UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

DEPARTAMENTO DE BIOQUÍMICA

ALTERAÇÕES METABÓLICAS INDUZIDAS PELO CONSUMO DE DIETAS HIPERLIPÍDICAS OU HIPERGLICÍDICAS ASSOCIADAS À HIDROCLOROTIAZIDA: POSSÍVEL PAPEL PROTETOR DO DISSELENETO DE DIFENILA EM RATOS

MARINEI CRISTINA PEREIRA RIBEIRO CAMARGO

Orientador: Prof. Dr. João Batista Teixeira da Rocha

Tese apresentada ao curso de Pós-graduação em Ciências Biológicas – Bioquímica, como requisito parcial à obtenção do grau de Doutor em Bioquímica.

Porto Alegre

2009

Dedico esta tese a minha família,

pelo amor, compreensão e apoio.

"Um homem não pode fazer o certo numa área da vida, enquanto está ocupado em fazer o errado em outra. A vida é um todo indivisível"
(Mahatma Gandhi)

“A coisa mais importante no mundo não é tanto onde nós chegamos,
como em qual direção estamos nos movendo”.
(Oliver Wendall Holmes)

AGRADECIMENTOS

A Deus, digno de toda a honra e louvor, por ser minha fortaleza e meu escudo nos momentos difíceis, por ser meu mais e fiel amigo de todas as horas. A Ele, minha eterna gratidão.

Aos grandes amores da minha vida, meu marido e minha filhinha. Ao Potiguara pelo amor, carinho, amizade, compreensão, paciência, apoio e palavras de incentivo para continuar o trabalho nas horas de alegria e também de dificuldade. A minha filha, que durante a gestação me oferece força, amor, compreensão e tem participação ativa na elaboração dessa tese.

Aos meus amados pais e irmão, Aldomiro, Zaíra e Robson, pelo ensinamento e cultivo dos verdadeiros valores da vida, pelo apoio, compreensão e amor incondicional. Não há palavras para agradecer...

Ao prof. João Batista, pela oportunidade de participar do seu grupo, continuar desenvolvendo a pesquisa e aprender com sua experiência.

Um agradecimento muito especial a Nilda, pela grande ajuda na realização de todos os meus trabalhos, pelas importantes sugestões e também pela amizade.

Aos colegas de laboratório que participaram da realização deste estudo: Roger, Lutiane, Daiana, Nilda, Viviane, Danúbia, Andreza, Robson, Gustavo, Verônica, Vanderlei, Juliano, Tiele e Marta.

Ao pessoal dos laboratórios dos professores João, Cristina e Gilson, pela troca de experiências científicas e pronta colaboração na solução das dificuldades que surgiram. Agradeço os bons momentos em que a amizade, companheirismo e alegria fizeram parte do nosso dia a dia no laboratório.

Aos queridos colegas do Serviço de Hemoterapia pelo auxílio, prestatividade, companheirismo e os inesquecíveis momentos de descontração.

Aos professores Maria Rosa Chitolina Schetinger, Andreza Fabro de Bem, Luiz Fernando Royes, Lisiane de Oliveira Porciúncula e Liane Nanci Rotta pela disponibilidade de fazer a leitura desta tese e compor sua comissão examinadora.

A Cléia, pela ajuda, eficiência e palavras de conforto e amizade.

A UFRGS e ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica pela oportunidade de continuar aprendendo e desenvolvendo a pesquisa.

Aos colegas, professores e funcionários do Setor de Bioquímica da UFSM, pelo apoio para a concretização deste estudo.

Enfim, a todos aqueles que com seus conhecimentos, comentários, sugestões e apoio tornaram possível a realização deste trabalho.

ÍNDICE

RESUMO

As dietas suplementadas com lipídios e/ou frutose têm sido associadas com o estresse oxidativo, a resistência à insulina e ao desenvolvimento da Síndrome Metabólica. Os diuréticos tiazídicos, como a hidroclorotiazida (HCTZ) são, frequentemente, usados por pacientes com esses distúrbios para o tratamento da hipertensão, mas também podem exacerbar essas alterações metabólicas. Então, com intenção de desenvolver um modelo animal para o estudo dos efeitos adversos da HCTZ, o objetivo desse trabalho foi investigar se a associação entre uma dieta hiperlipídica (HF) ou hiperglicídica (HFD) e o tratamento com hidroclorotiazida (HCTZ) produz uma influência sinérgica negativa na homeostase da glicose e em outros parâmetros bioquímicos associados ao desenvolvimento do Diabetes Mellitus (DM) tipo 2. Além disso, também foi avaliado se o disseleneto de difenila $(PhSe)_2$, um composto orgânico de selênio com propriedades antioxidantes, poderia reduzir as alterações metabólicas induzidas pelo consumo crônico da dieta hiperglicídica e/ou HCTZ. No modelo animal de alterações metabólicas induzidas pela dieta HF e/ou HCTZ, os ratos foram alimentados por 16 semanas com uma dieta controle ou com uma dieta HF, ambas suplementadas com diferentes doses de HCTZ (0,4; 1,0 e 4,0 g/kg de dieta). A HCTZ associada com uma dieta HF causou um aumento nos níveis de glicemia, frutosamina e também na peroxidação lipídica no tecido hepático e cerebral. Além disso, a ingestão da dieta HF foi associada com um aumento nos níveis de peroxidação lipídica cerebral, vitamina C e grupos tióis não-protéico (NPSH). Houve um aumento nos níveis de vitamina C e NPSH nos grupos tratados com HCTZ (1,0 e 4,0 g/kg) e HCTZ associada com dieta HF. A atividade da Na^+-K^+-ATPase foi inibida no cérebro dos animais tratados com HCTZ (4,0 g/kg) e HCTZ associada com a dieta HF. A ingestão de HCTZ e dieta HF produziram uma redução nos níveis de magnésio e potássio, bem como um aumento na peroxidação lipídica e vitamina C no fígado. Nesse contexto, a associação de HCTZ com a dieta HF causou uma exacerbação nos parâmetros bioquímicos relacionados à homeostase da glicose (particularmente, uma acentuada redução de magnésio) e um maior aumento no estresse oxidativo hepático e cerebral. Os dados indicam que a ingestão crônica de doses elevadas de HCTZ (4,0 g/kg) ou de uma dieta HF altera os índices bioquímicos de estresse oxidativo no cérebro de ratos. Assim, os resultados sugerem que a ingestão crônica de HCTZ ou dieta HF causa alterações metabólicas relacionadas à homeostase da glicose e que a associação de uma dieta HF com o tratamento com HCTZ pode exacerbar algumas dessas alterações bioquímicas. Portanto, pode-se sugerir que este modelo experimental pode ser usado para o estudo dos efeitos adversos da HCTZ. No modelo experimental de alterações bioquímicas causadas pela ingestão de dieta hiperglicídica e/ou HCTZ, os ratos foram alimentados com uma dieta controle (CT) ou com uma dieta enriquecida com frutose (HFD), ambas suplementadas com HCTZ (4,0 g/kg) e/ou $(PhSe)_2$ (3 ppm) durante 18 semanas. A HFD causou um aumento nos níveis de glucose, frutosamina, triglicerídios e colesterol dos animais, os quais não foram restaurados ao nível do controle pela suplementação com $(PhSe)_2$ ou potencializado pela HCTZ. No entanto, os níveis de colesterol e triglicerídios foram menores nos grupos que receberam HFD ou HCTZ suplementados com $(PhSe)_2$. A ingestão de HCTZ causou uma redução na atividade da catalase (CAT) hepática e da superóxido dismutase (SOD) renal, as quais foram restauradas pela suplementação com $(PhSe)_2$. No fígado, o $(PhSe)_2$ também foi efetivo no aumento dos níveis de vitamina C reduzidos pela ingestão de HFD e HFD associada a HCTZ. Além disso, o $(PhSe)_2$

aumentou *per se* a atividade de SOD hepática e renal e reduziu a oxidação de lipídios e proteínas causada pela HCTZ associada ou não com a ingestão de HFD. A associação entre HFD e HCTZ causou uma redução nos níveis de potássio e exacerbou a hipomagnesemia e a hipertrigliceridemia induzidas pela HCTZ. Esses resultados sugerem que algumas alterações bioquímicas podem ser potencializadas pela ingestão simultânea de HCTZ e HFD. Esses dados também demonstraram que a suplementação com $(PhSe)_2$ reduz os distúrbios metabólicos relacionados com o estresse oxidativo e que esse composto pode ser considerado um agente promissor para o tratamento dos distúrbios metabólicos induzidos pela HFD e pela HCTZ devido às suas propriedades antioxidantes.

ABSTRACT

High fat and/or high fructose diets have been associated with oxidative stress, insulin resistance and Metabolic Syndrome development. Thiazide diuretics, such as hydrochlorothiazide (HCTZ) are frequently used by patients with these disorders for treatment of hypertension, but they also can exacerbate these metabolic disturbances. Thus, in an attempt to develop a rodent model to study the adverse effects of HCTZ, the objective of this work was to investigate whether an association between a high fat (HF) or high-fructose diet (HFD) and HCTZ treatment produces a negative synergic influence on glucose homeostasis and in other biochemical parameters associated to type 2 Diabetes Mellitus (DM) development. Moreover, also was evaluated whether dietary diphenyl diselenide $(PhSe)_2$, a organoselenium compound with antioxidant properties, could reduce the metabolic alterations induced by chronic consumption of diets enriched with fructose and/or HCTZ. In animal model of metabolic alterations induced by HF diet and/or HCTZ, rats were fed for 16 weeks with a control diet or with an HF, both supplemented with different doses of HCTZ (0.4, 1.0, and 4.0 g/kg of diet). HCTZ associated with an HF diet caused an increase in blood glucose, fructosamine and lipid peroxidation levels in hepatic and cerebral tissues. In addition, HF ingestion was associated with an increase in cerebral lipid peroxidation, vitamin C and non-protein thiol groups (NPSH) levels. There was an increase in vitamin C as well as NPSH levels in HCTZ (1.0 and 4.0 g/kg of diet) and HF plus HCTZ groups. Cerebral Na^+-K^+-ATPase activity of HCTZ (4.0 g/kg of diet) and HCTZ plus HF-fed animals was inhibited. The intake of HCTZ and HF diet produced a reduction in magnesium and potassium levels as well as an increase in lipid peroxidation and vitamin C in liver. Importantly, the association of HCTZ with HF diet caused additional worsening of biochemical parameters related to glucose homeostasis (particularly accentuated magnesium depletion) and further increase in oxidative stress in hepatic and cerebral tissues. The data indicate that chronic intake of a high dose of HCTZ (4.0 g/kg of diet) or HF change biochemical indexes of oxidative stress in rat brain. Thus, results suggest that chronic intake of HCTZ or HF diet causes metabolic changes related to glucose homeostasis and that the association of HF diet and HCTZ treatment can exacerbate some of these biochemical alterations. Therefore, we can suggest that this experimental model can be used for studying the adverse effects of HCTZ. On experimental models of biochemical alterations caused by fructose and\or HCTZ intake, rats were fed with a control diet (CT) or with a high fructose diet (HFD), both supplemented with HCTZ (4.0 g/kg) and/or diphenyl diselenide (3 ppm) for 18 weeks. HFD diets caused an increase in the levels of glucose, fructosamine, triglycerides and cholesterol of animals, which were not restored to control levels by $(PhSe)_2$ supplementation or potentiated by HCTZ. However, the levels of cholesterol and triglycerides were lower in the groups that received HFD or HCTZ diet supplemented with $(PhSe)_2$. The ingestion of HCTZ caused a decrease in hepatic catalase (CAT) and renal superoxide dismutase (SOD) activities, which were restored by $(PhSe)_2$ supplementation. In liver, diphenyl diselenide was also effective in increasing vitamin C levels reduced by HFD and HFD plus HCTZ intake. Indeed, the compound increased *per se* hepatic and renal SOD activity and reduced the oxidation of the lipids and proteins caused by HCTZ associated or not with HFD intake. Furthermore, the association between HFD and HCTZ caused a decrease in potassium levels and aggravated the hypomagnesemia and the hypertriglyceridemia HCTZ-induced. Theses findings suggest

that some biochemical changes can be aggravated by ingestion simultaneous of HCTZ and HFD diet. In addition, data also demonstrate that $(PhSe)_2$ supplementation reduces metabolic disorders linked to oxidative stress and that this compound can be considered a promising agent for treatment of metabolic disturbances HFD and HCTZ-induced, via its antioxidant properties.

LISTA DE ABREVIATURAS

ADA: American Diabetes Association

ANOVA: Análise de variância

CAT: Catalase

DM: Diabetes mellitus

EROs: Espécies reativas de oxigênio

HCTZ: Hidroclorotiazida

HDL: Lipoproteína de alta densidade

HF: High fat

HFD: High fructose diet

LDL: Lipoproteína de baixa densidade

MDA: Malondialdeído

$(PhSe)_2$: Disseleneto de difenila

Se: Selênio

NPSH: Tióis não protéicos

SOD: Superóxido dismutase

TBARS: Espécies reativas ao ácido tiobarbitúrico

APRESENTAÇÃO

Esta tese apresenta os resultados sob a forma de artigo publicado (capítulo 1) e manuscritos submetidos à publicação (capítulos 2 e 3).

O item Discussão apresenta interpretação e comentários gerais dos resultados obtidos em todos os artigos que compõem esse trabalho.

O item Conclusões apresenta as conclusões finais do trabalho, considerando os artigos científicos (capítulos 1, 2 e 3).

As Referências Bibliográficas referem-se somente às citações que são apresentadas nos itens Introdução e Discussão.

INTRODUÇÃO

1. Dietas hiperlipídicas e hiperglicídicas

As dietas suplementadas com elevado teor de lipídios, elevado teor de carboidratos ou ambos estão associadas com intolerância à glicose, obesidade, doenças cardiovasculares, Diabetes Melitus (DM) tipo 2 (Feskens et al., 1991; Hill et al., 1992; Barnard et al., 1998; Liu & Manson, 2001), hipertensão e estresse oxidativo (Roberts et al., 2000; Girard et al. 2005; Roberts et al., 2005).

A nutrição e os tipos de dietas têm um papel muito importante nas alterações metabólicas relacionadas ao desenvolvimento do DM tipo 2, porém os fatores dietéticos específicos não estão claramente definidos. Ainda existem muitas controvérsias sobre a relação entre o risco de DM e a quantidade e os tipos de lipídios e de carboidratos que devem compor uma dieta adequada. No entanto, prevalece as recomendações dietéticas que sugerem dietas com baixas quantidades de lipídios e carboidratos para a prevenção do DM, doenças cardíacas e outras doenças crônicas (National Research Council, 1989; U. S. Department of Agriculture, 2000). Contudo, os lipídios e os carboidratos não são moléculas homogêneas, portanto diferentes tipos de lipídios e carboidratos têm efeitos diferenciados sobre a homeostase da glicose e a sensibilidade à insulina.

A obesidade está associada com a resistência à insulina e é um dos fatores mais importantes para o desenvolvimento do DM tipo 2 (Reaven, 1988; Pi-Sunyer, 1993), porém os ácidos graxos da dieta podem afetar a ação da insulina independente da existência de obesidade (Storlien et al., 1996). Nesse contexto, acredita-se que os efeitos dos ácidos graxos da dieta são mediados pela composição dos lipídios das membranas celulares (Pan

et al., 1995; Storlien et al., 1996; Vessby, 2000). O perfil específico dos lipídios nas membranas celulares pode influenciar a ação da insulina por vários mecanismos potentes, incluindo uma alteração na ligação ou na afinidade dos receptores de insulina e influenciando a permeabilidade a íons e a sinalização celular (Vessby, 2000). Assim, é o percentual de gordura saturada que parece ter maior importância, uma vez que a resistência à insulina está associada a uma maior proporção de gorduras saturadas e, a uma menor porcentagem de gorduras poliinsaturadas (Pan et al., 1995; Storlien et al., 1996).

A origem e o mecanismo das alterações metabólicas induzidas pela dieta não estão completamente esclarecidos na literatura (McDonald, 1995; Busserolles et al., 2002; Flanagan et al., 2008). No entanto, há evidências de que a ingestão crônica de dietas com alto teor de glicídios ou lipídios está associada com o dano oxidativo (McDonald, 1995; Folmer et al., 2002; Folmer et al., 2003; Brito et al., 2007), uma vez que essas dietas podem diminuir as defesas antioxidantes (Rayssiguier et al., 1981; Rayssiguier et al., 1993; Folmer et al., 2003; Brito et al, 2007) e causar um aumento na produção de espécies reativas ao oxigênio (EROs) (Rayssiguier et al., 1981; Rayssiguier et al., 1993; Folmer et al., 2002; Folmer et al., 2003, Fachineto et al., 2005; Brito et al., 2007). Neste contexto, estudos sugerem que a quantidade e os tipos de lipídios na dieta afetam a sensibilidade das células à peroxidação lipídica e ao dano oxidativo (Thomas & Rudel, 1996). Há evidências na literatura de que os ácidos graxos poliinsaturados podem sofrer oxidação e resultar em produtos que podem ser tóxicos às células (Halliwell & Chirico, 1993) e que os ácidos graxos saturados demonstram menor suscetibilidade à oxidação do que ácidos graxos insaturados (Nanji et al., 1995; Varghese & Oommen, 2000). Enfim, a composição da dieta em relação aos ácidos graxos pode afetar a composição da membrana celular (Sprecher, 1989) e, conseqüentemente alterar a suscetibilidade dessas células a agentes pró-oxidantes.

De acordo com isso, o trabalho realizado por Farina el al. (2003) mostrou que o efeito protetor do selenito contra a peroxidação lipídica induzida pelo mercúrio depende da saturação de gordura na dieta.

Vários estudos sobre a hiperinsulinemia e a hiperglicemia sugerem um efeito prejudicial da gordura saturada (Feskens & Kromhout, 1990; Maron et al., 1991; Parker et al., 1993; Feskens et al., 1994; Feskens et al., 1995; Marshall et al., 1997) e um efeito benéfico da gordura polinsaturada (Trevisan et al.,1990; Feskens et al., 1994; Salmeron et al., 2001). No entanto, outros estudos não confirmam esses resultados (Mayer et al., 1993; Mooy et al., 1995; Mayer-Davis et al., 1997). Assim, em estudos animais, ambos os tipos e quantidades de lipídios têm mostrado efeito na sensibilidade à insulina (Storlien et al., 1991). Em geral, os estudos relatam que o elevado teor de lipídios na dieta aumenta o ganho de peso, a intolerância à glicose e a resistência à insulina (Alsaif & Duwaihy, 2004). De acordo com a literatura, algumas das inconsistências nos resultados podem ter ocorrido pela falta de ajuste na composição da dieta ou ainda, por fatores de risco para o DM que não estejam relacionados à dieta. As divergências entre os resultados observados em diferentes populações podem ter ocorrido, porque os efeitos da dieta com alto teor de lipídio pode variar de acordo com as características da população estudada, tais como idade, sexo, índice de massa corporal e atividade física, que estão associados com sensibilidade à insulina (Paolisso et al., 1995; Ferrannini et al., 1997; Mayer-Davis et al., 1998).

Semelhante aos lipídios, os carboidratos não são homogêneos no que diz respeito à estrutura química e às funções biológicas, assim diferentes tipos de lipídios e carboidratos apresentam diferentes efeitos na homeostase da glicose e na resistência à insulina (Hu et al., 2001). Os carboidratos são classificados em simples ou complexos com base nas estruturas

químicas. As recomendações dietéticas têm enfatizado o uso de carboidratos complexos ou amidos e a limitação de carboidratos ou açúcares simples baseado na crença de que os carboidratos simples seriam digeridos e absorvidos mais rapidamente e, portanto, iriam induzir uma resposta mais rápida à glicemia pós-prandial. No entanto, numerosos estudos contestam essa opinião, pois é reconhecido que muitos alimentos amiláceos, como batatas cozidas e pão branco podem produzir respostas glicêmicas mais elevadas do que os carboidratos simples (Kalergis et al., 1998). Portanto, as recomendações dietéticas para a prevenção das alterações metabólicas associadas ao DM tipo 2 investem mais na qualidade dos lipídios e carboidratos do que, somente na quantidade e ainda, no balanceamento da energia total ingerida para evitar o sobrepeso e a obesidade (Hu et al., 2001).

Há vários modelos experimentais que utilizam animais para o estudo do DM. O DM tipo 1 pode ser induzido em animais por uma pancreatectomia parcial ou pela administração de drogas diabetogênicas. As drogas mais usadas para a indução de DM em roedores são o Aloxano e a estreptozotocina, as quais destroem seletivamente as células β das ilhotas de Langerhans no pâncreas (Oberley, 1988). Na literatura, há também diversos modelos experimentais para o estudo do DM tipo 2, incluindo a utilização de dietas suplementadas com elevadas quantidades ou tipos diferentes de carboidratos e/ou lipídios (Folmer et al., 2002, Folmer et al., 2003, Brito et al., 2007, Ribeiro et al., 2009). Nesse sentido, quantidades elevadas de sacarose e frutose têm sido usadas em modelos animais para induzir alterações metabólicas observadas na Síndrome Metabólica, uma desordem caracterizada por resistência à insulina, hipertensão, dislipidemia e alta incidência de doenças cardiovasculares (Reaven, 1988). Similarmente, as dietas com elevadas quantidades de lipídios têm sido muito utilizadas para o estudo dos processos que envolvem

a resistência à insulina e para investigação dos efeitos anti-obesidade e anti-diabetogênicos de algumas drogas. O modelo de ingestão de dietas suplementadas com lipídios é útil para o estudo da resistência à insulina branda porque ele é mais parecido com os animais normais do que com os animais diabéticos. Assim, se a ingestão calórica for controlada cuidadosamente para evitar a obesidade, esse modelo não exibe hiperglicemia mesmo depois de muitas semanas submetidos à dieta (Kraegen et al., 1986; Storlien et al., 1986). Portanto, a resistência à insulina desenvolve-se dentro de poucas semanas com hiperinsulinemia associada à intolerância à glicose, mas o desenvolvimento de hiperglicemia franca demora mais tempo.

2. Diabetes Mellitus

2.1 Histórico

O Diabetes Mellitus é um distúrbio metabólico, cuja existência e sintomatologia são relatadas há mais de 20 séculos. O papiro de Ebers, um dos documentos médicos mais antigos e importantes que se conhece e que foi escrito no antigo Egito, em data aproximada de 1550 a.C. relata uma doença caracterizada por micção freqüente, sintoma mais comum da DM. No ano 70 (d.C.), após estudos relacionados aos sintomas e ao quadro clínico apresentados pelos pacientes, o médico Areteu da Capadócia denominou “Diabetes”, palavra grega que significa sifão, ao conjunto de sintomas constituído por polidipsia, poliúria e polifagia (Dinsmoor, 1996). Durante muitos anos a pesquisa pouco avançou no estudo do Diabetes, portanto somente em 1670, o médico Thomas Wills observou que a

urina dos pacientes diabéticos tinha sabor adocicado (Dinsmoor, 1996). Em 1815, o médico Michel Chevreul confirmou que o açúcar específico presente na urina dos pacientes diabéticos tratava-se de glicose (Dinsmoor, 1996). Portanto, a partir dessa descoberta a doença passou a ser denominada "Diabetes Açucarada" ou "Diabetes Mellitus". Em estudos realizados em 1869, Paul Langerhans observou que o pâncreas continha dois grupos distintos de células, as células acinares, que secretam enzimas digestivas, e as células agrupadas em ilhas ou ilhotas, as quais poderiam ter função endócrina. Em 1889, essa função endócrina foi confirmada por dois médicos pesquisadores, Oskar Minkowski e Joseph Von Mering, após a realização de experimentos com cães pancreatomizados que desenvolveram uma síndrome semelhante ao DM nos seres humanos. Entre 1916 e 1920, Nicolas Paulesco demonstrou que extratos pancreáticos reduziam a glicemia e as cetonas urinárias. Em 1921, Frederick G. Banting e seu colaborador Charles H. Best descobriram a insulina (Banting et al., 1922; Minkowski, 1989). Esse achado rendeu ao mesmo o prêmio Nobel de Medicina e uma melhor qualidade de vida aos pacientes com DM.

2.2. Fisiopatologia

O DM é um grupo de doenças metabólicas caracterizado por hiperglicemia resultante de defeitos na secreção de insulina, ação da insulina, ou ambos. A hiperglicemia crônica do diabetes está associada a danos que ocorrem ao longo do tempo, disfunção e falência de vários órgãos, especialmente os olhos, rins, nervos, coração e vasos sanguíneos (ADA, 2008). Os sintomas dessa acentuada hiperglicemia são poliúria, polidipsia, perda de peso, polifagia, visão desfocada e aumento de risco para infecções (ADA, 2008; Hall & Davies, 2008). No entanto, a ausência dos mesmos é comum em muitos pacientes com DM

e não descarta a possibilidade de que exista um grau de hiperglicemia suficiente para causar alterações funcionais ou patológicas antes que o diagnóstico seja estabelecido.

A hiperglicemia crônica do DM é caracterizada por complicações que incluem retinopatia com perda potencial de visão, nefropatia levando à insuficiência renal, neuropatia periférica com risco de úlceras dos pés, amputações e danos nas articulações, e ainda neuropatia autonômica causando sintomas gastrointestinais, geniturinários e cardiovasculares (ADA, 2008). Os pacientes diabéticos têm uma incidência aumentada de doença cardíaca coronária, doença vascular cerebral e doença vascular periférica, que representam a principal causa de morbidade e mortalidade entre esses pacientes (ADA, 2008; Hall & Davies, 2008). Essas patologias ocorrem em uma idade muito mais jovem comparado com a população não diabética (Adisakwattana, 2005; Hall & Davies, 2008). A hipertensão e anormalidades no metabolismo de lipoproteínas também são complicações, freqüentemente, encontradas em pacientes diabéticos (Ferrannini et al., 1997; Hayden & Sowers, 2006; ADA, 2008).

2.3. Etiologia e Classificação

Segundo o "Expert Committee on the Diagnosis and Classification of Diabetes" da "American Diabetes Association" (ADA, 2008), a classificação etiológica do DM é a seguinte: DM tipo 1, DM tipo 2, outros tipos específicos de DM e DM gestacional. Na maioria dos casos os pacientes podem ser clinicamente, classificados como portadores de DM tipo 1 ou tipo 2. Os critérios da American Diabetes Association (ADA) para o diagnóstico de diabetes incluem os sintomas clássicos de hiperglicemia (poliúria, polidipsia e perda de peso inexplicada) e a concentração plasmática de glicose casual superior a 200

mg/dL (11,1 mmol), concentração plasmática de glicose em jejum igual ou superior a 126 mg/dL (7,0 mmol) ou concentração plasmática de glicose igual ou superior a 200 mg/dL (11,1 mmol/dL) dentro de 2 horas após a ingestão de uma carga de glicose oral (The Expert Committee on the Diagnosis and Classification of Diabetes, 2003; ADA, 2008).

2.3.1. Diabetes Mellitus tipo 1

No DM tipo 1, a causa é uma deficiência absoluta da secreção de insulina (ADA, 2008), provocada por uma redução na massa das células β do pâncreas. Assim, uma destruição das células β pancreáticas pode ocorrer por intermédio de três mecanismos que parecem estar interligados: suscetibilidade genética, ataque auto-imune e algum tipo de agressão ambiental (Bach, 1994). Os indivíduos que apresentam maior risco de desenvolver este tipo de diabetes podem ser identificados por intermédio de evidências sorológicas de um processo patológico auto-imune que ocorre nas ilhotas pancreáticas, e também por marcadores genéticos (ADA, 2008). Portanto, o DM tipo 1 pode ser classificado em:

- Diabetes Mellitus tipo 1 (causa imunológica): Esse tipo de DM representa apenas 5 a 10 % dos casos de DM, denominado, anteriormente, diabetes dependente de insulina, diabetes tipo I, ou diabetes de início juvenil e resulta de uma autodestruição das células β do pâncreas. Neste tipo de diabetes, a taxa de destruição das células β é bastante variável, sendo rápida em algumas pessoas (principalmente bebês e crianças) e lenta em outros (principalmente adultos). Alguns pacientes, principalmente crianças e adolescentes, podem apresentar a cetoacidose como primeira manifestação da doença. Outros apresentam modesta hiperglicemia em jejum que pode mudar rapidamente para hiperglicemia grave

e/ou cetoacidose na presença de infecção ou de outro estresse. Outros ainda, sobretudo adultos, podem apresentar uma função residual das células β suficiente para impedir cetoacidose durante muitos anos. Esses indivíduos, eventualmente, tornam-se dependente de insulina para sobreviver e estão em risco de cetoacidose. Neste último estágio da doença, há pouca ou nenhuma secreção de insulina. O DM causado por características imunológicas, geralmente, manifesta-se na infância e adolescência, mas pode ocorrer em qualquer idade. A autodestruição das células β tem múltiplas predisposições genéticas e está relacionada a fatores ambientais que ainda não estão bem definidos. Devido ao DM, esses pacientes tornam-se mais susceptíveis a outros distúrbios imunológicos. Os pacientes que apresentam esse tipo de DM, raramente, são obesos, mas a presença de obesidade não é incompatível com o diagnóstico.

❖ Diabetes Mellitus tipo 1 (causa idiopática): Algumas formas de DM tipo 1 não apresentam etiologia conhecida. Alguns destes doentes têm permanente insulinopenia e são propensos à cetoacidose, mas não apresentam evidências de auto-imunidade. Poucos pacientes têm DM tipo 1 que se enquadra nesta categoria, e dentre eles, a maioria são africanos ou de ascendência asiática. Os indivíduos com esta forma de DM sofrem com episódios de cetoacidose e apresentam diferentes graus de deficiência de insulina entre os episódios. Essa forma de DM está associada com características hereditárias, mas faltam evidências imunológicas para a auto-imunidade de células β.

2.3.2. Diabetes Mellitus tipo 2

Esse tipo de diabetes, que representa aproximadamente 90% dos pacientes diabéticos, denominado, anteriormente, diabetes não dependente de insulina, diabetes tipo

II ou diabetes de início adulto, abrange indivíduos que têm resistência à insulina e, geralmente possuem relativa (e não absoluta) deficiência de insulina. Inicialmente, e muitas vezes ao longo da sua vida útil, estes indivíduos não necessitam de tratamento com insulina para sobreviver. Há provavelmente muitas causas diferentes para essa forma de diabetes. Apesar de não se conhecer as etiologias específicas, a destruição auto-imune das células β não ocorre, e os pacientes não apresentam outras causas de diabetes que sejam conhecidas, atualmente.

A maioria dos pacientes com este tipo de diabetes é obeso, e a obesidade por si causa algum grau de resistência à insulina. Pacientes que não são obesos, segundo o critério tradicional para o peso, podem apresentar um aumento da percentagem de gordura corporal distribuída, predominantemente, na região abdominal. Nesse tipo de diabetes, a cetoacidose raramente ocorre, espontaneamente, mas quando ocorre, geralmente está associada com o estresse de outra doença como uma infecção. Este tipo de diabetes, freqüentemente não é diagnosticado por muitos anos, devido à hiperglicemia evoluir gradualmente e, em estágios iniciais da diabetes, pode não ser suficientemente grave para que o paciente perceba algum sintoma clássico de diabetes. No entanto, essa hiperglicemia provoca alterações patológicas e funcionais em diversos tecidos (Baynes & Thorpe, 1996) e, esses pacientes apresentam maior risco de desenvolver complicações microvasculares e macrovasculares (Hu & Tuomilehto, 2007).

A resistência à insulina pode ser beneficiada por redução do peso e/ou tratamento farmacológico da hiperglicemia, mas raramente é restaurada ao valor normal. O risco de desenvolver este tipo de diabetes aumenta com a idade, obesidade e a falta de atividade física. Ocorre mais, freqüentemente, em mulheres com DM gestacional prévia e em

indivíduos com hipertensão arterial ou dislipidemia, e sua freqüência varia entre os diferentes subgrupos étnicos raciais. Além disso, pode ser associado à predisposição genética. No entanto, as características genéticas dessa forma de diabetes são complexas e não estão claramente definidas.

