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Alterações metabólicas decorrentes de dieta hiperlipídica em modelo animal não resistente à insulina

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1 High fat-diet. 2. Metabolic syndrome. 3. Cardiovascular Disease. 4. Body weight. 5. Visceral adipose tissue.
Palavras chaves

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Dedicação

Dedico esta dissertação ao meu marido Rafael

e aos meus pais, Janete e Marco.

“O traço mais valioso de um homem é o senso crítico sobre aquilo em que não acreditar.”

Eurípedes

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Agradeço as amigas Luciana Antunes e Manoela Jornada pela dedicação incondicional.

LISTA DE ABREVIATURAS

I – DBSM: I Diretriz Brasileira de Diagnóstico e Tratamento da Síndrome Metabólica
AGL: Ácidos Graxos Livres
COBEA: Colégio Brasileiro de Experimentação Animal
DM: Diabetes Mellitus
GV: Gordura Vegetal
HDL-col: Lipoproteína de Alta Densidade (do inglês *High Density Lipoprotein*)
IDF: Federação Internacional de Diabetes (do inglês *International Diabetes Federation*)
IMC: Índice de Massa Corporal
LDL-col: Lipoproteína de Baixa Densidade (do inglês *Low Density Lipoprotein*)
NCEP-ATP III: National Cholesterol Education Program's - Adult Treatment Panel III
OMS: Organização Mundial de Saúde
PA: Pressão arterial
RI: Resistência à Insulina
SBC: Sociedade Brasileira de Diabetes
SM: Síndrome Metabólica
TG: Triglicerídeo

ÍNDICE

1. RESUMO

1. RESUMO

Objetivo: Sabe-se que trabalhadores noturnos tem preferência por lanches com maior concentração de gordura saturada. Trabalhadores de turno, particularmente trabalhadores noturnos, apresentam mais frequentemente hipertrigliceridemia e hiperglicemia, bem como menores níveis de HDL-colesterol quando comparados a trabalhadores diurnos, devido a alterações circadianas e estilo de vida, sugerindo uma predisposição para o desenvolvimento de doenças cardiovasculares. Estas alterações são conhecidas como Síndrome Metabólica (SM). Portanto, para entender as conseqüências de uma dieta hiperlipídica é importante a padronização de dietas para modelos animais que mimetizem a alimentação do trabalhador de turno. **Materiais e Método:** estudo experimental com 20 ratos Wistar dos quais 10 eram controle (CG) e 10 submetidos à dieta hiperlipídica (HFD). Utilizaram-se três dos cinco critérios do NCEP-ATP III para diagnóstico de SM - glicose, triglicerídeos (TG) e HDL-col. A quantidade de tecido adiposo visceral (TAV) foi avaliada bem como o peso do fígado e das glândulas adrenais. O peso ponderal foi avaliado semanalmente e o a ingestão alimentar e hídrica diariamente. Foi utilizado o *t*-test de Student's para amostras independentes. **Resultados:** não houve diferença significante entre os grupos para glicose, HDL-col e TG na medida basal. Após 15 semanas de intervenção, grupo HFD mostrou um aumento dos níveis de TG ($p=0,01$) e glicose ($p=0,01$) e diminuição de HDL-col ($p=0,009$) quando comparados com CG. Grupo HFD apresentou maior TAV ($p=0,005$) e peso do fígado ($p=0,01$). CG mostrou um aumento de ingestão alimentar e hídrica ($p<0,001$ e $p<0,001$ respectivamente) enquanto que o consumo energético foi maior no grupo HFD ($p<0,001$). Não foi encontrada diferença no peso das glândulas adrenais ($p=0,07$) e peso ponderal ($p=0,63$). **Conclusão:** os animais submetidos à dieta hiperlipídica apresentaram alterações metabólicas apesar da manutenção do peso corporal. Não foi encontrada correlação entre peso corporal e quantidade de TAV, sugerindo que o peso corporal não é preditor para quantidade de gordura corporal e que a composição da dieta influi diretamente nos marcadores de SM.

Palavras-chave: dieta hiperlipídica, síndrome metabólica, doença cardiovascular, peso corporal, tecido adiposo visceral.

ABSTRACT

Background and Aim: Shift workers, particularly night workers, more frequently present metabolic changes when compared to day workers suggesting a predisposition for cardiovascular disease. These changes are known as Metabolic Syndrome (MS). Therefore, to increase understanding of the consequences of a high-fat diet it is important to standardize a diet for experimental animals that mimics the shift workers' diet and its effects. **Methods and Results:** experimental study with 20 Wistar rats of which 10 were control group (CG) and 10 high-fat diet group (HFD). Three of five criteria for MS diagnosis from NCEP-ATP III were assessed. The amount of visceral adipose tissue was determined (VAT). Body weight was assessed weekly and food and water intake were measured daily. Student's *t*-test for independent samples was used. The groups were similar at baseline. After fifteen weeks of intervention, HFD group showed an increase in serum TG ($p=0.01$) and glucose ($p=0.01$) levels and a decrease in HDL ($p=0.009$) compared to CG. HFD showed increased VAT ($p=0.005$) and liver weight ($p=0.01$). Food intake and water intake were higher in CG ($p<0.001$ and $p<0.001$ respectively) while energy intake was increased in HFD ($p<0.001$). No difference was found in adrenal glands weight ($p=0.07$) and body weight ($p=0.63$). **Conclusion:** Animals submitted to HFD present significant metabolic alterations in spite of the maintenance of body weight. Body weight is not a predictive factor for the amount of VAT and that the quality of diet composition direct influences MS markers.

Keywords: high fat-diet, metabolic syndrome, cardiovascular disease, body weight, visceral adipose tissue

1. INTRODUÇÃO

2. INTRODUÇÃO

Nos últimos anos, a prevalência de doenças como obesidade, Diabetes Mellitus (DM) e doenças cardiovasculares tem apresentado significativo aumento. Fatores como hábitos nutricionais inadequados e estilo de vida são, muitas vezes, determinantes no aparecimento dessas patologias.

