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EFEITO DA FARINHA DE MANDIOCA SOBRE O PERFIL LIPÍDICO E REDOX DE RATOS (*Rattus norvegicus*) WISTAR DISLIPIDÊMICOS

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ABREVIATURAS

HDL – Lipoproteína de Alta Densidade

LDL – Lipoproteína de Baixa Densidade

VLDL – Lipoproteína de Muito Baixa Densidade

AST – Aspartato-aminotranferase

ALT – Alanina-aminotranferase

LDH – Lactato desidrogenase

TBARS – Espécies Reativas ao Ácido Tiobarbitúrico

SOD – Superóxido Dismutase

CAT – Catalase

NASH – Esteatose Hepática Não Alcoólica

DCNT – Doenças crônicas não transmissíveis

DCV – Doenças cardiovasculares

HAS – Hipertensão arterial sistêmica

DM2 – Diabetes Melitus tipo 2

DAC – Doença arterial coronariana

NADP – Nicotinamida Adenina Dinucleotídeo Fosfato

NO – Óxido nítrico

H_2O_2 – Peróxido de hidrogênio

$OH^{\bullet}$ – Radical Hidroxila

O_2 – Oxigênio

RESUMO

As doenças cardiovasculares são uma das principais causas de morte em países desenvolvidos e em desenvolvimento e um dos principais fatores de risco para essas doenças é o colesterol total elevado. Sugere-se que o aumento da produção de espécies reativas está associado à ingestão de gorduras, um dos fatores que contribuem para o desenvolvimento de aterosclerose, e consequentemente, doenças cardiovasculares. Sabe-se que alguns alimentos podem reduzir o colesterol plasmático, como a aveia. Um alimento similar à aveia quando comparamos o teor de fibras é a farinha de mandioca, que além de ter menor custo do que a primeira, não contém glúten. Diante disso, o objetivo deste estudo foi avaliar os efeitos da farinha de mandioca sobre o perfil lipídico e redox em ratos dislipidêmicos. Trinta ratos Wistar (oito semanas de idade) foram alocados em 3 grupos: controle, dieta rica em colesterol (HC) - contendo 40% de gordura + 1% de colesterol + 1% de ácido cólico, dieta rica em colesterol + 50g/kg de farinha de mandioca (CF) e foram tratados por 8 semanas. A massa corporal e consumo alimentar dos animais foram avaliados semanalmente. Após a eutanásia, foram realizadas análises do perfil bioquímico e de estresse oxidativo, além da análise histológica do fígado. O peso e o consumo dos animais (em calorias) foram estatisticamente maiores nos grupos HC e CF do que no grupo controle. Os parâmetros de perfil lipídico não apresentaram diferenças entre os grupos HC e CF. A ureia apresentou-se diminuída nos grupos HC e CF, enquanto a creatinina não teve diferença entre os três grupos estudados. O AST apresentou-se aumentado nos dois grupos tratados, enquanto o ALT apresentou-se aumentado somente no grupo CF, sendo semelhante entre os grupos HC e controle. Nas análises de TBARS e SOD os resultados apresentaram-se aumentados para o grupo HC quando comparados ao controle e CF. Na determinação da atividade da CAT, o grupo HC apresentou diminuição com relação ao grupo controle, e o grupo CF mostrou-se ainda menor quando comparado aos dois grupos. Os achados histológicos em fígado sugerem que a farinha de mandioca parece retardar a progressão da NASH (Esteatose Hepática Não Alcoólica). A farinha de mandioca foi capaz de proteger os animais da lipoperoxidação, de acordo com os resultados de TBARS e melhorar a atividade da SOD e o teor de tiol, porém, não conseguiu melhorar o perfil lipídico e CAT nos animais. Nesse contexto, mais estudos são necessários para complementar os achados do presente estudo, possivelmente utilizando maiores quantidades de farinha de mandioca na dieta.

Palavras-chave: hipercolesterolemia, colesterol, farinha de mandioca, estresse oxidativo, perfil lipídico

ABSTRACT

Cardiovascular Diseases are one of the leading causes of death in developed and developing countries and one of the major risk factors for these diseases is high total cholesterol. It is suggested that the increase in the production of reactive species is associated with the ingestion of fats, one of the factors that contribute to the development of atherosclerosis, consequently, cardiovascular diseases. It is known that some foods can reduce plasma cholesterol, such as oats. A food like oats when we compare the fiber content is cassava flour, which in addition to having lower cost than the first, does not contain gluten. Therefore, the objective of this study was to evaluate the effects of cassava meal on the lipid and redox profile in dyslipidemic rats. Thirty Wistar rats (eight weeks old) were divided into three groups: control, high cholesterol diet (HC) - 40% fat + 1% cholesterol + 1% cholic acid and high cholesterol diet + 50g / kg of cassava flour (CF) and were treated for 8 weeks. The weight and feed consumption of the animals were evaluated weekly. After euthanasia, analyzes of the biochemical profile and oxidative stress, besides the histological analysis of the liver, were performed. The weight and consumption of the animals (in calories) were statistically higher in the HC and CF groups than in the control group. The lipid profile parameters showed no differences between the HC and CF groups. Urea was decreased in the HC and CF groups, while creatinine had no difference between the three groups. AST was increased in both treated groups, while ALT was increased only in the CF group, being similar between HC and control groups. In TBARS and SOD analyzes the results were increased for the HC group when compared to the control and CF. In CAT determination, the HC group presented lower results in relation to the control group, and the CF group was even lower when compared to the two other groups. Cassava flour was possibly able to slow the progression of NASH according to liver histology. Cassava flour was able to protect animals from lipoperoxidation, according to TBARS results and improve SOD activity and thiol content, however failed to improve the lipid profile and CAT in the animals. Nevertheless, further studies are needed to complement the findings of the present study, possible using greater amounts of cassava flour on diet.

