UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso

AUMENTO DO CONSUMO DE SÓDIO SOBRE OS NÍVEIS DE PRESSÃO ARTERIAL E PARÂMETROS BIOQUÍMICOS EM RATOS WISTAR MACHOS

Tizye Lima Rizzo

Porto Alegre, novembro de 2014.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Faculdade de Farmácia

Disciplina de Trabalho de Conclusão de Curso

AUMENTO DO CONSUMO DE SÓDIO SOBRE OS NÍVEIS DE PRESSÃO ARTERIAL E PARÂMETROS BIOQUÍMICOS EM RATOS WISTAR MACHOS

Tizye Lima Rizzo

Trabalho de Conclusão de Curso de Farmácia

Prof. Dra. Iraci Lucena Da Silva Torres

Orientadora

MSc. Isabel Cristina Macedo

Co-orientadora

Porto Alegre, novembro de 2014.

Dedico esse trabalho

Ao meu irmão

Pelo carinho e incentivo,

Ao meu namorado

Pelo amor e compreensão,

A minha orientadora

Pela oportunidade,

A minha Co-orientadora

Pela paciência e entusiasmo e

Aos meus amigos pelo

Apoio e parceria

As dificuldades crescem à medida

que nos aproximamos

do nosso objetivo.

Goethe

Revisão bibliográfica

Obesidade

De acordo com a Organização Mundial da Saúde 1,5 bilhões de adultos estão acima do peso no mundo, dos quais 200 milhões são obesos. Estima-se que até 2015, aproximadamente 2,3 bilhões de adultos estarão com sobrepeso e mais de 700 milhões serão obesos. A obesidade está associada à predisposição genética, fatores psicológicos, sociais e hábitos alimentares inadequados, sendo considerada uma doença multifatorial (Pinto Jr et al. 2012; WHO, 2006). Esse distúrbio metabólico está associado ao excessivo consumo de alimentos semi-prontos que são altamente calóricos e contém altas quantidades de sódio e baixo valor nutricional. Nos últimos 40 anos a ingestão energética está acima das necessidades diárias e a disponibilidade de alimentos altamente palatáveis e de alto teor calórico contribuíram com a epidemia de obesidade (Downs, Chen et al. 2009; McAllister, Dhurandhar et al. 2009; Driessen, Cameron et al. 2014). A obesidade tem sido reconhecida como um fator que contribui significativamente para o desenvolvimento de várias doenças crônicas como coronariopatias, diabetes mellitus do tipo II, doença renal crônica, carcinomas (endométrio, mama e cólon), hipertensão, doenças hepáticas e da vesícula biliar, apneia do sono e problemas respiratórios, osteoartrite, síndrome dos ovários policísticos associada à infertilidade entre outras doenças ainda em estudo (CDC, 2014). Dietas constituídas por alto teor de alimentos processados ou com um elevado teor calórico normalmente também apresentam um alto conteúdo de sódio(Gray, Petersen et al. 2014).

Por isso é possível prever que o consumo de alimentos semi prontos e previamente processados além de contribuírem com a obesidade podem agravar outras comorbidades ligadas a esta doença.

Ingestão de Sódio e Controle da Pressão Arterial

A alta ingestão de sódio na dieta é um importante fator de risco para muitas doenças nas sociedades modernas (Doaei and Gholamalizadeh 2014). O sódio é o cátion mais abundante no meio extracelular, o principal determinante da osmolaridade do plasma e um dos eletrólitos mais importantes envolvidos no controle da homeostase de volume e pressão arterial. Devido à sua importância o balanço de sódio é fortemente regulado por vários eixos neuro-hormonais a fim de manter a constância do ambiente interno (Mc Causland, Waikar et al. 2013). Em indivíduos saudáveis, os rins são os principais órgãos que regulam a homeostase de sódio. O Centro de Promoção e Política Nutricional recomenda uma ingestão diária de sódio inferior a 2300 mg/dia para a população em geral e 1500mg/dia entre as pessoas com maior risco de doenças cardiovasculares (indivíduos com idade acima de 50 anos, afro-americanos, hipertensos, diabéticos, ou doentes renais crônicos) (DiNicolantonio, Niazi et al. 2013). As necessidades fisiológicas de sódio são 0,23 a 0,46 g/dia (0,58-1,17 g / sal dia), porém a ingestão de sódio em todo o mundo excede o preconizado, a maioria das populações adultas tem a ingestão de sódio maior que 2,3 g/dia (maior 5,85 g de sal/dia) (Batcagan-Abueg, Lee et al. 2013).

A *American Heart Association* enfatizou recentemente a meta de atingir "A Saúde Cardiovascular Ideal", em 2020, para que a ingestão diária de sódio seja inferior a 1,5g/dia (DiNicolantonio, Niazi et al. 2013). A capacidade renal para natriurese é ampla, sendo capaz de se adaptar a variabilidade na ingestão de sódio ajustando o volume plasmático circulante efetivo em situações de excesso ou deficiência de sódio (Mc Causland, Waikar et al. 2013). Porém a alta ingestão de sal na dieta representa um grande desafio para os rins excretar grandes quantidades de sal ingerido (Hall, 2011).

A regulação da pressão arterial (PA) é feita momento a momento por mecanismos neurais e reflexos (barorreceptores), e em longo prazo, por mecanismos neurais e hormonais conhecidos

como sistema renina angiotensina aldosterona (SRAA). A curto prazo, a PA é regulada pelo reflexo desencadeado pelos barorreceptores, que detectam o estiramento das artérias carótida e aórtica em resposta a elevação da PA. Os sinais detectados pelos barorreceptores desencadeiam a inibição do centro vasoconstitor bulbar e excitam o centro parassimpático vagal. Como resultado ocorre vasodilatação das veias e arteríolas periféricas, diminuição da frequência e força de contração cardíaca, resultando na queda da PA (Hall, 2011) (**figura 1).**

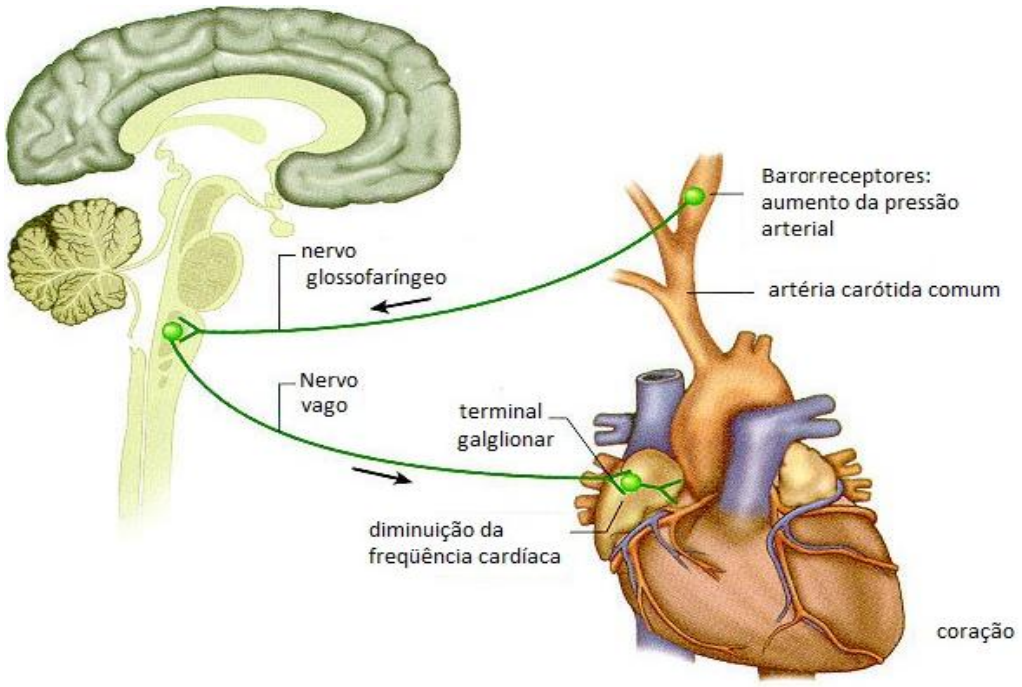


FIGURA 1. Regulação da pressão arterial em curto prazo. (adaptado de http://www.lookfordiagnosis.com/mesh_info.php?term=Sistema+Vasomotor&lang=3 acesso em 04 de novembro).