De acordo com a Organização Mundial de Saúde (OMS), 40,6% da população brasileira tem Índice de Massa Corporal (IMC) igual ou maior a 25 kg/m², enquanto que os Estados Unidos da América apresentam 66,9% da população nesta faixa. Nos EUA estima-se que 35% da população são diabéticos ou pré-diabéticos e, mundialmente, o número chega a 150 milhões. Em um estudo realizado entre 1994 e 2004 também nos EUA com mais de 8000 adultos foi encontrada a prevalência de 23,9% de SM de acordo com parâmetros do NCEP. Os dados são alarmantes e, segundo a OMS, o número de diabéticos deve duplicar nos próximos 20 anos.

Alterações metabólicas

A partir da década de 80, estudos envolvendo a obesidade central e alterações metabólicas como hiperglicemia e resistência à insulina (RI) foram aprofundados e formulou-se um novo conceito associando diversos fatores de risco para doenças cardiovasculares e DM. A esse conjunto de fatores de risco deu-se o nome de Síndrome X e, posteriormente, Síndrome Metabólica (SM).

SM é um transtorno complexo representado por um conjunto de fatores de risco cardiovascular relacionados à deposição central de gordura e à RI (I-DBSM, 2005). Apesar de não avaliar a resistência à insulina, os critérios utilizados para diagnóstico do NCEP-ATP III (quadro 1) (NCEP, 2001) são recomendados pela I Diretriz Brasileira de Diagnóstico e Tratamento da Síndrome Metabólica (I-DBSM) em razão de sua simplicidade e praticidade. O diagnóstico é firmado pela alteração dos níveis de três dentre quaisquer dos cinco componentes adotados que são: circunferência abdominal, triglicerídeos, lipoproteína de alta densidade (HDL), glicemia em jejum e pressão arterial (I-DBSM, 2005).

Quadro 1 - Componentes da síndrome metabólica segundo o NCEP-ATP III

Componentes	Níveis
Obesidade abdominal por meio de circunferência abdominal	
Homens	> 102 cm
Mulheres	> 88 cm
Triglicerídeos	≥ 150 mg/dL
HDL Colesterol	
Homens	< 40 mg/dL
Mulheres	< 50 mg/dL
Pressão arterial	≥ 130 mmHg ou ≥ 85 mmHg
Glicemia de jejum	≥ 110 mg/dL

A presença de *Diabetes mellitus* não exclui o diagnóstico de SM

Quadro 1 Fonte: I-DBSM, 2005

A presença da gordura visceral, verificada por meio de medida de circunferência abdominal, tem sido considerada melhor marcador para alterações metabólicas presentes na SM que a gordura corporal total (Després et al., 2006; Rexode et al., 1998). O perfil lipídico da SM é caracterizado por hipertrigliceridemia e diminuição de HDL. A insulina tem ação anti-lipolítica e de estímulo à lipase lipoproteica. Consequentemente, a resistência a este hormônio é determinante de lipólise e aumento de ácidos graxos livres (AGL) (Pankow et al., 2004).

Outro fator importante é a alteração na expressão ou funcionamento dos transportadores de glicose (GLUT) que podem estar envolvidos (Shepherd e Kahn, 1999). No tecido adiposo, ocorre uma redução na expressão de proteína transportadora de glicose 4 (GLUT4) que está associada à obesidade, desenvolvimento de RI, DM2 e também SM (Shepherd e Kahn, 1999). Associada à SM, também pode ser observada a ativação do eixo do sistema renina-angiotensina, determinando aumento de pressão arterial PA (Lopes e Egan, 2006).

Adipócito e Síndrome Metabólica

O adipócito recebe a influência de diversos sinais, como a insulina, cortisol e catecolaminas e, em resposta, secreta uma grande variedade de substâncias que atuam tanto local como sistemicamente, participando da regulação de diversos processos como a função endotelial, aterogênese, sensibilidade à insulina e regulação do balanço energético (Ribeiro Filho et al., 2006). Algumas dessas substâncias secretadas essencialmente pelo tecido adiposo como leptina, grelina e fator de necrose tumoral alfa

(TNF-α), apresentam papel fundamental na sensibilidade tecidual à insulina (Mohamed-Ali et al., 1998; Harmelen et al., 1998). Além disso, o adipócito, de acordo com sua localização, apresenta características metabólicas diferentes, sendo que a adiposidade intra-abdominal é a que apresenta maior impacto sobre a deterioração da sensibilidade à insulina (Giorgino et al., 2005).

Alimentação e Síndrome Metabólica

A SM afeta uma proporção substancial de adultos que ingerem calorias em excesso (Lopes, 2005). Hábitos alimentares inadequados constituem a principal causa do surgimento de dislipidemias, sendo que a gordura saturada leva ao aumento do colesterol e de TG (Fagherazzi et al., 2006). Estudos demonstram que gorduras saturadas e monoinsaturadas presentes na dieta apresentam também significativa correlação entre adiposidade visceral e percentual de gordura total (Pereira et al., 2003).

Os profissionais que trabalham em turnos, especialmente o trabalho noturno estão mais propensos a manifestar fadiga crônica, distúrbios digestivos e cardiovasculares (Duarte, 2001). Indivíduos com até 50 anos de idade, que trabalham à noite possuem níveis mais elevados de TG e de glicemia, assim como níveis mais baixos de HDL colesterol, quando comparados aos indivíduos que trabalham apenas durante o dia (Christi et al., 1996). Sabe-se que trabalhadores noturnos tem preferência por lanches que possuem maior concentração de gordura (Romon et al., 1993). Estudos prévios apontam que uma dieta com alta quantidade de gordura pode levar à hiperfagia, ganho de peso, aumento da adiposidade e à supressão inadequada da produção de glicose hepática estimulada pela insulina, levando ao desenvolvimento de hiperinsulinemia, RI e hiperglicemia. Este tipo de dieta altera tanto a atividade basal do eixo hipotálamo-hipófise-adrenal quanto à induzida por estresse, aumentando a produção de glicocorticóide nas glândulas adrenais em ratos. Este aumento de glicocorticóides pode levar à hipertrigliceridemia pela diminuição de lípase lipoproteica (Ghibaudi et al., 2002). Neste contexto se faz importante o estímulo a estudos que possibilitem a padronização de dietas para modelos animais que mimetizem a alimentação do trabalhador noturno.