Keywords: hypercholesterolemia, cholesterol, cassava flour, oxidative stress, lipid profile

SUMÁRIO

1. INTRODUÇÃO

As DCNTs são atualmente a maior causa de morte no mundo. Elas abrangem Diabetes Mellitus, Hipertensão Arterial, Obesidade, Doenças Cardiovasculares, entre outras.

As Doenças Cardiovasculares são também uma das principais causas de morte em países desenvolvidos e em desenvolvimento, e seus maiores fatores de risco modificáveis são uma alimentação inadequada, sedentarismo e tabagismo. Uma alimentação rica em gordura e colesterol pode levar à criação de placas ateroscleróticas, que por sua vez obstruem as artérias do coração, levando ao que chamamos de doença arterial coronariana (DAC).

Entre as modificações alimentares que podem ser feitas para prevenir a DAC está o aumento do consumo de fibras. Um alimento rico em fibras e que possui um potencial redutor de colesterol amplamente estudado é a aveia, porém a mesma pode conter traços de gluten e possui um preço elevado no Brasil. Por outro lado a farinha de mandioca, outro alimento rico em fibras, que não contém gluten e possui um baixo custo em nosso país até o momento tem sido pouco estudado. Tendo isso em vista, o presente estudo tem como objetivo verificar o efeito da farinha de mandioca sobre o perfil lipídico e redox de ratos Wistar.

1.1 Doenças Crônicas Não Transmissíveis (DCNT)

As DCNT são responsáveis por 70% das mortes no mundo, o que equivale a 38 milhões de mortes anuais. Desse número, cerca de 16 milhões acontecem prematuramente (em menores de 70 anos). Estão incluídas nas DCNT as doenças cardiovasculares e respiratórias crônicas, cânceres e diabetes (WHO 2012).

Estudos mostram que esse número de óbitos está relacionado ao crescimento dos fatores de risco dessas doenças, que incluem tabaco, inatividade física, uso indiscriminado de álcool e dietas não saudáveis (WHO, 2010). Os padrões alimentares adotados nas últimas décadas na sociedade ocidental podem ser prejudiciais de várias maneiras, e como por exemplo podemos citar o alto consumo de carne vermelha, de carne altamente processada e de ácidos graxos trans que está relacionado às doenças cardiovasculares e ao diabetes (WHO, 2011). Sabe-se que nem ¼ da população brasileira consome a quantidade de hortaliças e frutas que é recomendado (Azevedo et al. 2014), e isso também reflete na alta prevalência dessas doenças.

1.2 Doenças Cardiovasculares (DCV)

As doenças cardiovasculares são responsáveis por 17,3 milhões de mortes por ano em todo o mundo e estima-se que esse número possa duplicar nos próximos 15 anos (WHO, 2013). Nos países desenvolvidos e em desenvolvimento o número de mortes em função desse tipo de doença diminuiu significativamente nos últimos anos, porém em países subdesenvolvidos o número de óbitos só aumenta a cada ano (Dehghan et al. 2017).

No Brasil o número de mortes por DCV teve um aumento a partir da década de 1930, acompanhando a revolução industrial no país, e uma queda a partir dos anos 80 (Mansur et al. 2009). Uma revisão mostrou que a taxa de mortalidade teve sua maior diminuição nas regiões sul e sudestes do país, e a única região que obteve um aumento nessa taxa foi o nordeste (Mansur and Favarato 2016). Com esses resultados pode-se notar que as regiões sul e sudeste se comportaram como os países desenvolvidos, onde a taxa de mortalidade por DCV está diminuindo (Nichols et al. 2014).

Devido ao grande impacto das DCNT, dentre elas as DCV, na saúde da população e ao surgimento cada vez mais precoce, atualmente existe uma constante busca por alternativas dietéticas viáveis para a prevenção, controle e tratamento dessas doenças (Hickman et al. 1998).

Atualmente sabe-se que existe uma forte relação entre as doenças cardiovasculares e fatores genéticos, ambientais e de estilo de vida. Quando esses fatores coexistem com os fatores de risco para essas doenças, o risco de DAC aumenta exponencialmente (Castro et al. 2004). Os principais fatores de risco associados a essas doenças são HAS, colesterol total elevado, colesterol HDL reduzido, tabagismo, DM2 e idade (Wilson et al. 1998).

A DAC é caracterizada pela obstrução ou estreitamento das artérias coronárias, gerando uma insuficiência de irrigação sanguínea no coração. Essa condição está diretamente relacionada às placas ateroscleróticas, pois são essas que geram a obstrução ou estreitamento das artérias.

Um dos principais fatores de risco modificáveis da DAC é o nível elevado de colesterol circulante (Baigent et al. 2010; Lewington et al. 2007). O seu controle, principalmente do LDL-colesterol (lipoproteína de baixa densidade) pode alterar positivamente os desfechos cardiovasculares como infarto e morte (Baigent et al. 2010). A identificação precoce desses fatores e a melhora dos mesmos contribui para a redução da morbidade e mortalidade por essas doenças (Braga De Melo et al. 2018).

