats exhibited better balance between BW and consumption and increased expression of 5-HT_{2C} in response to increased energy demand. The increased expression of this receptor, which is intrinsically related to the melanocortin pathway suggest that these rats were better able to adapt to higher energy demands.

Keywords: SSRI, Programming, feeding behavior.

Funding: FACEPE, CNPq & CAPES-COFECUB.

LECTURES

Speaking-poster – (SP)

SP001

Protein malnourishment during early adulthood programs rat to increase fat tissue accretion and to imbalance autonomic nervous system activity in later life

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Nutritional insults during developmental plasticity have been linked with metabolic diseases such as diabetes in adulthood. We aimed to investigate whether a low-protein diet at the beginning of adulthood is able to program metabolic disruptions in rats. Control rats ate a normal-protein diet (23%, NP group) and treated animals were fed with a low-protein diet (4%, LP group) from 60- to 90-days-old. Following, normal-protein diet was offered until they were 150-day-old. Biochemical parameters, autonomous nervous system (ANS) and pancreatic islet function were then evaluated. Data were analyzed by Student t-test. LP rats displayed unchanged low body weight and reduced food intake during protein restriction period compared to NP group ($p<0.001$). After the equilibrated diet replacement, hyperphagia ($p<0.05$) and catch-up growth of 113% were found ($p<0.0001$). LP rats showed hyperglycemia, insulin resistance and higher fat accretion than the NP rats ($p<0.05$) at 120-day-old. Sympathetic tonus from LP rats was reduced by 28%; while the vagus tonus increased by 21% ($p<0.05$). Glucose insulinotropic effect, as well as cholinergic and adrenergic actions were unaltered in the islets from LP rats compared to NP rats. Protein restriction at the beginning of adulthood induced unbalanced ANS activity and fat tissue accretion later in life, even without functional disturbances in the pancreatic islets.

Keywords: Protein restriction, adulthood, metabolic programming.

Funding: CNPq, CAPES & Fundação Araucária.

SP002

Maternal treatment with metformin antidiabetic during lactation not induces resistance against tumor growth in adult offspring

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Nutritional insults early in life are key factors determining the metabolic programming on the offspring in adulthood. It is known that this malprogramming phenotype could be reverted by ensuring the maternal food flux, like vitamins and/or antioxidants supplementation. These considerations support the understanding that the maternal environment influences the offspring phenotype, either for health or for disease, which can be transmitted via milk, from dams to the offspring. Since the metformin displays anticancer activity, our aim was to determine whether the administration of metformin in lactating rats can programs offspring against the growth of cells of a breast carcinoma of rodents, the Walker 256 tumor. Lactating Wistar rats dams were gavaged with metformin (320mg/kg, metformin group) or water (water group), while other batch of dams were untreated (control group) throughout the lactation. At 21 days of age, the offspring of each experimental group were weaned. The weight gain and food intake were accompanied from weaning until 130-day-old, when the animals were grafted with tumor cells. After 14 days, the animals were sacrificed for evaluation of tumor growth and biometric parameters. The tumor weight of the animals treated with metformin was not attenuated when compared to the animals from water and/or control groups. The Lee index, subcutaneous fat and food intake of the animals treated with metformin were also no statistically different when compared to water and/or control groups. Treatment with metformin during lactation was not able to induce resistance to tumor growth, even though the perinatal period is likely to modulate functions in adult life, since our laboratory recently observed that chronic metformin treatment started after the weaning was able to induce a drastic inhibition of tumor growth even after the end of treatment.

Keywords: Metformin, Walker 256 tumor, maternal treatment.

Funding: CAPES.