3. REVISÃO DA LITERATURA

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Estratégias para localizar e selecionar informações

Para a introdução do tema central deste trabalho, foi realizada uma revisão sistemática na literatura. Os delineamentos escolhidos foram os estudos experimentais, observacionais, ensaios clínicos randomizados duplo-cegos, revisões sistemáticas e metanálises. As bases de dados MEDLINE e COCHRANE foram utilizadas. Adotaram-se artigos elaborados nos seguintes idiomas: inglês; português; italiano; francês e espanhol. Ao compilar esta dissertação, empregaram-se descritores como “high fat-diet”, “high fat-diet and Metabolic Syndrome”, “high fat-diet and cardiovascular disease”, “high fat-diet and body weight”, “high fat-diet and visceral adipose tissue”. Foram encontrados 352 artigos baseados na estratégia de busca acima descrita. Um total de 103 artigos foram selecionados por conter todas as especificações traçadas nos critérios de inclusão adotados, uma vez que, obrigatoriamente, deveria constar no resumo a relação entre dieta hiperlipídica e alterações metabólicas.

Os passos adotados para a estruturação dessa revisão da literatura estão presentes na figura 1.

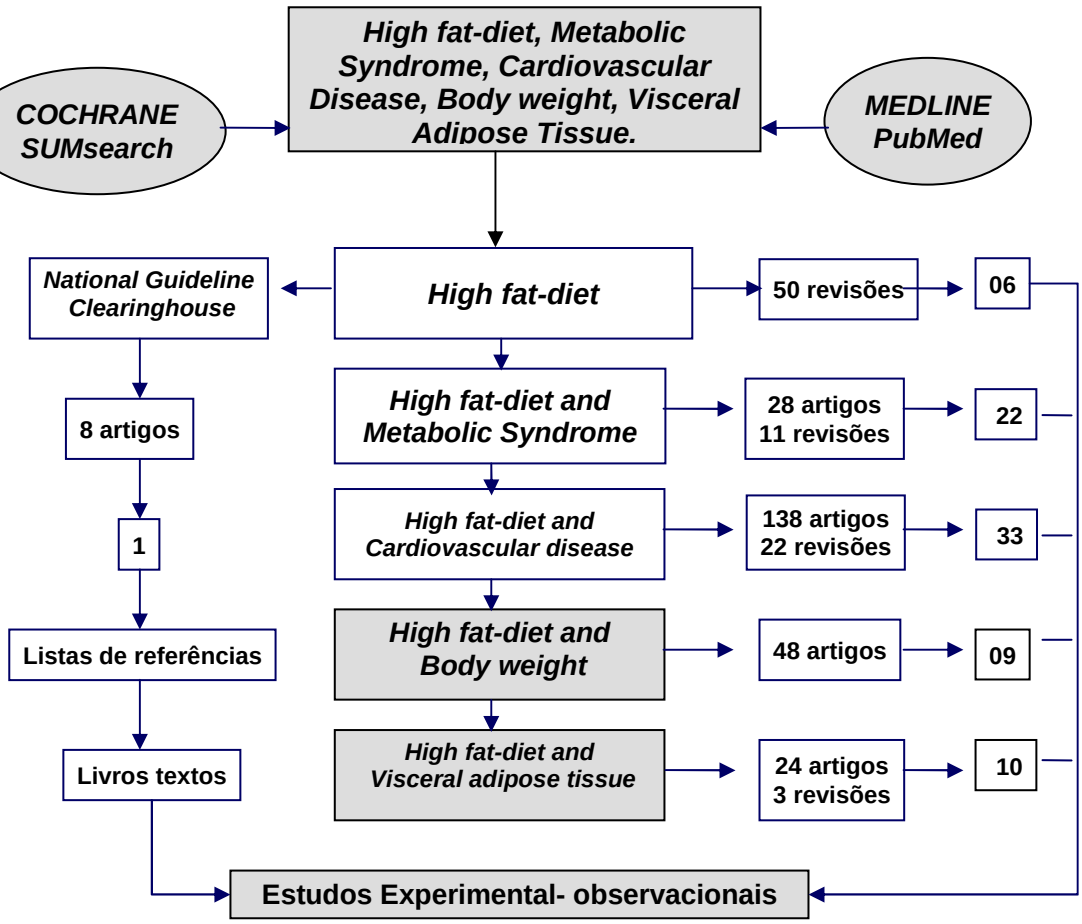

Figura 2: estratégias de busca de referências bibliográficas

Modelos de dieta hiperlipídicas

A literatura descreve alguns modelos de dietas animais conhecidas como *high-fat diet,* que induzem a um fenótipo de obesidade associado à hipertrigliceridemia (Buettner et al., 2006), hipercolesterolemia e hipertensão. Estes modelos de dietas podem variar em quantidade (15% a 60% de lipídios), qualidade dos ingredientes e duração de experimento. A gordura utilizada varia entre fonte animal e vegetal tais como toucinho, banha, margarina, gordura de boi, gordura de coco, óleo de palma e outros. Diversas dietas para induzir fatores de risco para cardiopatias e obesidade preconizam o aumento não só de lipídeos na ingestão diária de modelos animais de experimentação como também a adição de cloreto de sódio, frutose, sacarose, colesterol, fármacos e outros ingredientes e com diversas composições (Gajda et al., 2007). Estudos utilizando dietas hiperlipídicas tem reproduzido modelos experimentais de obesidade ou de fatores de risco a partir de três semanas e a duração do experimento depende de quais fatores se quer manipular. Sabe-se que o tipo de gordura utilizada tem influencia direto sob os marcadores que se deseja interferir.