1.3 Relação entre perfil lipídico e estado redox

O corpo humano possui um eficiente sistema antioxidante. Porém, quando ocorre um desequilíbrio entre os níveis de moléculas oxidantes e antioxidantes o resultado é a indução de dano oxidativo às estruturas celulares. Esse processo, chamado de estresse oxidativo, ocorre como consequência do aumento na produção de espécies reativas ou diminuição das defesas antioxidantes do organismo, ou ambos (Halliwell, B; Gutteridge, 2007).

Estudos tem demostrado relação entre a superprodução das espécies reativas e aterosclerose (Demir et al. 2014; Youn et al. n.d.). O aumento do dano oxidativo associado a defesas antioxidantes diminuídas podem levar a transtornos metabólicos e mudanças na sinalização celular (Roberts and Sindhu 2009).

Nesse caso, algumas dessas espécies reativas, tais como radical superóxido ($O_2^{\bullet-}$), radical hidroxila ($OH^{\bullet}$) e peróxido de hidrogênio (H_2O_2) podem produzir danos como a lipoperoxidação de lipídios insaturados das membranas celulares (Gomes Rodrigues et al. 2003). Os peróxidos lipídicos oxidam o colesterol aumentando a produção de LDL oxidada. A formação de radicais livres pode ser controlada pela ação de antioxidantes, destacando-se a glutationa peroxidase, que inibem a produção de LDL oxidada, favorecendo a síntese de colesterol HDL (Olszewer 1994). Estudos demonstram que a peroxidação lipídica aumentada está diretamente ligada ao desenvolvimento de aterosclerose (Jialal et al. 2015; Perez-martinez et al. 2013)

Sugere-se também que o aumento da produção de espécies reativas está associado à ingestão de gorduras, um dos fatores que contribuem para o desenvolvimento de aterosclerose (Tasset et al. 2008) e, consequentemente, doenças cardiovasculares.

Outra fonte importante de espécies reativas é a nicotinamida adenina fosfato dinucleotídeo (NADP) oxidase, uma enzima presente em tecido renal e cardiovascular, que catalisa a transferência de um único elétron para o O_2 produzindo ânion superóxido. O aumento de sua expressão está associado a níveis elevados de espécies reativas e, consequentemente, com estresse oxidativo (Youn et al. 2014). O estado pró-inflamatório e a hiperglicemia aumentam produção de espécies reativas, o que resulta na superativação do NADP oxidase, resultando na produção de $O_2^{\bullet-}$ e diminuição da biodisponibilidade de óxido nítrico (NO) (Hopps et al. 2010). Em um estudo realizado em células mononucleares humanas, foi observado que o aumento da atividade da NADPH oxidase nas células endoteliais vasculares está associado ao desenvolvimento de aterosclerose (Martinez-hervas et al. 2008).

1.4 Modelos animais de aterosclerose

Modelos animais têm sido cruciais para o entendimento da etiologia de doenças metabólicas em humanos. O principal motivo disso se dá ao fato dessas doenças poderem ser induzidas por dieta em animais de laboratório (Pellizzon and Ricci 2018). Estudos experimentais também proporcionam testes preliminares para novos tratamentos.

A primeira evidência de um modelo experimental para aterosclerose foi no ano de 1908, quando foi relatado um espessamento da íntima com formação de grandes células claras na aorta de coelhos alimentados com uma dieta rica em proteínas animais (carne, leite, ovos) (Ignatowski 1908). A partir desse momento, uma forte associação entre certos tipos de dislipidemia, incluindo hipercolesterolemia, hipertrigliceridemia e hiperlipidemia combinada e o desenvolvimento de lesões ateroscleróticas foi documentado por vários ensaios clínicos e estudos epidemiológicos e experimentais (Katz 1958; LN Katz; J Stamler 1953)

A "Teoria do colesterol" ainda permanece como centro do desenvolvimento da doença vascular aterosclerótica. O papel aterogênico do colesterol tem sido testado em um número cada vez maior de animais de laboratório, incluindo linhagens selvagens, modelos animais de aterosclerose naturalmente defeituosos ou geneticamente modificados. Os resultados de quase todos estes estudos demonstram que o aumento do nível de colesterol plasmático é um método confiável para a indução da aterogênese (Moghadasian 2002).

Nos últimos anos, ratos tem sido uma das espécies mais usadas, compreendendo aproximadamente metade de estudos com animais. A maioria das pesquisas utiliza uma linhagem selvagem de ratos, principalmente Sprague Dawley ou Wistar (Moghadasian 2002) Os ratos são geralmente hipossensíveis à dieta hipercolesterolêmica, por isso, hiperlipidemia e aterogênese só podem ser induzidas em ratos por dietas com alto teor de colesterol/gordura que contenham ácido cólico ou tiouracil, que diminuem a utilização de colesterol para a síntese de sais biliares. (Rats 1983).

1.5 Fibras dietéticas e prevenção das doenças cardiovasculares

A *Association of Official Analytical Chemists* (AOAC) conceitua fibra alimentar como a porção não digerível do alimento vegetal, a qual resiste à digestão e absorção intestinal, porém com fermentação completa ou parcial no intestino grosso (Mello and Laaksonen 2009). As fibras alimentares incluem polissacarídeos, oligossacarídeos, lignina e outras substâncias que promovem efeitos fisiológicos benéficos, como os laxativos, atenuação do colesterol sanguíneo e/ou atenuação da glicemia (Almaraz et al. 2015).