SP003

Beyond pain, buscopan inhibit the obesity onset

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Overfeeding during perinatal life is a risk factor to obesity onset, which is also associated to low birthweight indicating a risks to development of metabolic diseases. Muscarinic receptor (mAChR) has been associated with pancreatic beta-cell/insulin secretion dysfunction. The knockout mice to M3 subtype of mAChR display low insulinemia and a lean phenotype in adulthood. We investigated whether neonatal treatment with the muscarinic antagonist, buscopan, is able

to blocks metabolic dysfunction induced by early overfeeding in rat offspring. At birth, the litter size of rat offspring were adjusted to 9 (normal litter, NL) or 3 (small litter, SL) pups per dam. During the 12 first days of life, rats were intraperitoneally injected with buscopan [(B-NL and B-SL) at 0.5 mg/kg body weight (BW)], or equimolar saline solution (S-NL and S-SL). Diet intake and BW were measured each two days. After 85 days, all rats underwent intravenous glucose tolerance test (ivGTT) to farther glucose and insulin measurement. Retroperitoneal, periepididymal and visceral fat pad were removed and weighted. Data were submitted to one-way ANOVA or Student t-test with *GraphPad Prism* software version 6.01. Perinatal treatment with buscopan attenuated the accretion of BW, food intake and fat tissues in both group; however, the magnitude was bigger in B-SL than B-NL. Buscopan treatment had no effect on the glucose intolerance in the SL; however it induced an glucose intolerance state in NL rats. Blood insulin levels were decreased by buscopan treatment; however it was observed only in SL. On the same line, fasting insulinemia was also reduced in groups treated early in life with buscopan. Perinatal short-term treatment with buscopan programs rat offspring to a lean phenotype with metabolism dysfunction associated to poor insulin secretion. Finally, obesity programmed by reducing litter size is attenuated by early treatment with buscopan.

Key words: Buscopan, insulin, small litter, obesity

Funding: CNPq, CAPES & Fundação Araucária.

SP004

Soya beans isoflavone attenuates obesity induced by small litter in adult rats

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Reducing rat's litter size at birth can cause obesity development later in life, by offspring's overnutrition; indicating that lactation is a sensible window to provokes long life changes on the energy metabolism. Antioxidant isoflavone isolated from soybean has anti-obesity effects in rats exposed to surgical ablation of ovaries. The goal of this study was to evaluate whether isoflavone chronic treatment is able to attenuate obesity onset induced by SL, and whether it may improve the autonomous nervous system (ANS) activity in rats. The litter size were adjusted to 9 (normal litter, NL) or 3 (small litter, SL) pups per dam, three days after birth. From 60- to 90-day-old, rats from SL and NL were treated by gavage with isoflavone [1g/kg body weight (BW)/day]. BW and chow intake were taken each two days. Biochemical parameters, ANS activity and pancreatic islets function were evaluated. Data were analyzed by two-way ANOVA. Beyond, isoflavone treatment had no effect on the food intake and BW gain; it reduced fat tissue accretion by 40% in SL rats. While blood levels of cholesterol and triglycerides were decreased by 15%, HDL-cholesterol was increased by 62% in the SL rats treated with isoflavone. However, isoflavone did not alter the fasting moderate hyperglycemia; hyperinsulinemia was decrease by 38%, ameliorating also glucose intolerance and hyperinsulinemia during the glucose tolerance test in SL rats. High vagus as well as low sympathetic nerves activity observed in SL rats was normalized by isoflavone treatment. Islets from SL rats showed over glucose-induced insulin secretion, although it was corrected by isoflavone treatment. Short-term isoflavone treatment is able to ameliorate metabolic dysfunction in obese rats induced by early overfeeding, which may be associated to the improvement of the ANS and pancreatic beta-cell function.

Keywords: small litter, metabolic dysfunction, isoflavone soybean.

Funding: CNPq, CAPES & Fundação Araucária.

SP005

The role of the sympathetic nervous system and the sympathoadrenal installation of the metabolic syndrome in rats