Consequencias da dieta hiperlipídica

Há relatos de que em pouco tempo de intervenção (três semanas, por exemplo), já há uma resposta positiva para o aumento da quantidade dos tecidos adiposos retroperitoneal e epididimal (Duarte et al., 2001). Isto se deve ao fato de que o excesso de energia consumida na forma de lipídios é mais eficientemente armazenado na forma de gordura corporal (Kirk et al., 2000). Estes modelos de dieta levam também a distúrbios no perfil lipídico (Estadela et al., 2004) e alterações nas etapas iniciais da sinalização de insulina (Prada et al., 2005).

Estudos vem demonstrando o papel maléfico que a gordura saturada pode ter também sobre memória e doenças psiquiátricas. Camundongos recém nascidos alimentados com uma dieta rica em banha por nove semanas tiveram aumento de colesterol total e LDL-col e tiveram afetadas a memória e a capacidade de aprendizagem quando comparados com o grupo controle (Yu, 2009). A depressão também tem sido associada a hábitos alimentares em humanos. Em um estudo com aproximadamente 3.400 indivíduos de uma população mediterrânea foi constatado que um consumo de alimentos processados pode levar a depressão (Akbaraly, 2009). A dieta rica em gordura saturada tem capacidade para alterar a composição dos ácidos

graxos no cérebro, mostrando um aumento dos ácidos palmítico e esteárico, prejudicando as habilidades cognitivas.

A gordura vegetal tem sido utilizada em estudos para compulsão alimentar. Davis et al encontrou diferença significativa no peso corporal entre os grupos de animais alimentados com dieta hiperlipídica e dieta de GV hidrogenada *ad libitum* em comparação com o grupo controle ao final dos 60 dias de estudo e especula que os animais alimentados com a dieta hiperlipídica e dieta com GV apresentaram comportamento alimentar compulsivo. A dieta hiperlipídica também pode agir como um fator de estresse pois pode aumentar a liberação de glicocorticóides pelas glândulas adrenais através da estimulação do eixo hipotálamo-hipófise-adrenal, levando à hipertrigliceridemia através da diminuição da lipase lipoproteica.

Limitações do estudo

A espécie de animal a ser escolhido depende do objetivo da pesquisa e, muitas vezes, pode influenciá-lo. Sabe-se que ratos da espécie Swiss tem pré-disposição para obesidade, DM e RI quando expostos a dieta hiperlipídica. Os ratos da espécie Zucker tem propensão a desenvolver obesidade, RI e DM similar à DM tipo 2 dos humanos pois apresentam mutação no gene que codifica o receptor de leptina (Cintra et al., 2011). Para esta pesquisa, preferimos utilizar ratos Wistar que apresentam-se resistentes a alterações na insulinemia e portanto na glicemia, tornando o resultado mais interessante da ponto de vista do desenvolvimento da fisiopatologia da SM induzida unicamente por dieta. Talvez a essa resistência se deva o tempo de estudo alcançado, já que ratos Wistar podem levar mais tempo que outras espécies para mostrar as alterações metabólicas.

4. JUSTIFICATIVA

4. JUSTIFICATIVA

Nos últimos anos, houve um aumento significativo do número de doenças crônicas como obesidade, diabetes, doenças cardiovasculares. Alimentação inadequada e estilo de vida podem ser fatores determinantes no aparecimento dessas doenças. Os trabalhadores de turno estão predispostos à hipertrigliceridemia e hiperglicemia, assim como níveis mais baixos de HDL-colesterol, devido a alterações circadianas e estilo de vida. Essas alterações metabólicas compõem os critérios para diagnóstico de Síndrome Metabólica, manifestação comum nessa população. Estima-se que a prevalência da Síndrome Metabólica (SM) seja de 23,9% da população adulta dos Estados Unidos. A SM atingiu proporções de epidemia e, provavelmente, vai se tornar endêmica nos próximos anos em razão do aumento rápido na prevalência de obesidade. Não menos importante é o alerta de uma possível pandemia visto que tais fatores também têm ocorrido em países em desenvolvimento como o Brasil (Lopes e Egan, 2006). Deste modo, a prevalência, as tendências futuras, o impacto clínico e o significado econômico da SM, constituem grande problema de saúde e está, atualmente, em franco crescimento (Lopes e Egan, 2006). Em razão da gravidade desta patologia, devem-se investigar primeiramente suas causas e, posteriormente, mecanismos para evitar sua progressão. Considerando a crescente prevalência da SM e sua relação com morbimortalidade, faz-se importante a padronização de uma dieta modulada específica para indução desta patologia que poderá ser utilizada no desenvolvimento de estudos que abordem o seu aparecimento e a associação entre os principais marcadores envolvidos na regulação do balanço energético. Baseado neste entendimento, então, partir para o desenvolvimento de novas estratégias terapêuticas tanto farmacológicas quanto não-farmacológicas que visem diminuir a incidência de patologias relacionadas à Síndrome Metabólica.

5. OBJETIVO

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Objetivo Geral

Avaliar o modelo de dieta para indução de síndrome metabólica em ratos Wistar.

Objetivos específicos

Avaliar níveis de peso ponderal, quantidade de gordura visceral, triglicerídeos, glicose, HDL, peso do fígado, peso das glândulas adrenais e ingesta calórica.

6. ARTIGO

6. ARTIGO

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Title: METABOLIC DISTURBANCES INDEPENDENT OF WEIGHT GAIN IN A NON-RESISTANT INSULIN ANIMAL MODEL

Article Type: Brief Report

Keywords: metabolic syndrome; cardiovascular disease; body weight; visceral adipose tissue; wistar rats

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Abstract: Objective: Shift workers, particularly night workers, more frequently present metabolic changes when compared to day workers suggesting a predisposition for cardiovascular disease. These changes are known as Metabolic Syndrome (MS). Therefore, to increase understanding of the consequences of a high-fat diet it is important to standardize a diet for experimental animals that mimics the shift workers' diet and its effects. Methods: experimental study with 20 Wistar rats of which 10 were control group (CG) and 10 high-fat diet group (HFD). Three of five criteria for MS diagnosis from NCEP-ATP III were assessed. The amount of visceral adipose tissue was determined (VAT). Body weight was assessed weekly and food and water intake were measured daily. Student's t-test for independent samples was used. The groups were similar at baseline. Results: After fifteen weeks of intervention, HFD group showed an increase in serum TG ($p=0.01$) and glucose ($p=0.01$) levels and a decrease in HDL ($p=0.009$) compared to CG. HFD showed increased VAT ($p=0.005$) and liver weight ($p=0.01$). Food intake and water intake were higher in CG ($p<0.001$ and $p<0.001$ respectively) while energy intake was increased in HFD ($p<0.001$). No difference was found in adrenal glands weight ($p=0.07$) and body weight ($p=0.63$). Conclusion: Animals submitted to HFD present significant metabolic alterations in spite of the maintenance of body weight. Body weight is not a predictive factor for the amount of VAT and that the quality of diet composition direct influences MS markers.