As fibras podem ser classificadas quanto à sua solubilidade em água, em solúveis e insolúveis. A fibra solúvel é composta por pectinas, beta-glucanas, gomas, mucilagens e algumas hemiceluloses, enquanto a insolúvel é composta por lignina, pectinas insolúveis, celuloses e hemiceluloses (Tungland and Meyer 2002; Walker and Arvidsson 1953)

Desde o início da década de 50, quando se iniciou a industrialização de alimentos em diversos países, observou-se um aumento da incidência de doenças crônicas denominadas como “ocidentais” (DM2, DAC, obesidade), que até então eram menos frequentes (Gregorio, Areas, and Reyes 2001). Em função do aumento dessas enfermidades surgiu o interesse pelas fibras alimentares, a partir de estudos epidemiológicos realizados por Walker e Arvidsson em 1954, onde pela primeira vez a ingestão de alimentos contendo alto teor de fibras foi relacionada com níveis reduzidos de colesterol (Walker and Arvidsson 1953).

O mecanismo proposto para o efeito hipocolesterolêmico das fibras solúveis está relacionado a um efeito esponja, pois elas absorvem os sais biliares auxiliando na sua excreção nas fezes, fazendo com que o corpo produza mais desses sais, utilizando o colesterol presente no organismo (Bohm and Kulicke 1999). A diminuição do colesterol frente ao consumo de fibras solúveis já é bem conhecida e apresenta resultados significativos (Brug et al. 2008).

1.6 Farinha de mandioca e aveia

Exemplos de alimentos que estão associados a uma quantidade significativa de fibras alimentares são a aveia e a farinha de mandioca. Na aveia, a fibra alimentar total varia entre 9,62 a 13,86%, e na farinha de mandioca varia entra 3 e 10% (Gutkosky and Trombetta 1999; Montagnac, Davis, and Tanumihardjo 2009a).

Diversos estudos pré-clínicos já comprovaram o efeito hipolipidêmico da aveia (Charlton et al. 2012; Gu et al. 2015; Zhou et al. 2016). Anderson *et al.* foram os primeiros a provar esse benefício do alimento (Anderson et al. 1984). Em seu primeiro estudo mostram que o farelo de aveia pode reduzir os níveis de colesterol total em até 23% sem modificar os níveis de HDL-colesterol. Além disso, foi nesse estudo que surgiu a ideia de que a beta-glucana seria responsável por esse benefício (Anderson et al. 1984).

Um alimento que possui a composição físico-química similar à aveia no teor de fibras é a farinha de mandioca, segundo um estudo de Montagnac (Montagnac, Davis, and Tanumihardjo 2009a). A mandioca (*Manihot esculenta* Crantz) é uma das plantas cultivadas mais representativas da natureza (Albulquerque, T. T. O; Miranda, L. C. G.; Salim, J.; Quirino 1993). É totalmente aproveitável, desde as raízes às hastes e folhas. As suas raízes tuberosas ricas em amido são aproveitadas pelos mais diversos fins alimentícios ou industriais (Montagnac, Davis, and Tanumihardjo 2009).

A farinha de mandioca é amplamente consumida pelos brasileiros e possui um custo reduzido quando comparada à aveia. Além disso, é um alimento que não contém gluten, podendo ser consumida por indivíduos que possuam doença celíaca, enquanto diversas marcas de aveia podem conter traços dessa proteína (Montagnac, Davis, and Tanumihardjo 2009).

Em 2015 foi realizado um estudo que comparou as propriedades físico-químicas da aveia e da farinha de mandioca, como retenção de óleo e de água, além de soluções com pH 2, 7 e 9 e soluções de NaCl com concentrações de 4,5%, 9% e 14%. Em todos os resultados a farinha de mandioca se mostrou superior à aveia. Tendo em vista que o mecanismo de redução de colesterol por parte da aveia é feita em forma de "esponja", o estudo sugere que a farinha de mandioca possa ter o mesmo efeito. A análise com solução fosfato de diferentes pH foi adicionada ao estudo para que se evidenciassem os pH do sistema digestório do ser humano. O pH 2 é equivalente ao pH ácido do no estômago, o pH 7 é equivalente ao pH neutro, encontrado na boca e esôfago e o pH 9 é equivalente ao pH básico encontrado no intestino do ser humano. (Cunha et al, 2015).

Além disso, um ensaio clínico que utilizou a farinha de mandioca como placebo e farinha de nozes como intervenção não obteve diferença entre os dois grupos no colesterol total plasmático em 30 dias de estudo, e ambos tiveram diferença significativa com o grupo conrole, o que pode sugerir que a farinha de mandioca possua efeito hipocolesterolemiante (Carvalho et al. 2015). Isso pode acontecer mesmo que a farinha de mandioca apresente um percentual de fibras solúveis menor do que a aveia (Souza and Menezes 2004; Wood 1994), que foi considerada pela *American Dietetic Association* (ADA) um alimento funcional em função de sua capacidade de diminuição do colesterol plasmático (Thompson 2003). No entanto, não são encontrados na literatura estudos experimentais que utilizem a farinha de mandioca como intervenção quando é avaliada a modificação de perfil lipídico em animais.