2.3.3. Outros tipos Específicos de Diabetes

Nessa classificação são incluídos os defeitos genéticos da função das células β, os defeitos genéticos na ação da insulina, doenças exócrinas do pâncreas, endocrinopatias, indução por agentes químicos ou drogas, infecções, formas não comuns de diabetes mediadas por características imunológicas e outras síndromes genéticas associadas com o diabetes (ADA, 2008).

A indução de DM por agentes químicos ou drogas tem um importante significado para o nosso estudo, uma vez que usamos a hidroclorotiazida, um diurético tiazídico, em nosso modelo experimental. Nesse contexto, muitas drogas podem prejudicar a secreção de insulina, visto que estas não causam o DM, por si, mas podem precipitar o DM em indivíduos com resistência à insulina (ADA, 2008). Assim, estudos sugerem que os pacientes que desenvolvem o DM após o tratamento com diuréticos tiazídicos, provavelmente já tinham a doença, a qual foi exacerbada pela droga, pois o uso de diuréticos tiazídicos, raramente provoca hiperglicemia grave (ADA, 2008).

2.3.4. Diabetes Mellitus Gestacional (DMG)

O DM G é definido como qualquer grau de intolerância à glicose com início ou identificação durante a gravidez (ADA, 2008). Essa definição é aplicada, independentemente do uso de insulina ou de apenas uma modificação na dieta para tratamento ou ainda, se a condição persistir após a gravidez. A intolerância à glicose ocorre normalmente durante a gravidez, especialmente no terceiro mês de gestação (ADA, 2008).

O nível de glicose em jejum com resultado de 126 mg/dL (7,0 mmol/L) ou uma glicose plasmática aleatória com resultado de 200 mg/dL (11,1 mmol/L) significa que essa gestante encontra-se no limiar para o diagnóstico de diabetes

É recomendado que se realize uma triagem para o DMG em todas as gestações. A avaliação para o risco de desenvolvimento de DMG deve ser realizada na primeira consulta do pré-natal. Após, as mulheres com características clínicas compatíveis com um elevado risco para o DMG (obesidade, história pessoal de DMG, glicosúria ou uma história familiar de DM) devem fazer um teste de tolerância à glicose, ou seja, a gestante ingere 75g de glicose em jejum e, após são realizadas coletas de sangue em intervalos de tempo de 1 hora para a realização de dosagens de glicemia. Para as três primeiras coletas os valores máximos esperados são os seguintes: 95 mg/dL (jejum), 180 mg/dL (1h) e 155 mg/dL (2h) (ADA, 2008).

Intolerância à glicose e glicemia em jejum alterada

O Comitê de Peritos sobre o Diagnóstico e Classificação de Diabetes Mellitus (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997, 2003) reconhece um grupo de indivíduos cuja glicemia, embora não corresponda aos critérios

para a diabetes, é elevada para ser considerada normal. Este grupo apresenta nível de glicemia de jejum superior ou igual a 100 mg/dL (5,6 mmol/L), mas inferior a 126 mg/dL (7,0 mmol/L) ou concentração plasmática de glicose dentro de 2 horas após a ingestão de uma carga de glicose oral, no teste de tolerância a glicose superior a 140 mg/dL (7,8 mmol/dL), mas inferior a 200 mg/dL (11,1 mg/dL). Então, as categorias dos valores de glicose em jejum são as seguintes:

1. Normal: glicemia de jejum inferior a 100 mg/dL (5,6 mmol/L).
2. Tolerância à glicose diminuída: glicose de jejum apresenta valores de 100 – 125 mg/dL (5,6 – 6,9 mmol/L).
3. Diagnóstico provisório de diabetes: glicemia de jejum superior a 126 mg/dL (7,0 mmol/L).

O diagnóstico provisório é confirmado pelo teste de tolerância oral a glicose que apresenta as seguintes categorias:

1. Tolerância a glicose normal: concentração plasmática de glicose dentro de 2 horas após a ingestão de uma carga de glicose oral apresenta valores inferiores a 140 mg/dL (7,8 mmol/L).
2. Intolerância a glicose: concentração plasmática de glicose dentro de 2 horas após a ingestão de uma carga de glicose oral entre 140 - 199 mg/dL (7,8 -11,1 mmmol).
3. Diagnóstico provisório de diabetes: concentração plasmática de glicose dentro de 2 horas após a ingestão de uma carga de glicose oral apresenta valores superiores a 200 mg/dL (11,1 mg/dL).

2.4. Prevalência

O DM é um distúrbio metabólico crônico que tem um impacto significativo sobre a saúde, a qualidade de vida e a expectativa de vida dos pacientes, bem como sobre o Sistema de Saúde. A prevalência do diabetes para todas as faixas etárias em nível mundial foi estimada em 2,8% para o ano de 2000 e 4,4% para 2030 (Wild et. al., 2004), isso significa que 171 milhões de pessoas têm diabetes e que este valor, provavelmente, seja maior do que o dobro em 2030 (Wild et al., 2004). No mundo, cerca de 2,9 milhões de mortes no ano de 2000 foram atribuídas às complicações do diabetes, isso equivale a 5,2% de todas as mortes (Roglic et al., 2005).

Embora de caráter controlável, o diabetes vem despontando como uma epidemia de proporções graves. A sua prevalência está aumentando assustadoramente, como resultado do crescimento e envelhecimento da população, da urbanização e das alterações negativas no estilo de vida, como prevalência da obesidade e da inatividade física (Wild et al., 2004; Sicree & Shaw, 2007). De fato, em alguns países têm se divulgado a existência de uma epidemia de DM tipo 2, sendo a obesidade um dos principais fatores que contribui para aumentar a incidência dessa patologia (Wild et al., 2004; Sicree & Shaw, 2007). Por outro lado, embora a prevalência da obesidade se mantenha estável até 2030, o que parece improvável, é previsível que o número de pessoas com diabetes irá aumentar mais do que o dobro do número de diabéticos existentes, atualmente, como conseqüência do envelhecimento populacional e da urbanização (Wild et al., 2004; Sicree & Shaw, 2007). Portanto, a partir do aumento na prevalência da obesidade em muitos países do mundo e da importância da obesidade como fator de risco para o diabetes, o número de casos de diabetes em 2030 poderá ser superior a previsão dos estudos epidemiológicos (Wild et al.,

2004). Nesse contexto, as intervenções efetivas para a redução da prevalência do diabetes, incluindo mudanças na dieta, exercícios físicos ou tratamentos farmacológicos poderiam representar importantes fatores de prevenção.

A prevalência do diabetes é maior em homens do que em mulheres, mas existem mais mulheres do que homens com diabetes (Wild et al., 2004). Há uma projeção que revela que a população urbana, em países em desenvolvimento, apresentará no ano de 2030 o dobro da população existente no ano de 2000 (Wild et al., 2004). No entanto, a alteração demográfica mais importante para a prevalência do diabetes em todo o mundo parece ser o aumento na proporção de pessoas com mais de 65 anos de idade (Wild et al., 2004), uma vez que o DM tipo 2 é mais comum em idosos (Peter et al., 2006).

No Brasil, a diabetes atingiu cerca de 4,6 milhões de brasileiros no ano de 2000, ocupando o oitavo lugar entre os dez países com maior prevalência no mundo. O número estimado de diabetes em indivíduos adultos para o ano de 2030 é de, aproximadamente, 11,3 milhões (Malerbi & Franco, 1992; King & Rewers, 1993; King & Aubert, 1998; Wild et al., 2004). É notável que, aproximadamente, 40% dos indivíduos com DM desconhecem que possuem a doença e 90% deles são portadores de DM tipo 2 (Malerbi & Franco, 1992; King & Rewers, 1993; King & Aubert, 1998).

Alguns dados epidemiológicos relatam que o DM ocorre em cerca de 10% dos pacientes hospitalizados em qualquer unidade hospitalar no mundo, em cerca de 29% dos pacientes submetidos a cirurgia cardíaca e que a hiperglicemia ocorre em, aproximadamente, 38% dos pacientes hospitalizados, o que leva a maior tempo de internação e uma maior taxa de admissão na Unidade de Tratameto Intensivo (UTI) e, conseqüentemente, maior custo (Sociedade Brasileira de Diabetes, 2008). Portanto, uma iniciativa global é necessária para tratar a epidemia do DM.

3. Diuréticos tiazídicos

Os diuréticos aumentam o fluxo urinário e a excreção de sódio e são utilizados para ajustar o volume e/ou a composição dos líquidos corporais em uma variedade de situações clínicas, incluindo hipertensão, insuficiência cardíaca, insuficiência renal, síndrome nefrótica e cirrose. O mecanismo exato envolvido na redução da pressão arterial pelos diuréticos tiazídicos ainda não está totalmente elucidado. No entanto, estudos relatam que esses fármacos diminuem o volume extracelular através de sua interação com um co-transportador de Na-Cl sensível a tiazidas no rim. A depleção dos estoques de cloreto de sódio (NaCl) no corpo reduz a pressão arterial e o débito cardíaco. Entretanto, o efeito hipotensor é mantido durante a terapia a longo prazo, devido à redução da resistência vascular. Após algumas semanas, o débito cardíaco retorna aos valores anteriores ao tratamento (Goodman & Gilman, 2006; Parekh et al., 2008) e o volume extracelular retorna quase a seu valor normal, em conseqüência de respostas compensatórias, como a ativação do sistema renina-angiotensina (Goodman & Gilman, 2006). Não se sabe como esse processo ocorre, todavia os diuréticos tiazídicos promovem vasodilatação em vasos isolados de animais de laboratório e seres humanos (Goodman & Gilman, 2006).

Embora considerados seguros e eficazes, a sua utilização está associada com alterações metabólicas, incluindo dislipidemia, hiperglicemia, intolerância à glicose e um risco aumentado de desenvolvimento de DM tipo 2 (Wilcox, 1999; Huen & Goldfarb, 2007). Além disso, dados da literatura mostram que o uso de diuréticos tiazídicos também está associado com hiponatremia, hipocalemia e hipomagnesemia (Carlsen et al., 1999; Verdecchia et al., 2004). Portanto, tem sido questionada a segurança dos diuréticos para os diabéticos hipertensos.

O mecanismo pelo qual os diuréticos tiazídicos causam alteração na tolerância à glicose não está bem esclarecido, mas parece envolver uma redução da secreção de insulina, bem como alterações no metabolismo da glicose (Bonner, 1994). Nesse contexto, os efeitos dos diuréticos tiazídicos na secreção ou na sensibilidade à insulina podem ser responsáveis pela alteração no perfil lipídico, uma vez que a insulina ativa a lipoproteína lipase, a qual hidrolisa os triglicerídios em lipoproteínas de baixa densidade (LDL) (Grimm et al., 1981). Portanto, os diuréticos tiazídicos podem induzir aumento nos níveis séricos de colesterol total, triglicerídeos e LDL (Grimm et al., 1981; Perez-Stable & Carilis, 1983), além de alterações no metabolismo dos carboidratos, com conseqüente hiperglicemia. Mas esses efeitos adversos, em geral são dependentes da dose. Além disso, a redução da dose, além de modificações na dieta e do aumento da atividade física é suficiente, na maioria dos casos, para controlar tais alterações (Jones & Sands, 1994). Então, uma vez que os diuréticos tiazídicos, em doses baixas, não costumam produzir tais efeitos adversos (Huen et al., 2007), torna-se crescente a recomendação para o uso cada vez mais intenso desse fármaco, porém em doses cada vez menores.

Na literatura, há evidências de que os efeitos adversos dos diuréticos tiazídicos nos níveis de lipídios e na tolerância à glicose são, em parte, conseqüência da depleção de potássio (Helderman et al., 1983; Andersson et al., 1991), pois esses efeitos diminuem quando é administrado potássio simultaneamente com esses diuréticos (Wilcox et al., 1999). Por outro lado, a hiperglicemia na ausência de um bom controle metabólico, bem como a terapia com diuréticos tiazídicos podem aumentar a excreção urinária de magnésio (Barbagallo & Dominguez, 2007). O magnésio intracelular desempenha um papel fundamental na regulação da ação da insulina, na captação de glicose mediada pela insulina e no tônus vascular, portanto a insulina e a glicose são importantes reguladores do

metabolismo do magnésio (Barbagallo & Dominguez, 2007). Assim, a hipomagnesemia pode resultar em defeito na atividade da tirosina quinase no receptor de insulina, comprometimento da ação da insulina e desenvolvimento ou piora da resistência à insulina (Paolisso & Barbagallo, 1997; Barbagallo & Dominguez, 2007). Então, a deficiência de magnésio tem sido proposta como um possível mecanismo para o desenvolvimento da resistência à insulina causada pelos diuréticos tiazídicos. Neste contexto, o DM tipo 2 é caracterizado por deficiência celular e extracelular de magnésio, que é um importante co-fator para mais de trezentas reações enzimáticas. Evidências sugerem que a deficiência de magnésio está associada com um controle metabólico deficiente, aumento de dano tecidual oxidativo dependente de radicais livres e crônicas complicações em pacientes com DM tipo 2 (Rayssiguier et al., 1993; Lourdes et al., 1998; Gums, 2004).

3.1. Hidroclorotiazida (HCTZ)

A Hidroclorotiazida (HCTZ) (Figura 1) e seus similares (tiazídicos) são os diuréticos mais utilizados em todo o mundo para o controle da hipertensão arterial. A hidroclorotiazida (6-cloro-3, 4-dihidro-2H-1, 2, 4-benzotiadiazina-7-sulfonamida 1, 1-dióxido) é um diurético, derivado sulfonamida representante da classe das benzotiadiazinas, comumente conhecido como tiazida (Dollery, 1998).

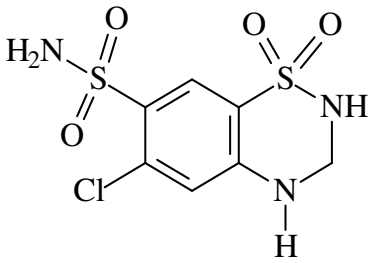


Figura 1. Estrutura química da Hidroclorotiazida

Vários estudos relatam a eficácia e segurança dos diuréticos tiazídicos na redução da morbidade e da mortalidade em pacientes hipertensos (The ALLHAT Study Group, 2002; Turnbull, 2003), uma vez que esses diuréticos têm demonstrado eficácia em diminuir a pressão arterial e uma comprovada capacidade de prevenir acidente vascular cerebral, infarto do miocárdio e insuficiência cardíaca congestiva (McInnes, 1992; The ALLHAT Study Group, 2002; Turnbull, 2003). Como resultado, os tiazídicos, assim como a HCTZ são recomendados como terapia de primeira linha para a hipertensão arterial (The ALLHAT Study Group, 2002). No entanto, o seu uso tem sido associado com anormalidades metabólicas, tais como distúrbios eletrolíticos, hiperlipidemia e comprometimento do metabolismo da glicose (Plavinik et al., 1992; Franse et al., 2000; Punzi & Punzi, 2004; Verdecchia et al., 2004). Portanto, esses efeitos adversos podem causar o desenvolvimento ou exacerbação de distúrbios metabólicos e DM tipo 2 (Reungjui et al., 2007), uma vez que muitos pacientes com hipertensão arterial apresentam algum tipo de distúrbio metabólico.

4. Estresse oxidativo

Um radical livre é qualquer átomo, grupo de átomos ou moléculas capaz de existir sob a forma independente e que contém um ou mais elétrons desemparelhados (Del Maestro, 1980; Southorn & Powis, 1988; Bergendi et al., 1999; Halliwell, 2006b; Halliwell & Gutteridge, 2006). O termo "Espécies Reativas de Oxigênio" (ROS) inclui os radicais de oxigênio pouco reativos como o $O_2^{\bullet-}$ e os radicais altamente reativos, como o $HO^{\bullet}$ (todos são radicais que contém um ou mais elétrons desemparelhados), e alguns não-radicais (sem elétrons desemparelhados), que são agentes oxidantes e/ou são facilmente convertidos em

radicais, como por exemplo, HOCl, HOBr, O_3, $ONOO^-$, $^1O_2$ e H_2O_2. Portanto, todos os radicais de oxigênio são ROS, mas nem todas as ROS são radicais de oxigênio (Halliwell, 2006a). Há também as Espécies Reativas de Nitrogênio (RNS), termo que inclui as espécies radicais como $NO^{\bullet}$ e $NO_2^{\bullet}$, bem como espécies não-radicais como HNO_2 e N_2O_4 (Halliwell, 2006a).

As EROs são produtos do metabolismo normal das células (Valko et al., 2007). No entanto, um aumento na produção destas espécies reativas ou uma diminuição das defesas antioxidantes pode levar ao estresse oxidativo, que está relacionado com a etiologia ou progressão de diversas doenças (Halliwell & Gutteridge, 2006; Valko et al., 2007). O excesso de produção de EROs pode causar dano em lipídios, proteínas e no ácido desoxirribonucléico (DNA) inibindo a sua função normal (Valko et al., 2007).

Os seres vivos dispõem de mecanismos protetores para evitar o acúmulo de EROs e de seus efeitos deletérios (Halliwell, 2006). Esses sistemas de defesa podem ser de origem enzimática ou não-enzimática. As principais enzimas antioxidantes são a superóxido dismutase, a catalase e a glutationa peroxidase. Essas enzimas evitam o acúmulo de radical superóxido, peróxido de hidrogênio e a conseqüente produção de radical hidroxil. As defesas não enzimáticas incluem os antioxidantes lipofílicos (tocoferóis, carotenóides e bioflavonóides) e hidrofílicos (glutationa e ascorbato) (Heffner & Repine, 1989; Cao et al., 1997; Halliwell, 2006; Halliwell & Gutteridge, 2006).

Os mecanismos moleculares que levam às complicações patológicas do diabetes não estão completamente esclarecidos na literatura, mas envolvem a participação de EROs (Maritim et al., 2003) devido à hiperglicemia crônica (Baynes & Thorpe, 1996). A elevada produção de EROs pode ocorrer em conseqüência da auto-oxidação da glicose, glicação

não-enzimática de proteínas e formação de produtos terminais de glicação avançada (PTGAs), então os níveis elevados de EROs e a redução simultânea nos mecanismos de defesa antioxidantes podem causar danos em organelas celulares, desenvolvimento de resistência à insulina (Maritim et al., 2003), alterações nas estruturas das proteínas, inativação de enzimas e aumento nos níveis de peroxidação lipídica em vários tecidos (Jain et al., 2001; Rosen et al., 2001; Folmer et al., 2002; Morgan et al., 2002; Maritim et al., 2003). Essa peroxidação lipídica leva à destruição oxidativa de ácidos graxos poli insaturados que constituem a membrana celular (Esterbauer et al., 1992; Cheeseman & Slater, 1993).

5. A enzima Na^+K^+-ATPase

A Na^+-K^+-ATPase (EC 3.6.1.37) é uma enzima que está incorporada na membrana das células e é responsável pelo transporte ativo de íons sódio (Na^+) e potássio (K^+) no sistema nervoso. Este processo regula a concentração celular de Na^+/K^+ e, conseqüentemente, os seus gradientes através da membrana plasmática, os quais são necessários para as funções vitais, tais como co-transporte pela membrana, regulação do volume celular e excitabilidade da membrana (Jorgensen, 1986; Doucet, 1988). Esta enzima dimérica existe em várias isoformas no cérebro e consome cerca de 40-50% do ATP gerado nesse tecido (Erecinska & Prata, 1994). A inativação de Na^+-K^+-ATPase conduz à despolarização parcial da membrana, permitindo a entrada excessiva de Ca^{2+} nos neurônios com conseqüente produção de eventos neurotóxicos (Beal, 1993). A Na^+-K^+-ATPase pode ser sensível à oxidação causada por agentes oxidantes (Carfagna et al., 1996;

Folmer et al., 2004), uma que possui grupos sulfidrilas, os quais são, altamente, suscetíveis ao estresse oxidativo (Yufu et al, 1993; Folmer et al., 2004).

Estudos mostram que o consumo crônico de dietas com grande quantidade de lipídios ou carboidratos pode promover o desenvolvimento de estresse oxidativo associado à hiperglicemia em vários tecidos, o que pode conduzir à inibição de enzimas como a δ-aminolevulinato desidratase (Folmer et al., 2002; Folmer et al., 2003) e a Na^+-K^+-ATPase (Morgan et al., 2002, Folmer et al., 2004). Nesse contexto, dados da literatura têm indicado que a atividade da Na^+-K^+-ATPase em eritrócitos pode ser inibida após exposição in vitro a altas concentrações de glicose (Jain & Lim, 2001). No entanto, estudos relativos aos efeitos de modelos experimentais de alterações metabólicas induzidas pela dieta ou por diuréticos tiazídicos, na atividade da Na^+-K^+-ATPase no tecido cerebral, são raros na literatura.

6. Selênio

O selênio é um elemento químico que foi descoberto em 1817 por Berzelius e está localizado no grupo VI da tabela periódica. Na forma de selenocisteína, este micronutriente encontra-se presente no sítio ativo de diversas enzimas que desempenham atividades antioxidantes nos sistemas biológicos como a glutationa peroxidase e a fosfolipídio hidroperóxido glutationa peroxidase (Flohe et al., 1973; Rotruck et al., 1973; Urisini et al., 1985).

O selênio em baixas concentrações é um elemento traço essencial aos mamíferos (Navarro-Alarcón & Lopes-Martinez, 2000; Rayman, 2000). No entanto, estudos mostram que altas concentrações de compostos de selênio orgânico ou inorgânico podem causar efeitos tóxicos e pró-oxidantes devido a sua habilidade de catalisar a oxidação de tióis e

produzir EROs (Nogueira et al., 2004; Schiar et al., 2009). Por outro lado, a deficiência de selênio na dieta está relacionada com a gênese e/ou progressão de diversas patologias como doenças cardiovasculares, disfunções imunológicas, câncer, diabetes e anormalidades metabólicas (Combs & Gray, 1998; Navarro-Alárcon & Lopez-Martinez, 2000; Rayman, 2000; Barbosa et al., 2006; Barbosa et al., 2008). Além disso, dados da literatura relatam que alguns compostos de selênio têm sido estudados em modelos clínicos e experimentais de diabetes (Bonnefont-Rousselot, 2004; Faure et al., 2007; Barbosa et al., 2008) e parecem ter efeitos benéficos na sensibilidade à insulina e na prevenção de lesão degenerativa vascular (Faure et al., 2007).

A atividade antioxidante exibida pelo selênio parece ser responsável pela sua eficácia no tratamento e prevenção das patologias que têm o estresse oxidativo como processo central no seu desenvolvimento. Assim, nas últimas décadas tem crescido muito o interesse em investigar o papel de compostos de selênio como possíveis agentes terapêuticos no tratamento de diversas patologias. No entanto, a dose de selênio a ser administrada constitui um fator crítico na atividade biológica do elemento, uma vez que a quantidade requerida, nutricionalmente é muito próxima da quantidade tóxica.

6.1. Disseleneto de difenila $(PhSe)_2$

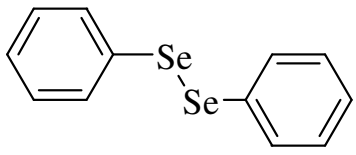


Figura 2. Estrutura química do Disseleneto de Difenila

O disseleneto de difenila (Figura 2) é um composto orgânico sintético de selênio que reage, eficientemente, com hidroperóxidos e peróxidos orgânicos através de reação similar a catalisada pela glutationa peroxidase (GPx). Vários estudos do nosso grupo de pesquisa têm demonstrado que o $(PhSe)_2$ apresenta importantes propriedades farmacológicas como antioxidante, anti-inflamatória, analgésica, neuroprotetora e anti-aterogênicas (Ghisleni et al. 2003; Nogueira et al., 2004; Zasso et al., 2005; Barbosa et al., 2006, Barbosa et al., 2008, Bem et al., 2008a; Bem et al., 2008b). Recentemente, dados do nosso laboratório mostraram que o tratamento crônico com $(PhSe)_2$ reduziu a hiperglicemia e outras alterações bioquímicas relacionadas ao estresse oxidativo em ratos tratados com estreptozotocina (Barbosa et al., 2006). No entanto, o efeito do $(PhSe)_2$ em alterações metabólicas induzidas por outros fatores como dietas suplementadas com carboidratos e/ou lipídios associadas ao tratamento com HCTZ não está disponível na literatura.

OBJETIVOS

OBJETIVO GERAL

O presente estudo visa entender melhor como o estresse oxidativo contribui para o estabelecimento do diabetes em modelos animais. Nesse trabalho também abordamos a análise dos efeitos toxicológicos relacionados à dose de HCTZ e a possível interação sinérgica entre a ingestão de dietas hiperlipídicas ou hiperglicídicas associadas ao tratamento com HCTZ, dois fatores de risco para o desenvolvimento de diabetes. Além disso, procuramos desenvolver um modelo animal para o estudo da toxicidade da HCTZ

associada aos sintomas bioquímicos do Diabetes Mellitus tipo 2 e avaliar o uso de $(PhSe)_2$, organocalcogênio antioxidante, como possível agente terapêutico nesse modelo animal.

OBJETIVOS ESPECÍFICOS

Capítulo 1

- Investigar a possível interação sinérgica entre a ingestão crônica de uma dieta hiperlipídica e o tratamento com HCTZ pela avaliação dos parâmetros bioquímicos relacionados ao estresse oxidativo no cérebro.

- Avaliar os efeitos toxicológicos de HCTZ no cérebro durante uma suplementação oral com diferentes doses desse anti-hipertensivo.

Capítulo 2

- Investigar se a associação entre uma dieta hiperlipídica e o tratamento com HCTZ apresenta uma influência sinérgica negativa na homeostase da glicose e nos parâmetros bioquímicos relacionados ao desenvolvimento de hiperglicemia, bem como investigar a possível relação entre essas alterações com o estresse oxidativo.

- Estudar os efeitos toxicológicos de HCTZ no fígado, pela avaliação dos parâmetros bioquímicos e marcadores de estresse oxidativo, durante uma suplementação oral com diferentes doses desse anti-hipertensivo.

- Desenvolver um modelo animal para o estudo da toxicidade da HCTZ associada aos sintomas bioquímicos do DM tipo 2.

Capítulo 3

- Estudar os efeitos da associação de uma dieta hiperglicídica com o tratamento com HCTZ nos parâmetros bioquímicos relacionados ao estresse oxidativo e aos marcadores bioquímicos de desenvolvimento de DM tipo 2, a fim de investigar se a HCTZ poderia exacerbar as alterações bioquímicas induzidas pela dieta hiperglicídica, uma vez que esses distúrbios são comuns em pacientes com hipertensão.

- Avaliar se a ingestão crônica de $(PhSe)_2$ pode prevenir e/ou reduzir as alterações bioquímicas causadas pela ingestão de uma dieta enriquecida com frutose e/ou hidroclorotiazida.

CAPÍTULO 1 – Artigo publicado.

High-fat diet and hydrochlorothiazide change biochemical indexes of oxidative stress in brain of rats

Marinei Cristina Pereira Ribeiro, Nilda Berenice de Vargas Barbosa, Roger Monteiro, Lutiane Mozzaquatro Parcianello, Verônica Bidinotto Brito, Juliano Perottoni, Daiana Silva de Ávila, João Batista Teixeira Rocha.

Cell Biochemistry & Function, 27: 473-478, 2009.

CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct 2009; **27**: 473–478.
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/cbf.1599

High-fat diet and hydrochlorothiazide increase oxidative stress in brain of rats

Marinei Cristina Pereira Ribeiro[1,2*], Nilda Berenice de Vargas Barbosa[2], Tielle Moraes de Almeida[2], Lutiane Mozzaquatro Parcianello[2], Juliano Perottoni[3], Daiana Silva de Ávila[2] and João Batista Teixeira Rocha[2*]

[1] *Hospital Universitário de Santa Maria, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil*
[2] *Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil*
[3] *Departamento de Zootecnia, Universidade Federal de Santa Maria - CESNORS, Palmeira das Missões, RS, Brazil*

This study evaluated the effect of possible synergic interaction between high fat diet (HF) and hydrochlorothiazide (HCTZ) on biochemical parameters of oxidative stress in brain. Rats were fed for 16 weeks with a control diet or with an HF, both supplemented with different doses of HCTZ (0.4, 1.0, and 4.0 g kg^{-1} of diet). HF associated with HCTZ caused a significant increase in lipid peroxidation and blood glucose levels. In addition, HF ingestion was associated with an increase in cerebral lipid peroxidation, vitamin C and non-protein thiol groups (NPSH) levels. There was an increase in vitamin C as well as NPSH levels in HCTZ (1.0 and 4.0 g kg^{-1} of diet) and HF plus HCTZ groups. Na^+–K^+-ATPase activity of HCTZ (4.0 g kg^{-1} of diet) and HCTZ plus HF-fed animals was significantly inhibited. Our data indicate that chronic intake of a high dose of HCTZ (4 g kg^{-1} of diet) or HF change biochemical indexes of oxidative stress in rat brain. Furthermore, high-fat diets consumption and HCTZ treatment have interactive effects on brain, showing that a long-term intake of high-fat diets can aggravate the toxicity of HCTZ. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS—high fat diet; hydrochlorothiazide; hyperglycemia; oxidative stress; neurotoxicity

ABBREVIATIONS—CT, control diet; HCTZ, hydrochlorothiazide; HF, high fat diet; NPSH, non-protein thiol groups; TBARS, thiobarbituric acid reactive substance; ROS, reactive oxygen species

INTRODUCTION

A high fat intake is considered to be an important factor in the development of insulin resistance[1,2] and oxidative stress.[3] Furthermore, results from both rodent and human studies provide evidence that chronic consumption of high-fat diets is associated with alterations in brain chemistry and structure, increased risk of cognitive decline and dementia.[4,5] One mechanism potentially linking high-fat diets to cognitive deficits is the development of insulin resistance and/or type 2 diabetes mellitus.[4] Therefore, chronic ingestion of high fat diet may have direct effects on neuronal function but at the same time can be a major contributor to other chronic diseases, including type 2 diabetes mellitus, cardiovascular disease and hypertension, all of which are considered independent risk factors for cognitive decline and dementia. Thus, it is unclear whether diet directly impacts on brain function or mediates its effects indirectly through of other chronic diseases.

Hydrochlorothiazide (HCTZ) belongs to the thiazide class of compounds used as diuretics in the treatment of hypertension, edema associated with congestive heart failure, and edema associated with hepatic cirrhosis.[6] However, its side effects include metabolic abnormalities, such as hypokalemia, hypercholesterolemia, and hyperglycemia.[7,8] Thus, a variety of studies have reported that thiazide diuretics therapy may impair glucose tolerance and decrease insulin sensitivity and thereby accelerate the development of diabetes mellitus.[7–9] However, few studies are available about the effect of HCTZ on brain.

Oxidative stress occurs in biological systems when there is an overproduction of reactive oxygen species (ROS) as well as a deficiency of enzymatic and non-enzymatic antioxidants. In other words, oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms.[10] In this context, brain is particularly vulnerable to oxidative damage because of the high oxygen utilization, the high content of unsaturated fatty acids (that are more liable to peroxidation), the presence of redox-active metals (Cu, Fe),[10,11] and a low reserve of antioxidant defences.[11] Interestingly, brain makes up about 2% of a person's mass but consumes 20% of their metabolic oxygen.

* Correspondence to: M. C. P. Ribeiro or J. B. T. Rocha, Hospital Universitário de Santa Maria, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. Tel: +55-55-3220-8513. Fax: 55-55-3220-8240. E-mail: mcpribeiro@yahoo.com.br or jbtrocha@yahoo.com.br

Received 22 April 2009
Revised 30 June 2009
Accepted 4 July 2009

The vast majority of this energy is used by neurons.[12] In this context, it has been shown that the oxidative stress increases neuronal death, which contributes to the neuropathology associated with diabetes.[13] In this way, enhanced formation of ROS occurs in tissues during hyperglycemia[14] and these oxidant radicals contribute to increase neuronal death through protein oxidation, DNA damage, and peroxidation of membrane lipids.[15,16] Since animal models of diabetes and insulin resistance can contribute to clarify the effects of diabetes on brain functioning, the role of oxidative stress in brain damage has been extensively studied in experimental diabetes and diabetic patients.[17–19]

In the present study, the possible negative synergic interaction between high fat intake and HCTZ treatment, two risk factors for the diabetes development, was assessed by measuring biochemical parameters related to oxidative stress in brain.