METABOLIC DISTURBANCES INDEPENDENT OF WEIGHT GAIN IN A NON-RESISTANT INSULIN ANIMAL MODEL

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ABSTRACT

Background and Aim: Shift workers, particularly night workers, more frequently present metabolic changes when compared to day workers suggesting a predisposition for cardiovascular disease. These changes are known as Metabolic Syndrome (MS). Therefore, to increase understanding of the consequences of a high-fat diet it is important to standardize a diet for experimental animals that mimics the shift workers' diet and its effects. **Methods and Results:** experimental study with 20 Wistar rats of which 10 were control group (CG) and 10 high-fat diet group (HFD). Three of five criteria for MS diagnosis from NCEP-ATP III were assessed. The amount of visceral adipose tissue was determined (VAT). Body weight was assessed weekly and food and water intake were measured daily. Student's *t*-test for independent samples was used. The groups were similar at baseline. After fifteen weeks of intervention, HFD group showed an increase in serum TG ($p=0.01$) and glucose ($p=0.01$) levels and a decrease in HDL ($p=0.009$) compared to CG. HFD showed increased VAT ($p=0.005$) and liver weight ($p=0.01$). Food intake and water intake were higher in CG ($p<0.001$ and $p<0.001$ respectively) while energy intake was increased in HFD ($p<0.001$). No difference was found in adrenal glands weight ($p=0.07$) and body weight ($p=0.63$). **Conclusion:** Animals submitted to HFD present significant metabolic alterations in spite of the maintenance of body weight. Body weight is not a predictive factor for the amount of VAT and that the quality of diet composition direct influences MS markers.

Keywords: high fat-diet, metabolic syndrome, cardiovascular disease, body weight, visceral adipose tissue

INTRODUCTION

Over the last few years, there has been a significant increase in the prevalence of chronic diseases such as obesity, diabetes, and cardiovascular diseases, and also in risk factors associated with inadequate nutrition. Certain metabolic changes comprise a set of risk factors for cardiovascular disease related to visceral fat deposition and insulin resistance, which are known as Metabolic Syndrome (MS); however, their criteria for diagnosis is still under discussion 1. MS is defined as the presence of three of five criteria which include hyperglycemia, hypertriglyceridemia, hypertension, decreased high density lipoprotein-cholesterol (HDL-cho) and increased abdominal circumference 2.

According to the World Health Organization (WHO) 3 in Brazil 40.6% of the population has a body mass index (BMI) equal to or greater than 25 kg/m², while in the USA 66.9% of the population fall into this category. Meanwhile, 23.9% of the American population was diagnosed with MS according to the National Cholesterol Education Program (NCEP) parameters 4. Furthermore, 35% of the same population is in a diabetic or prediabetic state 5. Metabolic alterations related to diet affect a considerable proportion of adults. Inadequate eating habits constitute one of the causes of dyslipidemia and a high intake of saturated fat leads to increased cholesterol and triglycerides, which are significantly correlated with visceral adiposity 6.

One particular population affected by inadequate nutrition is shift workers, particularly night workers, who more frequently present hypertriglyceridemia and hyperglycemia, as well as lower levels of HDL-cho, when compared to day workers 7 and this population is subject to an increased risk of obesity, MS, diabetes and cardiovascular disease. These diseases might be a result of physiological maladaptation

to chronically sleeping and eating at abnormal circadian times 8. It is known that night workers prefer snacks that contain high concentrations of saturated fat 9, 10. Previous studies have linked a high-fat diet with hyperphagia, weight gain, increased adiposity and inadequate suppression of hepatic glucose production stimulated by insulin. Later, such conditions may lead to hyperinsulinemia, insulin resistance (IR) and hyperglycemia. Furthermore, diets rich in unsaturated fatty acids can contribute to an increased risk of developing depression in humans 11, 12. Thus, it is important to encourage studies that seek to develop a standardized diet for experimental animals that mimics the food intake of shift workers.

The aim of this study was to create an animal model in which the composition of the diet was similar to the snacks consumed by shift workers in order to analyze the effects of such a diet on metabolism.

METHODS AND PROCEDURES

Animals

Twenty male Wistar rats were used, aged 2 months at the beginning of the study, obtained from CREAL (*Centro de Reprodução e Experimentação de Animais de Laboratório*; Universidade Federal do Rio Grande do Sul*)*. The animals were housed in polycarbonate cages, 5 animals per cage and maintained at 22 ± 2 °C with a 12:12 h light:dark cycle (lights on 07.00) according to the Guide to the Use and Care of Laboratory Animals 13, 14. One animal died during the experiment. The study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre, and was carried out in the Animal Experimental Unit of the same institution. All the procedures were performed in such a way as to minimize pain and discomfort.

Diet

All rats were allowed access *ad libitum* to food and water. The high-fat diet (HFD) consisted of 45.5% standard chow, 22.7% lard, 22.7% vegetable shortening and 9% sucrose, while the standard diet (control group; CG) comprised 100% chow Nuvital™. HFD was prepared every 4 days and stored in a refrigerator under controlled temperature (7°C $\underline{+}$ 2).