2. OBJETIVOS

2.1 Objetivo Geral

Este estudo teve como objetivo geral avaliar os efeitos da farinha de mandioca sobre o perfil lipídico e redox em ratos dislipidêmicos.

2.2 Objetivos Específicos

Avaliar, em animais suplementados, ou não, com farinha de mandioca os seguintes parâmetros:

1. Perfil lipídico plasmático através da determinação do colesterol total, colesterol-HDL, colesterol-LDL, colesterol-VLDL e triacilglicerois.
2. Glicemia.
3. Função renal e hepática por meio da determinação plasmática de ureia, creatinina, aspartato-aminotranferase (AST), alanina-aminotranferase (ALT) e lactato desidrogenase (LDH).
4. Estado redox por meio da determinação de espécies reativas ao ácido tiobarbitúrico (TBARS), conteúdo de sulfidrilas e avaliação da atividade das enzimas superóxido dismutase (SOD) e catalase (CAT) em fígado.
5. Aumento de massa corporal.
6. Histologia do fígado.

REFERÊNCIAS

Albulquerque, T. T. O; Miranda, L. C. G.; Salim, J.; Quirino, J. G. 1993. "Composição Centesimal Da Raiz de 10 Variedades de Mandioca (Manihot Esculenta Crantz) Cultivadas Em Minas Gerais." *Revista Brasileira de Mandioca*.

Almaraz, Rosalía Sánchez et al. 2015. "Indicaciones de Diferentes Tipos de Fibra En Distintas Patologías." *Nutricion Hospitalaria*.

Anderson, J. W. et al. 1984. "Hypocholesterolemic Effects of Oat-Bran or Bean Intake for Hypercholesterolemic Men." *American Journal of Clinical Nutrition* 40(December): 1146–55.

Azevedo, Edynara Cristiane de Castro, Fábia Morgana Rodrigues da Silva Dias, Alcides da Silva Diniz, and Poliana Coelho Cabral. 2014. "Consumo Alimentar de Risco e Proteção Para as Doenças Crônicas Não Transmissíveis e Sua Associação Com a Gordura Corporal: Um Estudo Com Funcionários Da Área de Saúde de Uma Universidade Pública de Recife (PE), Brasil." *Ciência & Saúde Coletiva* 19(5): 1613–22. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-81232014000501613&lng=pt&tlng=pt.

Baigent, C et al. 2010. "Efficacy and Safety of More Intensive Lowering of LDL Cholesterol : A Meta-Analysis of Data from 170 000 Participants in 26 Randomised Trials." *The Lancet* 376(9753): 1670–81. http://dx.doi.org/10.1016/S0140-6736(10)61350-5.

Bohm, N., and W. M. Kulicke. 1999. "Rheological Studies of Barley (1-3), (1-4)-b-Glucan in Concentrated Solution: Mechanistic and Kinetic Investigation of the Gel Formation." *Carbohydrate Research*.

Braga De Melo, Jorgileia et al. 2018. "Fatores de Risco Cardiovasculares Em Mulheres Climatéricas Com Doença Arterial Coronariana." *International Journal of Cardiovascular Sciences* 31(1): 4–11. http://www.onlineijcs.org.

Brug, Johannes et al. 2008. "Taste Preferences, Liking and Other Factors Related to Fruit and Vegetable Intakes among Schoolchildren: Results from Observational Studies." *British Journal of Nutrition*.

Carvalho, Roberta F et al. 2015. "Intake of Partially Defatted Brazil Nut Flour Reduces Serum Cholesterol in Hypercholesterolemic Patients- a Randomized

Controlled Trial.” *Nutrition Journal*.

Castro, Luiza Carla Vidigal, Sylvia Do Carmo Castro Franceschini, Sílvia Eloíza Priore, and Maria Do Carmo Gouveia Pelúzio. 2004. “Nutrição e Doenças Cardiovasculares: Os Marcadores de Risco Em Adultos.” *Revista de Nutricao*.

Charlton, Karen E. et al. 2012. “Effect of 6 Weeks’ Consumption of β-Glucan-Rich Oat Products on Cholesterol Levels in Mildly Hypercholesterolaemic Overweight Adults.” *British Journal of Nutrition*.

Dehghan, Mahshid et al. 2017. “Articles Associations of Fats and Carbohydrate Intake with Cardiovascular Disease and Mortality in 18 Countries from Five Continents (PURE): A Prospective Cohort Study.”

Demir, Bulent et al. 2014. “The Association between the Epicardial Adipose Tissue Thickness and Oxidative Stress Parameters in Isolated Metabolic Syndrome Patients : A Multimarker Approach.” 2014.

Gomes Rodrigues, Hosana et al. 2003. “Nutritional Supplementation with Natural Antioxidants: Effect of Rutin on HDL-Cholesterol Concentration.” 16(3): 315–20.

Gregorio, S R, M A Areas, and F G R Reyes. 2001. “Fibras Alimentares e Doença Cardiovascular.” *Soc. Bras. Alim. Nutr .= J. Brazilian Soc. Food Nutr* 22: 109–20.

Gu, Jiaojiao et al. 2015. “GC-TOF-MS-Based Serum Metabolomic Investigations of Naked Oat Bran Supplementation in High-Fat-Diet-Induced Dyslipidemic Rats.” *Journal of Nutritional Biochemistry*.