MATERIALS AND METHODS

Chemicals

Casein (technical grade), comassie brilliant blue G, sodium sulfate dodecyl (SDS), ethanol, reduced glutathione, ouabain, malondialdehyde (MDA), and thiobarbituric acid (TBA) were obtained from Sigma (St. Louis, MO, USA). Mono and dibasic potassium phosphate, acetic acid, ascorbic acid, *ortho*-phosphoric acid, tris buffer (tris[hydroxymethyl]aminomethane) and trichloroacetic acid were obtained from Merck (Rio de Janeiro, Brazil). Hydrochlorothiazide, were purchased from commercial sources cornstarch, lard, bone meal, wheat bran, soybean oil,

Animals and diets

Adult male Wistar rats (2 months old), weighing 250–300 g were used for the experiments. The animals were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21–25°C and humidity at roughly 56% and with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil.

Rats were randomly divided in eight experimental groups with five animals per group and fed for 16 weeks with: (1) control diet (CT); (2) CT plus HCTZ (0.4 g kg^{-1} of diet); (3) CT plus HCTZ (1.0 g kg^{-1} of diet); (4) CT plus HCTZ (4.0 g kg^{-1} of diet); (5) high fat diet (HF); (6) HF plus HCTZ (0.4 g kg^{-1} of diet); (7) HF plus HCTZ (1.0 g kg^{-1} of diet), and (8) HF plus HCTZ (4.0 g kg^{-1} of diet). HCTZ doses were selected on the basis of a previous study where a dose of 300 mg kg^{-1} of HCTZ was found to be the NOAEL for HCTZ in rats.[6] Here we have estimated a consumption of about 25 g day^{-1} rat^{-1}, which correspond to doses of HCTZ ranging from about 30 to 300 mg HCTZ per kg of body weight. The composition of the diets is shown in Table 1. Diets were prepared weekly and stored at 4°C. HCTZ was first mixed with the vitamin and mineral mixtures. Then, this new mixture was extensively mixed with casein and sequentially with sucrose, wheat bran and the other components of the diet until a homogenous mixture was obtained. Diet (25–30 g rat^{-1}) was offered daily and the leftovers were removed and weighted to calculate the daily food consumption (food consumption varied from about 20 to 25 g rat^{-1} day^{-1}).

Table 1. Composition of the diets (g kg^{-1})

Components	High fat diet	Control diet
Sucrose	200	200
Cornstarch	—	280
Casein	180	180
Albumin	22	22
Lard	280	—
Soybean oil	20	20
Bone's flour	60	60
Wheat bran	188	188
Mineral mixture*	40	40
Vitamin mixture†	10	10

*The mineral mixture contained (g kg^{-1}): bone meal (449); NaCl (38); KCl (134.2); $MgSO_4$ (20); $ZnCl_2$ (0.4); $CuSO_4$ (0.175); $MnSO_4$ (1.2); $FeSO_4$ (2), and cornstarch (355).
†The vitamin mixture (mg or IU g^{-1}) was composed of Vitamin A, 2000 IU; Vitamin D, 200 IU; tocopherol, 10 IU; menadione, 0.5 mg; choline, 200 mg; folic acid, 0.2 mg; *p*-aminobenzoic acid, 1.0 mg; inositol, 10 mg; calcium D-panthotenate, 4.0 mg; riboflavin, 0.8 mg; thiamin-HCl, 0.5 mg; pyridoxine-HCl, 0.5 mg; niacinamide, 0.3 mg; and biotin, 0.04.

Tissue preparation

At the end of the 16-week treatment, after 12 h of fasting, the animals were decapitated under mild ether anesthesia and blood was collected by cardiac puncture in heparinized tubes for the measurement of blood glucose levels. Brain was quickly removed, rinsed with saline, weighted, placed on ice, and homogenized in 10 volumes (w/v) in cold 50 mM Tris–HCl pH 7.4. The homogenate was centrifuged at 4000*g* at 4°C for 10 min to yield low-speed supernatant fraction (S1) that was used for biochemical assays.

Blood glucose levels

Blood glucose levels were measured by using commercial Kits (Labtest, Minas Gerais, Brazil).

Lipid peroxidation (LPO) levels

Lipid peroxidation was estimated by measuring TBA reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa *et al.*,[20] in which MDA, an end product of fatty acid peroxidation, reacts with TBA to form a colored complex. In brief, samples were incubated at 100°C for 60 min in acid medium containing 0.45% SDS, 1.27 mol L^{-1} acetic acid/270 mmol L^{-1} HCl, pH 3.5 and 0.8% TBA. After centrifugation, the reaction product was determined at 532 nm using 1,1,3,3-tetramethoxypropane as standard.

Vitamin C levels

Cerebral vitamin C (ascorbic acid) levels were determined by the method of Jacques-Silva *et al.*[21] Proteins of brain were precipitated with 1 volume of a cold 10% trichloroacetic acid followed by centrifugation. An aliquot of 300 µl of supernatants was mixed with 2,4-dinitrophenylhydrazine (4.5 mg ml^{-1}), $CuSO_4$ (0.075 mg ml^{-1}) and trichloroacetic acid 13.3% (final volume 1 ml) and incubated for 3 h at 37°C. Then, 1 ml of H_2SO_4 65% (v/v) was added to the medium. Ascorbic acid levels were measured spectrophotometrically at 520 nm and calculated using a standard curve (1.5–4.5 µmol L^{-1} ascorbic acid freshly prepared in sulfuric acid).

Non-protein thiol groups (NPSH) levels

Non-protein thiol groups content from brain were determined as described by Ellman.[22] For the NPSH determination the samples of S1 from brain were precipitated with 200 µl of 10% trichloroacetic acid followed by centrifugation. The colorimetric assay was carried out in phosphate buffer 1 M, pH 7.4. A standard curve using glutathione was constructed in order to calculate the non-protein thiol groups in the tissues samples.

Determination of Na^+–K^+-ATPase activity

Cerebral Na^+–K^+-ATPase activity was measured spectrophotometrically by determining the organic phosphate (Pi) released according to the method of Fiske and Subbarow.[23] Na^+–K^+-ATPase activity was calculated as the difference between the total Mg^{2+} ATPase activity (without ouabain) and Mg^{2+} ATPase activity determined in the presence 0.5 mmol L^{-1} of ouabain. Both activities were determined in the presence of 125 mmol L^{-1} NaCl and 20 mmol L^{-1} KCl.

Statistical analysis

All values obtained are expressed as mean ± standard error. Data were analyzed by one-way or two-way ANOVA analyses of variance followed by Duncan's multiple range tests when appropriate. Differences between groups were considered to be significant when $p < 0.05$.

RESULTS

Organ weight

Two-way ANOVA (2 diets × 4 HCTZ doses) revealed no significant main effect of diet ($p > 0.10$) or HCTZ ($p > 0.10$) or diet versus HCTZ interaction ($p > 0.10$).

Blood glucose levels

Two-way ANOVA of blood glucose levels revealed a significant main effect of the diet [$F(1, 32) = 9.74, p < 0.05$] and a significant main effect of the HCTZ treatment [$F(3, 32) = 4.73$, $p < 0.05$. HCTZ and HF treatment tended to increase blood glucose; however, post-hoc comparisons indicated that a significant increase in glucose levels occurred only after simultaneous ingestion of HF and HCTZ (1.0 and 4.0 g kg^{-1}; Figure 1, $p < 0.05$).

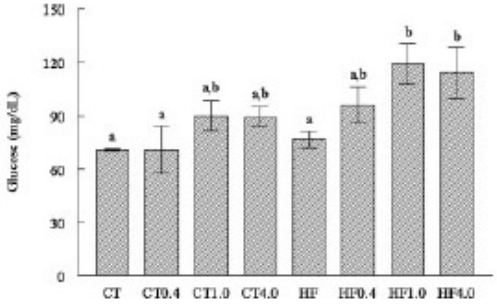


Figure 1. Effect of co-treatment with hydrochlorothiazide and control or high fat diet on glucose blood levels. Data are expressed as means ± SEM of five animals. [abc]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

Lipid peroxidation (LPO) levels

Two-way ANOVA of LPO levels revealed a significant main effect of the diet [$F(1, 32) = 15.43$, $p < 0.05$] and a significant main effect of the HCTZ treatment [$F(3, 32) = 5.56$, $p < 0.05$. HCTZ and HF treatment tended to increase LPO levels; however, post-hoc comparisons indicated that a significant increase in LPO levels occurred only after ingestion of 4.0 g kg^{-1} of HCTZ alone or after simultaneous ingestion of HF and HCTZ at 1 and 4 g kg^{-1} of HCTZ (Figure 2). Of particular importance, positive correlation was found between cerebral LPO and blood glucose levels ($r = 0.49$, $p < 0.05$).

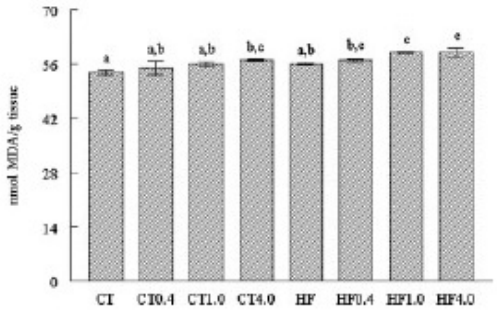


Figure 2. Thiobarbituric acid reactive substance levels in rats co-treated with hydrochlorothiazide and with control or high fat diets. Data are expressed as means ± SEM of five animals. [abc]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

Vitamin C levels

Two-way ANOVA of cerebral vitamin C levels revealed a significant main effect of diet [$F(1, 32) = 31.47$, $p < 0.05$] and of HCTZ treatment [$F(3, 32) = 24.72$, $p < 0.05$]. HCTZ caused a significant increase in the vitamin C levels in brain of animals. Diet × HCTZ treatment interaction was also significant [$F(3, 32) = 7.15$, $p < 0.05$]. Post-hoc comparisons revealed that the HCTZ caused a dose-dependent increase in cerebral vitamin C in rats fed with the CT, whereas only the highest dose of HCTZ caused a significant increase in vitamin C in rats fed with the high fat diet (Figure 3).

Non-protein thiol groups (NPSH) levels

Two-way ANOVA of cerebral NPSH levels revealed a significant main effect of the diet [$F(1, 32) = 21.89$, $p < 0.05$] and a significant diet versus HCTZ interaction [$F(3, 32) = 8.79$, $p < 0.05$]. Post-hoc comparisons indicated that a significant increase in NPSH levels occurred in brain from rats fed the CT only after of ingestion of 1.0 and 4.0 g kg^{-1} of HCTZ. In rats fed the HF, HCTZ caused an increase in NPSH at all doses (Figure 4).

Determination of Na^+–K^+-ATPase activity

Two-way ANOVA of cerebral Na^+–K^+-ATPase activity revealed a significant main effect of diet [$F(1, 32) = 47.10$, $p < 0.05$] and HCTZ treatment [$F(3, 32) = 13.44$, $p < 0.05$]. Post-hoc comparisons indicated that a significant decrease in Na^+–K^+-ATPase activity in rats fed the CT occurred after ingestion of 4.0 g kg^{-1} of HCTZ. In rats fed with the HF, HCTZ decreased Na^+–K^+-ATPase activity at all doses tested (Figure 5). In addition, negative correlation was found between the Na^+–K^+-ATPase activity and glucose levels ($r = -0.65$, $p < 0.05$).

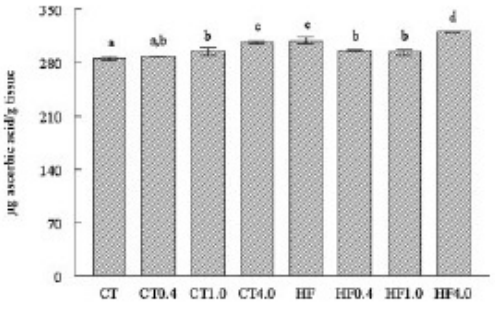


Figure 3. Vitamin C levels in rats co-treated with hydrochlorothiazide and with control or high fat diets. Data are expressed as means ± SEM of five animals. [abcd]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

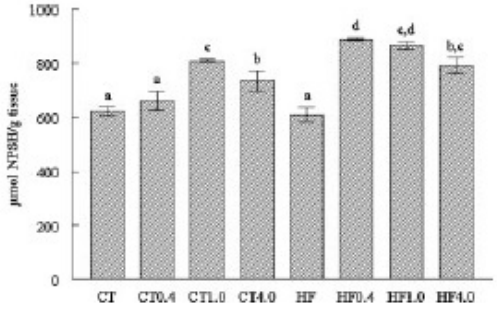


Figure 4. Non-protein thiol groups levels in rats co-treated with hydrochlorothiazide and with control or high fat diets. Data are expressed as means ± SEM of five animals. [abcd]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

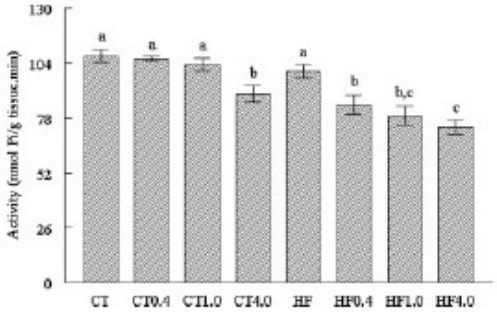


Figure 5. Effect of co-treatment with hydrochlorothiazide and with control or high fat diet on Na^+–K^+-ATPase activity. Data are expressed as means ± SEM of five animals. [abc]Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan)

DISCUSSION

Literature data have indicated that long-term consumption of high-fat diets[3] as well as HCTZ treatment[7] are important factors for the appearance of some metabolic changes related to type 2 diabetes. In this way, results of the present study indicate that high doses of HCTZ associated with HF caused an increase in blood glucose levels, which are compatible with the development of insulin resistance. Thus, we can suggested that simultaneous ingestion of high-fat diets and the use of thiazides as diuretics for the treatment of hypertension could potentiate the increase of blood glucose levels caused by this class of drug. The mechanism by which thiazide induces an increase in glucose levels and glucose intolerance is not completely understood. However, it has been implicated a decreased insulin secretion by pancreatic β cells and decreased tissue insulin sensitivity.[24]

In this context, it is well accepted that low to medium range doses of this diuretic is effective in lowering blood pressure with minimal side effects. When the dose is

increased, little contribution is observed regarding the control of blood pressure, whereas side effects increase substantially.[7,8,24] In fact, previous studies have indicated a clear correlation between the dose of thiazide and an increase in fasting glucose concentrations.[25] In the same vein, hydrochlorothiazide (at an average dose of 40 mg day^{-1}) caused hyperglycemia.[26] Recent data have indicated that prolonged treatment of hypertensive patients with a low dose of HCTZ (12.5 mg day^{-1}) improves arterial elasticity, but not in patients with type 2 diabetes mellitus or impaired fasting glucose. In addition, they have demonstrated that treatment with a full dose of HCTZ (25 mg day^{-1}) can aggravate metabolic parameters and arterial stiffness.[27] The doses tested here were higher than that commonly used for the treatment of hypertension; however, they are lower than the NOAEL of HCTZ for rats[6] and can indicate that a direct extrapolation of toxic doses from rats to human is not possible.

For years researchers have reported that ROS can cause cell degeneration, especially in brain,[18,19] since it is particularly vulnerable to oxidative stress due to limited antioxidant capacity.[12] In this context, it has been shown that chronic intake of a HF[3,28] as well as the hyperglycemia condition are linked to oxidative stress generation and that increased levels of ROS are involved in the development of insulin resistance[29–32] and diabetic neuropathy.[13,18,19,33] However, data about the potential facilitating effects of HCTZ in promoting insulin resistance and oxidative stress in animal models are scarce or lacking in literature. Herein we found that chronic HF consumption was associated with an increase in LPO levels in brain tissue that was aggravated by HCTZ treatment. In line with this, literature data have indicated that hyperglycemia causes an excessive non-enzymatic glycation of protein structures, with marked inactivation of enzymes, increased lipid peroxidation, and changes in antioxidant defense systems.[30,34]

In this study, we observed a small but statistically significant increase in cerebral vitamin C levels in rats fed with high doses of HCTZ alone or in combination with HF. Similarly, NPSH levels were increased in animals treated with 1.0 and 4.0 g kg^{-1} of HCTZ and the increase was proportionally higher in rats fed the HF diet. Accordingly, an increase on the antioxidant defense systems has been observed in a variety of experimental models of pathologies possibly as a compensatory response of the tissues to the presence of oxidative insults.[35,36] However, here the significance of the levels of Vitamin C remains unclear, particularly, in view of the fact that the actual differences in Vitamin C levels across the various groups were small.

Na^+–K^+-ATPase, a sulfhydryl-containing enzyme, is embedded in the cell membrane and is responsible for the active transport of sodium and potassium ions in the nervous system. This process regulates the cellular Na^+/K^+ concentrations and hence their gradients across the plasma membrane, which are required for vital functions such as membrane co-transports, cell volume regulation and membrane excitability.[37,38] This dimeric enzyme exists in several isoforms in brain and consumes the greater part of available ATP.[39] The inactivation of Na^+–K^+-ATPase leads to partial membrane depolarization allowing excessive Ca^{2+} entry inside neurons with resultant neurotoxic events.[40] In this study, we observed a significant inhibition in Na^+/K^+-ATPase activity in brain of the animals treated with high doses of HCTZ. HCTZ-induced enzyme inhibition may be associated with an increase in oxidative stress which can accelerate Na^+–K^+-ATPase denaturation.[41] In fact, –SH groups of this enzyme are highly susceptible to oxidative stress[42] and oxidizing agents.[43] Moreover, our data have indicated an interaction between HF and HCTZ effects on Na^+–K^+-ATPase activity in brain. Simultaneous co-treatment with HCTZ and the HF diet could cause additional pro-oxidative stress in this organ with concomitant Na^+–K^+-ATPase inhibition. However, further studies are necessary to understand the mechanism(s) involved in the interactive effects of diet and HCTZ on cerebral Na^+–K^+-ATPase.

In summary, our data indicate that chronic intake of the high doses of HCTZ or HF changes the biochemical parameters related to cerebral oxidative stress. The significant positive correlation between cerebral TBARS and blood glucose and the negative correlation between Na^+–K^+-ATPase and blood glucose levels may indicate a potential role for hyperglycemia, at least in part, in neurochemical changes after exposure to HCTZ and/or a high fat diet. In short, the results of the present investigation suggested that simultaneous consumption of high fat diets and HCTZ can exacerbate oxidative stress in brain.

ACKNOWLEDGEMENTS

The financial support by FINEP Research Grant ''Rede Instituto Brasileiro de Neurociência (IBN-Net)'' # 01.06.0842-00, FAPERGS, CAPES/SAUX, VITAE Fundation and CNPq and CNPQ-INCT is gratefully acknowledged. J.B.T.R. is the recipient of CNPq fellowships.

REFERENCES

1. Flanagan AM, Brown JL, Santiago CA, Aad PY, Spicer LJ, Spicer MT. High-fat diets promote insulin resistance through cytokine gene expression in growing female rats. *J Nutr Biochem* 2007; **19**: 505–513.
2. Ikehara O, Kawasaki N, Maezono K, Komatsu M, Konishi A. Acute and chronic treatment of L-isoleucine ameliorates glucose metabolism in glucose-intolerant and diabetic mice. *Biol Pharm Bull* 2008; **31**: 469–472.
3. Folmer V, Soares JCM, Gabriel DV, Rocha JBT. A high fat inhibits δ-aminolevulinate dehydratase and increases lipid peroxidation in mice (Mus musculus). *J Nutr* 2003; **133**: 2165–2170.
4. Greenwood CE, Winocur G. High-fat diets, insulin resistance and declining cognitive function. *Neurobiol Aging* 2005; **26**: 42–45.
5. Yehuda S, Rabinovitz S, Mostofsky DI. Mediation of cognitive function by high fat diet following stress and inflammation. *Nutr Neurosci* 2005; **8**: 309–315.
6. George JD, Price CJ, Tyl RW, Marr MC, Kimmerl CA. The evaluation of the developmental toxicity of hydrochlorothiazide in mice and rats. *Fundam Appl Toxicol* 1995; **26**: 174–180.
7. Pepine CJ, Cooper-DeHoff RM. Cardiovascular therapies and risk for development of diabetes. *J Am Coll Cardiol* 2004; **44**: 509–512.
8. Zee AM, Turner ST, Schwartz GL, Chapman AB, Klungel OH, Boerwinkle E. Demographic, environmental, and genetic predictors of metabolic side effects of hydrochlorothiazide treatment in hypertensive subjects. *Am J Hypertens* 2005; **18**: 1077–1083.

9. Bonner G. Hyperinsulinemia, insulin resistance, and hypertension. *J Cardiovasc Pharmacol* 1994; **24**: 39–49.
10. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; **39**: 44–84.
11. Çakatay U, Telci A, Kayali R, Tekeli F, Akçay T, Sivas A. Relation of oxidative protein damage and nitrotyrosine levels in the aging rat brain. *Exp Gerontol* 2001; **36**: 221–229.
12. Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: implications for neuroimaging. *Trends Neurosci* 2004; **27**: 489–495.
13. Greene DA, Stevens MJ, Obrosova I, Feldman EL. Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. *Eur J Pharmacol* 1999; **375**: 217–223.
14. Baydas G, Canatan H, Turkoglu A. Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus. *J Pineal Res* 2002; **32**: 225–230.
15. Luxford C, Dean RT, Davies MJ. Radicals derived from histone hydroperoxides damage nucleobases in RNA and DNA. *Chem Res Toxicol* 2000; **13**: 665–672.
16. Hawkins CL, Davies MJ. Generation and propagation of radical reactions on proteins. *Biochim Biophys Acta* 2001; **1504**: 196–219.
17. Baynes JW. Role of oxidative stress in the development of complications in diabetes. *Diabetes* 1991; **40**: 405–412.
18. Baydas G, Reiter RJ, Yasar A, Tuzcu M, Akdemir I, Nedzvetskii VS. Melatonin produces glial reactivity in the hippocampus, cortex, and cerebellum of streptozocin-induced diabetic rats. *Free Radical Biol Med* 2003; **35**: 797–804.
19. Baydas G, Donder E, Kiliboz M, *et al*. Neuroprotection by α-lipoic acid in streptozotocin-induced diabetes. *Biochemistry (Moscow)* 2004; **69**: 1001–1005.
20. Ohkawa H, Ohishi H, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351–358.
21. Jacques-Silva MC, Nogueira CW, Broch LC, Flores EM, Rocha JBT. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. *Pharmacol Toxicol* 2001; **88**: 119–125.
22. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; **82**: 70–77.
23. Fiske CF, Subbarow YJ. The colorimetric determination of phosphorous. *J Biol Chem* 1925; **66**: 375–381.
24. Grossman E, Messerli FH. Long-term safety of antihypertensive therapy. *Prog Cardiovasc Dis* 2006; **49**: 16–25.
25. Carlsen JE, Kober L, Torp-Pedersen C, Johansen P. Relation between dose of bendrofluazide, antihypertensive effect, and adverse biochemical effects. *Br Med J* 1990; **300**: 975–978.
26. Pollare T, Lithell H, Berne C. A comparison of the effect of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. *N Engl J Med* 1989; **321**: 868–873.
27. Zimlichman R, Shargorodsky M, Wainstein J. Prolonged treatment of hypertensive patients with low dose HCTZ improves arterial elasticity but not if they have NIDDM or IFG. Treatment with full dose HCTZ (25 mg/d) aggravates metabolic parameters and arterial stiffness. *Am J Hypertens* 2004; **17** (Suppl 1): 138.
28. Fachinetto R, Burger ME, Wagner C, *et al*. High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. *Pharmacol Biochem Behav* 2005; **81**: 585–592.
29. Folmer V, Soares JC, Rocha JBT. Oxidative stress in mice is dependent on the free glucose content in the diet. *Int J Biochem Cell Biol* 2002; **34**: 1279–1285.
30. Aksoy N, Vural H, Sabuncu T, Aksoy S. Effects of melatonin on oxidative-antioxidative status of tissues in streptozotocin-induced diabetic rats. *Cell Biochem Funct* 2003; **21**: 121–125.
31. Brito VB, Folmer V, Soares JCM, Silveira ID, Rocha JBT. Long-term sucrose and glucose consumption decreases the δ-aminolevulinate dehydratase activity in mice. *Nutrition* 2007; **23**: 818–826.
32. Maiese K, Morhan SD, Chong ZZ. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. *Curr Neurovasc Res* 2007; **4**: 63–71.
33. McCall AL. The impact of diabetes on the CNS. *Diabetes* 1992; **41**: 557–570.
34. Morgan PE, Dean RT, Davies MJ. Inactivation of cellular enzymes by carbonyls and protein-bound glycation glycoxidation products. *Arch Biochem Biophys* 2002; **403**: 259–269.
35. Barbosa NBV, Rocha JBT, Wondracek DC, Perottoni J, Zeni G, Nogueira CW. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. *Chem Biol Interact* 2006; **163**: 230–238.
36. Barbosa NBV, Rocha JBT, Soares JCM, *et al*. Dietary diphenyl diselenide reduces the STZ-induced toxicity. *Food Chem Toxicol* 2008; **46**: 186–194.
37. Doucet A. Function and control of Na^+-K^+-ATPase in single nephron segments of the mammalian kidney. *Kidney Int* 1988; **34**: 749–760.
38. Jorgensen PL. Structure, function and regulation of Na^+-K^+-ATPase in the kidney. *Kidney Int* 1986; **29**: 10–20.
39. Bertorello AM, Kats AL. Regulation of Na^+-K^+-pump activity: pathways between receptors and effectors. *NIPS* 1995; **10**: 253–259.
40. Beal MF, Hyman BT, koroshetz W. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases. *Trends Neurosci* 1993; **16**: 125–131.
41. Thevenod F, Friedmann JM. Cadmium-mediated oxidative stress in kidney proximal tubule cells induces degradation of Na^+/K^+-ATPase through proteasomal and endo-/lysosomal proteolytic pathways. *FASEB J* 1999; **13**: 1751–1761.
42. Yufu K, Itho T, Edamatsu R, Mori A, Hirakawa M. Effect of hyperbaric oxygenation on the Na^+-K^+-ATPase and membrane fluidity of cerebrocortical membranes after experimental subarachnoid hemorrhage. *Neurochem Res* 1993; **16**: 1033–1039.
43. Carfagna MA, Ponsler GD, Muhoberac BB. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. *Chem Biol Interact* 1996; **100**: 53–65.

CAPÍTULO 2 – Manuscrito 1.

Hydrochlorothiazide with a high fat diet hampers glucose homeostasis and increases oxidative stress in rats

Marinei Cristina Pereira Ribeiro, Roger Monteiro, Lutiane Mozzaquatro Parcianello, Daiana Silva de Ávila, Nilda Berenice de Vargas Barbosa e João Batista Teixeira Rocha.

Submetido à Life Sciences.

Hydrochlorothiazide with a high fat diet hampers glucose homeostasis and increases oxidative stress in rats

Marinei Cristina Pereira Ribeiro [a,b] *, Roger Monteiro [b],

Lutiane Mozzaquatro Parcianello [b], Daiana Silva Ávila [b], Nilda Berenice de Vargas Barbosa [b] and João Batista Teixeira Rocha [b,] *

[a] Hospital Universitário de Santa Maria,

Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil

[b] Departamento de Química, Centro de Ciências Naturais e Exatas,

Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil

[*] Corresponding author: Marinei Cristina Pereira Ribeiro or João Batista Teixeira da Rocha
Hospital Universitário de Santa Maria
Universidade Federal de Santa Maria
97105-900 Santa Maria, RS, Brazil
Tel.: +55 55 3220 8513; fax: +55 55 3220 8005
E-mail: mcpribeiro@yahoo.com.br or jbtrocha@yahoo.com.br

Abstract

Aims: This study was designed to investigate whether an association between a high fat (HF) diet and hydrochlorothiazide (HCTZ) produces a negative synergic influence on glucose homeostasis and in other biochemical parameters associated to hyperglycemia development.

Main methods: Rats were fed for 16 weeks with a control diet (CT) or with an HF diet supplemented or not with different doses of HCTZ (0.4, 1.0 and 4.0 g/kg of diet).

Key findings: In experimental trials, HCTZ associated with an HF diet caused a significant increase in blood glucose and fructosamine levels. The intake of HCTZ and HF diets produced a significant reduction in magnesium and potassium levels as well as an increase in lipid peroxidation and vitamin C in liver. Importantly, the association of HCTZ with HF diet caused additional worsening of biochemical parameters related to glucose homeostasis (particularly accentuated magnesium depletion) and further increase in hepatic oxidative stress.

Significance: Our results suggest that chronic intake of HCTZ or HF diet causes metabolic changes related to glucose homeostasis and that the association of HF diet and HCTZ treatment can exacerbate some of these biochemical alterations. Therefore, we can suggest that this experimental model can be used for studying the adverse effects of HCTZ.

Keywords: High fat diet, hydrochlorothiazide, hyperglycemia, oxidative stress, magnesium.

Introduction

Hydrochlorothiazide (HCTZ) is a diuretic that belongs to the thiazide class of compounds and is widely used for the treatment of hypertension (George et al. 1995). HCTZ safety and efficacy in reducing morbidity and mortality in hypertensive patients have been attested by numerous studies (JAMA 1991, BMJ 1992, Grossman and Messerli 2006), however, its use can be associated with the development of metabolic abnormalities such as hyperglycemia and type 2 diabetes mellitus (Weir and Moser 2000, Zee et al. 2005). Therefore, the intake of this class of diuretic in diabetic hypertensive patients has been questioned (Grossman 2006).

Similarly to HCTZ treatment, chronic intake of diets with a high proportion of fat can promote the appearance of hyperglycemic state, which ultimately leads to an increased risk of developing type 2 diabetes mellitus (Kamgang et al. 2005, Messier et al. 2007). Consequently, high fat diets have been commonly used in rodent to mimic experimentally human type 2 diabetes mellitus (Han et al. 1997, Hansen et al. 1998, Tremblay et al. 2001, Folmer et al. 2003, Kamgang et al. 2005, Shih et al. 2008).

Hyperglycemia, the primary clinical manifestation of diabetes mellitus, is associated with non enzymatic glycation of proteins and free radical generation (Maiese et al 2007). These processes can cause permanent chemical alterations in proteins and increase lipid peroxidation in a variety of tissues (Folmer et al. 2002, Brito et al. 2007, Maiese et al. 2007). In this context, excessive production of reactive oxygen species (ROS) or inadequate antioxidant protection facilitates the development and progression of diabetes and its complications (Rosen et al. 2001, Maritim et al. 2003).

Liver is one of the primary insulin-responsive organs and has a central role in modulating normal glucose homeostasis (Maiese et al. 2007). Literature reports have

demonstrated that hepatic damage induced by ROS can disrupt cellular homeostasis and aggravate metabolic syndrome features (Kohen and Nyska 2002, Raval et al. 2006). Thus, in an attempt to develop a rodent model to study the adverse effects of HCTZ, the main objective of this work was to investigate whether an association between HF diet and HCTZ could have a negative synergic influence on glucose homeostasis and on other biochemical parameters related to hyperglycemia development. Moreover, a possible relationship between these changes with oxidative stress was also investigated.

Materials and Methods

Chemicals

Casein (technical grade), comassie brilliant blue G, 2,4-dinitrophenylhydrazine, HCl, sodium sulphate dodecyl (SDS), heptane, acetate, ethanol, reduced glutathione, ouabain, malondialdehyde (MDA) and thiobarbituric acid (TBA) were obtained from Sigma, (St. Louis, MO., USA). Mono and dibasic potassium phosphate, acetic acid, ascorbic acid, ortho-phosphoric acid, tris buffer (tris[hydroxymethyl]aminomethane) and trichloroacetic acid were obtained from Merck (Rio de Janeiro, Brasil). Hydrochlorothiazide, cornstarch, lard, bone meal, wheat bran, soybean oil, vitamin and mineral complex were obtained from various commercial sources.