O sistema renina-angiotensina-aldosterona é conhecido por seu papel na regulação da pressão arterial e de fluidos e eletrólitos a longo prazo (Thethi, Kamiyama et al. 2012). Anatomicamente este sistema é composto por células especializadas localizadas na arteríola aferente de cada glomérulo, chamadas células justaglomerulares que secretam uma enzima

proteolítica, denominada renina. As alterações plasmáticas na concentração do íon Na^+ sinaliza para a liberação desta enzima. Uma vez na circulação ela reage com o angiotensinogênio formando a angiotensina I que tem poucas ações fisiológicas até agora descritas. A angiotensina I é convertida em angiotensina II (ANG II) que é fisiologicamente ativa. Esta reação é catalisada pela enzima conversora da angiotensina (ECA) existente na superfície luminal do endotélio vascular corporal, muito abundante no endotélio pulmonar. Os níveis aumentados de ANG II resultam em vasoconstrição, com maior constrição da arteríola eferente que da aferente, com consequente aumento da pressão hidrostática nos capilares glomerulares e manutenção da filtração glomerular. Como a ANG II causa aumento da filtração com consequente elevação da pressão oncótica peritubular desencadeia a reabsorção de sais e água, elevando a pressão. A liberação da aldosterona pela adrenal se faz principalmente por variações da osmolaridade do sangue e dos hormônios adrenocorticotrófico (ACTH) e ANG II. Nas células principais desse segmento a aldosterona estimula a reabsorção de sódio e a secreção de potássio (Aires, 2008).

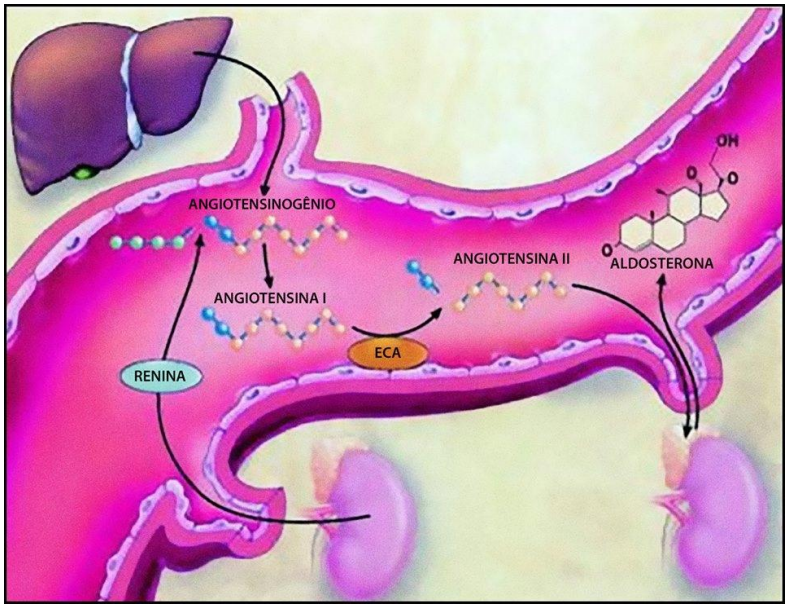


Figura 2. Sistema Renina Angiotensina Aldosterona (adaptado de www.google.com.br/search?q=sistema+renina+angiotensina+aldosterona, acesso em 04 de novembro de 2014).

A ativação do SRAA é comum na obesidade (Thethi, Kamiyama et al. 2012), que inicialmente provoca a vasodilatação renal e a hiperfiltração glomerular, que são mecanismos compensatórios para manter o equilíbrio de sódio. Essas compensações junto com a pressão arterial elevada e anormalidades metabólicas podem desencadear lesão glomerular e hipertensão (Hall, do Carmo et al. 2014).

Os rins são os órgãos responsáveis pela manutenção do volume e da composição do fluido extracelular do indivíduo dentro dos limites fisiológicos compatíveis com a vida. As funções mais importantes do rim são a filtração e a excreção de produtos residuais nitrogenados a partir do sangue (Basile, Anderson et al. 2012) **(figura 3)**.

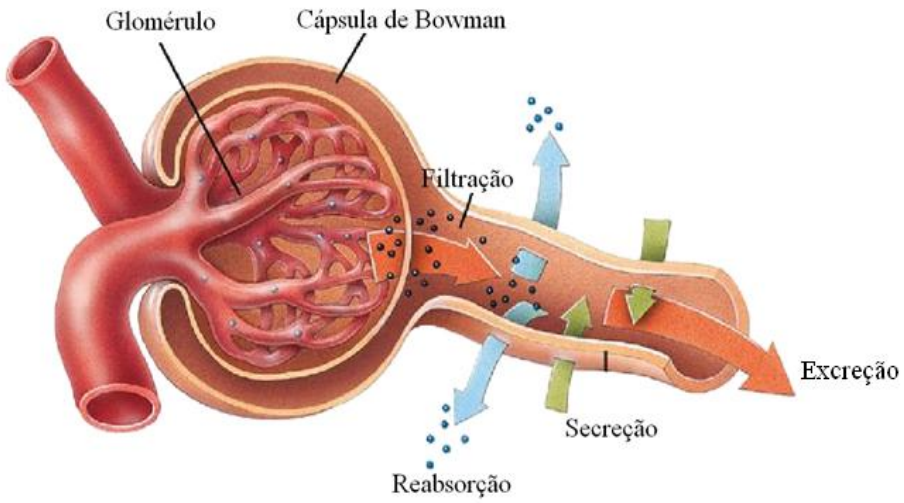


Figura 3. Processos renais(http://www.biomedicinapadrao.com.br/2013/12/entendendo-o-que-e-filtracao-reabsorcao.html acesso em 06 de novembro de 2014).

Marcadores de Função Renal

A dosagem da creatinina sérica ou plasmática é utilizada como método clínico de avaliação renal porque a depuração da creatinina apresenta boa correlação com a determinação da TFG pela inulina (marcador experimental de função renal, é 100% filtrada, não é reabsorvida nem excretada), sua excreção é relativamente constante durante o dia e sua determinação da creatinina sérica ou plasmática é relativamente simples, bem reproduzível e realizada na grande maioria dos laboratórios de análises clínicas (Abensur, 2014). A creatinina é um produto residual da creatina. A transformação de creatina em creatinina acontece no tecido muscular, no qual 1%-2% da creatina livre se converte espontânea e irreversivelmente em creatinina todos os dias. Logo, a quantidade de creatinina produzida é dependente da massa muscular e não apresenta grandes variações diárias. A creatinina é filtrada livremente no glomérulo e ativamente secretada em uma pequena parcela. A quantidade secretada não é constante e depende do indivíduo e da concentração plasmática desse analito, dificultando a determinação de uma constante de secreção. Em termos gerais, 7%-10% da creatinina presente na urina é secretada (Sodré et al. 2007). A dosagem de creatinina é amplamente utilizada na pesquisa epidemiológica como marcador de função renal e está associada à filtração glomerular, sendo utilizada como parâmetro para o cálculo da taxa de filtração glomerular (Odden, Shlipak et al. 2009). Além do mais os níveis de creatinina na urina pode também fornecer importante informação prognóstica.

A creatinina é gerada a uma taxa constante pelo músculo esquelético e depurado quase exclusivamente na urina. Assim, em situações normais a quantidade de creatinina produzida pelo músculo deve ser igual à quantidade excretada. A redução na taxa de excreção de creatinina na urina está associada com risco aumentado de mortalidade, eventos cardiovasculares e progressão da doença renal crônica (Carter, Gansevoort et al. 2012). Como a creatinina é um produto metabólico da degradação da creatina-fosfato no músculo, a sua produção é relativamente constante e depende da massa muscular. A ingestão de proteínas

em quantidade suficientemente grande pode aumentar potencialmente o nível sérico de creatinina (Borba et al. 2011).