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Obesity has become a worldwide syndrome that is closely associated to insulin resistance and type 2 diabetes. Insulin secretion is controlled by blood glucose levels and by the autonomous nervous system (ANS) action. Sympathoadrenal system, including sympathetic nervous system (SNS) and the chromaffin cells from the adrenal medulla, which secretes catecholamines into the bloodstream acts by inhibit insulin secretion. The relation between adipose tissue and the activity of the adrenal action on glucose metabolism regulation is an important field of study. We aimed to investigate the sympathoadrenal function on the obesity onset. At 60-day-old, male Wistar rats underwent a surgery intervention to sympathectomy (SYM), adrenodemedullation (ADMX) and/or both sympathectomy followed by adrenodemedullation (SYM/ADMX); while control rats were Sham-operated (CON). At 120-day-old, rats were submitted to intravenous glucose tolerant test (ivGTT) to posterior blood glucose and insulin levels measurement. After that, all rats were killed by anesthesia overdose, and tissue fat pad were isolated and weight. The SYM and ADMX groups gained 30% and 50% more fat tissue than the CON one, respectively; while the rats that underwent the double surgery, SYM/ADMX group, showed a reduction of these parameters ($p<0.001$). Although all of groups were normoglycemic, the SYM ones displayed glucose intolerance under the ivGTT, when compared to the CON group; by the other hand, the ADMX and SYM/ADMX groups presented a tendency to have hypoglycemia under the same condition. Either sympathectomy or adrenodemedullation, when isolated, were able to promote obesity in adult rats; however, when rats underwent the double surgery it was not observed, which may be associated to the decreasing of the sympathetic tonus in the pancreatic ends.

Keywords: sympathoadrenal, autonomous nervous system, catecholamine, glycemia, tissue fat accretion.

Funding: CNPq, CAPES & Fundação Araucária.

SP006

Antidiabetic drug metformin programs rat offspring to resist to Walker-256 tumor growth in adult life

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Stressful injuries in early life can determine the origins of metabolic diseases when babies become adults. However, it could be modulated by beneficial effects during the brain maturation. Metformin exerts protective effects against certain types of cancer; however, information regarding what is the phase of life when the treatment must be started are scarce. We aimed to study whether metformin treatment starting at weaning or after adolescence could give protection against cell tumor growth. Male rats were gaveged with metformin [320mg/kg of body weight (BW)/day] during 79 days. The treatment started at 21-day-old (M-21) or 60-day-old (M-60). Control rats received gavage of water (W-21 and W-60). After metformin treatment, rats were grafted with Walker-256 tumor cells and fourteen days after inoculation, the tumor growth was evaluated. The BW was measured to calculate cachexia. Fasting blood samples were taken to measure glucose and insulin levels. Another batch of rats was used to assess the metabolism. Intravenous glucose tolerance test (ivGTT) was used to evaluate the glucose homeostasis. Fat pad tissues were removed and weighed. Data were evaluated by one-way ANOVA. Fasting glycemia and insulinemia of M-21 and M-60 rats remained unchanged. Using ivGTT neither M-21 nor M-60 rats presented glucose intolerance and alterations on insulin levels. No decrease of fat tissue accretion was observed in rats treated with metformin independent of the beginning age of gavage. Tumor growth was reduced by 37% in M-21 rats; while, no changes were observed in M-60 rats when compared to animals that did not receive any drug treatment. Cachexia was inhibited by 20% in M-21 group, while it was not altered in M-60 animals. Early treatment with metformin, starting at weaning, partially protects adult rats against growth of Walker-256 tumor cells; however, when the treatment starts in adult rats, the antidiabetic drug does not show any antitumor effect.

Keywords: cancer; metformin; Walker-256 tumor; metabolic programming.

Funding: CNPq & CAPES.

SP007

Short-term aerobic exercise training protects young rats against full high-fat-diet-induced obesity onset