Animals and diets

Adult male Wistar rats (2 months old), weighing 250-300 g were used for the experiments. The animals were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21-25 ºC and humidity at roughly 56% and with free access to food

and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil.

Rats were randomly divided in eight experimental groups with five animals per group and fed for 16 wk with: (1) control diet (CT); (2) CT plus HCTZ (0.4 g/kg of diet); (3) CT plus HCTZ (1.0 g/kg of diet); (4) CT plus HCTZ (4.0 g/kg of diet); (5) high fat diet (HF); (6) HF plus HCTZ (0.4 g/kg of diet); (7) HF plus HCTZ (1.0 g/kg of diet) and (8) HF plus HCTZ (4.0 g/kg of diet). The composition of the diets is shown in Table 1. Diets were prepared weekly and stored at 4ºC. The food intake and the body weight of animals were measured daily and every week, respectively.

Blood samples and tissue preparation

At the end of the experimental period, after 12 h of fasting, the animals were sacrificed under mild ether anesthesia and blood was collected by cardiac puncture in heparinized tubes for vitamin C determination. A blood fraction was collected in tubes with sodium fluoride to determine glucose concentration. In addition, a parallel blood fraction was collected without anticoagulant for magnesium, potassium and fructosamine determination. The samples of liver were quickly removed, rinsed with saline, weighed, placed on ice and homogenized in 10 volumes (w/v) in cold 50 mM Tris-HCl pH 7.4. The homogenate was centrifuged at 4,000 x g at 4ºC for 10 min to yield low-speed supernatant fraction (S1) that was used for biochemical assays, except for measurement of protein carbonyl content (PCO), which was determined in samples of the homogenate. In addition, total adipose tissue deposits (total weight of perirenal, mesenteric, epididymal and subcutaneous fat-pads) content was also determined.

Biochemical analysis

Plasma glucose concentration was measured as previously described by Bergmeyer (1984) and fructosamine concentration was determined as described by Baker et al. (1985). The levels of magnesium were estimated according to the method of Bohuon (1962) and potassium levels were measured as previously described by Kavanagh and Mills (1997).

Lipid peroxidation (LPO) levels

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa et al. (1979), in which MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. In brief, samples were incubated at 100ºC for 60 min in acid medium containing 0.45% sodium dodecyl sulfate, 1.27 mol/L acetic acid/270 mmol/L HCl, pH 3.5 and 0.8% thiobarbituric acid. TBARS produced were measured at 532 nm and the absorbance was compared with the standard curve using malondialdehyde.

Protein carbonyl (PCO) content

The PCO content was determined as described by Levine et al. (1989) with some modifications. Briefly, homogenates were diluted to 750-800 µg/mL of protein in each sample, and 1 mL aliquots were mixed with 0.2 mL of 2,4-dinitrophenylhydrazine (DNPH, 10 mM) or 0.2 mL HCl (2 M). After incubation at room temperature for 1h in a dark ambient, 0.6 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 1.8 mL of heptane (99.5%) and 1.8 mL of ethanol (99.8%) were sequentially added,

mixed with vortex agitation for 40 sec and centrifuged for 15 min. The protein isolated from the interface was washed two times with 1 mL of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 mL of denaturing buffer. Each DNPH sample was read at 370 nm in a spectrophotometer against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000$M^{-1}cm^{-1}$. Protein was measured by method of Bradford (1976) using bovine serum albumin as standard.

Vitamin C levels

Total content of vitamin C (ascorbic acid) in plasma and liver was determined by the method of Jacques-Silva et al. (2001). Proteins of plasma and liver were precipitated with 1 vol. of a cold 10% trichloroacetic acid followed by centrifugation. An aliquot of 300 µL of the supernatants was mixed with 2,4-dinitrophenylhydrazine (4.5 mg/mL), $CuSO_4$ (0.075 mg/mL) and trichloroacetic acid 13.3% (final volume 1 mL) and incubated for 3 h at 37ºC. Then, 1 mL of H_2SO_4 65% (v/v) was added to the medium. Ascorbic acid levels were measured spectrophotometrically at 520 nm and calculated using a standard curve (1.5-4.5 µmol/L ascorbic acid freshly prepared in sulfuric acid).

Statistical analysis

All values obtained are expressed as mean ± standard error. Data were analyzed by one-way, two-way or three-way ANOVA analyses of variance followed by Duncan's multiple range tests when appropriate. Differences between groups were considered to be significant when $p < 0.05$.

Results

Body weight, organ weight and total adipose tissue deposit weight

Three-way ANOVA (2 diets x 4 HCTZ x 16 sampling times) revealed significant HCTZ x time interaction (Fig. 1A and 1B, $p < 0.05$), indicating that HCTZ treatment caused a reduction in body weight gain rate (Table 2). Two-way ANOVA of total adipose tissue weight revealed a significant main effect of HCTZ [$F(3,32)=10.75$, $p < 0.05$], indicating that HCTZ treatment decreased total adipose tissue deposits (Table 2). However, the proportion of lipid to body weight was significantly higher in the high fat diet group that was fed with 4.0 kg HCTZ than in other groups (data not shown).

Blood glucose levels

Two-way ANOVA of blood glucose levels revealed a significant main effect of the diet [$F(1,32)=9.74$, $p < 0.05$] and a significant main effect of the HCTZ treatment [$F(3,32)=4.73$, $p < 0.05$. HCTZ and HF treatment tended to increase blood glucose; however, post-hoc comparisons indicated that a significant increase in glucose levels occurred only after simultaneous ingestion of HF and HCTZ (1.0 and 4.0 g/kg; Table 3).

Fructosamine levels

Two-way ANOVA for fructosamine data revealed a significant main effect of the diet ($F(1,32)=32.23$, $p < 0.05$). In fact, the ingestion of the HF diet increased fructosamine levels in all groups (Table 3).

Magnesium and potassium determination

Two-way ANOVA of magnesium levels revealed a significant main effect of the diet [$F(1,32)=17,23$, $p < 0.05$] and a significant main effect of HCTZ [$F(3,32)=8.34$, $p < 0.05$]. Post-hoc treatment by Duncan's multiple range tests revealed that HF diet caused a significant reduction in magnesium level. Analyses also demonstrated that HCTZ caused further decrease in magnesium level in control and HF diets (Table 3). Of particular importance, negative correlations were found between the magnesium and glucose levels ($r = - 0.51$, $p < 0.05$) as well as between magnesium and fructosamine levels ($r = - 0.37$, $p < 0.05$).

Two-way ANOVA of potassium levels revealed a significant main effect of the diet ($F(1,32)= 20.8.$ $p < 0.05$) and of the HCTZ treatment [$F(3,32)=10,99$, $p < 0.05$] and a tendency for significant diet x HCTZ interaction [$F(3,32)=2.44$, $p < 0.10$]. Post-hoc comparisons indicated that HCTZ and HF caused a significant decrease in potassium levels when compared to CT group (Table 3). In addition, positive correlation was found between the potassium and magnesium levels ($r = 0.62$, $p < 0.05$).

Lipid peroxidation levels

Two-way ANOVA of LPO levels revealed a significant diet x HCTZ interaction [$F(3, 32)=45,93$, $p < 0.05$]. Post-hoc comparisons indicated that HCTZ caused a significant increase in hepatic TBARS levels that was higher in the HF + HCTZ (4.0 g/kg) group (Table 4) than in the corresponding CT group.

Protein carbonyl group content

No significant difference was observed in PCO levels in liver of animals (Table 4, $p > 0.05$).

Vitamin C levels

Two way ANOVA revealed that the intake of the HF diet [$F(1,32)=50.96$, $p < 0.05$] and HCTZ treatment [$F(3,32)=21.16$, $p < 0.05$] caused a significant increase in the vitamin C levels in liver of animals. However, the increase was proportionally higher in the HF groups treated simultaneously with HCTZ as evidenced by a significant interaction diet x HCTZ treatment [$F(3,32)=8.85$, $p < 0.05$] (Table 4). No significant difference was observed in vitamin C levels in plasma of animals (Table 4, $p > 0.05$).

Discussion

It has been shown that long-term consumption of HF diet as well as HCTZ treatment are important factors for the appearance of some metabolic changes related to hyperglycemia. In this way, in the present study we observed that HF supplemented with HCTZ caused an increase in blood glucose levels and, that HF diet, associated or not with HCTZ, enhanced the fructosamine levels, which are compatible with the development of hyperglycemic state. Thus, we can observe that the experimental model, with certain limitations, mimics the epidemiological data from literature and may indicate that simultaneous ingestion of high fat diets and the use of thiazides as diuretics for the treatment of hypertension could potentiate the adverse effects of this class of drug.

Literature data have reported that chronic intake of a HF diet (Folmer et al. 2003) as well as the hyperglycemia condition is linked to oxidative stress generation (Maiese et al. 2007, Lopes et al. 2008). However, data about the potential facilitating effects of HCTZ on these parameters in animal models are scarce or lacking in literature. In this context, we observed that chronic HF consumption caused a significant increase in hepatic TBARS levels that was potentialized by HCTZ treatment. The fact that PCO levels were not modified

indicates that the hepatic oxidative stress caused by HCTZ chronic intake was more restricted to biomembranes than proteins. In this way, HF diet produced an increase in glucose and fructosamine that triggered oxidative stress in hepatic tissue which was potentiated by HCTZ. In general, the non enzymatic glycation of proteins generates highly reactive products that could explain the relationship between hyperglycemia and lipid peroxidation (Lopes et al. 2008).

Another aspect that must be considered is that the treatment with HCTZ alone or in combination with HF diet increased vitamin C levels; a fact that could have occurred as a mechanism of protection against lipid peroxidation. Accordingly, an increase on the antioxidant defense systems has been observed in a variety of experimental models of pathologies possibily as a compensatory response of the tissues to the presence of oxidative insults (Barbosa et al. 2006, 2008).

It has been suggested that the depletion of potassium levels by thiazide is likely to have a role in impaired glucose metabolism (Rowe et al. 1980) and, that potassium supplementation can attenuate glucose intolerance induced by thiazides (Helderman et al. 1983). Intracellular magnesium also seems to play a key role in modulating glucose uptake (Barbagallo et al. 2003, Sontia and Touyz 2007). In fact, studies have evidenced a relationship between low magnesium levels with metabolic diseases such as type 2 diabetes mellitus, hypertension (Ma et al. 1995, Sontia and Touyz 2007) and with increased levels of free radical dependent-oxidative tissue damage (Lourdes 1998, Gums 2004). Accordingly, here we found a significant decrease in plasmatic magnesium and potassium levels as well as an increase in hepatic lipid peroxidation in rats fed with HF diet, associated or not with HCTZ. It is important to emphasize that the association of the HF diet with HCTZ (4.0 g/kg of diet) potentiated magnesium depletion and increased lipid peroxidation. Moreover, it has

been reported that a diet rich in saturated fats hinders magnesium absorption (Johnson 2001). In this context, we suggest that someone who eats a diet with high fat content may present loss of magnesium and potassium; a fact that may contribute to diabetes and oxidative stress development. Furthermore, the use of HCTZ by these patients may accelerate the development of diabetes and its complications. In this vein, the results here presented may indicate that magnesium and potassium play an important role in the adverse effects of HCTZ. Besides, the association of HCTZ and HF diet caused additional loss of magnesium, which may indicate that this element has a more fundamental role in HCTZ toxicity than potassium. Since biochemical changes were exacerbated by the combined consumption of HCTZ and HF diet, we can suppose that magnesium loss may have a central role in the adverse effects of HCTZ associated with high fat intake in rats. These results are in agreement with literature which indicates that intracellular magnesium plays a central role in glucose metabolism (Paolisso and Barbagallo 1997, Barbagallo 2000, 2001, 2003, 2007). Of particular importance, epidemiological studies have indicated that type 2 diabetes and hypertension can be associated with lowered intracellular magnesium (Paolisso and Barbagallo 1997, Barbagallo et al. 2000, 2001).

Conclusion

The data presented in this study show that the chronic intake of HCTZ or HF diet causes metabolic changes related to glucose homeostasis and that the association of the HF diet with HCTZ treatment can exarcebate some of these biochemical alterations. Besides, we can suggest that our experimental model can be used to study the adverse effects of HCTZ. Furthermore, in view of the fact that HF ingestion aggravated the effects of HCTZ, it would be important to investigate whether the incidence of type 2 diabetes by use of HCTZ is more

frequent in hypertensive patients who eat diets with high levels fat.

Acknowledgements

The financial support by FINEP Research Grant ‘‘Rede Instituto Brasileiro de Neurociência (IBN-Net)’’ # 01.06.0842-00, FAPERGS, CAPES/SAUX, VITAE Fundation and CNPq is gratefully acknowledged. J.B.T.R. is the recipient of CNPq fellowships.

References

Barbagallo M, Gupta R, Dominguez LJ, Resnick LM. Cellular ionic alterations with aging: relation to hypertension and diabetes. Jounal of the American Geriatrics Society 48, 1111-1116, 2000.

Barbagalo M, Dominguez LJ, Bardicef O, Resnick LM. Altered cellular magnesium responsiveness to hyperglycemia in hypertensive subjects. Hypertension 38, 612-615, 2001

Barbagallo M, Dominguez LJ, Galioto A, Ferlisi A, Cani C, Malfa L, Pineo A, Busardo A, Paolisso G. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Molecular Aspects of Medicine 24, 39-52, 2003.

Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. Archives of Biochemistry and Biophysics 458, 40-47, 2007.

Baker JR, Metcalf PA, Johnson RN, Newman D, Rietz P. Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. Clinical Chemistry 31, 1550-1554, 1985.

Barbosa NBV, Rocha JBT, Wondracek DC, Perottoni J, Zeni G. Nogueira, CW. Diphenyl diselenide reduces temporarily hyperglycemia: Possible relationship with oxidative stress. Chemico-Biological Interactions 163, 230-238, 2006.

Barbosa NBV, Rocha JBT, Soares JCM, Wondracek DC, Gonçalves JF, Schetinger MRC, Nogueira CW. Dietary diphenyl diselenide reduces the STZ-induced toxicity. Food and Chemical Toxicology 46, 186-194, 2008.

Bergmeyer HU. Methods of Enzimatic Analisys, 3ed, Deerfield Beach: Veriag Chemie, 6, 163-172, 1984.

Bohuon C. Microdetermination of magnesium in biological fluids. Clinica Chimica Acta 7, 811-817, 1962.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254, 1976.

Brito VB, Folmer V, Soares JCM, Silveira ID, Rocha, JBT. Long-term sucrose and glucose consumption decreases the δ-aminolevulinate dehydratase activity in mice. Nutrition 23, 818-826, 2007.

Folmer V, Soares JCM, Rocha JBT. Oxidative stress in mice is dependent on the free glucose content in the diet. The International Journal of Biochemistry & Cell Biology 34, 1279-1285, 2002.

Folmer V, Soares JCM, Gabriel D, Rocha BTR. A high fat diet inhibits δ-aminolevulinate dehydratase and increases lipid peroxidation in mice (Mus musculus). The Journal of Nutriton 133, 2165-2170, 2003.

George JD, Price CJ, Tyl RW, Marr MC, Kimmerl CA. The evaluation of the developmental toxicity of hydrochlorothiazide in mice and rats. Fundamental and applied toxicology 26, 174-180, 1995.

Grossman E, Messerli FH. Long-term safety of antihypertensive therapy. Progress in Cardiovascular Diseases 49, 16-25, 2006.

Gums JG. Magnesium in cardiovascular and other disorders. American Journal of Health-System Pharmacy 61, 1569-1576, 2004.

Han D-H., Hansen, PA, Host HH, Holloszy JO. Insulin resistance of muscle glucose transport in rats fed a high-fat diet: a reevaluation. Diabetes 46, 1761-1767, 1997.

Hansen PA, Dong HH, Marshall BA, Nolte LA, Chen MM, Mueckler M, Holloszy JO. A high fat diet impairs stimulation of glucose transport in muscle: Functional evaluation of potential mechanisms. The Journal of Biological Chemistry 273, 26157-26163, 1998.

Helderman JH, Elahi D, Andersen DK, Raizes GS, Tobin JD, Shocken D, Andres R. Prevention of the glucose intolerance of thiazide diuretics by maintenance of body potassium. Diabetes 32, 106-111, 1983.

Jacques-Silva MC, Nogueira CW, Broch LC, Flores EM, Rocha JBT. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharmacology & Toxicology 88, 119-125, 2001.

Johnson S. The multifaceted and widespread pathology of magnesium deficiency. Medical Hypotheses 56, 163-170, 2001.

Kamgang R, Mboumi RY, N'dille´ GPRM, Yonkeu JN. Cameroon local diet-induced glucose intolerance and dyslipidemia in adult Wistar rat. Diabetes Research and Clinical Practice 69, 224-230, 2005.

Kavanagh K, Mills JN. Measuring electrolytes: An evaluation of the IDEXX Vetlyte ion selective electrode method. Australian Veterinary Practice. 27, 97-102, 1997.

Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicologic Pathology 30, 620-650, 2002.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A, Ahn B, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymology 186, 464-478, 1989.

Lopes JP, Oliveira SM, Soares Fortunato J. Oxidative stress and its effects on insulin resistance and pancreatic beta-cells dysfunction: relationship with type 2 diabetes mellitus complications. Acta Médica Portuguesa 213, 293-302, 2008.

Lourdes LM, Crua T, Pousada JC, Rodrigues LE, Barbosa K, Canguru V. The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. Diabetes Care 21, 682-686, 1998.

Ma J, Folsom AR, Melnick SL, Eckfeldt JH, Sharrett AR, Nabulsi AA, Hutchinson RG, Metcalf PA. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin and carotid arterial wall thickness: the ARIC study. Journal of Clinical Epidemiology 48, 927-940, 1995.

Maiese K, Morhan SD, Chong ZZ. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. Current Neurovascular Research 4, 63-71, 2007.

Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: a review. Journal of Biochemical and Molecular Toxicology 17, 24-38, 2003 .

Medical Research Council trial of treatment of hypertension in older adults: Principal results. MRC working party. BMJ 304, 405-412, 1992.

Messier C, Whately K, Liang J, Du L, Puissant D. The effects of a high-fat, high-fructose, and combination diet on learning, weight, and glucose regulation in C57BL/6 mice. Behavioural Brain Research 178, 139-145, 2007.

Ohkawa H, Ohishi H, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95, 351-358, 1979.

Paolisso G, Barbagallo M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. American Journal of Hypertension 10, 346-355, 1997.

Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). SHEP cooperative research group. JAMA: the journal of the American Medical Association 265, 3255-3264, 1991.

Raval J, Lyman S, Nitta T, Mohuczy D, Lemasters JJ, Kim JS, Behrns KE. Basal reactive oxygen species determine the susceptibility to apoptosis in cirrhotic hepatocytes. Free Radical Biology & Medicine 41, 1645-1654, 2006.

Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a congress series sponsored by UNESCO-MCBN, the American Diabetes Association, and the German Diabetes Society. Diabetes/Metabolism Research and Reviews 17, 189-212, 2001.

Rowe JW, Tobin JD, Rosa RM, Andres R. Effect of experimental potassium deficiency on glucose and insulin metabolism. Metabolism 29, 498-502, 1980.

Shih C, Lin C, Lin W. Effects of Momordica charantia on insulin resistance and visceral obesity in mice on high-fat diet. Diabetes Research and Clinical Practice 81, 134-143, 2008.

Sontia B, Touyz RM. Role of magnesium in hypertension. Archives of Biochemistry and Biophysics 458, 33-39, 2007.

Tremblay F, Lavigne C, Jacques H, Marette A. Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (zeta/lambda) activities. Diabetes 50, 1901-1910, 2001.

Weir MR, Moser M. Diuretics and β-blockers: Is there a risk for dyslipidemia? American Heart Journal 139, 174-184, 2000.

Zee AM, Turner ST, Schwartz GL, Chapman AB, Klungel OH, Boerwinkle E. Demographic, environmental, and genetic predictors of metabolic side effects of hydrochlorothiazide treatment in hypertensive subjects. American Journal of Hypertension 18, 1077-1083, 2005.

Figure legends

Figure 1A: Effect of control diet supplemented with hydrochlorothiazide on body weight. Data are expressed as means ± S.E.M. of five animals.

Figure 1B: Effect of high fat diet supplemented with hydrochlorothiazide on body weight. Data are expressed as means ± S.E.M. of five animals. * Denoted $p < 0.05$ as compared to the HF group. (ANOVA/Duncan, $p < 0.05$).

Figure 1A

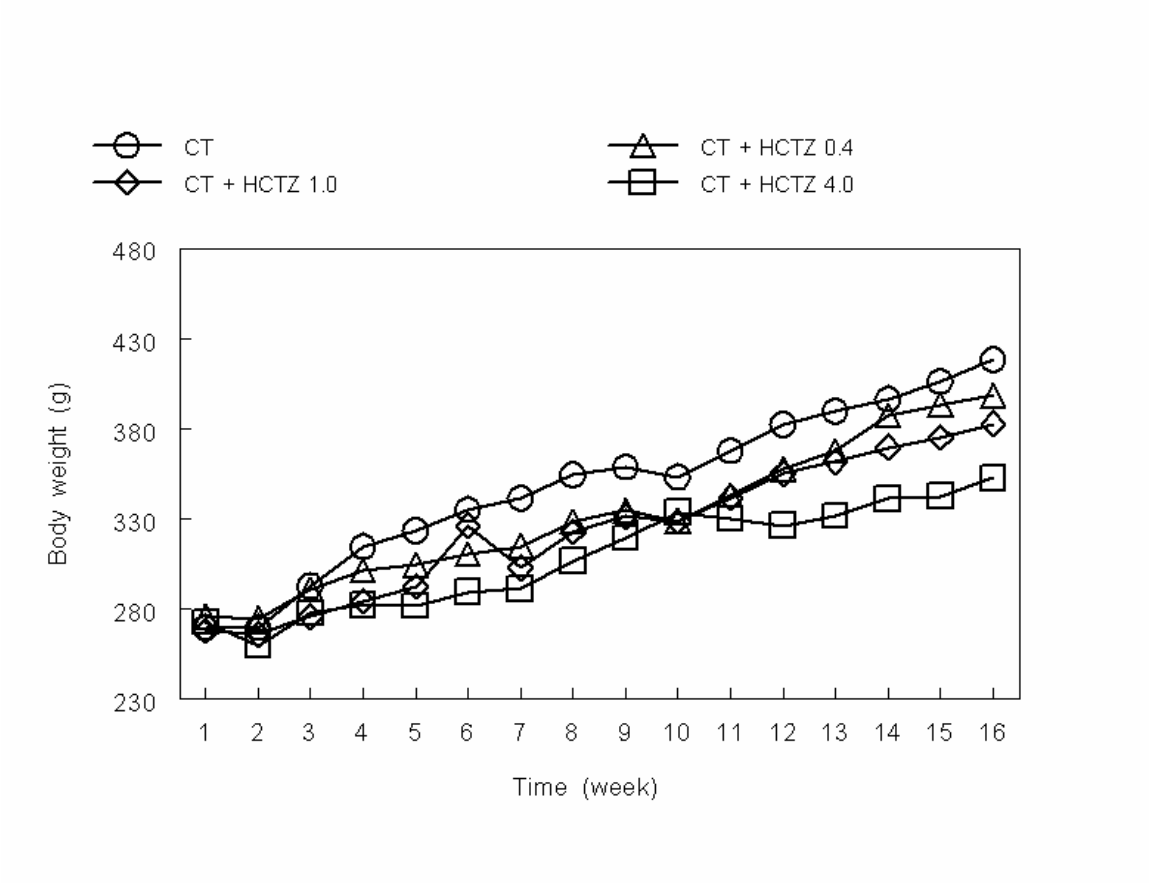

CT
CT + HCTZ 0.4
CT + HCTZ 1.0
CT + HCTZ 4.0
480
430
380
330
280
230
Body weight (g)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Time (week)

Figure 1B

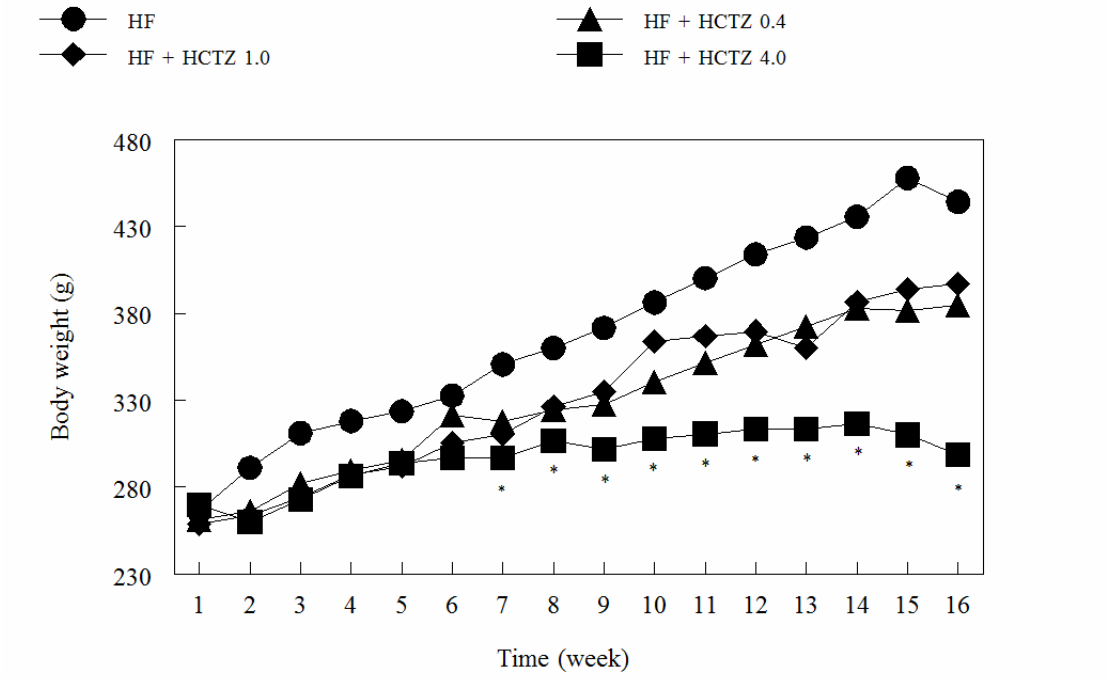

HF
HF + HCTZ 0.4
HF + HCTZ 1.0
HF + HCTZ 4.0
Body weight (g)
480
430
380
330
280
230
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (week)

Table 1: Composition of the diets (g/kg).

Components	High fat diet	Control diet
Sucrose	200	200
Cornstarch	-	280
Casein	180	180
Albumin	22	22
Lard	280	-
Soybean Oil	20	20
Bone's flour	60	60
Wheat bran	188	188
Mineral mixture [1]	40	40
Vitamin mixture [2]	10	10

[1] The mineral mixture contained (g/kg): bone meal (449); NaCl (38); KCl (134.2); $MgSO_4$ (20); $ZnCl_2$ (0.4); $CuSO_4$ (0.175); $MnSO_4$ (1.2); $FeSO_4$ (2), and cornstarch (355).

[2] The vitamin mixture (mg or IU/g) was composed of Vitamin A, 2000 IU; Vitamin D 200 IU; tocopherol, 10 IU; menadione, 0.5 mg; choline, 200 mg; folic acid, 0.2 mg; p-aminobenzoic acid, 1.0 mg; inositol, 10 mg; calcium D-panthotenate, 4.0 mg; riboflavin, 0.8 mg; thiamin-HCl, 0.5 mg; pyridoxine-HCl, 0.5 mg; niacinamide, 0.3 mg; and biotin, 0.04.

Table 2: Effect of hydrochlorothiazide associated with control or high fat diet on body weight, organ weight and total adipose tissue deposits weight.

	CT	CT + HCTZ (0.4 g/kg)	CT + HCTZ (1.0 g/kg)	CT + HCTZ (4.0 g/kg)	HF	HF + HCTZ (0.4 g/kg)	HF + HCTZ (1.0 g/kg)	HF + HCTZ (4.0 g/kg)
Initial body weight (g)	270.0±0.8	276.6±3.9	268.0±12.4	273.0±12.4	266.0±0.8	261.0±6.6	259.0±19.5	269.6±14.4
Final body weight (g)	418.6±17.3 [a,b]	399.0±10.2 [a,b]	383.0±18.5 [a,b]	353.0±21.9 [a,c]	444.2±17.3 [b]	384.6±22.1 [a,b]	397.0±31.3 [a,b]	298.6±39.1 [c]
Body weight gain (g)	148.5±16.4 [a,b]	122.5±7.9 [a,b]	115.0±26.2 [a,b]	80.0±14.1 [a,c]	178.2±17.7 [b]	123.5±15.5 [a,b]	138.0±35.6 [a,b]	29.0±28.3 [c]
Liver (g)	11.2±0.2	11.2±0.4	10.3±0.5	10.7±0.8	11.3±0.3	11.6±0.5	10.8±1.3	10.0±0.5
Total adipose tissue deposits (g) [1]	5.1±0.1 [a]	5.6±0.1 [a]	5.1±0.2 [a]	4.2±0.4 [b]	5.4±0.3 [a]	5.6±0.1 [a]	5.1±0.1 [a]	4.8±0.1 [b]

[1] The total weight of perirenal, mesenteric, epididymal and subcutaneous fat-pads.
Data are expressed as means ± S.E.M. of five animals.
[abc] Mean values within a row not sharing a common superscript letter were significantly different, $p < 0.05$.

Table 3: Effect of hydrochlorothiazide associated with control or high fat diet on biochemical parameters.

	CT	CT + HCTZ (0.4 g/kg)	CT + HCTZ (1.0 g/kg)	CT + HCTZ (4.0 g/kg)	HF	HF + HCTZ (0.4 g/kg)	HF + HCTZ (1.0 g/kg)	HF + HCTZ (4.0 g/kg)
Glucose *	70.8±0.5 [a]	70.8±13.1 [a]	90.0±8.3 [a,b]	89.2±5.8 [a,b]	76.6±4.8 [a]	95.8±10.0 [a,b]	119.2±11.2 [b]	114.0±14.4 [b]
Fructosamine **	0.99±0.13 [a]	1.0±0.01 [a]	1.1±0.02 [a]	1.0±0.07 [a]	1.62±0.07 [b]	1.32±0.06 [b]	1.43±0.04 [b]	1.40±0.2 [b]
Magnesium *	1.4±0.13 [a]	1.3±0.06 [a,b]	1.1±0.02 [b,c,d]	1.1±0.04 [b,c,d]	1.2±0.11 [b,c]	1.0±0.08 [c,d]	0.9±0.03 [d]	0.9±0.04 [d]
Potassium *	6.8±0.16	5.2±0.22 [a]	5.2±0.29 [a]	5.3±0.38 [a]	5.3±0.12 [a]	4.7±0.28 [a]	4.5±0.21 [a]	4.8±0.11 [a]

Data are expressed as means ± S.E.M. of five animals.
[abcd] Mean values within a row not sharing a common superscript letter were significantly different, $p < 0.05$.
* Data of glucose, magnesium and potassium levels are presented as mg/dL.
** Data of fructosamine levels are presented as mmol/L.

Table 4: Effect of hydrochlorothiazide associated with control or high fat diet on TBARS, PCO and vitamin C levels.