As proteínas são compostos indispensáveis ao funcionamento do organismo, representando a base da estrutura de células, tecidos e órgãos. Funcionam como catalisadores enzimáticos nas reações bioquímicas, carreadores de muitos constituintes do plasma e na defesa orgânica como anticorpos (Jain et al. 1993; Kaneko et al. 1997). Pelo significado biológico e múltiplas funções exercidas no sistema orgânico, a avaliação dos níveis séricos das proteínas totais representa um importante auxílio ao diagnóstico clínico (Kaneko et al. 1997). O metabolismo e a quantidade de proteínas presentes no soro podem sofrer influência de diversos fatores (Feldman et al. 2000). As deficiências nutricionais e a debilitação orgânica são situações que podem ocasionar redução das proteínas (Kerr, Southern et al. 2003).

Modelo animal de obesidade

Diversos modelos experimentais são utilizados para o estudo da obesidade, entre eles o emprego de animais geneticamente modificados (Drel, Mashtalir et al. 2006) ou animais que apresentam predisposição genética para o desenvolvimento de obesidade e diabetes, como os ratos Zucker (Shang, Chen et al. 2014). Muitos pesquisadores empregam com sucesso modelos experimentais de dietas obesogênicas que podem apresentam altos teores de gorduras e carboidratos (Bartolomucci, Cabassi et al. 2009). Um modelo bastante empregado para desencadear obesidade é a dieta de cafeteria, que mimetiza as refeições humanas conhecidas como *fast food* (Dietrich, Muller et al. 2007; Bartolomucci, Cabassi et al. 2009). Os alimentos que compõem esta dieta experimental são normalmente alimentos de consumo humano como salsicha, bolacha recheada, *waffer*, leite condensado, salgadinhos e refrigerante (Estadella, Oyama et al. 2004; Macedo, Medeiros et al. 2012). Este modelo de dieta pode desencadear hiperfagia e obesidade indexada pelo acúmulo de obesidade visceral e o acentuado ganho de peso. Além disso, desencadeia a desregulação nos níveis e no padrão de

liberação de leptina, que é um importante hormônio secretado pelo tecido adiposo para atuar na homeostase energética (Macedo, Medeiros et al. 2012; de Oliveira, Scarabelot et al. 2014). Além disso, já foi demonstrado em modelos animais que a dieta de cafeteria promove disfunção das ilhotas pancreáticas com desenvolvimento de hiperglicemia, hiperinsulinemia, inflamação e esteatose hepática (Sampey, Vanhoose et al. 2011).

Tabela 1: Quantidade de sódio em mg de cada 100g de alimento.

Nutrientes em 100g	SÓDIO (mg)
Coca-cola	5
Leite Condensado Elegê	90
Waffer morango Mabel	107,4
Salgadinho Fandangos	676
Salsicha Pena Branca	604
Biscoito Trakinas	256,66
Ração (Nuvilab CR-1)	270

Considerando os importantes achados relacionados ao consumo de dieta de cafeteria na desregulação metabólica, desencadeamento de obesidade e alto consumo de sódio, este trabalho de conclusão de curso tem como objetivo avaliar o consumo de sódio proporcionado por este modelo de dieta e sua relação com o consumo de líquidos, com a pressão arterial caudal e com marcadores de função renal.

Referências

Abensur H. Biomarcadores na Nefrologia e-book. Sociedade brasileira de nefrologia: <http://www.sbn.org.br/acesso em 04 de novembro>.

Aires, Margarida M. Fisiologia. 3 .ed. Rio de Janeiro: Guanabara Koogan, 2008.

Bartolomucci, A., A. Cabassi, et al. (2009). "Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress." PLoS One 4(1): e4331.

Basile, D. P., M. D. Anderson, et al. (2012). "Pathophysiology of acute kidney injury." Compr Physiol 2(2): 1303-1353.

Batcagan-Abueg, A. P., J. J. Lee, et al. (2013). "Salt intakes and salt reduction initiatives in Southeast Asia: a review." Asia Pac J Clin Nutr 22(4): 490-504.

Borba A.J; M.G. M Rocha; et al (2011) "Low-carbohydrate diet used for weight loss induces obesity in rats." Rev. Nutr. vol.24 no.4

Carter, C. E., R. T. Gansevoort, et al. (2012). "Influence of urine creatinine on the relationship between the albumin-to-creatinine ratio and cardiovascular events." Clin J Am Soc Nephrol 7(4): 595-603.

CDC- Centers for Desease Control and Prevention. http://www.cdc.gov/obesity/ acesso em 5 novembro de 2014.

de Oliveira, C., V. L. Scarabelot, et al. (2014). "Obesity and chronic stress are able to desynchronize the temporal pattern of serum levels of leptin and triglycerides." Peptides 51: 46-53.

Dietrich, M. O., A. Muller, et al. (2007). "Western style diet impairs entrance of blood-borne insulin-like growth factor-1 into the brain." Neuromolecular Med 9(4): 324-330.

DiNicolantonio, J. J., A. K. Niazi, et al. (2013). "Dietary sodium restriction: take it with a grain of salt." Am J Med 126(11): 951-955.

Doaei, S. and M. Gholamalizadeh (2014). "The association of genetic variations with sensitivity of blood pressure to dietary salt: A narrative literature review." ARYA Atheroscler 10(3): 169-174.

Downs, B. W., A. L. Chen, et al. (2009). "Nutrigenomic targeting of carbohydrate craving behavior: can we manage obesity and aberrant craving behaviors with neurochemical pathway manipulation by Immunological Compatible Substances (nutrients) using a Genetic Positioning System (GPS) Map?" Med Hypotheses 73(3): 427-434.

Drel, V. R., N. Mashtalir, et al. (2006). "The leptin-deficient (ob/ob) mouse: a new animal model of peripheral neuropathy of type 2 diabetes and obesity." Diabetes 55(12): 3335-3343.

Driessen, C. E., A. J. Cameron, et al. (2014). "Effect of changes to the school food environment on eating behaviours and/or body weight in children: a systematic review." Obes Rev.

Estadella, D., L. M. Oyama, et al. (2004). "Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats." Nutrition 20(2): 218-224.

Feldman, B.C. et al. Veterinary hematology. 5.ed. Philadelphia: Lippincott Williams & Wilkins, 2000. 1344p.

Gray, K. L., K. S. Petersen, et al. (2014). "Attitudes and beliefs of health risks associated with sodium intake in diabetes." Appetite 83C: 97-103.

Hall, John E. Tratado de Fisiologia Médica. 12ed, Elsevier, Rio De Janeiro 2011.

Hall, M. E., J. M. do Carmo, et al. (2014). "Obesity, hypertension, and chronic kidney disease." Int J Nephrol Renovasc Dis 7: 75-88.

Jain, N.C. Essentials of veterinary hematology. Philadelphia: Lea & Febiger, 1993. p.349-380.

Kaneko, J.J. et al. Clinical biochemistry of domestic animals. 5.ed. New York: Academic, 1997. p.932.

Kerr, B. J., L. L. Southern, et al. (2003). "Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition." J Anim Sci 81(12): 3075-3087.

Macedo, I. C., L. F. Medeiros, et al. (2012). "Cafeteria diet-induced obesity plus chronic stress alter serum leptin levels." Peptides 38(1): 189-196.

Mc Causland, F. R., S. S. Waikar, et al. (2013). "The relevance of dietary sodium in hemodialysis." Nephrol Dial Transplant 28(4): 797-802.

McAllister, E. J., N. V. Dhurandhar, et al. (2009). "Ten putative contributors to the obesity epidemic." Crit Rev Food Sci Nutr 49(10): 868-913.