Gutkosky, Luiz C., and Cassiana Trombetta. 1999. “Avaliação Dos Teores de Fibra Alimentar e de Beta-Glicanas Em Cultivares de Aveia (Avena Sativa L).” *Ciência e Tecnologia de Alimentos* 19(3): 387–90. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20611999000300016&lng=pt&nrm=iso&tlng=pt (June 18, 2017).

Halliwell, B; Gutteridge, J.M. 2007. *Free Radicals in Biology and Medicine*. New York: Oxford University Press.

Hickman, Tamy B et al. 1998. “Distributions and Trends of Serum Lipid Levels among United States Children and Adolescents Ages 4–19 Years: Data from the Third National Health and Nutrition Examination Survey 1.” *PREVENTIVE MEDICINE* 27: 879–90.

Hopps, E, D Noto, G Caimi, and M R Averna. 2010. "REVIEW A Novel Component of the Metabolic Syndrome : The Oxidative Stress." *Nutrition, Metabolism and Cardiovascular Diseases* 20(1): 72–77. http://dx.doi.org/10.1016/j.numecd.2009.06.002.

Ignatowski, A C. 1908. "Influence of Animal Food on the Organism of Rabbits." *Imp. Voyenno-Med. Akad.* 16: 154–73.

Jialal, Ishwarlal, Sridevi Devaraj, Beverley Adams-huet, and Xinpu Chen. 2015. "Increased Cellular and Circulating Biomarkers of Oxidative Stress in Nascent Metabolic Syndrome." 97(October): 1844–50.

Katz, L N; J Stamler; R Pick. 1958. "Nutrition and Atherosclerosis." *Lea and Febiger, Philadelphia*.

Lewington, Sarah et al. 2007. "Blood Cholesterol and Vascular Mortality by Age, Sex, and Blood Pressure: A Meta-Analysis of Individual Data from 61 Prospective Studies with 55 000 Vascular Deaths." *Lancet* 370: 1829–39.

LN Katz; J Stamler. 1953. "Experimental Atherosclerosis." *Charles C Thomas Publisher, Springfield*.

Mansur, Antonio De Padua et al. 2009. "Original Article Epidemiologic Transition in Mortality Rate from Circulatory Diseases in Brazil." : 468–72.

Mansur, Antonio De Padua, and Desidério Favarato. 2016. "Original Article Mortality Due to Cardiovascular Diseases in Women and Men in the Five Brazilian Regions , 1980-2012." : 137–46.

Martinez-hervas, Sergio et al. 2008. "Insulin Resistance and Oxidative Stress in Familial Combined Hyperlipidemia." 199: 384–89.

Mello, Vanessa D.de, and David E. Laaksonen. 2009. "Fibras Na Dieta: Tendências Atuais e Benefícios à Saúde Na Síndrome Metabólica e No Diabetes Melito Tipo 2." *Arquivos Brasileiros de Endocrinologia & Metabologia* 53: 1–10.

Moghadasian, Mohammed H. 2002. "Experimental Atherosclerosis A Historical Overview." 70: 855–65.

Montagnac, Julie A., Christopher R. Davis, and Sherry A. Tanumihardjo. 2009a. "Nutritional Value of Cassava for Use as a Staple Food and Recent Advances for Improvement." *Comprehensive Reviews in Food Science and Food Safety* 8(3): 181–94. http://doi.wiley.com/10.1111/j.1541-4337.2009.00077.x (June 18, 2017).

Nichols, Melanie, Nick Townsend, Peter Scarborough, and Mike Rayner. 2014. “Cardiovascular Disease in Europe 2014 : Epidemiological Update.”

Olszewer, Efrain. 1994. *O Perigoso Radical Livre*. São Paulo: Nova Linha Editorial.

Pellizzon, Michael A, and Matthew R Ricci. 2018. “The Common Use of Improper Control Diets in Diet-Induced Metabolic Disease Research Confounds Data Interpretation : The Fiber Factor.” : 1–6.

Perez-martinez, Pablo et al. 2013. “Oxidative Stress Is Associated with the Number of Components of Metabolic Syndrome : LIPGENE Study.” (March): 1–7.

Rats, Hypercholesterolemic. 1983. “Pathogenesis Aorta of Hypercholesterolemic Rats.” *Atherosclerosis*: 341–58.

Roberts, Christian K et al. 2006. “Oxidative Stress and Dysregulation of NAD (P) H Oxidase and Antioxidant Enzymes in Diet-Induced Metabolic Syndrome.” 55: 928–34.

Roberts, Christian K, and Kunal K Sindhu. 2009. “Oxidative Stress and Metabolic Syndrome.” *Life Sciences* 84(21–22): 705–12. http://dx.doi.org/10.1016/j.lfs.2009.02.026.

Souza, Maria Luzenira, and Hillary Castle de Menezes. 2004. “Processamento de Amêndoa e Torta de Castanha-Do-Brasil e Farinha de Mandioca: Parâmetros de Qualidade.” *Ciência e Tecnologia de Alimentos* 24(1): 120–28.

Tasset, I et al. 2008. “Fat Overload Aggravates Oxidative Stress in Patients.” 38: 510–15.

Thompson, T. 2003. “Oats and the Gluten-Free Diet.” *Journal of the American Dietetic Association* 103(3): 376–79.