	CT	CT+ HCTZ (0.4 g/kg)	CT+ HCTZ (1.0 g/kg)	CT + HCTZ (4.0 g/kg)	HF	HF + HCTZ (0.4 g/kg)	HF + HCTZ (1.0 g/kg)	HF + HCTZ (4.0 g/kg)
TBARS *								
Liver	68.7±0.9 [a]	75.2±0.2 [b,c]	77.4±0.3 [c]	73.3±0.7 [b]	75.1±0.4 [b,c]	75.3±1.6 [b,c]	76.0±1.2 [b,c]	90.5±0.1 [d]
Protein carbonylation **								
Liver	23.2±1.3	18.8±2.3	20.1±0.6	20.7±2.4	17.7±1.0	19.1±0.7	19.1±0.4	19.5±1.0
Vitamin C [#]								
Liver	498.0±20.0 [a]	717.6±32.4 [b,c]	721.1±55.7 [b,c]	696.0±50.9 [b]	684.0±17.4 [b]	811.4±69.1 [b,c]	842.8±59.7 [c]	1198.8±15.6 [d]
Plasma	5.3±0.8	5.8±0.2	5.5±0.8	5.4±0.4	5.9±0.4	7.1±0.6	7.0±0.2	6.0±0.5

Data are expressed as means ± S.E.M. of five animals.
[abcd] Mean values within a row not sharing a common superscript letter were significantly different, $p < 0.05$.
* TBARS is expressed as nmol/g tissue. TBARS: Thiobarbituric acid reactive substances.
** Protein carbonylation is expressed as nmol/mg protein.
[#] Vitamin C is expressed as µg ascorbic acid/g tissue.

CAPÍTULO 3 – Manuscrito 2.

Diphenyl diselenide supplementation reduces biochemical alterations associated to oxidative stress in rats fed with fructose and hydrochlorothiazide

Marinei Cristina Pereira Ribeiro, Roger Monteiro, Daiana Silva Ávila, Nilda Berenice de Vargas Barbosa, Viviane Patrícia Pires Schiar, Danúbia Bonfanti dos Santos, Marta Maria Medeiros Frescura Duarte, Robson Puntel e João Batista Teixeira Rocha.

Submetido à Chemico-Biological Interactions

Diphenyl diselenide supplementation reduces biochemical alterations associated to oxidative stress in rats fed with fructose and hydrochlorothiazide

Marinei Cristina Pereira Ribeiroa,b *, Roger Monteirob, Daiana Silva Ávila[b], Nilda Berenice de Vargas Barbosa[b], Viviane Patrícia Pires Schiar[b], Danúbia Bonfanti dos Santos[b], Marta Maria Medeiros Frescura Duarte[b], Robson Puntel[c] and João Batista Teixeira Rocha[b]

[a] Hospital Universitário de Santa Maria,

Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brasil

[b] Departamento de Química, Centro de Ciências Naturais e Exatas,

Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brasil

[c] Universidade Federal do Pampa, Campus Uruguaiana,

97500-970, Uruguaiana, RS, Brasil

* Corresponding author: Marinei Cristina Pereira Ribeiro or João Batista Teixeira da Rocha
Hospital Universitário de Santa Maria
Universidade Federal de Santa Maria
97105-900 Santa Maria, RS, Brazil
Tel.: +55 55 3220 8513, fax: +55 55 3220 8005
e-mail: mcpribeiro@yahoo.com.br or jbtrocha@yahoo.com.br

ABSTRACT

High fructose diets have been associated with oxidative stress, insulin resistance and metabolic syndrome development. Thiazides, such as hydrochlorothiazide (HCTZ), are frequently used by patients with these disorders for the treatment of hypertension; however they also can exacerbate metabolic disturbances. This study evaluated whether dietary diphenyl diselenide $(PhSe)_2$, a simple synthetic organoselenium compound with antioxidant properties, could reduce the biochemical alterations induced by chronic consumption of diets enriched with fructose and/or HCTZ. Rats were fed with a control diet (CT) or with a high fructose diet (HFD), both supplemented with HCTZ (4.0 g/kg) and/or $(PhSe)_2$ (3 ppm) for 18 weeks. HFD diet caused a significant increase in the levels of glucose, fructosamine, triglycerides and cholesterol of animals, which were not restored to control levels by $(PhSe)_2$ supplementation or potentiated by HCTZ. However, the levels of cholesterol and triglycerides were lower in the groups that received HFD or HCTZ diet supplemented with $(PhSe)_2$. The ingestion of HCTZ caused a decrease in hepatic catalase (CAT) and renal superoxide dismutase (SOD) activities, which were restored by $(PhSe)_2$ supplementation. In liver, $(PhSe)_2$ was also effective in increasing vitamin C levels reduced by HFD and HFD plus HCTZ intake. Indeed, the compound increased *per se* hepatic and renal SOD activity and reduced the oxidation of the lipids and proteins caused by HCTZ associated or not with HFD intake. Furthermore, the association between HFD and HCTZ caused a decrease in potassium levels and aggravated the hypomagnesemia and the hypertriglyceridemia HCTZ-induced. Our findings suggest that some biochemical changes can be aggravated by ingestion simultaneous of HCTZ and HFD diet. In addition, our data also demonstrate that $(PhSe)_2$ supplementation reduces metabolic disorders linked to oxidative stress and that this

compound can be considered a promising agent for treatment of metabolic disturbances HFD and HCTZ-induced, via its antioxidant properties.

Keywords: High fructose diet, hydrochlorothiazide, diphenyl diselenide, oxidative stress.

1. INTRODUCTION

High fructose diets have been commonly used in animal models to induce metabolic changes as those observed in metabolic syndrome, a disorder characterized by insulin resistance, hypertension and dyslipidemia, which are risk factors for cardiovascular diseases and type 2 diabetes mellitus [1,2]. The origin and mechanisms involved on detrimental consequences fructose-induced in animal models are not completely established; however, there are points of evidence that chronic fructose feeding is associated with oxidative damage [3]. In line with this, literature data indicate that high fructose intake can decrease antioxidant defenses and cause an overproduction of reactive oxygen species (ROS) [4,5].

Hydrochlorothiazide (HCTZ) is a diuretic, representative of the benzothiadiazine class of sulfonamide derivatives, usually known as thiazides [6]. The use of HCTZ is beneficial to patients with hypertension because it reduces both morbidity and mortality process [7,8]. In fact, this diuretic prevents strokes, myocardial infarction and congestive heart failure by its blood pressure-lowering efficacy [7-9]. Consequently, thiazides are frequently recommended as the first-line therapy for hypertension [8]. However, HCTZ can cause several adverse effects, such as electrolyte disorders (hypokalemia, hyponatremia, and hypomagnesemia), hyperlipidemia, and impairment of glucose metabolism [10-12]. Moreover, these adverse effects can result in the development or exacerbation of metabolic syndrome and type 2 diabetes, since many patients with hypertension have metabolic disturbances [13].

Selenium is a nutritionally essential trace element to mammals [14,15]. Importantly, selenium plays a crucial role as an integral component of several enzymes with antioxidant properties, including glutathione peroxidase isoforms [16-18]. In addition, its deficiency

has been linked to an increase in the incidence of cardiovascular diseases, immune dysfunctions, cancer, diabetes and metabolic abnormalities [14,15,19]. Of particular importance, literature reports have indicated that some selenium compounds can attenuate diabetes complications via its insulin-mimetic and anti-glycating properties [20,21]. Furthermore, selenium compounds have been documented as pharmacological agents in insulin sensitivity dysfunctions and vascular degenerative lesions in both experimental and clinical diabetes [22,23].

The interest on chemistry and biochemistry of organoselenium compounds have increased in the last three decades mainly due the antioxidant activity exhibited by several selenium organic forms [16]. In this context, diphenyl diselenide $(PhSe)_2$, the simplest of the synthetic diaryl diselenides, has glutathione peroxidase-like activity and other antioxidant properties [24]. Importantly, this compound also displays neuroprotective, anti-nociceptive, anti-hipercolesterolemic and anti-inflammatory activities in different experimental models of human pathologies [16,25-29]. Regarding to metabolic disorders, recent data from our group have demonstrated that chronic treatment with $(PhSe)_2$ reduces hyperglycemia and biochemical changes associated to oxidative stress in streptozotocin-treated rats [26,30]. However, the effect of $(PhSe)_2$ in metabolic disturbances induced by other factors as diets enriched with carbohydrates and/or by HCTZ treatment is not available in literature. Hence, the present study was designed to evaluate whether $(PhSe)_2$ supplementation could prevent or reduce biochemical alterations caused by fructose and\or HCTZ intake. Furthermore, we were interested to know whether thiazides consumption could exacerbate these features, since metabolic disorders are common in patients with hypertension.

2. MATERIALS AND METHODS

2.1. Chemicals

Casein (technical grade), coomassie brilliant blue G, 2,4-dinitrophenylhydrazine, HCl, sodium sulphate dodecyl, heptane, acetate, ethanol, malondialdehyde (MDA) and thiobarbituric acid (TBA) were obtained from Sigma, (St. Louis, MO., USA). Mono and dibasic potassium phosphate, acetic acid, ascorbic acid, ortho-phosphoric acid, tris buffer (tris[hydroxymethyl]aminomethane), fructose and trichloroacetic acid were obtained from Merck (Rio de Janeiro, Brazil). Diphenyl diselenide compound was synthesized by method described by Paulmier [31]. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(PhSe)_2$ (99.9%) was determined by GC/HPLC. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2 Animals and diets

Adult male Wistar rats (2 months old), weighing 250-300 g were used for the experiments. The animals were kept on a 12 h light/12 h dark cycle, in a room with the temperature regulated to 21-25 ºC and humidity at roughly 56% with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil.

Rats were fed for 18 wk and randomly divided into eight experimental groups (n=6): (1) control (CT); (2) CT + HCTZ (4.0 g/kg); (3) CT + $(PhSe)_2$ (3 ppm); (4) CT + HCTZ + $(PhSe)_2$; (5) high fructose diet (HFD); (6) HFD + HCTZ; (7) HFD + $(PhSe)_2$ and (8) HFD + HCTZ + $(PhSe)_2$. The composition of the diets is shown in Table 1. Diphenyl diselenide was dissolved in soybean oil and mixed in the diet with a food mixer to insure

uniform distribution. This diet provided 3 μg selenium/g of diet/per day, which is considered acceptable amount for this element [23]. The diets were prepared weekly and stored at 4 ºC.

2.3. Blood samples and tissue preparation

At the end of the experimental period, rats were fasted 12 h, anesthetized with ether and sacrificed by decapitation. A blood fraction was collected without anticoagulant and was centrifuged at 4000 × g for 10 min to yield serum samples that were used for fructosamine, total cholesterol, triglycerides, magnesium, potassium, urea and creatinine determination. A parallel blood fraction was collected in tubes with sodium fluoride to determine glucose concentration.

The samples of the tissues were quickly removed, rinsed with saline, weighted, placed on ice and homogenized in 10 volumes (w/v) in cold 50 mM Tris-HCl pH 7.4. The homogenate was centrifuged at 4000 x g at 4ºC for 10 min to yield low-speed supernatant fraction (S1) that was used for biochemical assays, except for measurement of protein carbonylation, which was determined in samples of the homogenates. In addition, adipose tissue deposit (total weight of perirenal, mesenteric, epididymal and subcutaneous fat-pads) content was also determined.

2.4. Measurements

Food and water consumption were measured daily at the same time (9:00 to 10:00 h). The body weights were determined once a week.

2.5. Biochemical analysis

Plasma glucose concentration was measured as previously described by Bergmeyer [32] and fructosamine concentration was determined as described by Baker et al. [33]. Serum total cholesterol concentration was measured as previously described by Alain et al. [34] and triglycerides concentration was measured as described by Bucolo and David [35]. The levels of magnesium were estimated according to the method of Bohuon [36] and potassium levels were measured as previously described by Kavanagh and Mills [37]. In addition, serum urea and creatinine were determined by method of Bergmeyer [38] and, Yatzidis [39], respectively. The biochemical assays were done using a Johnson & Johnson:Vitros 750XRC chemistry analyzer.

2.6. Lipid peroxidation (LPO) levels

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa et al. [40], in which MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. In brief, samples were incubated at 100ºC for 60 min in acid medium containing 0.45% sodium dodecyl sulfate, 1.27 mol/L acetic acid/270 mmol/L HCl, pH 3.5 and 0.8% thiobarbituric acid. TBARS produced were measured at 532 nm and the absorbance was compared with the standard curve using malondialdehyde.

2.7. Protein carbonyl (PCO) content

The PCO content was determined as described by method of Levine et al. [41] with some modifications. Briefly, homogenates were diluted to 750-800 μg/mL of protein in

each sample, and 1 mL aliquots were mixed with 0.2 mL of 2,4-dinitrophenylhydrazine (DNPH, 10 mM) or 0.2 mL HCl (2 M). After incubation at room temperature for 1h in a dark ambient, 0.6 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 1.8 mL of heptane (99.5%) and 1.8 mL of ethanol (99.8%) were sequentially added, and mixed with vortex agitation for 40 sec and centrifuged for 15 min. The protein isolated from the interface was washed two times with 1 mL of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 mL of denaturing buffer. Each DNPH sample was read at 370 nm in a spectrophotometer against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000$M^{-1}cm^{-1}$. Protein was measured by method of Bradford [42] using bovine serum albumin as standard.

2.8. Vitamin C levels

Hepatic and renal vitamin C (ascorbic acid) levels were determined by method of Jacques-Silva et al. [43]. Proteins of liver and kidney were precipitated with 1 vol. of a cold 10% trichloroacetic acid followed by centrifugation. An aliquot of 300 µL of the supernatants were mixed with 2,4-dinitrophenylhydrazine (4.5 mg/mL), $CuSO_4$ (0.075 mg/mL) and trichloroacetic acid 13.3% (final volume 1 mL) and incubated for 3 h at 37ºC. Then 1 mL of H_2SO_4 65% (v/v) was added to the medium. Ascorbic acid levels were measured spectrophotometrically at 520 nm and calculated using a standard curve (1.5-4.5 µmol/L ascorbic acid freshly prepared in sulfuric acid).

2.9. Catalase (CAT) activity

The measurement of CAT activity from liver and kidney was determined as described by method of Aebi [44]. Samples of S1 from liver and kidney were added to a

cuvette and the reaction was started by the addition of 70 µL of freshly prepared 300 mM H_2O_2 in phosphate buffer (50 mM, pH 7.0; total volume of incubation: 1 mL). The rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm during 120 s. One unit of the enzyme is considered as the amount of enzyme which decomposes 1 µmol H_2O_2/min at pH 7.0.

2.10. Superoxide dismutase (SOD) activity

Hepatic and renal SOD activity in S1 was determined as described by Misra and Fridovich [45]. This method is based on the capacity of SOD in inhibiting autoxidation of adrenaline to adrenochrome. The color reaction was measured at 480 nm. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26°C. The S1 was diluted 1:10 (v/v) for determination of SOD activity in the test day. Aliquots of supernatant were added in a glycine buffer 50 mM pH 10.3. Enzymatic reaction was started by adding of epinephrine. One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 26°C. The enzymatic activity was expressed as Units U/mg protein.

2.11. Statistical analysis

All values obtained are expressed as mean ± standard error. Data were analyzed by one-way, two-way or three-way ANOVA analysis of variance, followed by Duncan's multiple range tests when appropriate. Differences between groups were considered to be significant when $p < 0.05$.

3. RESULTS

3.1. Body weight, organ weight and total adipose tissue deposits weight

The data of table 2 show that the intake of HFD, HCTZ or $(PhSe)_2$ alone did not modify neither body weight gain nor final body weight of the rats when compared to control group (Table 2, $p > 0.05$). On the other hand, three-way ANOVA (2 diets (CT or HFD) x 2 (CT or HCTZ) x 2 (CT or $(PhSe)_2$) revealed significant HCTZ x HFD interaction, indicating that HCTZ plus HFD caused a reduction in final body weight (Table 2, $p < 0.05$).

The consumption of $(PhSe)_2$ diet caused a significant increase in the organ weight for liver and kidney when compared to control group (Table 2, $p < 0.05$).

HCTZ caused *per se* a significant reduction in total adipose tissue deposits weight when compared to control group (Table 2, $p < 0.05$), that were not restored by $(PhSe)_2$ supplementation or modified in HCTZ plus HFD group.

3.2. Water and food consumption

Water consumption of animals fed with HFD or HCTZ alone was significantly greater than animals fed with control diet (Table 3, $p < 0.05$). Moreover, three-way ANOVA (2 diets (CT or HFD) x 2 (CT or HCTZ) x 2 (CT or $(PhSe)_2$) revealed significant HCTZ x HFD interaction (Table 3, $p < 0.05$), showing that HCTZ and HFD caused a increase in water consumption. The animals supplemented with HFD diet also had a significant reduction in food consumption when compared to control groups (Table 3, $p < 0.05$).

3.3. Blood glucose and fructosamine levels

The results of table 4 demonstrate that HCTZ and $(PhSe)_2$ intake did not change the levels of glucose and fructosamine of rats. Different, HFD consumption caused a significant increase in blood glucose and fructosamine contents of animals when compared to values found in control group (Table 4, $p < 0.05$). However, $(PhSe)_2$ supplementation was not effective in restoring the levels of glucose and fructosamine enhanced by HFD intake (Table 4, $p > 0.05$).

3.4. Total cholesterol and triglycerides levels

The animals fed with HFD diets had a significant increase in total cholesterol levels when compared to values found in control group (Table 4, $p < 0.05$). Statistical analysis revealed again that the intake of HCTZ and HFD diets caused a significant increase in the levels of triglycerides and that this enhance was proportionally higher in the HFD groups. The data of table 4 also demonstrate that triglycerides and cholesterol levels were lower in the groups that received HFD and HCTZ diet supplemented with $(PhSe)_2$.

3.5. Magnesium and potassium levels

HCTZ intake caused a significant decrease in magnesium levels, which were not prevented by $(PhSe)_2$ supplementation when compared to control group (Table 4, $p < 0.05$). Furthermore, the reduction in magnesium levels HCTZ-induced was higher in the groups that received HCTZ associated to HFD. However, in this reduction in the magnesium levels did not modify by $(PhSe)_2$ supplementation. Similarly, the animals fed with HCTZ plus HFD had an decrease in the content of potassium, which were not prevented by $(PhSe)_2$ supplementation when compared to values found in control group (Table 4, $p < 0.05$). In

these parameters, three-way ANOVA (2 diets (CT or HFD) x 2 (CT or HCTZ) x 2 (CT or $(PhSe)_2$) revealed a significant HCTZ x HFD interaction (Table 4, $p < 0.05$), indicating that HCTZ plus HFD caused a reduction in magnesium and potassium levels. Of particular importance, negative correlations were found between the magnesium and glucose levels ($r = - 0.18$, $p < 0.05$); magnesium and fructosamine levels ($r = - 0.09$, $p < 0.05$), as well as potassium and glucose levels ($r = - 0.18$, $p < 0.05$) and between potassium and fructosamine levels ($r = - 0.05$, $p < 0.05$). Indeed, positive correlation was found between the potassium and magnesium levels ($r = 0.42$, $p < 0.05$).

3.6. Urea and creatinine levels

No significant difference was observed in the levels of urea and creatinine of groups (Table 4, $p > 0.05$).

3.7. Lipid peroxidation (LPO) levels

HFD, HCTZ and HFD plus HCTZ intake caused a significant increase on hepatic and renal lipid peroxidation (Fig. 1A and 1B). Moreover, three-way ANOVA of LPO levels in liver and kidney revealed a significant HFD x HCTZ interaction ($p < 0.05$). In both tissues, $(PhSe)_2$ supplementation was effective in reducing the LPO induced by consumption of HFD plus HCTZ diet (Fig. 1A and 1B, $p < 0.05$).

3.8. Protein carbonyl content

HCTZ, HFD and HCTZ plus HFD intake caused a significant increase in hepatic and renal protein oxidation (Fig. 2A and 2B, $p < 0.05$), which were restored to control levels by $(PhSe)_2$ supplementation (Fig. 2A and 2B, $p < 0.05$).

3.9. Vitamin C levels

The data of figure 3A show that HCTZ, HFD and HCTZ plus HFD intake caused a significant decrease in hepatic vitamin C levels, which were restored to control levels by $(PhSe)_2$ supplementation only in HFD-fed rats (Fig. 3A, $p < 0.05$). In liver, three-way ANOVA of vitamin C levels revealed a significant HFD x HCTZ interaction ($p < 0.05$). Contrary, no significant difference was observed in renal vitamin C content between groups (Fig. 3B, $p > 0.05$).

3.10. Catalase and SOD activities

HCTZ and HCTZ plus HFD diet caused a significant reduction in hepatic catalase activity that was restored to control levels by $(PhSe)_2$ supplementation (Fig. 4A, $p < 0.05$). Different, no change was observed in renal catalase activity of groups (Fig. 4B, $p > 0.05$).

The consumption of HCTZ, HFD or HCTZ plus HFD did not modify hepatic SOD activity (Fig. 5A, $p < 0.05$). On the other hand, all groups supplemented with $(PhSe)_2$ had an increase in SOD activity of liver. In kidney, HCTZ alone or associated to HFD caused a significant decrease in renal SOD activity of rats that was restored to control values by $(PhSe)_2$ supplementation (Fig. 5B, $p < 0.05$).

4. DISCUSSION

Thiazide diuretics are frequently used in patients with metabolic disorders as well as in type 2 diabetes for treatment of hypertension. However, the chronic use of thiazides also may exacerbate theses metabolic alterations [7-13]. Indeed, literature data have reported that long-term consumption of HFD as well as HCTZ treatment are important factors for the appearance of some metabolic changes related to type 2 diabetes [46-49]. According, in

the present study we observe that HFD intake, associated or not with HCTZ, increased glucose, fructosamine, total cholesterol and triglycerides levels, which are factors related with the development of insulin resistance. Thus, we can suggest that the experimental protocol used in this work, with certain limitations, mimics the epidemiological data from literature and may indicate that simultaneous ingestion of HFD and thiazides as diuretics for the treatment of hypertension potentiates the adverse effects of this class of drug.

It has been suggested that the oxidative stress is partly responsible for metabolic disorders induced by diets enriched with carbohydrates and that antioxidants are potential therapeutic agents by preventing or reducing the oxidative damage [46-49]. In this way, numerous studies have showed that selenium supplementation increase antioxidant defenses; decrease lipid peroxidation levels and up-regulated mRNA expression for antioxidant enzymes in patient and animal diabetics [50-52]. In line with this, our results showed that $(PhSe)_2$ supplementation provided a protective effect against the alterations in antioxidant defenses induced by HFD and HCTZ intake. In fact, the long-term $(PhSe)_2$ intake restored the decrease in hepatic vitamin C levels caused by association between HCTZ and HFD as well as reduced the lipids and proteins oxidation in liver and kidney of animals fed with HCTZ associated or not with HFD. Indeed, $(PhSe)_2$ dietary was able on reversing the reduction in CAT (liver) and SOD activities (kidney) HCTZ-induced. The antioxidant potential of $(PhSe)_2$ can be explained, at least in part, by its glutathione peroxidase-like activity [24]. Interestingly, this compound increased *per se* hepatic and renal SOD activity.

Another point that we have observed was that $(PhSe)_2$ intake did not attenuate the increase of glycemia induced by HFD consumption. The lack of anti-hyperglycemic activity of compound in this protocol model can be related with the route used. This

hypothesis is supported by studies showing that chronic administration of $(PhSe)_2$, via subcutaneous, promotes a significant decrease in plasma glucose levels of streptozotocin-induced diabetic rats [26].

The chronic intakes of an HFD as well as hyperglycemia condition are linked to oxidative stress generation and increased levels of ROS with the development of insulin resistance [3,4,53]. In this context, fructose is considered a potent agent involved in the glycoxidation process, therefore may intensify oxidative stress [54]. In general, the nonenzymatic glycation of proteins has been postulated to explain the relationship between hyperglycemia and oxidant events as lipid peroxidation [55]. This process generates radicals and highly reactive oxidants from the glycated proteins under physiologic conditions [55]. However, data about the potential facilitating effects of HCTZ in promoting insulin resistance and oxidative stress in animal models is scarce or lacking in literature. Regarding to, our results suggest that HFD associated or not with HCTZ elevates glucose and fructosamine levels, which can trigger oxidative damage in hepatic and renal tissues, since TBARS and protein carbonylation were enhanced in these tissues.

It has been showed that long-term treatment with thiazide diuretics may impair glucose tolerance and decrease insulin sensitivity and thereby accelerate the development of diabetes mellitus [56]. In addition, diuretic therapy has been associated with increases in serum total cholesterol and LDL-cholesterol of up to 10% of baseline levels. Moreover, triglyceride concentrations can increase by 5% to 15% [57]. Different, here we observed an increase in the total cholesterol levels only when HCTZ was associated with HFD in rats. Furthermore, our results demonstrated that HCTZ caused an increase in triglycerides levels that was aggravated by association between HFD and HCTZ. Moreover, $(PhSe)_2$ intake

attenuated the increase of triglycerides and cholesterol levels caused by HFD or HCTZ intake.

It is important to emphasize that HCTZ caused a decrease in magnesium levels and that the association between HFD and HCTZ aggravated the hypomagnesemia condition. Through these data we can suggest that hypertensive patients that use HCTZ and eat a diet with high fructose content may present loss of magnesium, a fact that may contribute to metabolic disorders and oxidative stress development. Furthermore, the use of HCTZ by these patients could precipitate further the onset of diabetes and its complications. In fact, several studies suggest that intracellular magnesium may play a key role in modulating insulin-mediated glucose uptake and vascular tone [58,59]. Accordingly, points of evidence indicate an association between low magnesium levels and metabolic diseases, including the type 2 diabetes mellitus, hypertension [58,59] and increased free radical dependent-oxidative tissue damage [59]. In addition, has been reported that hypokalemia may be an important factor in hyperglycemia, hyperinsulinemia, and insulin resistance in patients receiving HCTZ, because these features can be restored with potassium supplements [13]. Our results also show that potassium depletion is significantly associated with exacerbation of hyperglycemia as well as hypomagnesemia. Thus, we can suggest that the model experimental developed in this work can be used for further studies regarding HCTZ toxicity, as well as to clarify the role of magnesium and potassium in insulin resistance associated with HCTZ treatment and high fructose ingestion. Besides, this model may also allow the investigations whether the incidence of metabolic disturbances as well as type 2 diabetes HCTC-induced is more frequent in hypertensive patients who eat diets with high fructose content. Indeed we suggest that the reduction in electrolytes is related with HCTZ, since the animals treated with fructose or $(PhSe)_2$ did not show these changes.

5. CONCLUSIONS

In conclusion, the results obtained in this study suggest that some biochemical changes can be aggravated by ingestion simultaneous of HCTZ and HFD diet. In addition, our data demonstrate that $(PhSe)_2$ supplementation reduces metabolic disorders linked to oxidative stress and that this compound can be considered a promising agent for treatment of metabolic disturbances by antioxidant therapy. Nevertheless, it will be important to determine whether similar protection can be provided by this way in humans.

Acknowledgements

The financial support by FINEP Research Grant ‘‘Rede Instituto Brasileiro de Neurociência (IBN-Net)’’ # 01.06.0842-00, FAPERGS, CAPES/SAUX, VITAE Fundation and CNPq is gratefully acknowledged. J.B.T.R. is the recipient of CNPq fellowships.

REFERENCES

[1] G.M. Reaven, Banting lecture. Role of insulin resistance in human disease, Diabetes 37 (1988) 1595-1607.

[2] G.M. Reaven, A. Laws, Insulin resistance, compensatory hyperinsulinaemia and coronary heart disease, Diabetologia 37 (1994) 948-952.

[3] R.B. McDonald, Influence of dietary sucrose on biological aging, Am. J. Clin. Nutr. 62 (1995) 284-293.

[4] Y. Rayssiguier, E. Gueux, D. Weiser, Effect of magnesium deficiency on lipid metabolism in rats fed a high carbohydrate diet, J. Nutr. 111 (1981) 1876-1883.

[5] V.B. Brito, V. Folmer., J.C.M. Soares, I.D. Silveira, J.B.T. Rocha, Long-term sucrose and glucose consumption decreases the δ-aminolevulinate dehydratase activity in mice, Nutrition 23 (2007) 818-826.

[6] C. Dollery, Therapeutic Drugs, 2nd ed., Churchill Livingstone, 1998.

[7] F. Turnbull, Effects of different blood-pressure-lowering regimens on major cardiovascular events: Results of prospectively-designed overviews of randomized trials, Lancet 362 (2003) 1527-1535.

[8] The ALLHAT Study Group, Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium-channel blocker vs. diuretic, JAMA 288 (2002) 2981-2997.

[9] G.T. McInnes, W.W. Yeo, L.E. Ramsey, M. Moser, Cardiotoxicity and diuretics: much speculation-little substance, J. Hypertens. 10 (1992) 317-335.

[10] F.L. Plavinik, C.I. Rodrigues, M.T. Zanella, A.B. Ribeiro, Hypokalemia, glucose intolerance, and hyperinsulinemia during diuretic therapy, Hypertension 19 (1992) 1126-1129.

[11] H.A. Punzi, C.F. Punzi, Antihypertensive and Lipid-Lowering Heart Attack Trial Study; Trinity Hypertension Research Institute: Metabolic issues in the Antihypertensive and Lipid-Lowering Heart Attack Trial Study, Curr. Hypertens. Rep. 6 (2004) 106-110.

[12] P. Verdecchia, G. Reboldi, F. Angeli, C. Borgioni, R Gattobigio, L Filippucci, S. Norgiolini, C. Bracco, C. Porcellati, Adverse prognostic significance of new diabetes in treated hypertensive subjects, Hypertension 43 (2004) 963-969.

[13] S. Reungjui, C.A. Roncal, W. Mu, T.R. Srinivas, D. Sirivongs, R.J. Johnson, T. Nakagawa, Thiazide Diuretics Exacerbate Fructose-Induced Metabolic Syndrome, J. Am. Soc. Nephrol. 18 (2007) 2724-2731.

[14] M. Navarro-Alarcón, M.C. Lopes-Martinez, Essentiality o selenium in the human body: relationship with different diseases, Sc. Total Environ. 249 (2000) 347-371.

[15] M.P. Rayman,. The importance of selenium to human health, Lancet 356 (2000) 233-241.

[16] C.W. Nogueira, G. Zeni, J.B.T. Rocha, Organoselenium and organotellurium compounds: toxicology and pharmacology, Chem. Rev. 104 (2004) 6255-6285.

[17] L. Flohe, W.A. Gunzler, H.H. Schock, Glutathione peroxidase: a selenoenzyme, FEBS. Lett. 32 (1973) 132-134.

[18] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase, Science 179 (1973) 588-590.

[19] G.F. Combs, P.G. Gray, Chemopreventive agents: selenium, Pharmacol. Ther. 79 (1998) 179-192.

[20] O. Ezaki, The insulin-like effects of selenate in rat adipocytes J. Biol. Chem. 265 (1990) 1124-1130.

[21] J.A. Vinson, T.B. Howard, Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients, J. Nutr. Biochem. 7 (1996) 659-663.

[22] D. Bonnefont-Rousselot, The role of antioxidant micronutrients in the prevention of diabetic complications, Treat. Endocrinol. 3 (2004) 41-52.

[23] P. Faure, D. Barclay, M. Joyeux-Faure, S. Halimi, Comparison of the effects of zinc alone and zinc associated with selenium and vitamin E on insulin sensitivity and oxidative stress in high-fructose-fed rats, J. Trace Elem. Med. Biol. 21 (2007) 113-119.

[24] S.R. Wilson, P.A. Zucker, R.R.C. Huang, A. Spector, Development of synthetic compounds with glutathione peroxidase activity, J. Am. Chem. Soc. 111 (1989) 5936-5939.

[25] G. Ghisleni, L.O. Porciuncula, H. Cimarostia, J.B.T. Rocha, C.G. Salbego, D.O. Souza, Diphenyl diselenide protects rat hippocampal slices submitted to oxygen-glucose deprivation and diminishes inducible nitric oxide synthase immunocontent, Brain Res. 986 (2003) 196-199.

[26] N.B.V. Barbosa, J.B.T. Rocha, D.C. Wondracek, J. Perottoni, G. Zeni, C.W. Nogueira, Diphenyl diselenide reduces temporarily hyperglycemia: Possible relationship with oxidative stress, Chem. Biol. Interact. 163 (2006) 230-238.

[27] F.B. Zasso, C.E.P. Gonçales, E.A.C. Jung, D. Araldi, G. Zeni, J.B.T. Rocha, C.W. Nogueira, On the mechanisms involved in antinociception induced by diphenyl diselenide, Environ. Toxicol. Pharmacol. 19 (2005) 283-289.