Experimental Procedures

The rats were randomized and allowed a 1-week period of adaptation to the laboratory conditions and chow diet. After this, the animals were divided in two groups of 10 animals each: HFD and CG. The treatment lasted 15 weeks. Food and water intake were measured daily. The body weight was determined weekly. At the beginning of the treatment period blood samples were collected to establish baseline values. The samples were obtained from the retro orbital plexus after sedating the animals with

inhaled isoflurane. At the end of the treatment, the rats were killed by decapitation to facilitate blood collection and no drugs were used before this procedure. Epididymal and retroperitoneal fats forming visceral adipose tissue (VAT), liver and adrenal glands were removed and weighed on a Marte balance model AS5500C, which was also used to determine food, water and animal body weight. Trunk blood was collected for analysis of the glucose, HDL-cho and triglycerides.

Metabolite Determinations

The diagnosis of Metabolic Syndrome was made using three of the five criteria of NCEP-ATP III: serum levels of glucose, high-density lipoprotein cholesterol (HDL-cho) and triglycerides (TG). Serum from trunk blood was assayed for TG and glucose using assay kits from Roche Diagnostics (Mannheim, Germany) by enzymatic colorimetric assay. Levels of HDL-cho were determined by homogeneous enzymatic colorimetric assay using kits also from Roche Diagnostics.

Statistical Analysis

Data were expressed as mean $\pm$ SEM and analyzed using Student's *t*-test for independent samples. A *P*-value of less than 0.05 was considered to be statistically significant. All of the statistical analysis was carried out with the software SPSS 16.0.

RESULTS

This study examined two groups of rats (n=20), which were maintained for 15 weeks on a HFD (45.5% chow) diet that sought to mimic the diet of shift workers, or Control (100% chow).

Baseline - Data for serum glucose, TG and HDL-cho (mean±SEM) are shown in Table 1. No significant difference was observed between the control and HFD groups at baseline for body weight (CG=224.10±6.21, HFD=217.90±7.55; p=0.53), serum glucose (CG=152.40±4.11, HFD=166.30±7.13; p=0.11), HDL-cho (CG=49.60±1.40, HFD=47.20±1.32; p=0.23) and TG (CG=88.40±8.68, HFD=108.00±9.21; p=0.14).

Metabolic Parameters - Data for serum glucose, TG and HDL-cho (mean±SEM) after 15 weeks of intervention are presented in Table 2. The HFD group showed a significant increase in levels of serum glucose (CG=166.88±3.64, HFD=183.40±4.89; p=0.01), TG (CG=157.33±21.51, HFD=299.10±42.36; p=0.01) and a significant decrease in HDL-cho (CG=49.66±2.19, HFD=40.40±2.22; p=0.009).

Organs and Tissues – The weights of VAT, liver and adrenal glands (mean±SEM) can be seen in Table 2, while the body weights of the CG and HFD groups are compared in Figure 1a. In the HFD group the VAT (CG=17.12±1.76, HFD=34.22±4.70; p=0.005) and liver weight (CG=11.33±1.50, HFD=13.17±0.52; p=0.01) were found to be significantly higher than in the CG. No difference was found between the HFD and CG groups regarding body weight (CG=402.73±13.48, HFD=391.24±19.08; p=0.63) and the weight of adrenal glands (CG=0.05±0.005, HFD=0.07±0.005; p=0.07).

Food, Water and Energy Intake – Food and water intake in the two groups are shown in Figures 1b and 1c. Both food and water intake in the HFD group (151.16±2.77 and 226.56±4.71, respectively; p<0.001) were lower than control (239.60±3.26,

390.0±5.64; p<0.001). The energy intake was significantly higher in the HFD group (CG=78.8±1.20, HFD=91.4±2.75; p=<0.001).

DISCUSSION

In this study the model diet caused metabolic changes. As far as we are aware this is the first study designed to create an animal model diet containing lard and shortening, both saturated fatty acids, which are the two most commonly found types of fat in processed food. In this diet, 45% of energy was supplied by fat. The metabolic changes found matched with the MS diagnostic criteria confirming that the quality of diet composition can have a direct influence on these markers 15, 16.

It is interesting to note that body weight *per se* may not be a good predictor for metabolic change. Thus, there was no significant difference between the two groups concerning body weight, although there was an increase in VAT. Since the animals showed no correlation between body weight and the amount of VAT, they demonstrated that there is a non-causal relationship between these variables. Controversy surrounds the results from studies in the literature that show an increase in body weight on a high-fat diet. In Sampey et al. 17 Wistar rats were fed for 15 weeks with a lard-based 45% fat diet and had higher food intake than the standard-diet group, as well as higher body weight. It may be that the variety of ingredients used in manipulated, high fat diets, and their flavors, are important in determining higher or lower food intake and weight gain. Therefore, we propose that, in animal experiments, the amount of VAT might be used as another criterion for MS diagnosis, especially since studies suggest that intra-abdominal adiposity is a better risk marker for MS and cardiovascular disease than body weight 18.

In our study animals on the high-fat diet did not show hyperphagia because they not only maintained their body weight the same as that of the CG, but also had a lower food intake, in contrast with what was seen in other studies in which rats were fed with

a HFD 19, 20. The HFD group ate a lower amount of food (~15 g/day/rat) than CG (~26 g/day/rat). However, the energy intake was higher in HFD, demonstrating that a high fat and low sucrose diet does not cause addictive feeding behavior. These data suggests that despite the fact that sucrose is considered palatable, this model diet does not lead to compulsive-like feeding behavior. Such behavior has been described in studies with obese rats where striatal dopamine D2 receptors (D2Rs) were downregulated by a palatable high-fat food, triggering food addiction in the same way as drugs 21. Moreover, we found that a higher energy density in a meal can decrease the food intake, and the key to this mechanism may be linked to central and peripheral satiety factors. According to Erlanson-Albertsson 22 a high-fat diet can up-regulate the expression of hunger and satiety signals and at the same time blunt the response to satiety signals. On the other hand, it has been reported that rats fed with a higher calorie diet than that required spontaneously increase basal energy expenditure, stimulating thermogenesis as a compensatory mechanism in an attempt to maintain body weight 23, 24.