Tungland, B.C., and D. Meyer. 2002. “Nondigestible Oligo- and Polysaccharides (Dietary Fiber): Their Physiology and Role in Human Health and Food.” *Comprehensive Reviews in Food Science and Food Safety* 1(3): 90–109. http://doi.wiley.com/10.1111/j.1541-4337.2002.tb00009.x.

Walker, By Alexander R P, and Ulla B Arvidsson. 1953. “Fat Intake, Serum Cholesterol Concentration, and Atherosclerosis in the South African Bantu. Part I. Low Fat Intake and the Age Trens of Serum Cholesterol Concentration in the South African Bantu.” (20): 1358–65.

WHO. 2010. *Global Status Report on Noncommunicable Diseases 2010*.
WHO. 2011. *Closing the Gap: Policy into Practice on Social Determinants of Health: Discussion Paper for the World Conference on Social Determinants of Health*.
WHO. 2012. *Health Statistics and Information Systems: Estimates for 2000-2012*.
WHO. 2013. “65th World Health Assembly Closes with New Global Health Measures.”
Wilson, P. W. F. et al. 1998. “Prediction of Coronary Heart Disease Using Risk Factor Categories.” *Circulation*.
Wood, Peter J. 1994. “Evaluation of Oat Bran as a Soluble Fibre Source. Characterization of Oat β-Glucan and Its Effects on Glycaemic Response.” *Carbohydrate Polymers* 25(4): 331–36. http://www-sciencedirect-com.ez41.periodicos.capes.gov.br/science/article/pii/0144861794900590 (September 19, 2017).
Youn, Ji-youn et al. *Role of Vascular Oxidative Stress in Obesity and Metabolic Syndrome*.
Zhou, Xianrong et al. 2016. “Hypolipidaemic Effects of Oat Flakes and β-Glucans Derived from Four Chinese Naked Oat (Avena Nuda) Cultivars in Wistar-Lewis Rats.” *Journal of the Science of Food and Agriculture*.

4. MANUSCRITO EM INGLÊS

MODELING HYPERCHOLESTEROLEMIA IN RATS USING HIGH CHOLESTEROL DIET

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Modeling hypercholesterolemia in rats using high cholesterol diet

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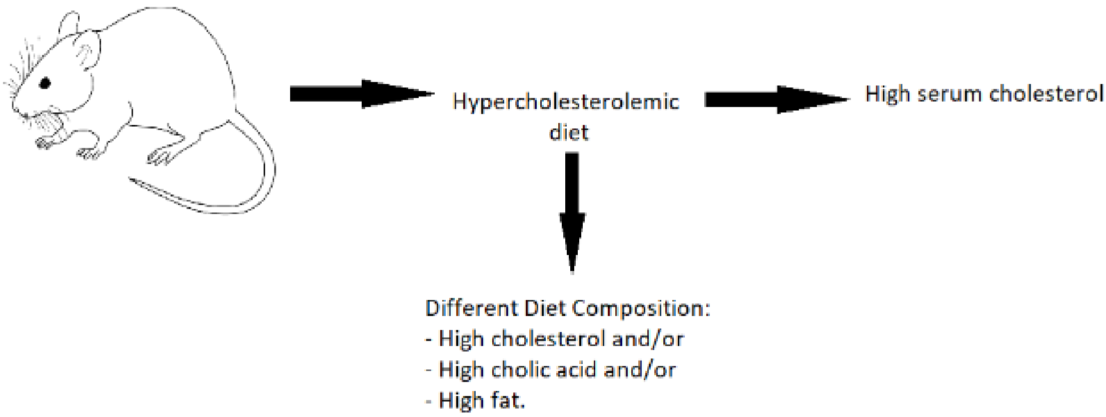
Abstract

Hypercholesterolemia is a complex condition with multiple causes, including lifestyle and genetic. It is also a risk factor for cardiovascular diseases (CVD), which are responsible for 17,2 million deaths/year. Although the outlines for hypercholesterolemia are known, there are many critical questions to be answered so new therapeutics can be developed. To elucidate the pathobiology of this condition, animal models can mimic the pathology of human hypercholesterolemia. One example of animal model is the hypercholesterolemic diet in Wistar rats. The present review first summarizes the current understanding of the metabolic profile involved in hypercholesterolemia in humans. Besides, there is no consensus about which hypercholesterolemia induction protocol should be used. Considering this, the present work aimed to review experimental studies which induced hypercholesterolemia in rats Wistar and not judge the best model, since they all achieved the goal that was to induce increased serum cholesterol.

Graphic Abstract



Highlights

List of induction models of hypercholesterolemia in Wistar rats.

Impacts of hypercholesterolemic diet in Wistar rats.

Metabolic profile of hypercholesterolemic humans.

Abbreviations

CVD – Cardiovascular diseases

DM2 – Diabetes Mellitus type 2

SAH – Sistemic Arterial Hypertension

LDL – Low Density Lipoprotein

HDL – High Density Lipoprotein

VLDL – Very Low Density Lipoprotein

Keywords

Hypercholesterolemia, Wistar Rats

Declarations of interest

none

Introduction

Cardiovascular diseases (CVD) are responsible for 17,2 million deaths/year all over the world (WHO, 2013). There is a constant search for alternative dietetic treatments, once this kind of disease have been seen at early age (Hickman et al., 1998). The CVD are included in the group of chronic non-communicable diseases, which also includes obesity, systemic arterial hypertension (SAH), diabetes mellitus type 2 (DM2), dyslipidemia and cancer (Duncan et al., 2012).