[28] A.F. de Bem, M. Farina, R.L. Portella, C.W. Nogueira, T.C.P. Dinis, J.A.N. Laranjinha, L.M. Almeida, J.B.T. Rocha, Diphenyl diselenide, a simple glutathione peroxidase mimetic, inhibits human LDL oxidation in vitro, Atherosclerosis 201 (2008) 92-100

[29] A.F. de Bem, R.L. Portella, E. Colpo, M.M.M.F. Duarte, A. Frediane, P.S. Taube, C.W. Nogueira, M. Farina, E.L. Silva, J.B.T. Rocha, Diphenyl Diselenide Decreases

Serum Levels of Total Cholesterol and Tissue Oxidative Stress in Cholesterol-fed Rabbits, Basic Clin. Pharmacol. Toxicol. 105 (2008) 17-23.

[30] N.B.V. Barbosa, J.B.T. Rocha, J.C.M. Soares, D.C. Wondracek, J.F. Gonçalves, M.R.C. Schetinger, C.W. Nogueira, Dietary diphenyl diselenide reduces the STZ-induced toxicity, Food Chem. Toxicol. 46 (2008) 186-194.

[31] C. Paulmier, Selenium Reagents and Intermediates in Organic Synthesis, Pergamon Books, Oxford, UK, 1986.

[32] H.U. Bergmeyer, Methods of Enzimatic Analisys, 3ed, Deerfield Beach:Veriag Chemie, 6 (1984) 163-172.

[33] J.R. Baker, P.A. Metcalf, R.N. Johnson, D. Newman, P. Rietz, Use of protein-based standards in automated colorimetric determinations of fructosamine in serum, Clin. Chem. 31 (1985) 1550-1554.

[34] C.C. Allain, L.S. Poon, C.S.G. Chan, W. Richmond, P.C. Fu. Enzymatic determination of total serum-cholesterol Clin. Chem. 20 (1974) 470-475.

[35] G. Bucolo, H. David, Quantitative-determination of serum triglycerides by use of enzymes, Clin. Chem. 19 (1973) 476-482.

[36] C. Bohuon, Microdetermination of magnesium in biological fluids, Clin. Chim. Acta 7 (1962) 811-817.

[37] K. Kavanagh, J.N. Mills, Measuring electrolytes: An evaluation of the IDEXX Vetlyte ion selective electrode method, Aust. Vet. Pract. 27 (1997).97-102.

[38] H.U. Bergmeyer, Methods of Enzymatic Analisys, 3 ed, Deerfield Beach, 8 (1985) 444-449.

[39] H. Yatzidis, New method for direct determination of true creatinine, Clin. Chem. 20 (1974) 1131-1134.

[40] H. Ohkawa, H. Ohishi, K. Yagi, Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351-358.

[41] R.L. Levine, D. Garland, C.N. Oliver, A Amici, I. Climent, A. Lenz, B. Ahn, S. Shaltiel, E.R. Stadtman, Determination of carbonyl content in oxidatively modified proteins, Methods Enzymol. 186 (1989) 464-478.

[42] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-254.

[43] M.C. Jacques-Silva, C.W. Nogueira, L.C. Broch, E.M. Flores, J.B.T. Rocha, Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice, Pharmacol. Toxicol. 88 (2001) 119-125.

[44] H. Aebi, Catalase ‘‘in vitro’’, Methods Enzymol. 105 (1984) 121-127.

[45] H.P. Misra, I. Fridovich, A peroxide-dependent reduction of cytochrome-*C* by NADH, Biochim. Biophys. Acta 3 (1972) 815-824.

[46] S.S. Reddy, P. Ramatholisamma, R. Karuna, D. Saralakumari, Preventive effect of Tinospora cordifolia against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats, Food Chem. Toxicol. 47 (2009) 2224-2229.

[47] E. Ho, N. Quan, Y.H. Tsai, W. Lai, T.M. Bray, Dietary zinc supplementation inhibits NF-kappa B activation and protects against chemically induced diabetes, Exp. Biol. Med. 226 (2001) 103-111.

[48] P.N. Chander, O. Gealekman, S.V. Brodsky, S. Elitok, A. Tojo, M. Crabtree, S.S. Gross, S. Goligorsky, Nephropathy in zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen, J. Am. Soc. Nephrol. 15 (2004) 2391-2403.

[49] M. Miranda, M. Muriach, J. Roma, F. Bosh-Morell, J.M. Genoves, J. Barcia, J. Araiz, M. Diaz-Llospis, F.J. Romero, Oxidative stress in a model for experimental diabetic retinopathy: the utility of peroxinitrite scavengers, Arch. Soc. Esp. Oftalm. 81 (2006) 27-32.

[50] M. Naziroglu, M. Cay, Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanisms in rats with diabetes induced by streptozotocin, Biol. Trace Elem. Res. 79 (2001) 149-159.

[51] P. Faure, Protective effects of antioxidant micronutrients (vitamin E, zinc and selenium) in type 2 diabetes mellitus, Clin. Chem. Lab. Med. 41 (2003) 995-998.

[52] P. Faure, O. Ramon, A. Favier, S. Halimi, Selenium supplementation decreases nuclear factor-kappa B activity in peripheral blood mononuclear cells from type 2 diabetic patients, Eur. J. Clin. Invest. 7 (2004) 475-481.

[53] K. Maiese, S.D. Morhan, Z.Z. Chong, Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus, Curr. Neurovasc. Res. 4 (2007) 63-71.

[54] C.J. Mullarkey, D. Edelstein, M. Brownlee, Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes, Biochem. Biophys. Res. Commun. 173 (1990) 932-939.

[55] J.P. Lopes, S.M. Oliveira, J.S. Fortunato, Oxidative stress and its effects on insulin resistance and pancreatic beta-cells dysfunction: relationship with type 2 diabetes mellitus complications, Acta Med. Port. 213 (2008) 293-302.

[56] G. Bonner, Hyperinsulinemia, insulin resistance, and hypertension, J. Cardiovasc. Pharmacol. 24 (1994) 39-49.

[57] P. Weidmann, M. Courten, P. Ferrari, Effects of diuretics on the plasma lipid profile, Eur. Heart J. 13 (1992) 61-67.

[58] M. Barbagallo, L.J. Dominguez, A. Galioto, A. Ferlisi, C. Cani, L. Malfa, A. Pineo, A. Busardo, G. Paolisso, Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X, Mol. Aspects Med. 24 (2003) 39-52.

[59] L.M. Lourdes, T. Crua, J.C. Pousada, L.E. Rodrigues, K. Barbosa, V. Canguru, The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes, Diabetes Care 21 (1998) 682-686.

Figures legends

Figure 1. Thiobarbituric acid-reactive species content in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake. Data are expressed as means ± SEM of six animals. [abcd] Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan). CT, control; HFD, high fructose diet; HCTZ, hydrochlorothiazide; Se, diphenyl diselenide; MDA, malondialdehyde.

Figure 2. Protein carbonylation levels in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake. Data are expressed as means ± SEM of six animals. [abc] Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan). CT, control; HFD, high fructose diet; HCTZ, hydrochlorothiazide; Se, diphenyl diselenide.

Figure 3. Vitamin C levels in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake. Data are expressed as means ± SEM of six animals. [abcd] Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan). CT, control; HFD, high fructose diet; HCTZ, hydrochlorothiazide; Se, diphenyl diselenide.

Figure 4. Catalase activity in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake. Data are expressed as means ± SEM of six animals. [a] Mean values that do not share a common superscript letter were significantly

different, $p < 0.05$ (ANOVA/Duncan). CT, control; HFD, high fructose diet; HCTZ, hydrochlorothiazide; Se, diphenyl diselenide.

Figure 5. Superoxide dismutase activity in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake. Data are expressed as means ± SEM of six animals. [abc] Mean values that do not share a common superscript letter were significantly different, $p < 0.05$ (ANOVA/Duncan). CT, control; HFD, high fructose diet; HCTZ, hydrochlorothiazide; Se, diphenyl diselenide.

Figures

Figure 1A

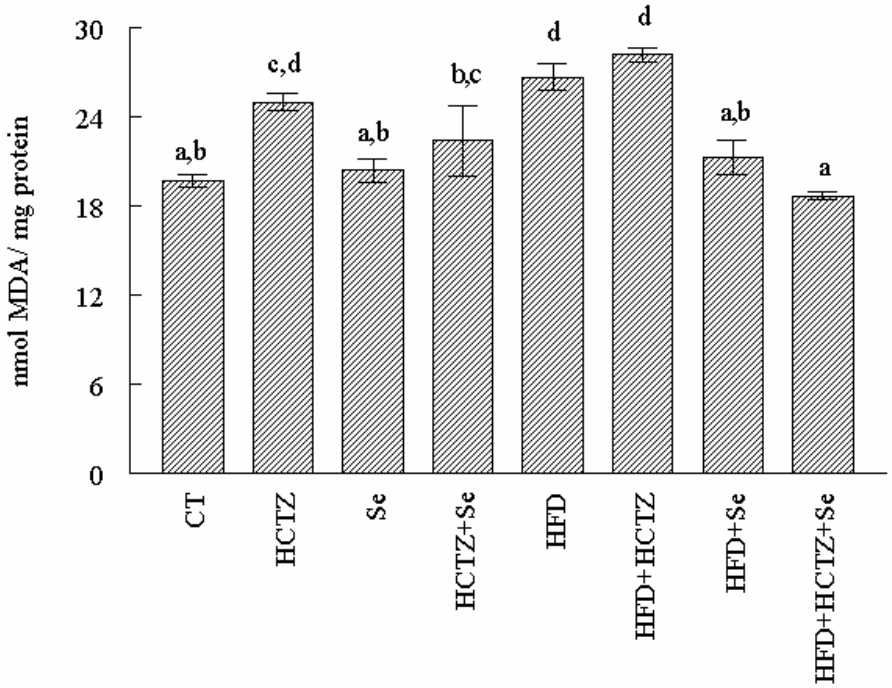


Figure 1B

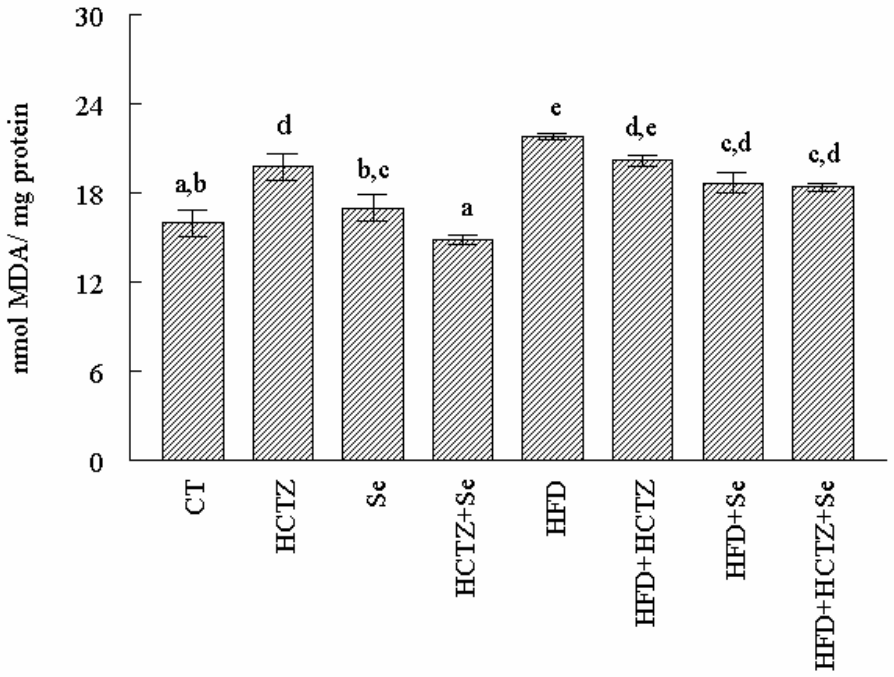

30
24
18
12
6
0
nmol MDA/ mg protein
a,b
d
b,c
a
e
d,e
c,d
c,d
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 2A

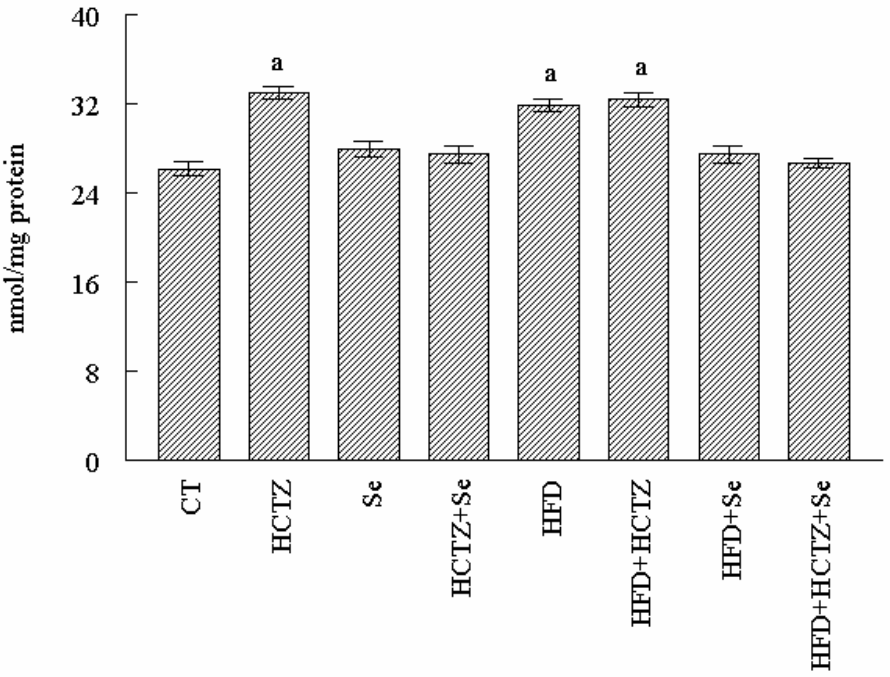

40
32
24
16
8
0
nmol/mg protein
a
a
a
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 2B

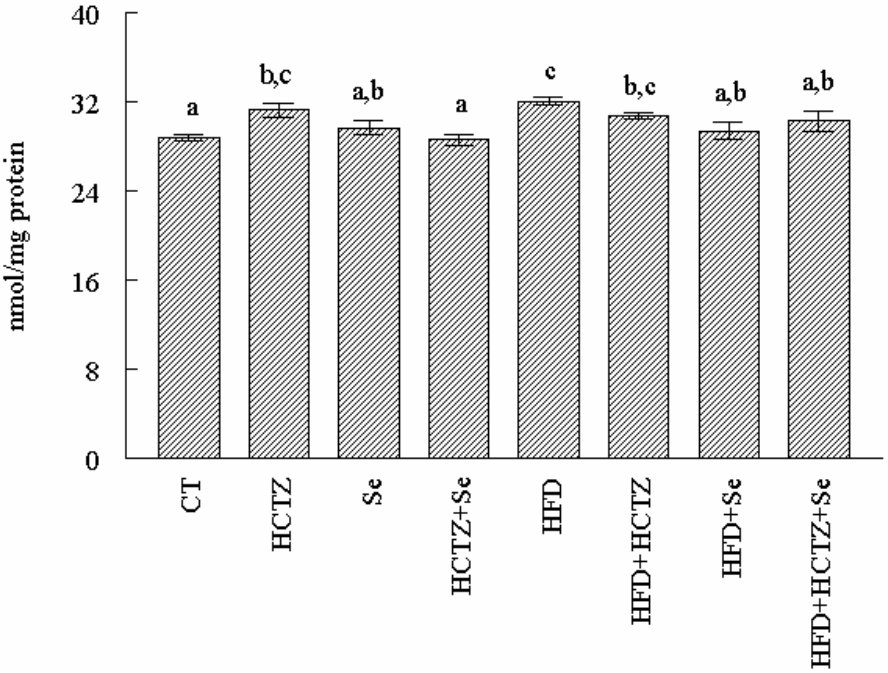

40
32
24
16
8
0
nmol/mg protein
a
b,c
a,b
a
c
b,c
a,b
a,b
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 3A

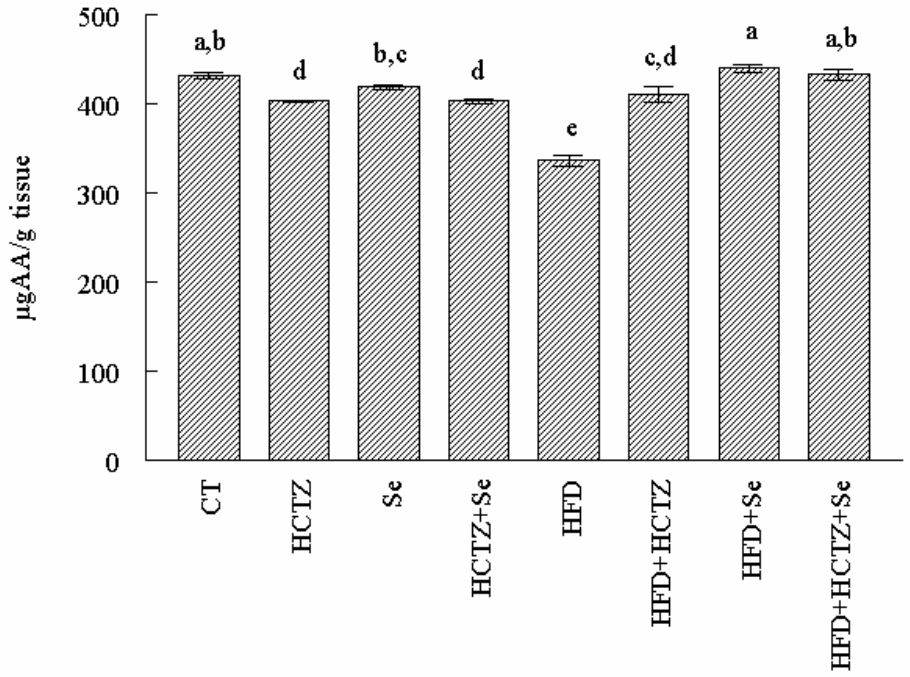

500
400
300
200
100
0
µgAA/g tissue
a,b
d
b,c
d
e
c,d
a
a,b
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 3B

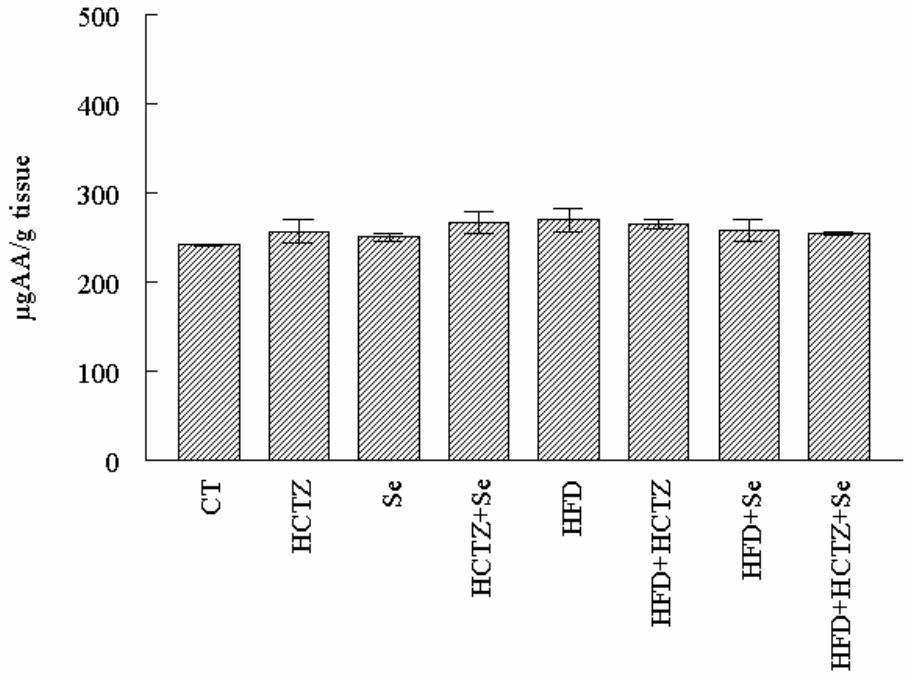

500
400
300
200
100
0
µgAA/g tissue
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 4A

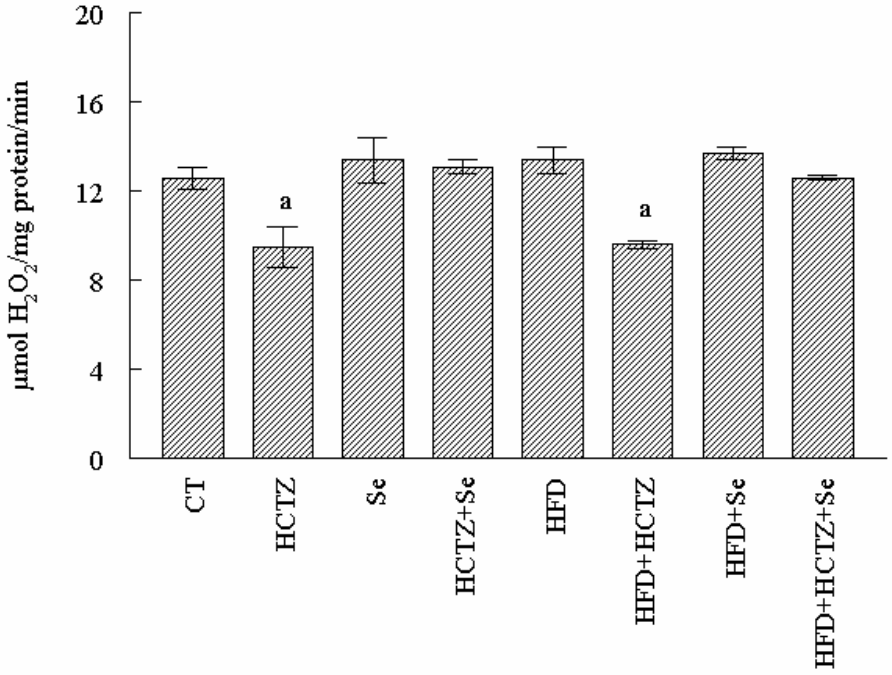

20
16
12
8
4
0
µmol H_2O_2/mg protein/min
a
a
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 4B

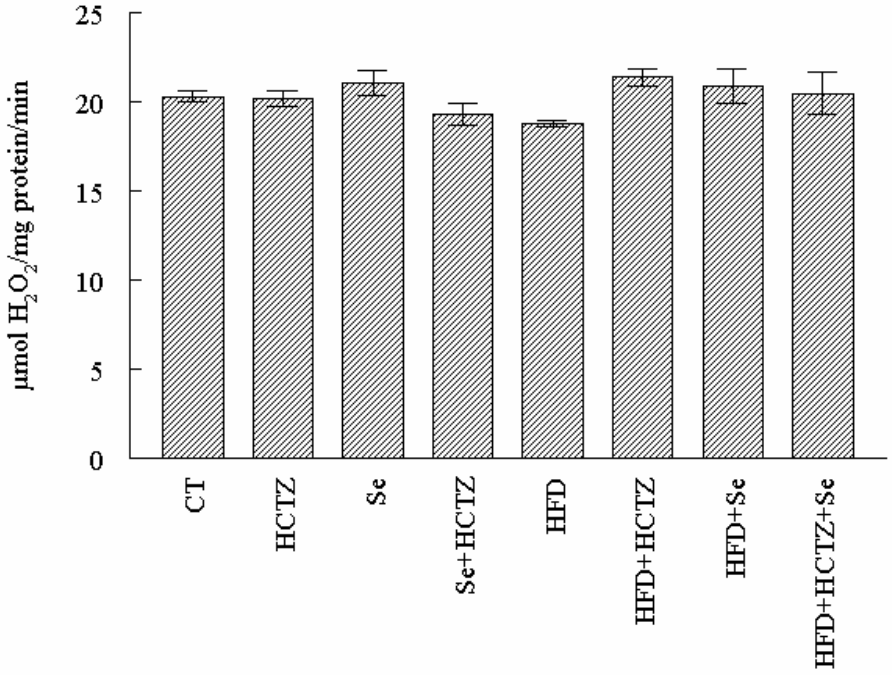

25
20
15
10
5
0
µmol H_2O_2/mg protein/min
CT
HCTZ
Se
Se+HCTZ
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 5A

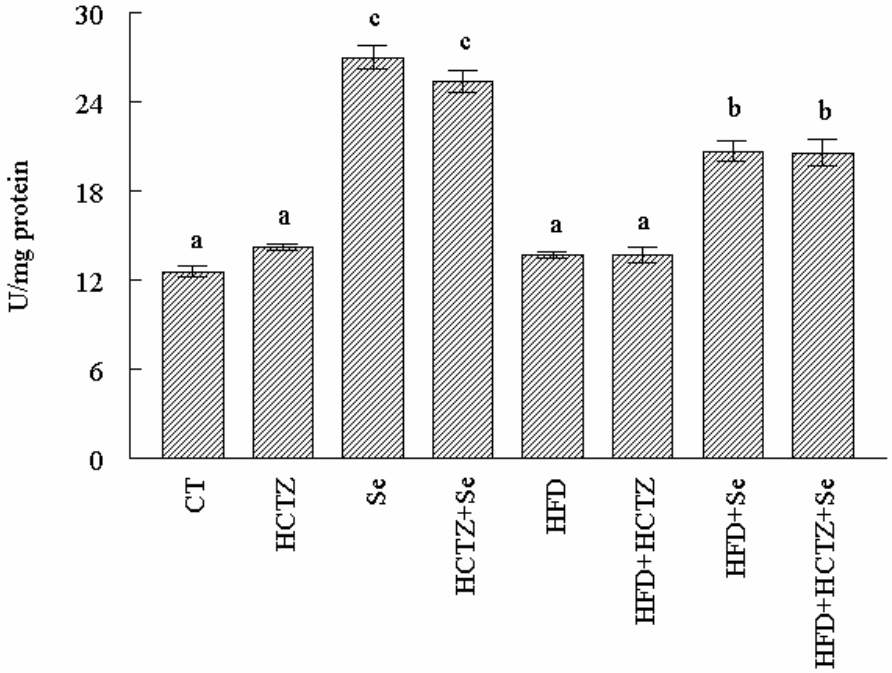
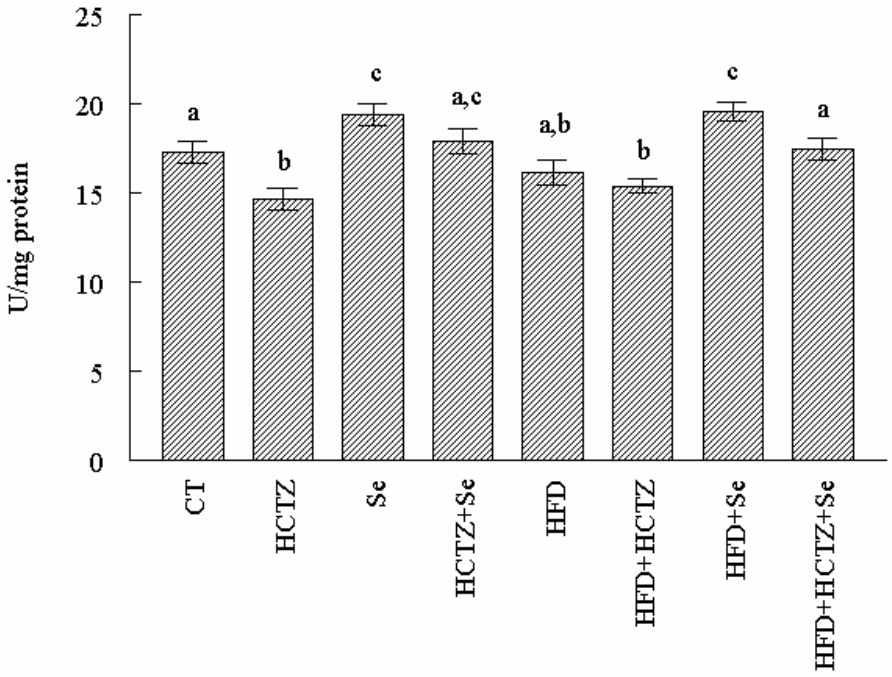

30
24
18
12
6
0
U/mg protein
a
a
c
c
a
a
b
b
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Figure 5B


25
20
15
10
5
0
U/mg protein
a
b
c
a,c
a,b
b
c
a
CT
HCTZ
Se
HCTZ+Se
HFD
HFD+HCTZ
HFD+Se
HFD+HCTZ+Se

Tables

Table 1 - Composition of the diets (g/kg)

Components	Control diet	Diet enriched with fructose
Fructose	-	600
Casein	150	150
Soybean Oil	50	50
Lard	50	50
Wheat flour	105	105
Cornstarch	600	-
Mineral mixture [1]	35	35
Vitamin mixture [2]	10	10

[1] The mineral mixture contained (g/kg): bone meal (449); NaCl (38); KCl (134.2); $MgSO_4$ (20); $ZnCl_2$ (0.4); $CuSO_4$ (0.175); $MnSO_4$ (1.2); $FeSO_4$ (2), and cornstarch (355).

[2] The vitamin mixture (mg or IU/g) was composed of Vitamin A, 2000 IU; Vitamin D 200 IU; tocopherol, 10 IU; menadione, 0.5 mg; choline, 200 mg; folic acid, 0.2 mg; p-aminobenzoic acid, 1.0 mg; inositol, 10 mg; calcium D-panthotenate, 4.0 mg; riboflavin, 0.8 mg; thiamin-HCl, 0.5 mg; pyridoxine-HCl, 0.5 mg; niacinamide, 0.3 mg; and biotin, 0.04.

Diphenyl diselenide (3 ppm) was dissolved in soybean oil and mixed in the diet in a food mixer to insure uniform distribution.

Wheat flour (4g/kg of diet) was substituted by HCTZ.

Table 2. Body weight, organ weight and total adipose tissue deposits (g) changes in diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

Parameters	Groups							
	Control	**HCTZ**	**Se**	**Se + HCTZ**	**HFD**	**HFD + HCTZ**	**HFD + Se**	**HFD + HCTZ +Se**
Initial body weight	254.0±18.4	259.3±19.8	254.7±17.4	258.7±19.0	264.0±11.8	256.7±15.6	251.3±21.2	257.3±18.6
Final body weight	357.3±13.1 a	359.3±5.1 a	355.3±3.6 a	340.0±17.2 a	330.7±8.8 a,b	271.3±2.2 c	306.3±10.3 b	277.3±8.4 c
Body weight gain	103.3±31.2 a	100.0±17.2 a	100.7±20.7 a	81.3±29.7 a,b	66.7±6.6 a,b,c	14.7±13.5 c	54.7±10.9 a,b,c	20.0±24.0 b,c
Liver	8.8±0.3 a,b	8.9±0.2 a,b	10.5±0.1 c	8.6±0.2 a,b	8.9±0.2 a,b	8.2±0.2 a	8.9±0.3 a,b	9.2±0.4 b
Kidney	2.0±0.03 a,b	1.9±0.03 a	2.3±0.04 d	2.1±0.06 b,c	2.2±0.02 b,c,d	2.1±0.02 b,c	2.2±0.05 c,d	2.3±0.03 d
Total adipose tissue deposits *	6.7±0.3 a	4.6±0.1 b,c	5.9±0.8 a,b	5.3±0.7 a,b,c	5.9±0.4 a,b	3.7±0.6 c	5.2±0.7 a,b,c	4.2±0.7 b,c

Data are expressed as means ± S.E.M. of six animals per group.
[abcd] Mean values within a row not sharing a common superscript letter were significantly different, $p < 0.05$.
* The total weight of perirenal, mesenteric, epididymal and subcutaneous fat-pads.