In this study the experimental group presented a decrease in water intake (HFD~26 ml/day/rat and CG~39 ml/day/rat). More studies on water intake and satiety mechanisms are needed especially regarding the control of diabetes. Cholecystokinin (CCK) is related to satiety for food and water intake; in addition, a decrease in the proopiomelanocortin (POMC) level has been reported to decrease water intake, and one or both of these mediators may be involved in our findings 25, 26.

In this study the experimental group presented lower serum HDL-cho and higher triglycerides concentrations than the control group. We can raised to hypothesis linked to these results. First, lipoprotein lipase (LPL) reduction activity may explain the decreased HDL-cho in these animals because LPL supplies lipid components from chylomicrons and very low density lipoprotein (VLDL-cho) for HDL-cho generation

and maturation. HDL-cho is seen to be increased when a diet rich in unsaturated fatty acids is supplied 27. Second, the fact that glucocorticoids can promote lipogenesis, other peripheral regulators are also involved in energy balance, such as leptin, which activates lipolysis but appears to cause hyperleptinemia in animals that consumed HFD, and insulin, which is capable of affecting the body weight and is affected by the amount of VAT.

The deposit of TG commences in subcutaneous adipose tissue, increasing IR and leading to its accumulation in the visceral area. From there, TG will migrate to other organs causing IR in ectopic sites 28. The adipocytes from visceral tissue produce higher amounts of free fatty acids (FFA), overloading the capacity of the liver. This increased FFA flow to the liver stimulates TG synthesis. Thereafter, TNF-α and IL-6, produced by adipose tissue, can inhibit LPL, which reduces TG hydrolysis leading to the onset of hypertriglyceridemia.

This TG is accumulated in the liver and can cause hepatomegaly. Steatosis and hepatic insulin resistance can lead to nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of MS. Also, a HFD can act as a stressor, increasing glucocorticoid levels through stimulation of the hypothalamus–pituitary–adrenal (HPA) axis, which leads to hypertriglyceridemia by decreasing LPL 29, 30. Lard can increase the plasma level of corticosterone more than any other fat source 31 because this fat is rich in stearic fatty acid and this stimulates the adrenal cortex, thereby inducing an increase in corticosterone release. In such a way, a HFD can act as a stress factor and increase the weight of the adrenal glands 32. The animals in our study did not present a significant change in this parameter, although there was a trend towards an increase. With this in mind it should be considered that length of exposure to the diet may have been insufficient to bring about an increase in the weight of the adrenal glands.

VAT secretes many adipokines and is chronically inflamed 33. Inflammatory signals can damage the insulin response through the c-Jun N-terminal kinase (JNK), which acts as central mediator of IR, or adipose tissue macrophages (ATM), which can promote IR and through the deficiency of chemokine receptors reduces ATM accumulation, while T lymphocytes, natural killer T cells, mast cells, and B cells may be involved in the physiology of macrophages and adipocytes 34, 35. Consequently, these immune cells may play an important role in changes in VAT and the development of MS.

In other work a diet designed to induce obesity and diabetes mellitus 36 reached its aim within a few weeks, although those authors used a high-fat diet combined with streptozotocin injection. In our research the latency to the development of MS was 15 weeks due to our attempt to trigger the most natural response in MS parameters. This longer period allows a longer exposure to this type of food, as is seen in shift workers. The length of the study is supported by the later response of HDL, since rats have *per se* higher levels of this lipoprotein compared to humans 37. We chose to feed the rats on a diet that was not high in sucrose, demonstrating that the increase in glucose levels maybe was due to the IR caused by a high fat diet. In contrast to the above-mentioned study 36, hyperglycemia was induced only by food, which makes this model most similar to the natural course of MS physiopathology. Lard and shortening are saturated fatty acids (SFAs) and these SFAs appear to be more harmful than polyunsaturated fatty acids (PUFAs) since in this study SFAs significantly increased the VAT. SFAs are poorly used for energy production, being acylated into TG and stored in adipose tissue whereas PUFAs and monounsaturated fatty acids (MUFAs) are quickly used for energy and stored less 38 besides presenting antiinflammatory properties. In contrast, fat stored

in the visceral area promotes an increase in the release of proinflammatory cytokines such as IL-1 and TNF-α.

This study shows that feeding experimental animals for a sustained period with a high-fat diet can lead to changes in metabolic parameters, in spite of there was no significant increase concerning body weight, although there was an increase in VAT. The body weight *per se* may not be a good predictor for metabolic change. These metabolic changes underlie the development of chronic diseases such as a prediabetic state, type 2 diabetes, obesity and nonalcoholic fatty liver disease (NAFLD). Since meal composition may be involved in the genesis of metabolic syndrome, studies employing such a diet can contribute to a better understanding of the MS physiopathology. This hypothesis is robust as no difference was observed between control and HFD groups at baseline, indicating that the animals were a homogeneous group at the beginning of the study. In conclusion, the proposed experimental diet is effective for MS induction, measured by changes in serum glucose, triglycerides and HDL-cho levels according to NCEP-ATP III.

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The authors declare no conflict of interest

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TABLES

Table 1. Sample characteristics at baseline. Data presented as mean (±SEM). Student's *t*-test for two independent samples.