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Elevated intake of high-fat diets (HFD), at any age, could induces metabolic dysfunctions, such as obesity, type 2 diabetes and cardiovascular diseases; on the other hand, it can be ameliorated by the aerobic exercises. It has been observed that body weight tends to recover after interruption of training. However, could the aerobic exercise give to trained animals some resistance to metabolic dysfunctions onset due to the ingestion of diets with high fat content. At 60-day-old, rats were submitted to an aerobic exercise program with moderate intensity for 30 days, 3 sessions/week. At 90-day-old, either trained or sedentary rats received a HFD for 30 days (HFD-EXE or HFD-SED group, respectively). While another group of animals, both exercised and sedentary rats were fed a normal-fat-diet (NFD) (NFD-EXE and NFD-SED group, respectively). At 120-day-old, rats were submitted to intravenous glucose tolerance test (ivGTT) for glucose and insulin measurement. Direct electrical activities from the superior vagus and sympathetic branch nerves were recorded. Under anesthesia (thiopental) rats were sacrificed and the periepididimal, retroperitoneal and visceral fat pads were removed and weighed. Data were submitted to one-way ANOVA or Student t-test. The body weight gain of HFD-rats was prevented by prior exercise; however, it was not observed any difference regarding diet intake. Fat tissue accretion was bigger in HFD-SED compared with the NFD-SED group. Early exercise prevented partially the fat tissue accretion in both HFD-rats and NFD-rat. Even 30 days after the end of the exercise training, fasting glycemia and insulinemia of HFD-rats were normalized compared with the NFD groups. HFD-rats also showed glucose intolerance observed during ivGTT; however, it was not observed in trained rats. HFD-rats presented high vagus nerve activity, whereas it was avoided by exercise. On the other hand, HFD-EXE and NFD-EXE rats presented similar increase of the sympathetic nerve activity, compared with the sedentary groups. Previous short-term aerobic exercise training, programs adult rats against HFD-induced obesity, which could be associated to autonomous nervous system activity improvement.

Keywords: Exercise, High Fat Diet, Obesity.

Funding: CAPES.

SP008

Hypoglycemic effect of insulin-cyclodextrin complex gel applied in the testing of wound healing in rats

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Changes in skin, like wound healing disturbances, has been associated with metabolic alterations such as insulin resistance, obesity and type 2 diabetes. Topical insulin application has been showed by stimulates keratinocytes and vascular endothelial cells migration through the insulin receptor-mediated signal pathway, thus accelerating reepithelialization and angiogenesis in several animal models. Chronic administration of large doses of a cyclodextrin (CD) mixture, such as α-CD, β-CD, γ-CD have been reported by reducing weight gain, fat deposition, and serum triacylglycerol concentrations. Thus, the aim of this work was to study whether chronic treatment with CD associated to insulin could influencing the blood glucose homeostasis in rats subjected to skin injury. Male Wistar rats (n=12) weighing 180–200g were anesthetized to farther experimental procedure. After that, rats underwent a slight surgery to induce skin injury, while control animals were sham-operated. A batch of rats from both, operate and sham-operate groups, were treated with hydroxypropyl-β-cyclodextrin (HP-β-CD) gel or with gel base-insulin during different periods (4, 7, 10 and 14 days). After the period of gel treatment, fed rats were killed to evaluate the blood post-prandial glucose concentration by the glucose oxidase method. All of data were submitted to one-way ANOVA for statistical analyzes. In relation to all of the period of treatment, pot-prandial glucose alterations was observed just in rats treated by 10 days. When compared to sham-operated (172.3±8.81mg/dL) and/or HP-β-CD groups (172.8±20.59mg/dL), a reduction in the glycemia was observed just in rats treated during 10 days with the gel base-insulin (114.8 ± 24.21mg/dL). Our data suggest that the complex insulin-CD incorporated into a base gel does not affect glucose levels. However, it is known that CD is a carbohydrate, it might keep cellular homeostasis during the modified insulin release in the wound healing and into the bloodstream.

Keyword: glucose, cyclodextrin, insulin, postprandial glycemia, wound healing.

Funding: CNPq & CAPES

SP009

Chronic metformin treatment can program rat metabolism against the aggressiveness of Walker 256 tumor