Odden, M. C., M. G. Shlipak, et al. (2009). "Serum creatinine and functional limitation in elderly persons." J Gerontol A Biol Sci Med Sci 64(3): 370-376.

Pinto Jr DAC. Seraphin PM.Dieta de cafeteria por quatorze semanas pode causar obesidade e resistência insulínica em ratos WistarRev. Nutr., Campinas, 25(3):313-319, maio/jun., 2012.

Sampey, B. P., A. M. Vanhoose, et al. (2011). "Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet." Obesity (Silver Spring) 19(6): 1109-1117.

Shang, J., Z. Chen, et al. (2014). "Zucker Diabetic Fatty rats exhibit hypercoagulability and accelerated thrombus formation in the Arterio-Venous shunt model of thrombosis." Thromb Res 134(2): 433-439.

Sodré FL; Costa JCB; Lima JCC. Evaluation of renal function and damage: a laboratorial challenge. J. Bras. Patol. Med. Lab. vol.43 no.5 Rio de Janeiro Sept./Oct. 2007

Thethi, T., M. Kamiyama, et al. (2012). "The link between the renin-angiotensin-aldosterone system and renal injury in obesity and the metabolic syndrome." Curr Hypertens Rep 14(2): 160-169.

WHO - World health statistics 2006, Communicable Diseases, World Health Organization.<http://www.who.int/gho/publications/world_health_statistics/whostat2006_erratareduce.pdf> acesso em 11 de novembro.

Faz parte deste Trabalho de Conclusão de Curso uma revisão bibliográfica elaborada pela autora que foi utilizada para fins de estudo durante a elaboração do manuscrito.

Este manuscrito foi elaborado seguindo as orientações para os autores da revista "*International Journal of Food Sciences and Nutrition*" apresentadas ao final em anexo.

Este Trabalho de Conclusão de Curso esta vinculado a um projeto de doutorado com aprovação pelo comitê de ética do CEUA-HCPA conforme consta na carta de aprovação apresentada ao final em anexo.

INCREASE OF SODIUM INTAKE UPON BLOOD PRESSURE AND BIOCHEMICAL PARAMETERS IN MALE WISTAR RATS

Rizzo TL[1,3]; Macedo IC[1,2,3]; Torres ILS[1,2,3*]

[1] Pain and Neuromodulation Laboratory, Department of Pharmacology, Universidade Federal do Rio Grande do Sul Institute of Basic Health Sciences, Porto Alegre, RS 90050-170, Brazil.

[2] Graduate Program in Biological Sciences – Physiology, Universidade Federal do Rio Grande do Sul Institute of Basic Health Sciences, Porto Alegre, RS 90050-170, Brazil.

[3] Animal Experimentation Unit and Graduate Research Group, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS 90035-003, Brazil.

[*]CORRESPONDING AUTHOR:

Departamento de Farmacologia - ICBS, UFRGS.

Rua Sarmento Leite, 500 sala 202.

90050-170 - Porto Alegre, RS, Brazil.

Phone: 0055-51 3308 3183; FAX: 0055-51 3308 3121.

Abstract

Important changes in human dietary pattern occurred in recent decades, especially in developing countries characterized by increased intake of processed foods lead to consequently obesity. This disease is related with development of various chronic diseases such as cardiovascular disease, type 2 diabetes mellitus, stroke, hypertension chronic kidney disease, among others. The prevalence of hypertension has dramatically increased in recent years, and the marked increase in sodium intake contributes for this disease. In healthy individuals, the kidneys are the primary end-organs that regulate sodium homeostasis. Methods: this study aims to evaluate the obesity development, sodium and liquid intake, renal function parameters (creatinine and total protein) and systolic blood pressure in an animal model of obesity. As obesity parameters was used the weekly weight and the systolic pressure was measured by tail cuff apparatus. Results: this study showed that cafeteria diet o induced obesity, and lead to significant increased of sodium intake and consequently increased of liquid intake, although of increase in this parameters was not observed significant difference on systolic pressure measure. Moreover, obese animals had increase creatinine serum, but not showed differences on creatinine urine or total protein serum levels.

Key words: Cafeteria diet; systolic pressure; obesity, creatinine; total proteins.

Introduction

Important changes in human dietary pattern occurred in recent decades, especially in developing countries characterized by increased intake of processed foods. This diets were characterized by excessive consumption of energy-dense, nutrient-poor foods and drinks and it is a key cause of weight gain and obesity (McAllister, Dhurandhar et al. 2009; Driessen, Cameron et al. 2014). Obesity has long been acknowledged as a significant contributing factor in the development of various chronic diseases such as cardiovascular disease, type 2 diabetes mellitus, stroke, hypertension, among others (Adeboye, Bermano et al. 2012).

Hypertension is a major worldwide risk factor for cardiovascular diseases (CVDs) such as heart attack, congestion, heart failure, stroke, and peripheral vascular disease. The prevalence of hypertension has dramatically increased in recent years. High dietary sodium intake is the most prevalent risk factor in modern societies (Doaei and Gholamalizadeh 2014), because the high dietary intake of sodium is associated with elevated blood pressure, a major risk factor for cardiovascular disease (Mozaffarian, Fahimi et al. 2014). Guidelines by the Center for Nutrition Policy and Promotion recommend a daily sodium intake to be less than 2300 mg/d in the general population and 1500 mg/d among people at greater risk of cardiovascular diseases (ie, individuals aged >50 years, African Americans, and those who have hypertension, type 2 diabetes mellitus, or chronic kidney disease). The American Heart Association has recently emphasized goals to achieve “Ideal Cardiovascular Health” by 2020, and one of the dietary metrics is daily sodium intake of less than 1500 mg/day (DiNicolantonio, Lucan et al. 2014). The body’s physiological need for sodium is 0.23 to 0.46 g/day (0.58 to 1.17 g/day salt), but sodium intakes around the world exceed this recommendation and most adult populations have mean sodium intakes >2.3 g/day (>5.85 g/day salt) (Batcagan-Abueg, Lee et al. 2013).

Sodium is the most abundant extracellular cation, the major determinant of plasma osmolality and one of the most important electrolytes involved in the homeostatic control of extracellular volume and blood pressure in humans. Due its importance the control of sodium

balance is tightly regulated by several neurohormonal axes in order to maintain the constancy of the internal environment. In healthy individuals, the kidneys are the primary end-organs that regulate sodium homeostasis. Renal capacity for natriuresis varies across a broad range so as to be able to account for variability in dietary sodium intake and effective circulating volume status (Mc Causland, Waikar et al. 2013). High dietary salt intake presents a major challenge to the kidneys to excrete large amounts of salt administered (Ha 2014).

Considering the importance of this issue this study aims to evaluate renal function parameters (creatinine and total protein) and systolic blood pressure in an animal model of obesity. The diet used to induce obesity is cafeteria diet, based on the consumption of food for human rich in sugar, fat and sodium (Macedo, Medeiros et al. 2012).

Materials and methods

Animals and experimental design

Thirty sixty-day-old male Wistar rats (weight 200–250 g) were randomized by weight and housed in Polypropylene cages (49x34x16cm). The animals were housed in groups of five animals per cage. All animals were kept on a standard 12-hour light/dark cycle (lights on at 7.00 a.m. and lights off at 7.00 p.m.), in a temperature-controlled environment (22±2°C), and had access to water and chow *ad libitum* (standard diet or cafeteria diet). All experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol No. 09231) and were compliant with Brazilian guidelines involving use of animals in research (Law No. 11.794). Vigorous attempts were made to minimize animal suffering and decrease external sources of pain and discomfort, as well as to use only the number of animals that was essential to produce reliable scientific data. Rats were allowed 1 week to acclimate to their settings before the start of the experiment. The animals were divided into two groups: standard diet and cafeteria diet. The animals were weighed weekly and food intake was recorded daily. This experiment was carried for 6 weeks.