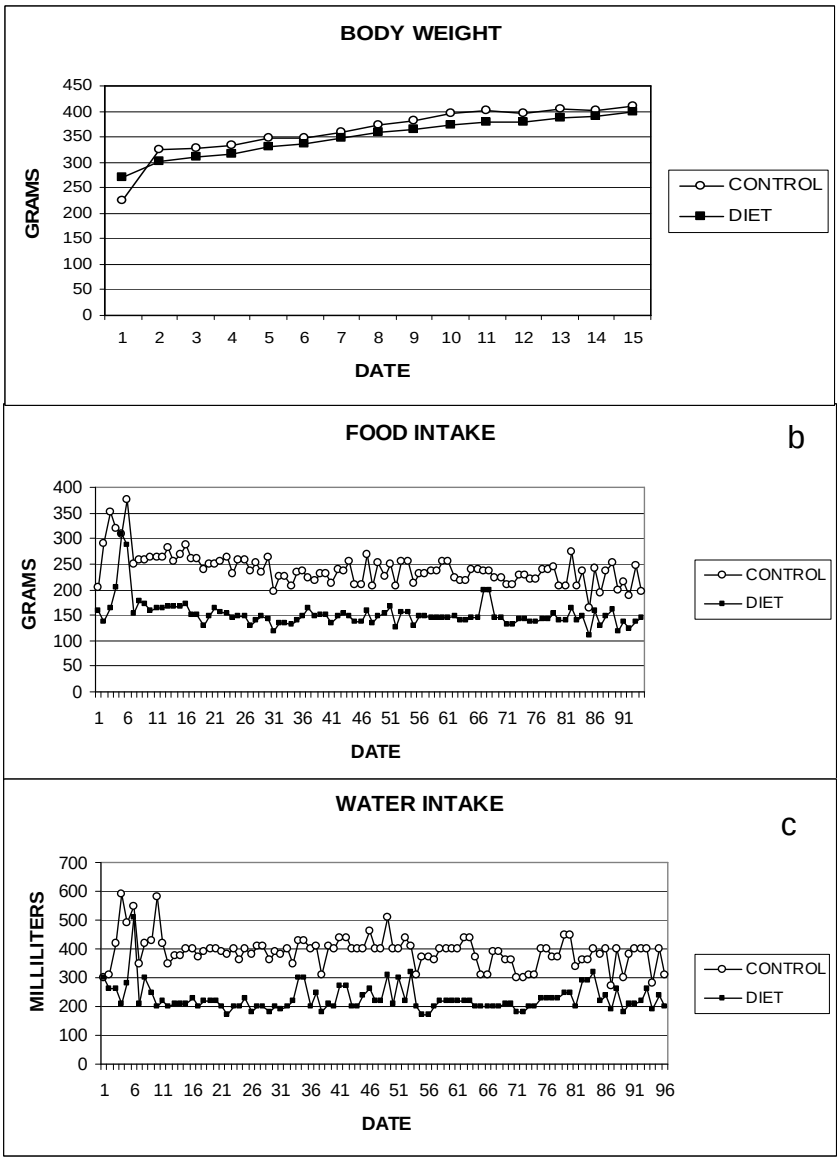
	Control group (n=10)	**HFD group (n=10)**	**T**	**P**
Body weight (g)	224.10 (±6.21)	217.90 (±7.55)	0.63	0.53
Serum glucose (mg/dL)	152.40 (±4.11)	166.30 (±7.13)	-1.68	0.11
HDL-cholesterol (mg/dL)	49.60 (±1.40)	47.20 (±1.32)	1.24	0.23
Triglycerides (mg/dL)	88.40 (±8.68)	108.00 (±9.21)	-1.54	0.14

Table 2. Sample characteristics after fifteen weeks of intervention. Data presented as mean (±SEM). Student's *t*-test for two independent samples.

	Control group (n=09)	HFD group (n=10)	T	P
Serum glucose (mg/dL)	166.88 (±3.64)	183.40 (±4.89)	-2.65	0.01**
HDL-cholesterol (mg/dL)	49.66 (±2.19)	40.40 (±2.22)	2.95	0.009**
Triglycerides (mg/dL)	157.33 (±21.51)	299.10 (±42.36)	-2.88	0.01*
Visceral adipose tissue (g)	17.12 (±1.76)	34.22 (±4.70)	-3.26	0.005**
Liver weight (g)	11.33 (±1.50)	13.17 (±0.52)	2.62	0.01**
Adrenal gland weight(g)	0.05 (±0.005)	0.07 (±0.005)	1.89	0.07
Body weight (g)	402.73 (±13.48)	391.24 (±19.08)	0.48	0.63
Food intake (g)	239.60 (±3.26)	151.16 (±2.77)	20.62	<0.001***
Water intake (mL)	390.0 (±5.64)	226.56 (±4.71)	22.23	<0.001***
Energy intake (kcal/day/rat)	78.8 (±1.20)	91.4 (±2.75)	-4.22	<0.001***

For significant p values: *p<0.05; **p<0.01; ***p<0.001.

FIGURE 1



Legend: figure 1.a shows HFD group and CG body weight through 15 weeks; 1.b shows HFD group and CG food intake through 15 weeks; 1.c shows HFD group and CG water intake through 15 weeks. HFD=high-fat diet, CG=control group

7. CONCLUSÃO

7. CONCLUSÃO

Os animais submetidos à dieta hiperlipídica desenvolveram SM em 15 semanas e as alterações metabólicas encontradas compatíveis com o diagnóstico de SM evidenciaram que a qualidade da composição da dieta tem influência direta nos marcadores desta patologia. Também podemos reforçar a idéia de que a quantidade de gordura abdominal parece estar mais intimamente relacionada com o risco aumentado para doenças cardiovasculares do que o IMC sendo esta, portanto, melhor marcador para ser utilizado como critério em estudos com animais que estejam relacionados à saúde cardiovascular.

8. REFERÊNCIAS DA REVISÃO DE LITERATURA

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9. PERSPECTIVAS FUTURAS

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Sabemos que a dessincronização circadiana a qual estão expostos os trabalhadores de turno por si só traz efeitos maléficos à saúde. Estudos indicam que um desajuste do ritmo circadiano pode ser fator causal para uma relação positiva entre o trabalho noturno e SM, obesidade e DM. O trabalho noturno tem sido relacionado a desajustes nos biomarcadores de doenças cardiovasculares tais como índice de massa corporal (IMC), PA diastólica, TG e circunferência abdominal. Hormônios envolvidos no balanço energético parecem estar envolvidos com estas patologias, porém poucos estudos abordam esta associação levando em consideração alterações do ritmo provocadas por uma inversão do claro-escuro. É interessante analisar a relação do trabalhador de turno em relação à sua alimentação, já que esta pode potencializar esses reflexos nocivos.