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Experimental and epidemiological data demonstrate that the metformin could affect tumor growth and reduce the risk of several types of cancer; however, the mechanisms underlying the anticancer effects are under debate. There are two major hypotheses related with anticancer effect of metformin, a direct effect on cell proliferation and an indirect mechanism targeting metabolism. We aimed to test the action of metformin chronic treatment on the Walker 256 tumor growth in rats with metabolic syndrome. Pre-diabetic hyperinsulinemic rats were obtained by neonatal treatment with monosodium L-glutamate (MSG). The animals were chronically treated every day, from weaning to 100-day-old, with metformin (320 mg/kg body weight). After the end of metformin treatment, the control and MSG-rats, treated or untreated with metformin, were grafted with tumor cells and euthanized after 14 days. Tumors were removed and weighted to evaluation of the tumor growth and detection of apoptotic cells by hematoxylin-eosin (H&E) staining and TUNEL methods. The cachexia, fasting glycemia and insulinemia were also evaluated. Pancreatic islets were isolated to evaluate the beta-cell response on glucose-induced insulin secretion. Metformin improved glucose intolerance, insulin resistance and hyperinsulinemia, and decreased the fat tissue accretion in MSG-rats. Meanwhile, metformin had no effect in the glucose insulinotropic effect on pancreatic islets. Metformin was able to inhibit the tumor growth by 37% in both control and MSG-rats. The H&E and TUNEL positive staining reactions were remarkably increased in tumor cells from rats treated with metformin. The anticancer effect of metformin is not related to its role in correct metabolism imbalance, such as hyperinsulinemia. However, the chronic metformin treatment increased programmed cell death and necrosis of tumoral tissues, attenuating the growth of Walker 256 carcinoma by programming the rat organism.

Keywords: Metformin, Walker 256 tumor, Metabolic Syndrome

Funding: CAPES

SP010

Effects of maternal physical exercise and/or isoflavone supplementation as deprogramming tool in adult rat's offspring from lactating dams fed a high-fat diet

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It has been reported that the developmental plasticity of the energy regulatory pathways in the hypothalamus, in rodents, occurs mainly during the first weeks of lactation. As the largest neuronal plasticity stage, it makes the hypothalamus very vulnerable to external stressful factors that can permanently alter the set point of energy balance, predisposing offspring to metabolic syndrome. Exercise training as well as functional food and/or isoflavones diet-supplementation have been reported by exert beneficial effects on the metabolic syndrome disturbances. This study investigate whether physical exercise training and/or isoflavones-enriched diet to lactating mothers can prevents the metabolic syndrome installation in rat's offspring from dams subjected to a high-fat diet. At delivery, Wistar rat dams were randomly divided in the following groups: lactating dams that were fed a high- or normal-fat diet, supplemented or not with isoflavone (HF, NF, HF/Iso and NF/Iso groups, respectively). All of these groups were subjected or not to the physical exercise protocol (exercise group: HF-exe, NF-exe, HF/Iso-exe and NF/Iso-exe; while other batch of animals were sedentary: HF-sed, NF-sed, HF/Iso-sed and NF/Iso-sed). At weaning, just male rat

offspring were used to the experimental protocol. At 100-day-old, rat offspring underwent intravenous glucose tolerance test. Body weight, epididymal and retroperitoneal fat pads and brown adipose tissue were evaluated to obesity assessment. Our data show that high-fat diet to lactating dams promoted glucose intolerance and high increase in the body weight and fat pads in the offspring at adulthood. Both physical exercise and isoflavones-diet supplementation did not prevent the obesity output in the offspring from lactating dams fed a high-fat diet; however, it was able to improve the glucose intolerance on these rat offspring.

Keywords: Isoflavone, high-fat diet, obesity, physical exercise.

SP011

BAT to WAT – Early programming obesity induces brown adipose tissue hypoactivity in rats

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Overfeeding in early postnatal life leads human and animal to obesity. Brown adipose tissue (BAT) activation has been considered a potential anti-obesity mechanism, because it is able to expend energy through thermogenesis. On the other hand, white adipose tissue stores energy contributing to obesity. We investigated whether early programming obesity changes interscapular BAT structure in adulthood and its effect on thermogenesis in rats. Rats pups birth was consider day 0. At day 2, litter size was adjusted to normal (9 pups) and to small (3 pups)per litter. At day 21, litters were weaned. Eighty-one days old animals had a temperature transponderimplanted underneath interscapular BAT pads; local temperature was measured at light and dark periods between days 87 and 90. At day 91, animals were euthanized; tissues and blood samples were collected for further analysis. Vagus and retroperitoneal sympathetic nerves activity was recorded in 90 days old rats. Small litter rats presented lower interscapular BAT temperature during light ($p<0.001$) and dark ($p<0.001$) periods compared to control. Histology showed, in small litters, lower lipids droplet number in the tissue center, while in the periphery they were bigger. Small litter vagus nerve presented higher activity than control ($p<0.01$) and no difference was observed in sympathetic nerve activity. Small litter rats was heavier in adulthood, compared to control ($p<0.001$) and also presented higher glycemia ($p<0.05$), insulinemia ($p<0.05$) and corticosteronemia ($p<0.01$). Early programming obesity changes interscapular BAT structure on adulthood leading to local thermogenesis hypoactivity, which can reduce energy expenditure and may contribute to obesity in adult life.