Obesity model

The standard rat diet (Nuvilab CR-1, NUVITAL®, Curitiba, PR, Brazil) was composed of 55.0% carbohydrates, 22.0% protein, 4.5% lipids, and 18.5% other constituents (fiber and vitamins); the diet totaled 2.93 kcal/g (information provided by the manufacturer). The palatable high-calorie diet (cafeteria diet) consisted of about 60.0% carbohydrates, 20.0% lipids, 15.0% protein, and 5.0% other constituents (sodium, calcium, vitamins, preservatives, minerals, etc.); the diet totaled 4.18 kcal/g (included of soda - 0.42 kcal/mL). The calories calculations were based on information provided by the manufacturer on the package label). The palatable diet was adapted based on a diet known as cafeteria diet or Western diet (Estadella, Oyama et al. 2004). Foods in the cafeteria diet are crackers, wafers, sausages, chips, condensed milk, and soda (Macedo, Medeiros et al. 2012). Both the standard chow and the experimental diet were replaced daily with fresh food. All the animals had access to standard chow and water, including one who received cafeteria diet. Comparison between the compositions of the standard diet and cafeteria diet are the **Table 1.**

---------- *Insert Table1*----------

Weekly weight (g), sodium intake (mg), Liquid intake (mL) and

The animals were weighed weekly using a semi-analytical balance and the data were expressed as grams (g). The sodium content was calculated based on information provided by the manufacturer on the package label and the data were expressed as grams (g). The liquid intake was recorded daily. The liquid intake was measured every day from the difference found between the liquid and the leftovers offered. Was considered consumption of water and the soda and the data were expressed as milliliters (mL).

Systolic blood pressure (SBP)

The systolic blood pressure measurements were carried out using a photoelectric sensor connected to a signal amplifier (IITC Life Science Inc., Woodland Hills, CA) connected to a computer, which allows to measure the pressure in the caudal artery in rats in a non-invasive. The animals were kept for 30 minutes at acrylic contensores in a room with temperature between 27-28°C. A blood pressure reading is given by the record pulsations artery flow and pressure in the cuff placed on separate channels, the systolic pressure is determined as the pulsations reappear during gradual cuff deflation. Each measure was obtained by averaging five individual readings and results shown as a measure of systolic blood pressure in mmHg weekly. The animals were habituated to the equipment for 3 days before the test, for 1 hour per day. During habituation and testing the retainer was heated to 30°C.

Serum and Urine Creatinine Levels (mg/dL)

Creatinine was measured using a Labtest® kit (Creatinina, Ref. 25, ANVISA – 10009010034, MG, Brazil). The determination of creatinine in urine and blood samples is tests of renal function. Creatinine and other serum components react with the solution picrate in alkaline medium to form a red colored complex is measured photometrically. The addition of an acidulant lowers the pH to 5.0, promoting the decomposition of creatinine picrate, unchanged color derived from chromogens, which is also measured photometrically. The difference between the two readings gives the value of creatinine.

Total proteins in serum (g/dL)

Total proteins were measured using a Labtest® kit (Total Proteins, Ref. 99, ANVISA - 10009010080, MG, Brazil). This method uses copper (Cu^{+}), present in the biuret reagent that reacts with the peptide bonds of the serum proteins, forming a purple color that is measured at a maximum absorbance wavelength of 545 nm, being proportional to the protein concentrations in

the sample. The kit contains biuret, which must be stored between 15-30ºC, and contains sodium hydroxide 600mmol/L, copper sulfate 12mmol/L, preservative and antioxidant. The product is of corrosive nature, and need to be manipulated with care, never using the mouth to sample it; and a standard 4,0g/dL, that contains bovine albumin 4g/dL and azide sodium 15, 4 mmol/L. The standard must be stored between 15-30ºC and sealed to avoid evaporation. The product must be used with care, as azide sodium is a toxic chemical, and reacts with metals forming explosive compounds. Thus, discarding it with great volumes of water is recommended. Samples were read in a spectrophotometer, and the absorbance of the test and standard were measured in 545 nm or under green filter (430-550 nm), using the blank as zero. The color is stable for 60 minutes.

Statistical Analysis

The results were expressed as the mean ± standard error of the mean (S.E.M.). The data analysis and interactions was evaluated using Student's *t*-test or *Paired-Samples T test* when necessary. The repeated measures ANOVA followed by a Bonferroni's test (effect of cafeteria diet, effect of time or effect of cafeteria diet x time) when necessary. Data and interactions differences were considered signficant at $P<0.05$.

Results

Weekly weight (g), sodium intake (mg) and liquid intake (mL)

The weekly weight parameters were used to confirm the presence of obesity induced by cafeteria diet. The groups were randomized by weight, and the Student's *t*-test not showed significant differences between groups on baseline weight. The results of repeated measures ANOVA followed by *Bonferroni,* (effect of time or cafeteria diet) demonstrated that cafeteria diet increased of weekly weight ($F_{(6,28)=}2.249$, $P= 0.001$) from third week of treatment. On other hand the animals play, as expected, increased of weight over time, showed interaction between time

and cafeteria diet ($F_{(6,28)=}$9.967 *P*=0.004). This effect suggests that both factors (time and cafeteria diet) contribute to the increase the weight of animals (Figure 1, panel A).

The results of repeated measures ANOVA followed by *Bonferroni test* demonstrated an effect of cafeteria diet ($F_{(1,28)=}$ 773.666, *P*=0.001), of time ($F_{(5,28)=}$ 2.859, *P*=0.02) and interactions between time and cafeteria diet ($F_{(6,28)=}$ 2.859, *P*=0.02) with both factors increasing the sodium intake (Figure 1, panel B). In liquid intake occurred effect of cafeteria diet ($F_{(1,28)=}$ 147.04, *P*=0.001) and time ($F_{(5,28)=}$3.996, *P*=0.003) (Fig. 2 Panel C), and a was not observed interactions between time and cafeteria diet (P>0.05).

---------- *Insert Figure 1, Panel A, B and C* ----------

Systolic blood pressure (SBP) (mmHg)

The *Students T test* not showed differences between groups on systolic pressure measure (*P*>0.05) (Figure 3).

---------- *Insert Figure2* ----------

Total proteins in serum (g/dL), serum and urine creatinine levels (mg/dL)

The *Students T test* not showed differences between groups on total proteins levels (*P*>0.05). While the cafeteria diet exposure lead to increased on creatinine serum levels (*P*=0.002), but this effect not was observed on creatinine urine levels (*P*>0.05) (Figure 3 panel A, B and C).

---------- *Insert Figure 3, Panel A, B and C* ----------

Discussion

This study, showed that cafeteria diet-induced obesity significant increased of sodium intake and consequently increase of liquid intake, although of increase in this parameters were not related to difference on systolic pressure measure. Importantly the obese animals not increased serum levels of creatinine, but not showed differences on urine levels of creatinine or total protein serum levels.

In this study we show that the rats exposed to a cafeteria diet has significant increase of weight over time. These results corroborate with a previous study of our group using the same cafeteria diet protocol for six weeks, which proved that this period was enough to trigger obesity in the animals and alter parameters that lead to hyperphagia and results in increases fat pad mass and marked weight gain (Macedo, Medeiros et al. 2012). Several genetic models of obesity are used, such as *ob/ob* mice, *db/db* mice, Zucker *fa/fa* obese rats, Agouti yellow mice, and melanocortin 4 receptor knockout mice among others (Drel, Mashtalir et al. 2006; Sampey, Vanhoose et al. 2011; Li, Zhai et al. 2014)**.** On other hand, studies using rodent models of diet-induced obesity with administration of high-fat diets or high caloric diets. In this study was used the cafeteria diet that is the robust model for obesity achievement. This diet consists of a simple exchange of carbohydrate-derived calories with fat-derived calories when compared to low fat or chow control diets. This is experimental rodent diet model that more accurately reflects the variety of highly palatable, energy dense foods that are prevalent in Western society and associated with the current obesity pandemic. In this model, animals are allowed free access to standard chow and water while concurrently offered highly palatable, energy dense, unhealthy human foods *ad libitum* (Sampey, Vanhoose et al. 2011). Several studies agree that dietary pattern has contributed with overweight and obesity incidence around the world and provide

poor eating habits. Moreover, this palatable diet due its pleasurable experience leads to satisfaction and makes it prone to abuse (Lenard, Zheng et al. 2010) and (Pinto Jr. et al. 2012).