Table 3. Water and food consumption (months) changes in diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

Parameters	Groups							
	Control	**HCTZ**	**Se**	**HCTZ + Se**	**HFD**	**HFD + HCTZ**	**HFD + Se**	**HFD + HCTZ + Se**
Water consumption								
1	15.1±1.6	23.7±1.4 a	14.6±1.1	18.5±0.3	26.3±1.5 a	23.9±1.1 a	25.7±2.1 a	22.9±1.9 a
2	23.9±1.3 a,b	31.8±1.3 c	21.6±1.3 a	23.8±1.7 a,b	33.9±0.8 c	35.4±0.9 c	27.5±2.0 b	26.4±0.8 b
3	21.9±1.3 a	31.0±2.3 b,c	22.7±1.3 a	22.7±1.5 a	28.6±1.8 b,c	33.9±1.9 c	27.9±1.6 b	29.9±2.0 b,c
4	22.9±0.9 a	35.6±1.7 c	22.9±2.1 a	20.9±1.9 a	31.7±1.4 b,c	32.9±2.5 b,c	29.2±2.3 b	29.5±0.5 b
Food consumption								
1	22.9±0.9 a	22.2±0.5 a	22.9±0.8 a	21.0±0.8 a	12.9±0.8 b	11.3±0.6 b,c	11.2±0.6 b,c	10.8±0.6 c
2	18.9±0.9 a	20.6±0.8 a	20.4±0.9 a	20.9±0.8 a	12.9±0.6 b	12.4±0.5 b,c	12.3±0.7 b,c	10.7±0.5 c
3	19.3±0.7	18.7±1.0	18.4±0.7	18.5±0.6	12.9±0.4 a	12.7±0.6 a	11.5±0.4 a	12.2±0.6 a
4	16.9±0.9	16.6±0.9	17.4±0.9	15.5±0.6	11.7±0.6 a	10.5±0.4 a	11.1±0.4 a	10.9±0.5 a

Data are expressed as means ± S.E.M. of six animals per group.
[abc] Mean values within a row not sharing a common superscript letter were significantly different, $p < 0.05$.
Data of water and food consumption are presented as months.

Table 4. Effect of diphenyl diselenide supplementation on biochemical parameters associated to fructose and HCTZ intake.

Parameters	Groups							
	Control	**HCTZ**	**Se**	**HCTZ + Se**	**HFD**	**HFD + HCTZ**	**HFD + Se**	**HFD + HCTZ + Se**
Glucose	84.4±1.5 a	84.3±0.8 a	85.6±0.3 a,b	89.2±2.9 a,b,c	92.0±3.2 c	93.3±1.1 c	91.0±2.9 b,c	93.2±0.4 c
Fructosamine	100.7±0.9 a	98.5±0.8 a	97.2±3.1 a	102.5±4.1 a,b	113.0±1.3 c	108.5±1.6 b,c	107.8±2.4 b,c	108.5±0.5 b,c
Cholesterol	94.3±1.2 a	91.2±2.2 a	89.7±3.4 a	88.3±3.0 a	151.3±3.3 c	102.3±1.4 b	101.2±2.1 b	101.7±0.4 b
Triglycerides	86.3±1.9 a	110.0±1.2 b,c	96.0±7.7 a,b	103.0±4.8 a,b,c	144.7±3.1 d	141.7±9.1 d	117.7±0.3 c	140.8±9.5 d
Magnesium	2.6±0.12 a	2.1±0.06 b,c,d	2.4±0.10 a,b	2.1±0.08 c,d	2.6±0.15 a	1.6±0.03 e	2.3±0.07 b,c	1.9±0.05 d
Potassium	6.9±0.20	6.8±0.07	6.7±0.21	6.9±0.16	6.9±0.16	5.8±0.55 a	6.7±0.10	5.6±0.47 a
Urea	48.3±0.3	49.7±1.0	44.6±3.7	45.2±3.1	49.7±2.5	44.0±1.2	44.2±1.4	44.4±0.4
Creatinine	0.8±0.02	0.7±0.01	0.9±0.07	0.7±0.05	0.9±0.03	0.7±0.06	0.8±0.04	0.8±0.04

Data are expressed as means ± S.E.M. of six animals per group.
[abcd] Mean values within a row not sharing a common superscript letter were significantly different, $p < 0.05$.
Data of glucose, fructosamine, cholesterol, triglycerides, magnesium, potassium, urea and creatinine levels are presented as mg/dL.

DISCUSSÃO

O consumo crônico de dietas hiperlipídicas e/ou hiperglicídicas (Folmer et al., 2002; Folmer et al., 2003; Brito et al., 2007; Faure et al., 2007), assim como o tratamento com o anti-hipertensivo, HCTZ (Pepine & Cooper-DeHoff, 2004) são fatores importantes para o aparecimento de algumas alterações metabólicas relacionadas ao DM tipo 2. A partir dessas evidências, associou-se a HCTZ com modelos de distúrbios metabólicos relacionados ao desenvolvimento de DM tipo 2 induzidos pelas dietas hiperlipídicas e hiperglicídicas, uma vez que essas desordens metabólicas são comuns em pacientes com hipertensão e, portanto seria importante determinar se os diuréticos tiazídicos poderiam exacerbar esses alterações metabólicas. Dessa maneira, um dos objetivos desta pesquisa foi avaliar o efeito de uma dieta hiperlipídica suplementada com diferentes concentrações de hidroclorotiazida, em ratos, com a intenção de investigar uma possível interação sinérgica desses dois fatores de risco para o desenvolvimento do DM tipo 2 sobre os parâmetros bioquímicos relacionados ao estresse oxidativo. Os resultados obtidos nesse trabalho mostram que altas doses de HCTZ associadas com dietas hiperlipídicas causaram um aumento nos níveis de glicose sangüínea (artigo 1 e manuscrito 1), e que a dieta hiperlipídica, associada ou não com a HCTZ, aumentou os níveis de frutosamina (manuscrito 1), fatos que são compatíveis com o desenvolvimento de resistência à insulina. Então, pode-se sugerir que a ingestão simultânea de dietas hiperlipídicas e o uso de HCTZ como diurético para o tratamento da hipertensão pode potencializar o aumento da glicemia causada por essa classe de drogas. O mecanismo pelo qual os diuréticos tiazídicos induzem aumento na glicemia e intolerância à glicose não está bem esclarecido. No entanto, algumas evidências da literatura sugerem esses diuréticos

causam uma redução na secreção de insulina pelas células β do pâncreas e uma diminuição na sensibilidade à insulina pelos tecidos (Grossman & Messerli, 2006).

Há estudos que relatam que os diuréticos tiazídicos, em doses baixas e médias, são eficazes na redução da pressão sanguínea e causam mínimos efeitos colaterais. Porém, quando a dose é aumentada, uma pequena contribuição é observada em relação ao controle da pressão arterial, enquanto é significativo o aumento de efeitos colaterais (Pepine & Cooper-DeHoff, 2004; Zee et al., 2005; Grossman & Messerli, 2006). De fato, trabalhos prévios indicaram uma clara correlação entre a dose de HCTZ e o aumento nas concentrações de glicemia em jejum (Carlsen et al., 1990) e, que a HCTZ, em dose média de 40 mg/dia, causou hiperglicemia (Pollare, 1989). Recentes dados têm indicado que o tratamento prolongado de pacientes hipertensos com uma dose baixa de HCTZ (12,5 mg/dia) melhora a elasticidade arterial, mas não em pacientes com DM tipo 2 ou com glicemia de jejum alterada. Além disso, esses estudos demonstraram que o tratamento com uma dose de 25 mg/dia de HCTZ agravou os parâmetros metabólicos e a rigidez arterial (Zimlichman et al., 2004). De acordo com essas considerações, as doses testadas nesse trabalho foram maiores do que aquelas, comumente, utilizadas para o tratamento da hipertensão, no entanto, são inferiores às do Nível sem Efeito Adverso Observado (NOAEL) para HCTZ em ratos (George et al., 1995) e pode indicar que uma extrapolação direta de doses tóxicas de ratos para humanos não é possível.

Dados da literatura têm relatado que a ingestão crônica de uma dieta hiperlipídica (Folmer et al., 2003; Fachineto et al., 2005), bem como a hiperglicemia estão ligados à produção de estresse oxidativo e que o aumento dos níveis de EROs estão envolvidas no desenvolvimento de resistência à insulina (Folmer et al., 2002; Aksoy et al., 2003; Brito et

al., 2007; Maiese et al., 2007) e neuropatia diabética (McCall, 1992; Greene et al., 1999; Baydas et al., 2003; Baydas et al., 2004). Entretanto, dados sobre os efeitos da HCTZ na promoção da resistência à insulina e estresse oxidativo em modelos animais são raros na literatura.

Vários pesquisadores relataram que as EROs podem causar degeneração celular, especialmente no cérebro (Baydas et al., 2003; Baydas et al., 2004), uma vez que é particularmente vulnerável ao estresse oxidativo, devido à sua limitada capacidade antioxidante (Shulman et al., 2004). Por outro lado, o fígado é o órgão que apresenta papel central na modulação da homeostase da glicose (Maiese et al., 2007). Assim, estudos demonstraram que o dano hepático induzido pelas EROs pode prejudicar a homeostase celular e potencializar as características da Síndrome Metabólica (Kohen & Nyska, 2002; Raval et al., 2006). Em nosso trabalho, constatou-se que o consumo crônico da dieta hiperlipídica produziu um aumento na glicose (artigo 1 e manuscrito1) e na frutosamina (manuscrito 1), as quais provocaram estresse oxidativo nos tecidos cerebral e hepático, evidenciado pelo aumento dos níveis de peroxidação, estes que foram exacerbados pelo tratamento com HCTZ (artigo 1 e manuscrito 1). Em consonância com este resultado, dados da literatura têm indicado que a hiperglicemia provoca uma excessiva glicação não enzimática de proteínas, com acentuada inativação de enzimas, aumento na peroxidação lipídica e alterações no sistema de defesa antioxidante (Morgan et al., 2002; Aksoy et al., 2003). Em geral, a glicação não enzimática de proteínas gera produtos altamente reativos, fato que poderia explicar a relação entre a hiperglicemia e a peroxidação lipídica (Lopes et al., 2008).

Neste estudo, observou-se um significativo aumento nos níveis de vitamina C, no tecido hepático e cerebral, dos ratos alimentados com doses elevadas de HCTZ, quando

foram administradas isoladamente ou em combinação com a dieta hiperlipídica (artigo 1 e manuscrito 1). Da mesma forma, os níveis de grupos tiol não-protéico (NPSH) foram aumentados em animais tratados com 1,0 e 4,0 g/kg de HCTZ e o aumento foi, proporcionalmente, maior nos ratos alimentados com a dieta hiperlipídica (artigo 1). De acordo com esses resultados, um aumento nos sistemas de defesa antioxidante foi observado em uma variedade de modelos experimentais de patologias, possivelmente como uma resposta compensatória dos tecidos à presença de danos oxidativos (Barbosa et al., 2006; Barbosa et al., 2008).

Neste estudo, observou-se uma inibição significativa da atividade da Na^+-K^+-ATPase no cérebro dos animais tratados com altas doses de HCTZ. A inibição dessa enzima induzida pela HCTZ pode estar associada a um aumento no estresse oxidativo, o qual pode acelerar a desnaturação da Na^+-K^+-ATPase (Thevenod & Friedmann, 1999). De fato, os grupos -SH da enzima são altamente suscetíveis ao estresse oxidativo (Yufu et al., 1993) e a agentes oxidantes (Carfagna et al., 1996). Além disso, os dados obtidos nesse estudo indicaram uma interação entre os efeitos da dieta hiperlipídica e da HCTZ na atividade da Na^+-K^+-ATPase no cérebro. Assim, torna-se razoável sugerir que simultânea ingestão de uma dieta hiperlipídica com o co-tratamento com HCTZ pode causar efeitos pró-oxidativos adicionais a este órgão com inibição da atividade da Na^+-K^+-ATPase. No entanto, mais estudos são necessários para entender o mecanismo envolvido nos efeitos interativos da dieta e hidroclorotiazida na atividade da Na^+K^+-ATPase. A significativa correlação positiva entre o TBARS cerebral e a glicemia, assim como a correlação negativa entre Na^+-K^+-ATPase e níveis de glicose sanguínea podem indicar um papel potencial para a hiperglicemia, pelo menos em parte, nas alterações neuroquímicas após a exposição à HCTZ e/ou uma dieta hiperlipídica (artigo 1).

Há evidências de que a diminuição dos níveis de potássio causada pelos diuréticos tiazídicos pode ter um papel importante no metabolismo da glicose (Rowe et al., 1980), e que uma dieta suplementada com potássio pode atenuar a intolerância à glicose induzida pelas tiazidas (Helderman et al., 1983). Neste contexto, o magnésio intracelular, também parece desempenhar um papel fundamental no metabolismo da glicose (Paolisso & Barbagallo, 1997; Barbagallo et al., 2000; Barbagallo et al., 2001; Barbagallo et al., 2003, Barbagallo & Dominguez, 2007; Sontia & Touyz 2007), uma vez que estudos epidemiológicos têm indicado que as doenças metabólicas, tais como o DM tipo 2 e a hipertensão podem ser associados com a redução do magnésio intracelular (Ma et al., 1995; Paolisso & Barbagallo, 1997; Barbagallo et al., 2000; Barbagallo et al., 2001; Sontia & Touyz, 2007) e aumento no dano oxidativo em vários tecidos (Lourdes, 1998; Gums, 2004). Assim, os dados obtidos no manuscrito 1 mostram uma diminuição significativa nos níveis de magnésio e potássio plasmáticos, bem como um aumento na peroxidação lipídica hepática em ratos alimentados com uma dieta hiperlipídica associada ou não à HCTZ. É importante ressaltar que a associação da dieta hiperlipídica com a HCTZ (4,0 g/kg de ração) potencializou a depleção de magnésio e aumentou a peroxidação lipídica. Então, considerando que as dietas suplementadas com gorduras saturadas dificultam a absorção de magnésio (Johnson, 2001), sugere-se que indivíduos que ingerem uma dieta suplementada com lipídios do tipo saturado podem apresentar perda de magnésio e potássio, fato que pode contribuir para o desenvolvimento de diabetes e estresse oxidativo. Nesse sentido, os resultados apresentados nesse trabalho podem indicar que o magnésio e o potássio desempenham um papel importante nos efeitos adversos da HCTZ e, uma vez que as alterações bioquímicas foram exacerbadas pelo consumo combinado de HCTZ com uma dieta hiperlipídica, pode-se supor que a perda de magnésio pode ter um papel central nos

efeitos adversos da hidroclorotiazida associada com a ingestão de dietas hiperlipídicas em ratos.

Enfim, os dados apresentados no artigo 1 e no manuscrito 1, indicam que a ingestão crônica de doses elevadas de HCTZ ou dieta hiperlipídica provoca alterações metabólicas relacionadas com a homeostase da glicose e com o estresse oxidativo no tecido cerebral e hepático. Além disso, os resultados também sugerem que a associação da dieta hiperlipídica com o tratamento com HCTZ pode exacerbar algumas destas alterações bioquímicas. Assim, podemos sugerir que o nosso modelo experimental pode ser usado para o estudo dos efeitos adversos da HCTZ.

Atualmente, tem sido intensa a procura por compostos naturais ou sintéticos efetivos no tratamento da diabetes e suas complicações. Assim, compostos com propriedades antioxidantes e hipoglicemiantes são usados com relativo sucesso no controle da hiperglicemia e do estresse oxidativo. Nesse contexto, nas últimas três décadas, os estudos químicos e bioquímicos dos compostos de selênio têm aumentado, principalmente devido ao fato de que uma variedade de formas orgânicas de selênio possui atividade antioxidante (Andersson et al., 1994; Nogueira et al., 2004). Similarmente, a suplementação com formas inorgânicas de selênio como o selenito e selenato de sódio, são bastante usadas no tratamento de pacientes e de animais com diabetes por apresentarem estas propriedades (Berg et al., 1995; Mukherjee et al., 1998; Stapleton, 2000; Faure, 2004). Baseando-se nestas evidências e pelo fato de que não existem dados na literatura que avaliem o efeito de compostos de selênio sobre as alterações metabólicas induzidas por outros fatores como dietas suplementadas com carboidratos e/ou tratamento com HCTZ, os objetivos do manuscrito 2 foram avaliar se o $(PhSe)_2$ poderia evitar ou reduzir as alterações bioquímicas provocadas pela ingestão de uma dieta hiperglicídica e/ou HCTZ, bem como investigar se o

consumo de tiazidas poderia exacerbar essas alterações, uma vez que esses distúrbios metabólicos são comuns em pacientes com hipertensão.

Os resultados obtidos no manuscrito 2 demonstraram que a ingestão da dieta hiperglicídica, associada ou não com a HCTZ, causou aumento na glicemia, frutosamina, colesterol total e triglicerídios, que são fatores relacionados com o desenvolvimento da resistência à insulina. Assim, pode-se sugerir que a ingestão simultânea de dietas hiperglicídicas com o tratamento com tiazidas potencializa os efeitos adversos desta classe de drogas.

Vários estudos sugerem que o estresse oxidativo é parcialmente responsável por distúrbios metabólicos induzidos por dietas suplementadas com carboidratos e que os antioxidantes são agentes terapêuticos efetivos para prevenir ou reduzir esse dano oxidativo (Ho et al., 2001; Chander et al., 2004; Di Leo et al., 2004; El-Dermerdasch et al., 2005; Miranda et al., 2006). De acordo com essas evidências, os dados obtidos no manuscrito 2 mostram um efeito protetor da suplementação com $(PhSe)_2$ contra as alterações nas defesas antioxidantes induzidas pela dieta hiperglicídica e pelo tratamento com a HCTZ. De fato, o $(PhSe)_2$ restaurou a diminuição dos níveis de vitamina C hepática causada pela associação entre HCTZ e dieta hiperglicídica, bem como reduziu a oxidação de lipídios e proteínas no fígado e rins de animais tratados com HCTZ associada ou não com a dieta hiperglicídica. Além disso, o $(PhSe)_2$ foi capaz de reverter a redução das atividades da catalase (fígado) e da superóxido dismutase (SOD) (rim) induzidas pela HCTZ. De particular importância, este composto também causou um aumentou *per se* na atividade da SOD hepática e renal. Esses resultados estão de acordo com outros estudos que mostram que a suplementação com selênio aumenta as defesas antioxidantes, diminui os níveis de peroxidação lipídica e incrementam a expressão de RNAm para as enzimas antioxidantes em pacientes diabéticos

e modelos animais (Naziroglu & Cay, 2001; Faure, 2003; Faure et al., 2004; El-Dermerdasch et al., 2005). Pode-se sugerir que o potencial antioxidante do $(PhSe)_2$ pode ser explicado, pelo menos em parte, pela sua atividade mimética a glutationa peroxidase (Wilson et al., 1989).

Nesse trabalho (manuscrito 2) observou-se que a ingestão de $(PhSe)_2$ não atenuou o aumento da glicemia induzida pelo consumo da dieta hiperglicídica. Portanto, sugere-se que a falta de atividade anti-hiperglicemiante desse composto neste modelo experimental pode estar relacionado com a via de administração utilizada, uma vez que estudos mostrando que a administração crônica de $(PhSe)_2$, via subcutânea, promove uma diminuição significativa nos níveis plasmáticos de glicose em ratos diabéticos (Barbosa et al., 2006).

A ingestão crônica de uma dieta hiperglicídica, bem como a hiperglicemia está associada à geração de estresse oxidativo e com o desenvolvimento da resistência à insulina (Rayssiguier et al., 1981; Rayssiguier et al., 1993; McDonald, 1995; Maiese et al., 2007). Neste estudo, observou-se que a dieta hiperglicídica associada ou não com a HCTZ (que provoca um efeito *per se*) elevou os níveis de glicose e frutosamina, fato que pode ter causado danos oxidativos nos tecidos hepático e renal, uma vez que os níveis de peroxidação lipídica e carbonilação de proteínas foram aumentados nesses tecidos.

Há evidências de que o tratamento crônico com diuréticos tiazídicos pode prejudicar a tolerância à glicose e diminuir a sensibilidade à insulina e, assim, acelerar o desenvolvimento de DM (Bonner, 1994). Além disso, a terapia com diuréticos tem sido associada com o aumento nos níveis de colesterol e triglicerídios. Neste trabalho, observou-se um aumento nos níveis de colesterol total, somente quando a HCTZ foi associada com a dieta hiperglicídica. Além disso, também foi observado um aumento nos níveis de

triglicerídios causado pela HCTZ, o qual foi exacerbado pela associação entre a dieta hiperglicídica e HCTZ. Nesse aspecto, a ingestão de $(PhSe)_2$ atenuou o aumento dos triglicerídios causados por HCTZ e o aumento dos níveis de colesterol e triglicerídios causados pela ingestão da dieta hiperglicídica.

É importante enfatizar que a HCTZ causou uma diminuição nos níveis de magnésio (manuscritos 1 e 2) e que a associação entre a dieta hiperglicídica e a HCTZ agravou a condição de hipomagnesemia (manuscrito 2). Esse resultado sugere que os pacientes hipertensos que usam HCTZ e ingerem dietas hiperlipídicas e hiperglicídicas podem apresentar perda de magnésio, fato que pode contribuir para o desenvolvimento de distúrbios metabólicos e dano oxidativo. Além disso, pode-se sugerir que o uso de HCTZ por esses pacientes pode ainda precipitar o aparecimento do diabetes e suas complicações.

Os resultados dos manuscritos 1 e 2 mostram que a redução nos níveis de potássio foi, significativamente, associada com o aumento da hiperglicemia e da hipomagnesemia, fato que indica que os modelos experimentais desenvolvido neste trabalho podem ser usado para o estudo dos efeitos adversos da HCTZ e para esclarecer o papel do magnésio e do potássio na resistência à insulina associada com a ingestão de dietas hiperlipídicas ou hiperglicídicas associadas à HCTZ. Além disso, com o uso deste modelo pode-se também investigar se a incidência de distúrbios metabólicos como DM tipo 2, pelo uso de HCTZ é mais freqüente em pacientes hipertensos que consomem esses tipos de dieta.

Os resultados obtidos no manuscrito 2 sugerem que algumas alterações bioquímicas induzidas pela ingestão de dietas hiperglicídicas pode ser agravada pelo uso simultâneo de HCTZ. Além disso, outro aspecto muito importante desse estudo foi evidenciado pela capacidade do $(PhSe)_2$ contribuir para a prevenção das alterações metabólicas relacionadas ao estresse oxidativo. Assim, sugere-se que este composto pode ser considerado um agente

promissor para o tratamento de distúrbios metabólicos pela terapia antioxidante. Baseado nessas considerações, os resultados obtidos neste estudo contribuem para um melhor entendimento das bases toxicológicas e farmacológicas da aplicabilidade clínica do $(PhSe)_2$. Neste contexto pode-se inferir que o $(PhSe)_2$ é um composto com atrativas propriedades farmacológicas relacionada à atividade anti-diabetogênica.

CONCLUSÕES

De acordo com os resultados apresentados nesse trabalho pode-se concluir que:

Capítulo 1

A ingestão crônica de doses altas de HCTZ ou de uma dieta hiperlipídica altera os índices bioquímicos de estresse oxidativo, fato que demonstra a possível contribuição do aumento da glicemia para o dano cerebral. Além disso, o consumo de dietas hiperlipídicas e o tratamento com HCTZ apresentam efeitos interativos no cérebro, indicando que, à longo prazo, a ingestão crônica dessas dietas pode acentuar a toxicidade da HCTZ.

Capítulo 2

A ingestão crônica de HCTZ ou dieta hiperlipídica causa alterações metabólicas relacionadas à homeostase da glicose e a associação entre a dieta hiperlipídica e o tratamento com HCTZ pode exacerbar algumas dessas alterações bioquímicas. Portanto, pode-se sugerir que este modelo experimental pode ser usado para estudos dos efeitos toxicológicos da HCTZ.

Capítulo 3

A ingestão crônica de uma dieta hiperglicídica exacerbou algumas alterações bioquímicas causadas pela HCTZ, nesse modelo experimental. Portanto, esse trabalho foi validado como modelo viável para o estudo das interações entre desordens metabólicas e o tratamento com HCTZ, droga usada para a terapia anti-hipertensiva, mas que em determinadas concentrações e condições patológicas individuais pode causar sérios danos

aos pacientes. Assim, esse estudo é também importante para auxiliar a saúde e bem estar desses pacientes. Além disso, os resultados deste trabalho sugerem que a suplementação com $(PhSe)_2$ contribui para a prevenção e/ou redução de alterações metabólicas relacionadas ao estresse oxidativo e, que este composto pode ser considerado um agente promissor para o tratamento de distúrbios metabólicos pela terapia antioxidante.

PERSPECTIVAS

A partir dos promissores resultados obtidos nesta tese, poderíamos aprofundar ainda mais esses estudos pela concretização dos seguintes objetivos:

- Investigar o mecanismo envolvido nos efeitos interativos das dietas hiperlipídicas ou hiperglicídicas associadas à HCTZ nos parâmetros bioquímicos relacionados ao estresse oxidativo.

- Investigar se a incidência de DM tipo 2, pelo uso de HCTZ é mais freqüente em pacientes hipertensos que consomem dietas hiperlipídicas e/ou hiperglicídicas.

- Testar a HCTZ em via de administração e tempo de tratamento diferente associada com dietas hiperlipídicas ou hiperglicídicas, a fim de investigar os efeitos toxicológicos desse anti-hipertensivo.

- Investigar os efeitos de dietas hiperlipídicas e/ou hiperglicídicas associadas ao tratamento com a HCTZ na população humana.

- Testar o $(PhSe)_2$ em concentrações, via de administração e tempo de tratamento diferentes, a fim de comparar a sua efetividade e estabelecer uma dose/resposta.

- Procurar identificar o metabólito responsável pelo efeito farmacológico do $(PhSe)_2$, uma vez que a via de administração modifica a eficácia do tratamento.

- Testar outros compostos orgânicos de selênio com o propósito de desenvolvimento de novos fármacos com ação antioxidantes para o uso na terapêutica de alterações metabólicas relacionadas à DM (e talvez de outras doenças que envolvam espécies reativas de oxigênio e nitrogênio).

REFERÊNCIAS BIBLIOGRÁFICAS

ADA, American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. **Diabetes Care**, 31, 55-60, 2008.

Adisakwattana, S.; Roengsamran, S.; Hsu, W. H.; Yibchok-anun, S. Mechanisms of antihyperglycemic effect of *p*-methoxycinnamic acid in normal and streptozotocin-induced diabetic rats. **Life Sci.** 78, 406-412, 2005.

Aksoy, N.; Vural, H.; Sabuncu, T.; Aksoy, S. Effects of melatonin on oxidative-antioxidative status of tissues in streptozotocin-induced diabetic rats. **Cell Biochem. Funct.** 21, 121-125, 2003.

Alsaif, M. A.; Duwaihy, M. M. S. Influence of dietary fat quantity and composition on glucose tolerance and insulin sensitivity in rats. **Nutr. Res.** 24, 417-425, 2004.

Andersson, C. M.; Hallberg, A.; Linden, M.; Brattsand, R.; Moldeus, P.; Cotgreave, I. Antioxidant activity of some diarylselenides in biological-systems. **Free Radic. Biol. Med.** 16, 17-28, 1994.

Andersson, O. K.; Gudbrandsson, T.; Jamerson, K. Metabolic adverse effects of thiazide diuretics: the importance of normokalaemia. **J. Intern. Med. Suppl.** 735, 89-96, 1991.

Anonymous. Propranolol or hydrochlorothiazide alone for the initial treatment of hypertension. IV. Effect on plasma glucose and glucose tolerance. Veterans Administration Cooperative Study Group on Antihypertensive Agents. **Hypertension** 7, 1008-1016, 1985.

Bach, J. F. Insulin-dependent diabetes mellitus as an autoimmune disease. **Endocr. Rev.** 15, 516-542, 1994.

Banting, F. G.; Best, C. H.; Collip, J. B.; Campbell, W. R.; Fletcher, A. A. Pancreatic extracts in the treatment of diabetes mellitus. **Can. Med. Assoc. J.** 12, 141-146, 1922.

Barbagallo, M.; Dominguez, L. J. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. **Arch. Biochem. Biophys.** 458, 40-47, 2007.

Barbagalo, M.; Dominguez, L. J.; Bardicef, O.; Resnick, L. M. Altered cellular magnesium responsiveness to hyperglycemia in hypertensive subjects. **Hypertension** 38, 612-615, 2001.

Barbagallo, M.; Dominguez, L. J.; Galioto, A.; Ferlisi, A.; Cani, C.; Malfa, L.; Pineo, A.; Busardo, A.; Paolisso, G. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. **Mol. Aspects Med.** 24, 39-52, 2003.

Barbagallo, M.; Gupta, R.; Dominguez, L. J.; Resnick, L. M. Cellular ionic alterations with aging: relation to hypertension and diabetes. **J. Am. Geriatr. Soc.** 48, 1111-1116, 2000.

Barbosa, N. B. V.; Rocha, J. B. T.; Soares, J. C. M.; Wondracek, D. C.; Gonçalves, J. F.; Schetinger, M. R. C.; Nogueira, C. W. Dietary diphenyl diselenide reduces the STZ-induced toxicity. **Food Chem. Toxicol.** 46, 186-194, 2008.

Barbosa, N. B. V.; Rocha, J. B. T.; Wondracek, D. C.; Perottoni, J.; Zeni, G.; Nogueira, C. W. Diphenyl diselenide reduces temporarily hyperglycemia: Possible relationship with oxidative stress. **Chem. Biol. Interact.** 163, 230-238, 2006.

Barnard, R. J.; Roberts, C. K.; Varon, S. M.; Berger, J. J. Diet-induced insulin resistance precedes other aspects of the metabolic syndrome. **J. Appl. Physiol.** 84, 1311-1315, 1998.

Baydas, G.; Donder, E.; Kiliboz, M.; Sonkaya, E.;Tuzcu, M.; Yasar, A.; Nedzvetskii, V. S. Neuroprotection by a-lipoic acid in streptozotocin-induced diabetes. **Biochemistry Mosc.** 69, 1001-1005, 2004.

Baydas, G.; Reiter, R. J.; Yasar, A.; Tuzcu, M.; Akdemir, I.; Nedzvetskii, V. S. Melatonin produces glial reactivity in the hippocampus, cortex, and cerebellum of treptozocin-induced diabetic rats. **Free Radic. Biol. Med.** 35, 797-804, 2003.

Baynes, J. W. Thorpe, S. R. The role of oxidative stress in diabetic complications. **Curr. Opin. Endocrinol.** 3, 277-284, 1996.

Beal, M. F.; Hyman, B. T.; koroshetz, W. Do defects in mitochondrial energy metabolism underlie the pathology of neurodegenerative diseases. **Trends Neurosci.** 16, 125-131, 1993.

Bem, A. F.; Farina, M.; Portella, R. L.; Nogueira, C. W. ; Dinis, T. C. P. ; Laranjinha, J. A. N. ; Almeida, L. M. Rocha, J. B. T. Diphenyl diselenide, a simple glutathione peroxidase mimetic, inhibits human LDL oxidation in vitro. **Atherosclerosis** 201, 92-100, 2008a.

Bem, A. F.; Portella, R. L.; Colpo, E.; Duarte, M. M. M. F.; Frediane, A.; Taube, P. S.; Nogueira, C. W.; Farina, M.; Silva, E. L.; Rocha, J. B. T. Diphenyl Diselenide Decreases Serum Levels of Total Cholesterol and Tissue Oxidative Stress in Cholesterol-fed Rabbits. **Basic Clin. Pharmacol. Toxicol.** 105, 17-23, 2008b.

Berg, E. A.; Wu, J. Y.; Campbell, L.; Kagey, M.; Stapleton, S. R. Insulin-like of vanadate and selenate on the expression of glucose 6- phosphate dehydrogenase and fatty acid synthase in diabetic rats. **Biochimie** 77, 919-924, 1995.

Bergendi, L.; Benes, L.; Durackova, Z; Ferencik, M. Chemistry, physiology and pathology of free radicals. **Life Sci.** 65, 1865-1874, 1999.

Bonnefont-Rousselot, D. The role of antioxidant micronutrients in the prevention of diabetic complications. **Treat. Endocrinol.** 3, 41-52, 2004.