Keywords: perinatal overfeeding, thermogenesis, trans-differentiation.
Funding: CNPq & CAPES

SP012

Experimental obesity and fiber supplementation in the diet of rats: effects on plasma parameters and liver enzymes

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The food quality has suffered important changes: whole foods have been replaced by refined, the consumption of fat and calories increased and fiber's consumption decreased, contributing to the development of obesity. The cafeteria diet causes obesity similarly to humans; that is characterized by high calorie and low protein and fiber intake, promoting increased body weight gain by around 30–40%. Dietary fibers play roles in the metabolism of lipids and carbohydrates, and promote satiety. The aim of this study was to evaluate the effect of cafeteria diet with and without soluble fiber supplementation on plasma parameters and liver enzymes in rodents. Male Wistar rats were divided into the following 4 groups: rats fed a standard diet (CO); rats fed a cafeteria diet (CA group; constituted by mortadella, soda, sausage, etc.), and rats COF and CAF, that were fed a diet with soluble fiber supplementation (*Fiber Mais Nestlé*; 4g fiber/20ml distilled water) by the experimental period of 14 weeks. At 158 days of age, the animals were euthanized, blood was collected for measurement of total cholesterol (TC), triglycerides, glucose, total protein, HDL and aminotransferases liver enzymes (AST and ALT). After laparotomy, the retroperitoneal, periepididymal and mesenteric fats were removed and weighed. Positive effects of fiber supplementation were found for COF rats, on which reduced body weight, Lee index, total protein and fat pads were observed; however, AST and ALT were increased. The cafeteria diet was able on increases fat pad depots, Lee index and triglycerides. By the other hand, the inclusion of fibers proved to be effective in reducing total cholesterol and fat pad. The cafeteria diet installed an obesity pattern in animals and the inclusion of fibers was able to reduce body fat and total cholesterol.

Keywords: obesity, diet cafeteria, fiber supplementation
Funding: CAPES & *Fundação Araucária*

SP013

Reduction in the number of goblet cells in the ileum of mice with high-fat diet-induced obesity

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Diets with high lipid content, called high-fat diets (HFDs) have been associated with obesity development in both humans and animals, with implications to organs and body systems. Considering that diet is one of the factors that can direct or indirectly affect the intestinal epithelial cells, the aim of this study was to evaluate the goblet cells population in the ileum mucosal epithelium of mice fed a HFD. Male Swiss mice, at 42-day-old, were fed a standard rodent chow or a HFD rich in saturated fat, for 8 weeks. Distal ileum samples were submitted to histological processing and *Periodic Acid Schiff* (PAS) staining. The goblet cells present in the villi were evaluated by quantifying the total number of cells and the goblet cells proportion in the region, totaling approximately 2,500 cells per animal. The HFD induced obesity on the rodents, confirmed by the higher body weight gain (17.6%) and retroperitoneal, mesenteric and periepididymal visceral fat weight (155.8%). On the small intestine, there was a reduction in organ length (9.2%) and ileum diameter (29.2%). The HFD also decreased the proportion of goblet cells in the ileum by 17.7%, and goblet cells in both groups were more prominent in the ileum than in other proximal segments. With the smaller number of goblet cells in the ileum of HFD-fed animals, the mucus production and the formation of the intestinal mucosa protective barrier may have been changed.

Keywords: obesity, goblet cells, ileum, high-fat diet.