The obesity model used here also shows a high content of sodium that exceeds the limits recommended by the guidelines. The increased sodium intake is associated with the development of cardiovascular diseases include increased blood pressure (Wright and Cavanaugh 2010; McMahon, Bauer et al. 2012). Our study showed that animals that were exposed to the cafeteria diet consumed more sodium and consequently also had a higher consumption of liquid. The association between increased sodium intake and thirst is well established. Sodium intake increases the plasma sodium, and then the thirst is stimulated with increase in liquid intake in order to maintain homeostasis (Grimes, Riddell et al. 2013; Grimes, Wright et al. 2013).

Obesity is a great risk factor for hypertension and other comorbidities that may contribute to the development of chronic renal damage (Thethi, Kamiyama et al. 2012; Hall, do Carmo et al. 2014). High sodium intake increases blood pressure, induces glomerular hyperfiltration and affects renal function (Koo, Kim et al. 2014). First obesity causes vasodilation and renal glomerular hyperfiltration that act as compensation mechanisms to maintain homeostasis of the body, to keep the sodium balance even though the increase tubular reabsorption, this mechanism becomes vicious because these offsets along with the increase in blood pressure can lead to glomerular injury (Hall, do Carmo et al. 2014). The renin-angiotensin-aldosterone system is activated in obesity, which makes it an important mechanism in hypertension (Thethi, Kamiyama et al. 2012). Although there is a strong relationship between sodium intake and hypertension our study not showed increased pressure in rats feed with cafeteria diet for 6 weeks. Probably due to small number of sample size (n=5), what we consider one of the main limitations of our study. We speculate that perhaps the duration of the treatment was insufficient to trigger hypertension in animals. Another study with cafeteria diet showed a robust

increase in systolic blood pressure, but at 10 weeks of exposure to cafeteria diet (Pons, Guerrero et al. 2014).

The most important function of kidney is filtration and excretion of nitrogenous compounds from blood (Basile, Anderson et al. 2012). Creatinine is a good parameter for assessing renal function, since their production only depends on the muscle cell metabolism and is almost entirely eliminated by glomerular filtration (Odden, Shlipak et al. 2009; Basile, Anderson et al. 2012; Carter, Gansevoort et al. 2012), but also by tubular secretion. In our study the animals underwent the cafeteria diet had higher serum creatinine levels, but this effect was not observed in urine levels. This result is very important because suggest that obesity can be triggering incipient kidney damage. In this regard we believe it is an initial process because we did not observe compensatory tubular secretion, which would lead to increased levels of urinary creatinine. Importantly, the animals fed with cafeteria diet showed a significant increase in food consumption (data not shown) with high total protein intake. It is known that the protein intake in large quantities can lead to decline in renal function with a progressive loss of renal capacity (Martin, Armstrong et al. 2005). Although that metabolism and the amount of protein present in the serum may be influenced by several factors (Feldman et al. 2000), in this study the total protein levels did not show difference between the groups.

In this study the results showed that obesity triggered by exposure to cafeteria diet can change a important serum marker of renal function (creatinine). Considering that this dietary pattern shows an increasing and indiscriminate use worldwide, it is important to detect early problems generated by it. Further studies are required to elucidate the extent of kidney damage triggered by this type of diet over time.

Acknowledgements

This research was supported by the following Brazilian funding agencies: National Council for Scientific and Technological Development - CNPq (Dr. ILS Torres; Dr. Caumo W);

Brazilian Federal Agency for Support and Evaluation of Graduate Education - CAPES (IC Macedo); Graduate Research Group of Hospital de Clínicas de Porto Alegre - GPPG (ILS Torres – Grant 110455) PIBIC HCPA/CNPq (Rizzo TL).

Competing interest

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

Legends

Table 1. Standard diet and cafeteria diet composition

Figure 1, panel A, B and C: Weekly weight, sodium intake (mg), liquid intake (ml) and systolic pressure (mmHg). SD: standard diet (receiving standard chow) and CD: cafeteria diet (receiving cafeteria diet). **(*)** Significant effect of cafeteria diet. **(#)** Significant effect of time. (&) Interaction between cafeteria diet and time. Data expressed as mean ± SEM (repeated measures ANOVA/Bonferroni's test for weekly weight, sodium intake and liquid intake, n= 15 animals/group; *Students T test* for systolic pressure, n=5 animals/group). SD: standard diet (receiving standard chow) and CD: cafeteria diet (receiving cafeteria diet). **(*)** Significant effect of diet. **(#)** Significant effect of time. Data expressed as mean ± SEM (repeated measures ANOVA/Bonferroni's test, n= 15 animals/group).

Figure 2: Systolic pressure (mmHg). SD: standard diet (receiving standard chow) and CD: cafeteria diet (receiving cafeteria diet). Data expressed as mean ± SEM (*Students T test*, n= 5 animals/group).

Figure 3, panel A, B and C: Creatinine serum levels and creatinine urine levels (mg/dL). Total proteins serum levels (g/dL). SD: standard diet (receiving standard chow) and CD: cafeteria diet (receiving cafeteria diet). **(*)** Significant effect of cafeteria diet. Data expressed as mean ± SEM (*Students T test*, n= 7 animals/group).

Table 1

	Standard diet (%)	*Cafeteria diet (%)*
Carbohydrates	55	60
Protein	22	20
Lipids	4.5	15
Other constituents (fibers, vitamins)	18.5	5