Bonner, G. Hyperinsulinemia, insulin resistance, and hypertension, **J. Cardiovasc. Pharmacol.** 24, 39-49, 1994.

Brito, V. B., Folmer, V., Soares, J.C.M., Silveira, I.D., Rocha, J.B.T. Long-term sucrose and glucose consumption decreases the δ-aminolevulinate dehydratase activity in mice. **Nutrition** 23, 818-826, 2007.

Busserolles, J.; Zimowska, W.; Rock, E.; Rayssiguier, Y.; Mazur, A. Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. **Life Sci.** 71, 1303-1312, 2002.

Cao, G.; Sofic, E.; Pryor, R. L. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. **F. Radic. Biol. Med.** 22, 749-760, 1997.

Carfagna, M. A.; Ponsler, G. D.; Muhoberac, B. B. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. **Chem. Biol. Interact.** 100, 53-65, 1996.

Carlsen, J. E.; Kober, L.; Torp-Pedersen, C.; Johansen, P. Relation between dose of bendrofluazide, antihypertensive effect, and adverse biochemical effects. **Br. Med. J.** 300, 975-978, 1990.

Chander, P. N.; Gealekman, O.; Brodsky, S. V.; Elitok, S.; Tojo, A.; Crabtree, M.; Gross, S. S.; Goligorsky, S. Nephropathy in zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen. **J. Am. Soc. Nephrol.** 15, 2391-2403, 2004.

Cheeseman, K. H.; Slater, T. F. An introduction to free radical biochemistry. **Br. Med. Bull.** 49, 481-493, 1993.

Combs, G.F.; Gray, P.G. Chemopreventive agents: selenium. **Pharmacol. Ther.** 79, 179-192, 1998.

Del Maestro, R. F. An approach and to free radicals in medicine and biology. **Acta Physiol. Scand.** 492, 153-167, 1980.

Di Leo, M. A. S.; Santinim, S. A.; Gentiloni, N.; Giardina, B.; Franconi, F.; Ghirlanda, G. Long-term taurine supplementation reduces mortality rate in streptozotocin-induced diabetic rats. **Amino Acids** 27, 187-191, 2004.

Dinsmoore, R. The history of Diabetes. Publicado em **C.F.K. Magazine Fall**, 1996 (informação disponível na internet na seguinte página: http:/www.jdf.org/kids.

Dollery, C. **Therapeutic Drugs**, 2nd ed., Churchill Livingstone, 1998.

Doucet, A. Function and control of Na^+-K^+-ATPase in single nephron segments of the mammalian kidney. **Kidney Int.** 34, 749-760, 1988.

El-Dermerdasch, F. M.; Yousef, M. I.; Abou El-Naga, N. I. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. **Food Chem. Toxicol.** 43, 57-63, 2005.

Erecinska, M.; Silver, I.A. Ions and energy in mammalian brain. **Prog. Neurobiol.** 16, 37-71, 1994.

Esterbauer, H.; Waeg, G.; Puhl, H.; Dieber-Rotheneder, M.; Tatzber, F. Inhibition of LDL oxidation by antioxidants. **EXS** 62, 145-157, 1992.

Fachinetto, R.; Burger, M. E.; Wagner, C.; Wondraceka, D. C.; Brito, V. B.; Nogueira, C. W.; Ferreira, J.; Rocha, J. B. T. R. High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. **Pharmacol. Biochem. Behav.** 81, 585-592, 2005.

Farina, M.; Soares, F. A.; Feoli, A.; Roehring, C.; Brusque, A.M.; Rotta, L.; Perry, M. L.; Souza, D. O.; Rocha, J. B. T. In Vitro Effects of Selenite and Mercuric Chloride on Liver Thiobarbituric Acid–Reactive Substances and Non-Protein Thiols From Rats: Influences of Dietary Cholesterol and Polyunsaturated and Saturated Fatty Acids. **Nutrition** 19, 531-535, 2003.

Faure, P. Protective effects of antioxidant micronutrients (vitamin E, zinc and selenium) in type 2 diabetes mellitus. **Clin. Chem. Lab. Med.** 41, 995-998, 2003.

Faure, P.; Barclay, D.; Joyeux-Faure, M.; Halimi, S. Comparison of the effects of zinc alone and zinc associated with selenium and vitamin E on insulin sensitivity and oxidative stress in high-fructose-fed rats. **J. Trace Elem. Med. Biol.** 21, 113-119, 2007.

Faure, P.; Ramon, O.; Favier, A.; Halimi, S. Selenium supplementation decreases nuclear factor-kappa B activity in peripheral blood mononuclear cells from type 2 diabetic patients. **Eur. J. Clin. Invest.** 7, 475-481, 2004.

Ferrannini, E.; Natali, A.; Bell, P.; Cavallo-Perin, P.; Lalic, N.; Mingrone, G. Insulin resistance and hypersecretion in obesity: European Group for the Study of Insulin Resistance (EGIR). **J. Clin. Invest.** 100, 1166-1173, 1997.

Feskens, E.; Bowles, C. H.; Kromhout, D. Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. **Diabetes Care** 14, 935-941, 1991.

Feskens, E. J.; Kromhout, D. Habitual dietary intake and glucose tolerance in euglycaemic men: the Zutphen Study. **Int. J. Epidemiol.** 19, 953-959, 1990.

Feskens, E. J.; Loeber, J. G.; Kromhout, D. Diet and physical activity as determinants of hyperinsulinemia: the Zutphen Elderly Study. **Am. J. Epidemiol.** 140, 350-360, 1994.

Feskens, E. J.; Virtanen, S. M.; Rasanen, L.; Tuomilehto, J.; Stengård, J.; Pekkanen, J.; Nissinen, A.; Kromhout, D. Dietary factors determining diabetes and impaired glucose tolerance: a 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. **Diabetes Care** 18, 1104-1112, 1995.

Flanagan, A. M.; Brown, J. L.; Santiago, C. A.; Aad, P. Y.; Spicer, L. J.; Spicer, M. T. High-fat diets promote insulin resistance through cytokine gene expression in growing female rats. **J. Nutr. Biochem**. 19, 505-513, 2008.

Flohe, L.; Gunzler, W. A.; Schock, H. H. Glutathione peroxidase: a selenoenzyme. **FEBS. Lett.** 32, 132-134, 1973.

Folmer, V.; Santos, F.W.; Savegnago, L.; Brito, V.B.; Nogueira, C.W.; Rocha, J.B.T. High sucrose consumption potentiates the sub-acute cadmium effect on Na+-K+-ATPase but not on and δ- aminolevulinate dehydratase in mice. **Toxicol. Lett.** 153, 333-341, 2004.

Folmer, V.; Soares, J. C. M.; Gabriel, D.; Rocha, J. B. T. A High Fat Diet Inhibits δ - Aminolevulinate Dehydratase and Increases Lipid Peroxidation in Mice (*Mus musculus).* **J. Nutr.** 133, 2165-2170, 2003.

Folmer, V.; Soares, J. C. M.; Rocha, J. B. T. Oxidative stress in mice is dependent on the free glucose content of the diet. **Int. J. Biochem. Cell Biol.** 34, 1279-1285, 2002.

Franse, L. V.; Pahor, M.; Di Bari, M.; Shorr, R. I.; Wan, J. Y.; Somes, G. W.; Applegate, W. B. Serum uric acid, diuretic treatment and risk of cardiovascular events in the Systolic Hypertension in the Elderly Program. **J. Hypertens.** 18, 1149-1154, 2000.

George, J. D.; Price, C. J.; Tyl, R. W.; Marr, M. C.; Kimmerl, C. A. The evaluation of the developmental toxicity of hydrochlorothiazide in mice and rats. **Fundam. Appl. Toxicol.** 26, 174-180, 1995.

Ghisleni, G.; Porciuncula, L. O.; Cimarostia, H.; Rocha, J. B. T.; Salbego, C. G.; Souza, D. O. Diphenyl diselenide protects rat hippocampal slices submitted to oxygen-glucose deprivation and diminishes inducible nitric oxide synthase immunocontent. **Brain Res.** 986, 196-199, 2003.

Girard, A.; Madani, S.; El Boustani, E. S.; Belleville, J.; Prost, J. Changes in lipid metabolism and antioxidant defense status in spontaneously hypertensive rats and Wistar rats fed a diet enriched with fructose and saturated fatty acids. **Nutrition** 21, 240-248, 2005.

Goodman, L. S.; Gilman, A. **As bases farmacológicas da terapêutica**, 11ª edição. Rio de Janeiro: McGraw-Hill Interamericana do Brasil, 2006.

Greene, D. A.; Stevens, M. J.; Obrosova, I.; Feldman, E. L. Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy. **Eur. J. Pharmacol.** 375, 217-223, 1999.

Grimm, R. H. Jr.; Leon, A. S.; Hunninghake, D. B.; Lenz, K.; Hannan, P.; Blackburn, H. Effects of thiazide diuretics on plasma lipids and lipoproteins in mildly hypertensive patients: a double-blind controlled trial. **Ann. Intern. Med.** 94, 7-11, 1981.

Grossman, E.; Messerli, F. H. Long-term safety of antihypertensive therapy. **Prog. Cardiovasc. Dis.** 49, 16-25, 2006.

Gums, J. G. Magnesium in cardiovascular and other disorders. **Am. J. Health Syst. Pharm.** 61, 1569-1576, 2004.

Hall, A. P.; Davies, M.J. Assessment and management of diabetes mellitus. **Found. Years** 4, 224-229, 2008.

Halliwell, B. Phagocyte-derived reactive species: salvation or suicide? **TRENDS in Biochem. Sci.** 31, 509-515, 2006a.

Halliwell, B. Reactive Species and Antioxidants. Redox Biology Is a Fundamental Theme of Aerobic Life. **Plant Physiol.** 141, 312-322, 2006b.

Halliwell, B.; Chirico, S. Lipid peroxidation: its mechanism, measurement, and significance. **Am. J. Clin. Nutr.** 57, 715-724, 1993.

Halliwell, B. & Gutteridge, J. M. C. **Free Radic. Biol. Med.**, 4ª ed., Claredon Press, 2006.

Hayden, M. R.; Sowers, J. R. Hypertension in Type 2 Diabetes Mellitus. **Insulin**, 1, 22-37, 2006.

Heffner, J. E. & Repine, J. E. Pulmonary strategies of antioxidant defense. **Am. Rev. Respir. Dis.** 140, 531-554, 1989.

Helderman, J. H.; Elahi, D.; Andersen, D. K.; Raizes, G. S.; Tobin, J. D.; Shocken, D.; Andres, R. Prevention of the glucose intolerance of thiazide diuretics by maintenance of body potassium. **Diabetes** 32, 106-111, 1983.

Hill, J. O.; Lin, D.; Yakubu, F.; Peters, J. C. Development of dietary obesity in rats: influence of amount and composition of dietary fat. **Int. J. Obes. Relat. Metab. Disord.** 16, 321-333, 1992.

Ho, E.; Quan, N.; Tsai, Y. H.; Lai, W.; Bray, T. M. Dietary zinc supplementation inhibits NF-kappa B activation and protects against chemically induced diabetes. **Exp. Biol. Med.** 226, 103-111, 2001.

Hu, F. B.; van Dam, R. M.; Liu, S. Diet and risk of Type II diabetes: the role of types of fat and carbohydrate. **Diabetologia**, 44, 805-817, 2001.

Hu, G.; Tuomilehto, J. Lifestyle and outcome among patients with type 2 diabetes. **Int. Congr. Ser.** 1303, 160-171, 2007.

Huen, S. C. & Goldfarb D. S. Adverse Metabolic Side Effects of Thiazides: Implications for Patients With Calcium Nephrolithiasis. **J. Urol.** 177, 1238-1243, 2007.

Jain, S. K.; Lim, G. Pyridoxine and pyridoxiamine inhibits superoxide radicals and prevents lipid peroxidation, protein glycosylation, and (Na^+K^+)-ATPase activity reduction in high glucosetreated human erythrocytes. **Free Radic. Biol. Med.** 30, 232–237, 2001.

Johnson, S. The multifaceted and widespread pathology of magnesium deficiency. Med. **Hypotheses** 56, 163-170, 2001

Jones, D. W.; Sands, C. D. Lack of effect of hydrocholorthiazide on total cholesterol. **Clin. Exp. Hypertens.** 16, 675-689, 1994.

Jorgensen, P. L. Structure, function and regulation of Na^+-K^+-ATPase in the kidney. **Kidney Int.** 29, 10-20, 1986.

Kalergis, M.; Pacaud, D.; Yale, J-F. Attempts to control the glycemic response to carbohydrate in diabetes mellitus: Overview and practical implications. **Can. J. Diabetes Care** 22, 20-29, 1998.

King, H.; Aubert, R. E.; Herman, W. H. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. **Diabetes Care** 21, 1414-1431, 1998.

King, H.; Rewers, M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. **Diabetes Care** 16, 157-177, 1993.

Kohen, R.; Nyska, A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. **Toxicol. Pathol.** 30, 620-650, 2002.

Kraegen, E. W.; James, D. E.; Storlien, L. H.; Burleigh, K. M.; Chisholm, D. J. In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. **Diabetologia** 29, 192-198, 1986.

Liu, S.; Manson, J. E. Dietary carbohydrates, physical inactivity, obesity, and the 'metabolic syndrome' as predictors of coronary heart disease. **Curr. Opin. Lipidol.** 12, 395-404, 2001.

Lopes, J. P.; Oliveira, S. M.; Fortunato, J. S. Oxidative stress and its effects on insulin resistance and pancreatic beta-cells dysfunction: relationship with type 2 diabetes mellitus complications. **Acta Med. Port.** 213, 293-302, 2008.

Lourdes, L. M.; Crua, T.; Pousada, J.C.; Rodrigues, L.E.; Barbosa, K.; Canguru, V. The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. **Diabetes Care** 21, 682-686, 1998.

Ma, J.; Folsom, A. R.; Melnick, S. L.; Eckfeldt, J. H.; Sharrett, A. R.; Nabulsi, A. A.; Hutchinson, R. G.; Metcalf, P. A. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin and carotid arterial wall thickness: the ARIC study. **J. Clin. Epidemiol.** 48, 927-940, 1995.

Maiese, K.; Morhan, S. D.; Chong, Z. Z. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. **Curr. Neurovasc. Res.** 4, 63-71, 2007.

Malerbi, D. A.; Franco, L. J. Multicenter study of the prevalence of diabetes mellitus and impaired glucose tolerance in the urban Brazilian population aged 30-69 yr. The Brazilian Cooperative Group on the Study of Diabetes Prevalence. **Diabetes Care** 15, 1509-1516, 1992.

Maritim, A. C.; Sanders, R. A.; Watkins, J. B. Diabetes, Oxidative Stress, and Antioxidants: A Review. **J. Biochem. Mol. Toxicol.** 17, 24-38, 2003.

Maron, D. J.; Fair, J. M.; Haskell, W. L. Saturated fat intake and insulin resistance in men with coronary artery disease: the Stanford Coronary Risk Intervention Project Investigators and Staff. **Circulation** 84, 2020-2027, 1991.

Marshall, J. A.; Bessesen, D. H.; Hamman, R.F. High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. **Diabetologia** 40, 430-438, 1997.

Mayer-Davis, E. J.; D'Agostino, R. Jr.; Karter, A. J.; Haffner, S. M.; Rewers, M. J.; Saad, M.; Bergman, R. N. Intensity and amount of physical activity in relation to insulin sensitivity: the Insulin Resistance and Atherosclerosis Study. **JAMA** 279, 669-674, 1998.

Mayer-Davis, E. J.; Monaco, J. H.; Hoen, H. M.; Carmichael, S.; Vitolins, M. Z.; Rewers, M. J.; Haffner, S. M.; Ayad, M. F.; Bergman, R. N.; Karter, A. J. Dietary fat and insulin sensitivity in a triethnic population: the role of obesity: the Insulin Resistance Atherosclerosis Study (IRAS). **Am. J. Clin. Nutr.** 65, 79-87, 1997.

Mayer, E. J.; Newman, B.; Quesenberry, C. P. Jr.; Selby, J. V. Usual dietary fat intake and insulin concentrations in healthy women twins. **Diabetes Care** 16, 1459-1469, 1993.

McCall, A. L. The impact of diabetes on the CNS. **Diabetes** 41, 557-570, 1992.

McDonald R. B. Influence of dietary sucrose on biological aging. **Am. J. Clin. Nutr.** 62, 284-293, 1995.

McInnes, G. T.; Yeo, W. W.; Ramsey, L. E.; Moser, M. Cardiotoxicity and diuretics: much speculation-little substance. **J. Hypertens.** 10, 317-335, 1992.

Ministério da Saúde/SVS. **URL:http://www.datasus.gov.br.** Acessado em setembro de 2009.

Minkowski, O. Historical development of the theory of pancreatic diabetes (introdution and translation by Rachimiel Levine). **Diabetes** 38, 1-6, 1989.

Miranda, M.; Muriach, M.; Roma, J.; Bosh-Morell, F.; Genoves, J. M.; Barcia, J.; Araiz, J.; Diaz-Llospis, M.; Romero, F. J. Oxidative stress in a model for experimental diabetic retinopathy: the utility of peroxinitrite scavengers. **Arch. Soc. Esp. Oftalm.** 81, 27-32, 2006.

Mooy, J. M.; Grootenhuis, P. A.; de Vries, H.; Valkenburg, H. A.; Bouter, L. M.; Kostense, P. J.; Heine, R. J. Prevalence and determinants of glucose intolerance in a Dutch Caucasian population: the Hoorn Study. **Diabetes Care** 18, 1270-1273, 1995.

Morgan, P. E.; Dean, R. T.; Davies, M. J. Inactivation of cellular enzymes by carbonyls and protein-bound glycation glycoxidation products. **Arch. Biochem. Biophys.** 403, 259-269, 2002.

Mukherjee, B.; Anbazhagan, S.; Roy, A.; Ghosh, R.; Chatterjee, M.; Novel implications of the potential role of selenium on antioxidant status in streptozotocin-induced diabetic mice. **Biomed. Pharmacother.** 52, 89-95, 1998.

Nanji, A. A.; Sadrzadeh, S. M.; Yang, E. K.; Fogt, F.; Meydani, M.; Dannenberg, A. J. Dietary saturated fatty acids: a novel treatment for alcoholic liver disease. **Gastroenterology** 109, 547-554, 1995.

National Research Council - Committee on diet and Health. Diet and Health: Implications for Reducing Chronic Disease Risk. **National Academy Press**, Washington, 1989.

Navarro-Alarcón, M.; Lopes-Martinez, M. C. Essentiality o selenium in the human body: relationship with different diseases. **Sc. Total Environ.** 249, 347-371, 2000.

Naziroglu, M.; Cay, M. Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanisms in rats with diabetes induced by streptozotocin. **Biol. Trace Elem. Res.** 79, 149-159, 2001.

Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. Organoselenium and organotellurium compounds: toxicology and pharmacology. **Chem. Rev.** 104, 6255-6285, 2004.

Oberley, L. W. Free radicals and diabetes. **Free Radic. Biol. Med.** 5, 113-124, 1988.

Pan, D. A.; Milner, M. R.; Lillioja, S.; Storlien, L. H. Muscle lipid composition is related to body fatness and insulin action in humans. **Int. J. Obes.** 19, 213-117, 1995.

Paolisso, G.; Barbagallo, M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. **Am. J. Hypertens.** 10, 346-355, 1997.

Paolisso, G.; Scheen, A.; Lefevre, P. Glucose handling, diabetes and ageing. **Horm. Res.** 43, 52-57, 1995.

Parekh, S. A.; Pudageb, A.; Joshib, S. S.; Vaidyaa, V. V.; Gomes, N. A.; Kamata, S. S. Simultaneous determination of hydrochlorothiazide, quinapril and quinaprilat in human plasma by liquid chromatography-tandem mass spectrometry. **J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.** 873, 59-69, 2008.

Parker, D. R.; Weiss, S. T.; Troisi, R.; Cassano, P. A.; Vokonas, P. S.; Landsberg, L. Relationship of dietary saturated fatty acids and body habitus to serum insulin concentrations: the Normative Aging Study. **Am. J. Clin. Nutr.** 58, 129-136, 1993.

Pepine, C. J.; Cooper-DeHoff, R. M. Cardiovascular therapies and risk for development of diabetes. **J. Am. Coll. Cardiol.** 44, 509-512, 2004.

Perez-Stable, E.; Caralis, P. V. Thiazide-induced disturbances in carbohydrate, lipid, and potassium metabolism. **Am. Heart J.** 106, 245-251, 1983.

Peter, P.; Martin, U.; Sharma, A. Effect of treatment with nebivolol on parameters of oxidative stress in type 2 diabetics with mild to moderate hypertension. **J. Clin. Pharm. Ther.** 31, 153-159, 2006.

Pi-Sunyer, F. X., Medical hazards of obesity. **Ann. Intern. Med.** 119, 655-660, 1993.

Plavinik, F. L.; Rodrigues, C.I.; Zanella, M. T.; Ribeiro, A.B. Hypokalemia, glucose intolerance, and hyperinsulinemia during diuretic therapy, **Hypertension** 19, 1126-1129, 1992.

Pollare, T.; Lithell, H.; Berne, C. A comparison of the effect of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. **N. Engl. J. Med.** 321, 868-873, 1989.

Punzi, H.A.; Punzi, C.F. Antihypertensive and Lipid-Lowering Heart Attack Trial Study; Trinity Hypertension Research Institute: Metabolic issues in the Antihypertensive and Lipid-Lowering Heart Attack Trial Study. **Curr. Hypertens. Rep.** 6, 106-110, 2004.

Raval, J.; Lyman, S.; Nitta, T.; Mohuczy, D.; Lemasters, J. J.; Kim, J. S.; Behrns, K. E. Basal reactive oxygen species determine the susceptibility to apoptosis in cirrhotic hepatocytes. **Free Radic. Biol. Med.** 41, 1645-1654, 2006.

Rayman, M. P. The importance of selenium to human health. **Lancet** 356, 233-241, 2000.

Rayssiguier, Y.; Durlach, J.; Gueux, E.; Rock, E.; Mazur, A. Magnesium and ageing: experimental data importance of oxidative damage. **Magnes. Res.** 6, 369-378, 1993.

Rayssiguier, Y.; Gueux, E.; Bussière, L.; Mazur, A. Copper deficiency increases the susceptibility of lipoproteins and tissues to peroxidation in rats. **J. Nutr.** 123, 1343-1348, 1993.

Rayssiguier, Y.; Gueux, E.; Weiser, D. Effect of magnesium deficiency on lipid metabolism in rats fed a high carbohydrate diet. **J. Nutr.** 111, 1876-1883, 1981.

Reaven, G. M. Role of insulin resistance in human disease. **Diabetes** 37, 1595-1607, 1988.

Reungjui, S.; Roncal, C. A.; Mu, W.; Srinivas, T. R.; Sirivongs, D.; Johnson, R. J.; Nakagawa, T. Thiazide Diuretics Exacerbate Fructose-Induced Metabolic Syndrome. **J. Am. Soc. Nephrol.** 18, 2724-2731, 2007.

Ribeiro, M. C. P.; Barbosa, N. B. V.; Parcianello, L. M.; Perottoni, J.; Ávila, D.S.; Rocha, J. B. T. High-fat diet and hydrochlorothiazide increase oxidative stress in brain of rats. **Cell Biochem. Funct.** 27, 473-478, 2009.

Roberts, C. K.; Barnard, R. J.; Sindhu, R. K.; Jurczak, M.; Ehdaie, A.; Vaziri, N. D. A high-fat, refined-carbohydrate diet induces endothelial dysfunction and oxidant/antioxidant imbalance and depresses NOS protein expression. **J. Appl. Physiol.** 98, 203-210, 2005.

Roberts, C. K.; Vaziri, N. D.; Wang, X. Q.; Barnard, R. J. Enhanced NO Inactivation and Hypertension Induced by a High-Fat, Refined-Carbohydrate Diet. **Hypertension** 36, 423-429, 2000.

Roglic, G.; Unwin, N.; Bennett, P. H.; Mathers, C.; Tuomilehto, J.; Nag, S.; Connolly, V.; King, H. The Burden of Mortality Attributable to Diabetes - Realistic estimates for the year 2000. **Diabetes Care** 28, 2130-2135, 2005.

Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G. Hoekstra, W. G. Selenium: biochemical role as a component of glutathione peroxidase. **Science** 179, 588-590, 1973.

Rosen, P.; Nawroth, P. P.; King, G.; Moller, W.; Tritschler, H. J.; Packer, L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a congress series sponsored by UNESCO-MCBN, the American Diabetes Association, and the German Diabetes Society. **Diabetes Metab. Res. Rev.** 17, 189-212, 2001.

Rowe, J. W.; Tobin, J. D.; Rosa, R. M.; Andres, R. Effect of experimental potassium deficiency on glucose and insulin metabolism. **Metab.** 29, 498-502, 1980.

Salmeron, J.; Hu, F. B.; Manson, J. E.; Stampfer, M. J.; Colditz, G. A.; Rimm, E. B.; Willett, W. C. Dietary fat intake and risk of type 2 diabetes in women. **Am. J. Clin. Nutr.** 73, 1019-1026, 2001.

Sarafidis, P. A.; Bakris, G. L. The antinatriuretic effect of insulin: an unappreciated mechanism for hypertension associated with insulin resistance? **Am. J. Nephrol.** 27, 44-54, 2007.

Schiar, V. P. P. ; Santos, D. B. ; Paixão, M.W., Nogueira, C. W. ; Rocha, J.B.T.; Zeni, G. Human erythrocyte hemolysis induced by selenium and tellurium compounds increased by GSH or glucose: A possible involvement of reactive oxygen species. **Chem. Biol. Interact.** 177, 28-33, 2009.

Shulman, R. G.; Rothman, D. L.; Behar, K. L.; Hyder, F. Energetic basis of brain activity: implications for neuroimaging. **Trends Neurosci.** 27, 489-495, 2004.

Sicree, R.; Shaw, J. Type 2 diabetes: An epidemic or not, and why it is happening. **Diabetes Metab. Syndr. Clin. Res. Rev.** 1, 75-81, 2007.

Sociedade Brasileira de Diabetes. **URL: http://www.diabetes.org.br.** Acessado em setembro de 2009.

Sontia, B.; Touyz, R. M. Role of magnesium in hypertension. **Arch. Biochem. Biophys.** 458, 33-39, 2007.

Southorn, P. A. & Powis, G. Free radicals in medicine I. Chemical nature and biologic reactions. **Mayo Clin. Proc.** 63, 381-389, 1988.

Sprecher, H. Interactions between the metabolism of n-3 and n-6 fatty acids**. J. Intern. Med.** 225, 5-9, 1989.

Stapleton, S. R. Selenium: an insulin-mimetic. **Cell. Mol. Life Sci.** 57, 1874-1879, 2000.

Storlien, L. H.; Baur, L. A.; Kriketos, A. D.; Pan, D. A.; Clooney, G. J.; Jenkins, A. B.; Calvert, G. D.; Campbell, L. V. Dietary fats and insulin action. **Diabetologia** 39, 621-631, 1996.

Storlien, L. H.; James, D. E.; Burleigh, K. M.; Crisholm, D. J.; Kraegen, E. W. Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. **Am. J. Physiol.**, 251, 576-583, 1986.

Storlien, L. H.; Jenkins, A. B.; Chisholm, D. J.; Pascoe, W. S.; Khouri, S.; Kraegen, E. W. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. **Diabetes** 40, 280-289, 1991.

The ALLHAT Study Group, Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium-channel blocker vs. diuretic. **JAMA** 288, 2981-2997, 2002.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Follow-up report on the diagnosis of diabetes mellitus. **Diabetes Care** 26, 3160-3167, 2003.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. **Diabetes Care** 20, 1183-1197, 1997.

Thevenod, F.; Friedmann, J. M. Cadmium-mediated oxidative stress in kidney proximal tubule cells induces degradation of Na^+/K^+-ATPase through proteasomal and endo-/lysosomal proteolytic pathways. **FASEB J.** 13, 1751-1761, 1999.

Thomas, M. J.; Rudel, L. L. Dietary fatty acids, low density lipoprotein composition and oxidation and primate atherosclerosis. **J. Nutr.** 126, 1058-1062, 1996.

Trevisan, M.; Krogh, V.; Freudenheim, J.; Blake, A.; Muti, P.; Panico, S.; Farinaro, E.; Mancini, M.; Menotti, A.; Ricci, G. Consumption of olive oil, butter, and vegetable oils and coronary heart disease risk factors: the Research Group ATS-RF2 of the Italian National Research Council. **JAMA** 263, 688-692, 1990.

Turnbull, F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: Results of prospectively-designed overviews of randomized trials. **Lancet** 362, 1527-1535, 2003.

U. S. Department of Agriculture - U. S. Department of Health and Human Services. Nutrition and your health: Dietary guidelines for Americans. **Homes Gard. Bull.**, No. 232, U. S. Printing Office, Washington, 2000.

Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human disease. **Int. J. Biochem. Cell Biol.** 39, 44-84, 2007.

Varghese, S.; Oommen, O.V. Long-term feeding of dietary oils alters lipid metabolism, lipid peroxidation, and antioxidant enzyme activities in a teleost (*Anabas estudineus* Bloch). **Lipids** 35, 757-762, 2000.

Verdecchia, P. ; Reboldi, G.; Angeli, F.; Borgioni, C. ; Gattobigio, R.; Filippucci, L.; Norgiolini, S.; Bracco, C.; Porcellati, C. Adverse prognostic significance of new diabetes in treated hypertensive subjects. **Hypertension** 43, 963-969, 2004.

Vessby, B. Dietary fat and insulin action in humans. **Br. J. Nutr.** 83, 91-93, 2000.

Wilcox C. Metabolic and adverse effects of diuretics. **Semin. Nephrol.** 19, 557-568, 1999.

Wild, S.; Roglic, R.; Green, A.; Sicree, R.; King, H. Global Prevalence of Diabetes - Estimates for the year 2000 and projections for 2030. **Diabetes Care** 27, 1047-1053, 2004.

Wilson, S. R.; Zucker, P. A.; Huang, R. R. C.; Spector, A. Development of synthetic compounds with glutathione peroxidase activity. **J. Am. Chem. Soc.** 111, 5936-5939, 1989.

Yufu, K.; Itho, T.; Edamatsu, R.; Mori, A.; Hirakawa, M. Effect of hyperbaric oxygenation on the Naþ-Kþ-ATPase and membrane fluidity of cerebrocortical membranes after experimental subarachnoid hemorrhage. **Neurochem. Res.** 16, 1033-1039, 1993.

Zasso, F. B.; Gonçales, C.E.P.; Jung, E. A. C.; Araldi, D. ; Zeni, G.; Rocha, J. B. T.; Nogueira, C. W.On the mechanisms involved in antinociception induced by diphenyl diselenide. **Environ. Toxicol. Pharmacol.** 19, 283-289, 2005.

Zee, A. M.; Turner, S. T.; Schwartz, G. L.; Chapman, A. B.; Klungel, O. H.; Boerwinkle, E. Demographic, environmental, and genetic predictors of metabolic side effects of hydrochlorothiazide treatment in hypertensive subjects. **Am. J. Hypertens.** 18, 1077-1083, 2005.

Zimlichman, R.; Shargorodsky, M.; Wainstein, J. Prolonged treatment of hypertensive patients with low dose HCTZ improves arterial elasticity but not if they have NIDDM or IFG. Treatment with full dose HCTZ (25 mg/d) aggravates metabolic parameters and arterial stiffness. **Am. J. Hypertens.** 17, 138, 2004.

LISTA DE FIGURAS

INTRODUÇÃO

ARTIGO 1

MANUSCRITO 1

MANUSCRITO 2

Figura 1: Thiobarbituric acid-reactive species content in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

Figura 2: Protein carbonylation levels in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

Figura 3: Vitamin C levels in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

Figura 4. Catalase activity in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

Figura 5. Superoxide dismutase activity in the liver (A) and kidney (B) of diphenyl diselenide-fed rats associated to fructose and HCTZ intake.

LISTA DE TABELAS

ARTIGO 1

MANUSCRITO 1

MANUSCRITO 2