SP014

Morphometry of the general population of myenteric neurons in the small intestine of mice fed high-fat diet

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Consumption of diets rich in lipids, or high-fat diets (HFDs), for long periods can easily induce obesity, which is considered a risk factor for other chronic diseases, such as hypertension, cardiovascular diseases, type 2 diabetes, fatty liver disease and gastrointestinal motility disorders. The intestinal functions are regulated and coordinated by the enteric nervous system, in which the myenteric plexus are controlling the gastrointestinal motility. This study evaluated the effects of a HFD in the morphometry of the general neuronal myenteric population on the small intestine of obese mice. Male Swiss mice, 42 days old, were fed a standard rodent chow or a HFD, rich in saturated fat, for 17 weeks. Samples of the duodenum, jejunum and distal ileum were fixed, dissected and subjected to immunofluorescence technique (myosin-V antibody). The morphometric analysis was assessed by measuring the area of 100 neuronal cell bodies per animal. The HFD consumption promoted increase in body weight and visceral fat deposition. The length of the small intestine did not differ from the controls. The neuronal area of the general population increased 6% in the duodenum of HFD group, while in the ileum there was an increase of 21.3%. In the jejunum, neuronal area did not change with the HFD consumption. The distinct changes in the neuronal profile of the intestinal segments suggest an adaptation to diet and changes in the intestinal wall, as a result of functional differences between these regions of the small intestine.

Keywords: myenteric plexus, myosin-V, small intestine, high-fat diet, immunofluorescence.

SP015

High-fat diet induces changes in the population of enteroendocrine cells of the small intestine of mice

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The high rates of obesity are attributed to behavioral changes in modern society, such as increased consumption of foods rich in fat. High-fat diets (HFDs) promote obesity and its associated conditions, including gastrointestinal disorders, which may be relevant on this field, considering the regulatory function of the gastrointestinal system in food intake, digestion and absorption. Thus, this study evaluated the effects of a HFD on the enteroendocrine cells population of the mucosal epithelium in the small intestine of mice. Male Swiss mice, 42 days old, received a standard rodent chow or a HFD rich in saturated fat for 8 weeks. Samples of the duodenum, jejunum and distal ileum were subjected to histological processing and the Grimelius technique. Enteroendocrine cells in the crypt-villus axis were evaluated by the quantification of the total number of cells and the proportion of endocrine cells, totaling approximately 2,500 cells per animal. With the HFD, animals had higher body weight (17.6%) and visceral fat gain (155.8%), confirming the obesity installation. The length of the small intestine and the ileum diameter were reduced in the HFD group. The proportion of enteroendocrine cells, representing approximately 0.5% of epithelial cells in the villi and crypts, changed between groups only in the ileum, with a 30.2% increase in the HFD group. The HFD affected, only the enteroendocrine cells of the ileum and may have influenced the secretion of hormones that contribute to digestive and absorptive efficiency.

Keywords: obesity, enteroendocrine cells, small intestine, high-fat diet.

SP016

Assessment of in vivo and in vitro genotoxicity of glibenclamide in eukaryotic cells

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Glibenclamide is an oral hypoglycemic drug commonly prescribed for the treatment of type 2 diabetes mellitus, whose anti-tumor activity has been recently described in several human cancer cells. The mutagenic potential of this antidiabetic drug and the glibenclamide recombinogenic activity in eukaryotic cells were evaluated, the later for the first time. The mutagenic potential of glibenclamide in therapeutically plasma concentrations (0.6μM, 1.2μM and 2.4μM) was assessed by the in vitro micronuclei test in human lymphocyte cultures. In addition, assuming that the loss of heterozygosity (LOH) arising from allelic recombination is an important biologically significant consequence of oxidative damage, the glibenclamide recombinogenic activity at 1μM, 10μM and 100μM concentrations was evaluated by the *in vivo* homozygotization assay. Glibenclamide failed to alter the frequency of micronuclei and CBPI rates. These results reveal the lack of mutagenic and cytotoxic/cytostatic effects of glibenclamide. In the homozygotization assay, the homozigotization indices for diploids obtained by the three concentrations of glibenclamide were lower than 2.0 for the analyzed markers and demonstrated the lack of recombinogenic activity of glibenclamide. Since glibenclamide has been proposed as a potential anticancer agent either alone or in combination with standard chemotherapeutic drugs, data in current study demonstrate that glibenclamide, in different analysis systems, is devoid of significant genotoxic effects. This fact encourages further investigations on the use of this antidiabetic agent as a chemotherapeutic drug.