Figures

Figure 1, Panel A

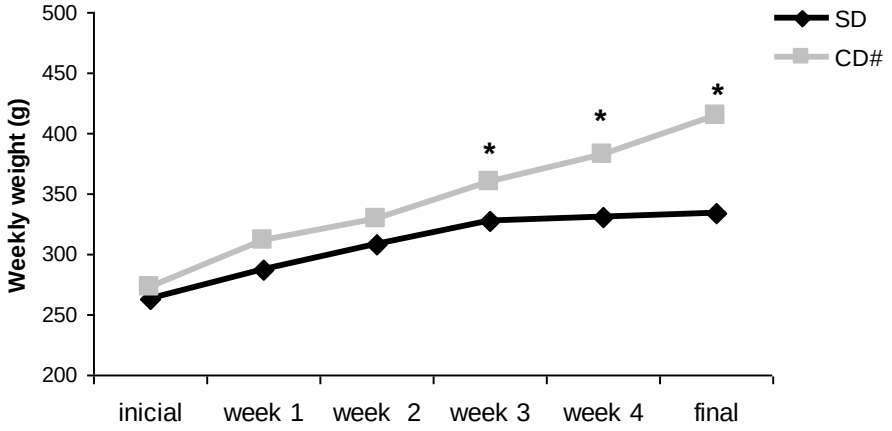


Figure 1, Panel B

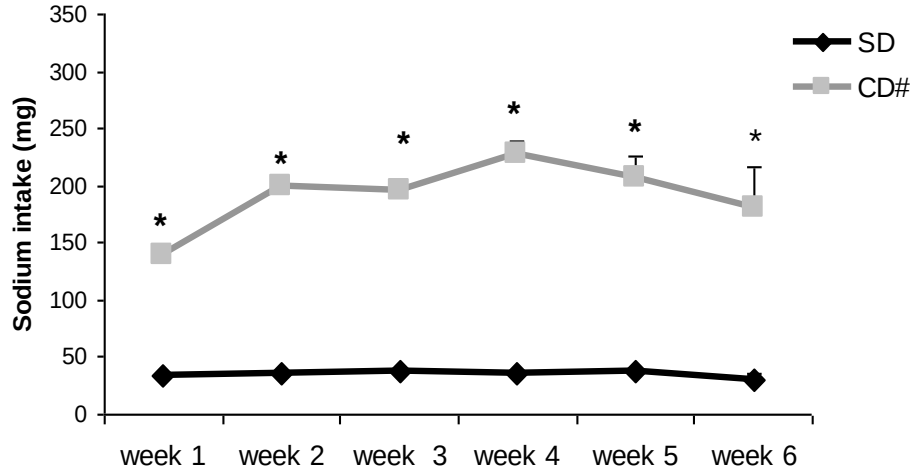


Figure 1, Panel C

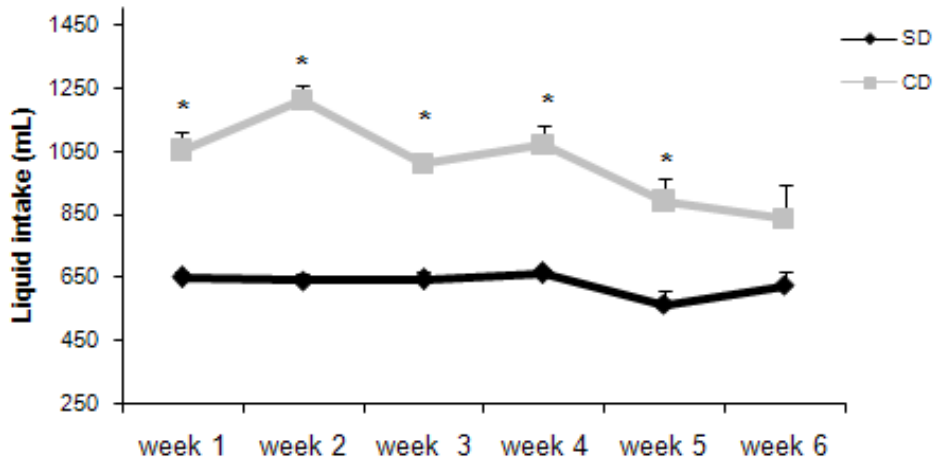


Figure 2

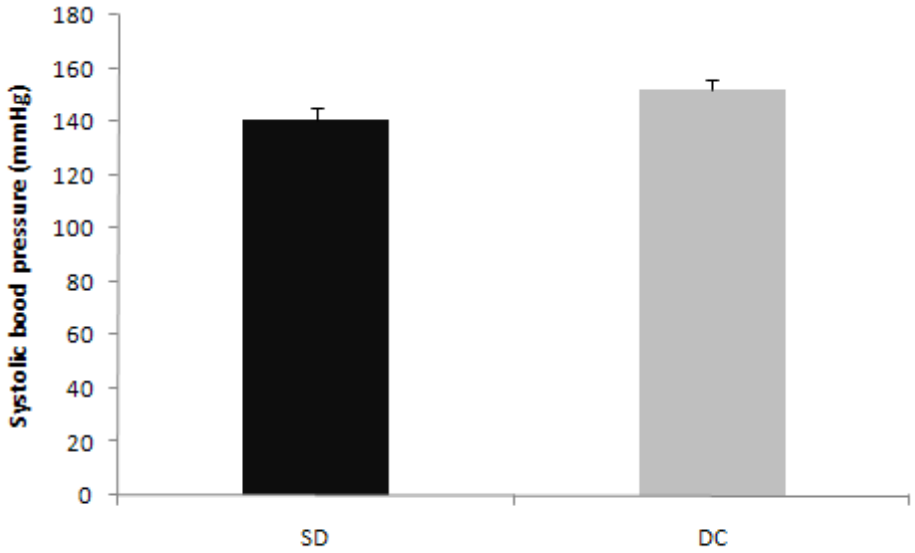


Figure 3, Panel A

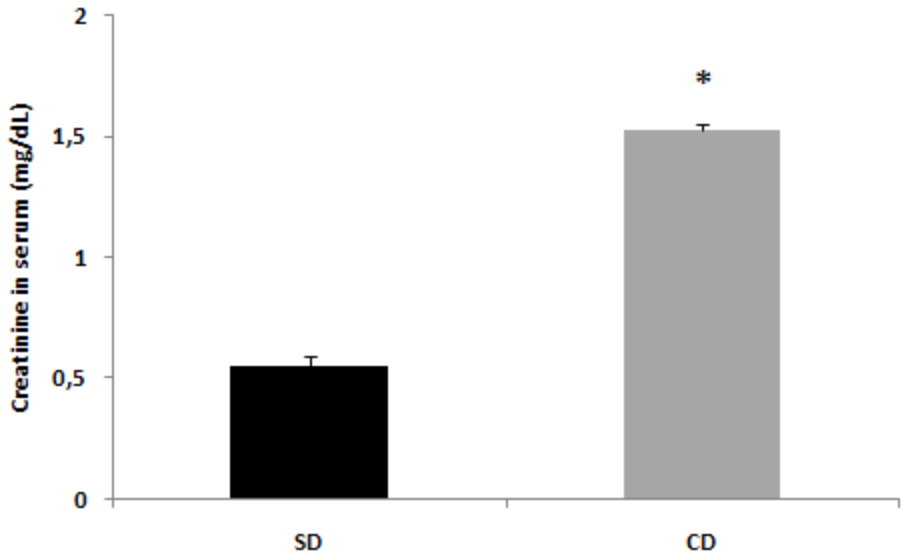


Figure 3, Panel B

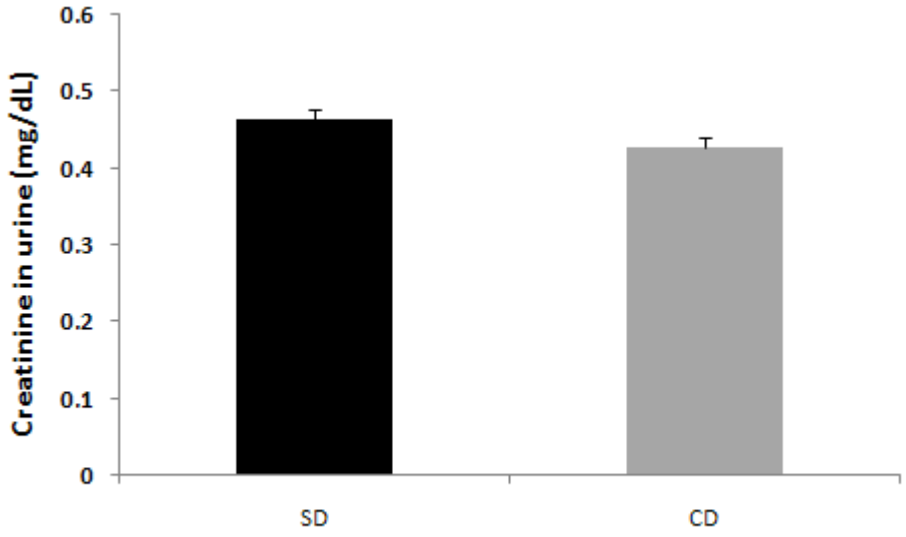
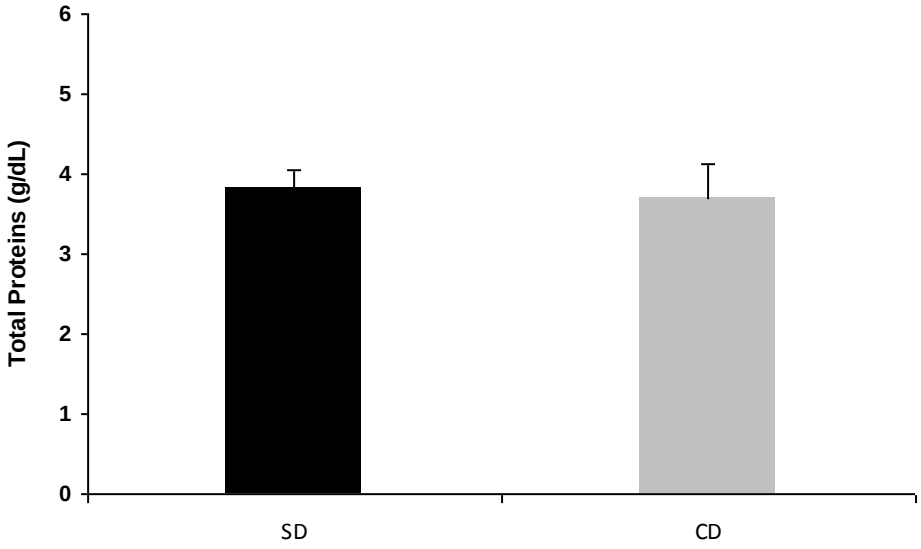


Figure 3, Panel C



References

Adeboye, B., G. Bermano, et al. (2012). "Obesity and its health impact in Africa: a systematic review." Cardiovasc J Afr **23**(9): 512-521.