Keywords: genotoxicity, homozygotization assay, antidiabetic drug, micronucleus test, Aspergillus nidulans.

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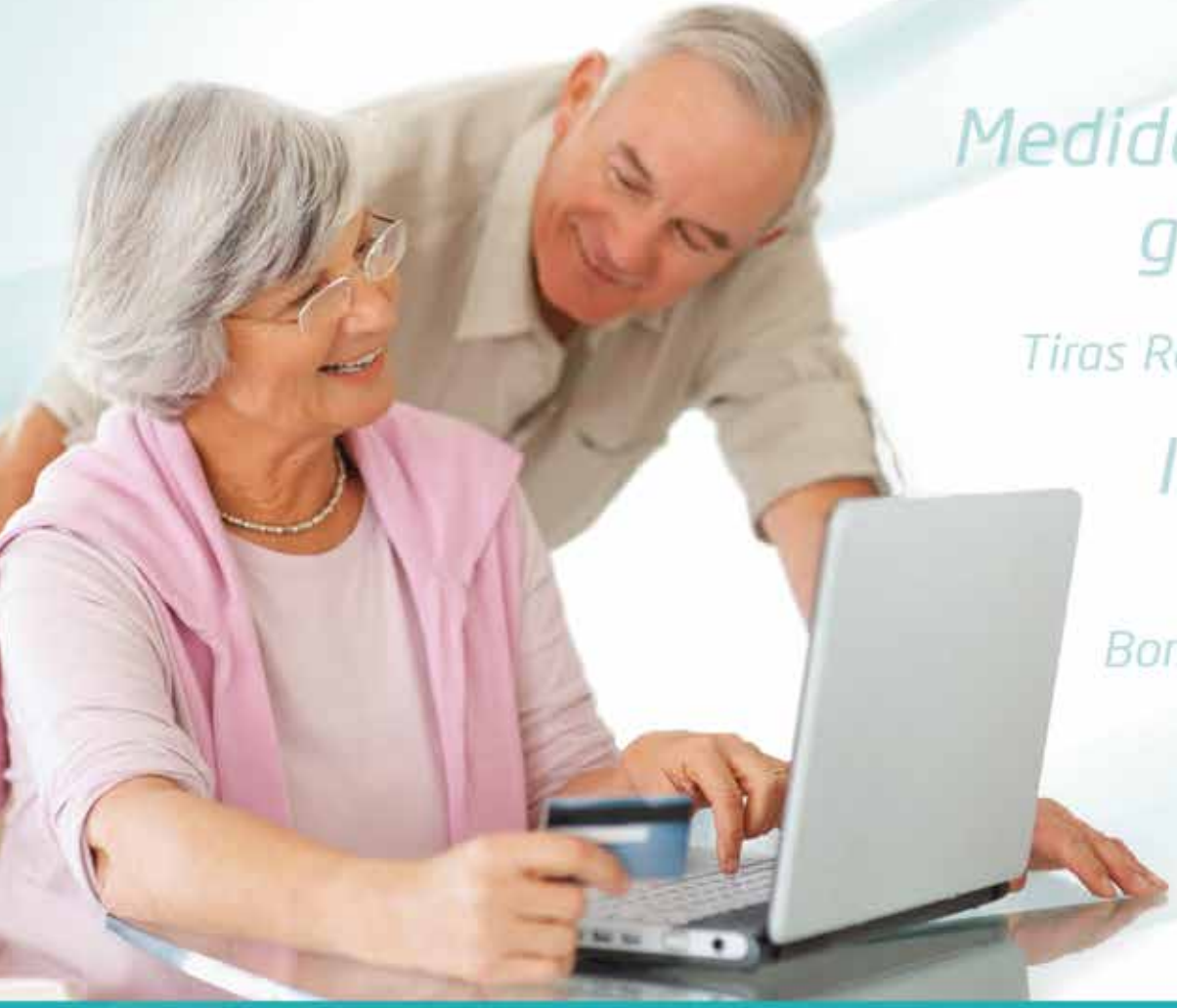

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