Bartolomucci, A., A. Cabassi, et al. (2009). "Metabolic consequences and vulnerability to diet-induced obesity in male mice under chronic social stress." PLoS One **4**(1): e4331.

Basile, D. P., M. D. Anderson, et al. (2012). "Pathophysiology of acute kidney injury." Compr Physiol **2**(2): 1303-1353.

Batcagan-Abueg, A. P., J. J. Lee, et al. (2013). "Salt intakes and salt reduction initiatives in Southeast Asia: a review." Asia Pac J Clin Nutr **22**(4): 490-504.

Carter, C. E., R. T. Gansevoort, et al. (2012). "Influence of urine creatinine on the relationship between the albumin-to-creatinine ratio and cardiovascular events." Clin J Am Soc Nephrol **7**(4): 595-603.

de Oliveira, C., V. L. Scarabelot, et al. (2014). "Obesity and chronic stress are able to desynchronize the temporal pattern of serum levels of leptin and triglycerides." Peptides **51**: 46-53.

Dietrich, M. O., A. Muller, et al. (2007). "Western style diet impairs entrance of blood-borne insulin-like growth factor-1 into the brain." Neuromolecular Med **9**(4): 324-330.

DiNicolantonio, J. J., S. C. Lucan, et al. (2014). "Dietary sodium restriction: still searching for the grains of truth. The reply." Am J Med **127**(6): e17.

DiNicolantonio, J. J., A. K. Niazi, et al. (2013). "Dietary sodium restriction: take it with a grain of salt." Am J Med **126**(11): 951-955.

Doaei, S. and M. Gholamalizadeh (2014). "The association of genetic variations with sensitivity of blood pressure to dietary salt: A narrative literature review." ARYA Atheroscler **10**(3): 169-174.

Downs, B. W., A. L. Chen, et al. (2009). "Nutrigenomic targeting of carbohydrate craving behavior: can we manage obesity and aberrant craving behaviors with neurochemical pathway manipulation by Immunological Compatible Substances (nutrients) using a Genetic Positioning System (GPS) Map?" Med Hypotheses **73**(3): 427-434.

Drel, V. R., N. Mashtalir, et al. (2006). "The leptin-deficient (ob/ob) mouse: a new animal model of peripheral neuropathy of type 2 diabetes and obesity." Diabetes **55**(12): 3335-3343.

Driessen, C. E., A. J. Cameron, et al. (2014). "Effect of changes to the school food environment on eating behaviours and/or body weight in children: a systematic review." Obes Rev.

Estadella, D., L. M. Oyama, et al. (2004). "Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats." Nutrition **20**(2): 218-224.

Feldman, B.C. et al. Veterinary hematology. 5.ed. Philadelphia: Lippincott Williams & Wilkins, 2000. 1344p.

Gray, K. L., K. S. Petersen, et al. (2014). "Attitudes and beliefs of health risks associated with sodium intake in diabetes." Appetite **83C**: 97-103.

Grimes, C. A., L. J. Riddell, et al. (2013). "Dietary salt intake assessed by 24 h urinary sodium excretion in Australian schoolchildren aged 5-13 years." Public Health Nutr **16**(10): 1789-1795.

Grimes, C. A., J. D. Wright, et al. (2013). "Dietary sodium intake is associated with total fluid and sugar-sweetened beverage consumption in US children and adolescents aged 2-18 y: NHANES 2005-2008." Am J Clin Nutr **98**(1): 189-196.

Ha, S. K. (2014). "Dietary salt intake and hypertension." Electrolyte Blood Press **12**(1): 7-18.

Hall, M. E., J. M. do Carmo, et al. (2014). "Obesity, hypertension, and chronic kidney disease." Int J Nephrol Renovasc Dis **7**: 75-88.

Kerr, B. J., L. L. Southern, et al. (2003). "Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition." J Anim Sci **81**(12): 3075-3087.

Koo, H. S., Y. C. Kim, et al. (2014). "Analysis of Correlation between 24-Hour Urinary Sodium and the Degree of Blood Pressure Control in Patients with Chronic Kidney Disease and Non-Chronic Kidney Disease." J Korean Med Sci **29 Suppl 2**: S117-122.

Lenard, N. R., H. Zheng, et al. (2010). "Chronic suppression of mu-opioid receptor signaling in the nucleus accumbens attenuates development of diet-induced obesity in rats." Int J Obes (Lond) **34**(6): 1001-1010.

Li, S., X. Zhai, et al. (2014). "Therapeutic effect of vagus nerve stimulation on depressive-like behavior, hyperglycemia and insulin receptor expression in zucker Fatty rats." PLoS One **9**(11): e112066.

Macedo, I. C., L. F. Medeiros, et al. (2012). "Cafeteria diet-induced obesity plus chronic stress alter serum leptin levels." Peptides **38**(1): 189-196.

Martin, W. F., L. E. Armstrong, et al. (2005). "Dietary protein intake and renal function." Nutr Metab (Lond) **2**: 25.

Mc Causland, F. R., S. S. Waikar, et al. (2013). "The relevance of dietary sodium in hemodialysis." Nephrol Dial Transplant **28**(4): 797-802.

McAllister, E. J., N. V. Dhurandhar, et al. (2009). "Ten putative contributors to the obesity epidemic." Crit Rev Food Sci Nutr **49**(10): 868-913.

McMahon, E. J., J. D. Bauer, et al. (2012). "The effect of lowering salt intake on ambulatory blood pressure to reduce cardiovascular risk in chronic kidney disease (LowSALT CKD study): protocol of a randomized trial." BMC Nephrol **13**: 137.

Mozaffarian, D., S. Fahimi, et al. (2014). "Global sodium consumption and death from cardiovascular causes." N Engl J Med **371**(7): 624-634.

Odden, M. C., M. G. Shlipak, et al. (2009). "Serum creatinine and functional limitation in elderly persons." J Gerontol A Biol Sci Med Sci **64**(3): 370-376.

Pons, Z., L. Guerrero, et al. (2014). "Effect of low molecular grape seed proanthocyanidins on blood pressure and lipid homeostasis in cafeteria diet-fed rats." J Physiol Biochem **70**(2): 629-637.

Pinto Jr DAC. Seraphin PM.Dieta de cafeteria por quatorze semanas pode causar obesidade e resistência insulínica em ratos WistarRev. Nutr., Campinas, 25(3):313-319, maio/jun., 2012.

Sampey, B. P., A. M. Vanhoose, et al. (2011). "Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet." Obesity (Silver Spring) 19(6): 1109-1117.

Shang, J., Z. Chen, et al. (2014). "Zucker Diabetic Fatty rats exhibit hypercoagulability and accelerated thrombus formation in the Arterio-Venous shunt model of thrombosis." Thromb Res **134**(2): 433-439.

Thethi, T., M. Kamiyama, et al. (2012). "The link between the renin-angiotensin-aldosterone system and renal injury in obesity and the metabolic syndrome." Curr Hypertens Rep **14**(2): 160-169.

Wright, J. A. and K. L. Cavanaugh (2010). "Dietary sodium in chronic kidney disease: a comprehensive approach." Semin Dial **23**(4): 415-421.