There is a strong relation between cardiovascular diseases and genetic, environmental factors and lifestyle. When these conditions are allied to risk factors to CVD, the possibility of coronary artery disease increases exponentially (Castro et al., 2004). The main risk factors to CVD are high total cholesterol, SAH, reduced LDL cholesterol, DM2 and age (Wilson et al., 1998).

Increased serum total cholesterol and low LDL cholesterol are components of hypercholesterolemia. Besides being a risk factor for cardiovascular diseases, these alterations can also ally to several other harms to the organism. Hypercholesterolemia can be classified as primary when the lipid disorder has genetic influence, or secondary, caused by inadequate diet and lifestyle, as well as medications (Cardiologia, 2017).

The decrease in serum cholesterol levels can be achieved by consumption of appropriate food, specific foods and/or drugs. Foods and drugs presenting hypocholesterolemic action should be tested in vitro and in vivo before being consumed by humans to verify their efficacy and safety. (Xie et al., 2015; L. Yang et al., 2013). Before being consumed by humans, these foods and their hypocholesterolemic potential should be tested in vitro and in vivo, so that their efficacy can be proven.

Hypercholesterolemia, as one of the main risk factors for CVD, has been strongly studied in animal models, especially searching new therapeutic approaches.

However, there is no consensus about which hypercholesterolemia induction protocol should be used. Considering this, the present work aimed to review experimental studies which induced hypercholesterolemia in rats Wistar.

Metabolic profile of hypercholesterolemic humans

Before studying hypercholesterolemic diet effects in animals, it's important to understand the cholesterol metabolism in humans, so that later the similarity with animals can be verified.

Cholesterol is a vital compound to human organism, essential in cell membranes formation, sex hormones production, bile salts formation, among several other parts of the organism (Santos et al., 2013). It can be found in animal fats, basically in its free form. Its main food sources are egg yolk, milk and milk derivates, shrimp, beef, bird skin and viscera. Some classic studies shown strong association between high cholesterol consumption and increased incidence of atherosclerosis (Keys et al., 1965; Walker and Arvidsson, 1953). Despite that, it is known that 56% of dietetic cholesterol is absorbed, and other fatty acids also have influence on plasmatic cholesterol concentration (Santos et al., 2013).

Cholesterol circulates in the bloodstream in structures called lipoproteins, formed by lipids and proteins. These are classified according to their composition (chylomicrons and VLDL- rich in triglycerides, LDL- rich in cholesterol, HDL- rich in cholesterol and proteins). When cholesterol is in excess in the body, it can form plaques, which we call atherosclerotic plaques and can harm the health of the individual (Cardiologia, 2017).

Atherosclerosis can be classified as a chronic inflammatory disease of multifactorial origin, which mainly affects medium and large caliber arteries reducing the blood flow. The initial injuries are formed in childhood and are characterized by cholesterol accumulation in macrophages. The deposition of lipoproteins in the arterial wall, key process at the beginning of atherogenesis, occurs proportionally to the plasma lipoprotein concentration. The severity of atherosclerosis is related to classic risk factors, as dyslipidemia, diabetes, tabagism, hypertension, and others. At cellular level, cholesterol crystals, microfilaments released by neutrophils, ischemia and changes in hemodynamic drag pressure have been implicated in the activation of inflammatory process, which is associated to atherosclerotic plaque rupture or endothelial erosion (Ridker and Medical, 2016).

This implications leads the tecidual factor of the vascular intima to interact with the circulating VIIa factor, leading to thrombin generation, platelet activation and thrombus formation, determining the main complications of atherosclerosis, acute myocardial infarction and cerebrovascular accident (stroke) (Cardiologia, 2017).

Considering physiology and metabolism, Wistar rats seems to be a promising animal model to evaluate comorbidities of Metabolic Syndrome because their similarity to humans(Bunnoy et al., 2015).

Data collection

Articles were found using the keywords “Hypercholesterolemia OR Hyperlipidemias AND Diet AND Rats, Wistar” in PubMed and “Hypercholesterolemia OR Hyperlipidemias AND Diet AND Rats Wistar” in BVS. We included data from experimental studies which efficiently induced hypercholesterolemia by diet in Wistar rats from 2013 until 2017. We excluded articles that induced hypercholesterolemia by injection, drug tests and reviews. Were also excluded studies that did not have a control group, because in that case we could not prove and quantify the induction. The data collection had three phases: title evaluation, abstract evaluation and full text evaluation. The final number of articles was 36. The findings are summarized in Table A1.

Impact of the Hypercholestolemic diet

The regulation of bile acids in the body depends on cholesterol, and this causes cholesterol to modify the composition of bile salts in various parts of the organism, including fecal excretion (González-Peña et al., 2017b; Katsarou et al., 2016). Wang et al. demonstrated in their study a smaller fecal bolus in the high cholesterol diet group (Wang et al., 2014), but there is no evidence of this finding in other studies.

The induction of hypercholesterolemia can lead to obesity, even when the amount of lipids in control and hypercholesterolemic groups is the same (Fidèle et al., 2017; González-Peña et al., 2017b; Wang et al., 2014). Santos-Lopez et al. observed that animals fed with high cholesterol diet presented a weight loss, even without significant difference in energy consumption when compared to control group animals (Santos-López et al., 2017).

Hypercholesterolemia induced by diet also affects animal's liver, increasing its weight and causing damage. This alterations probably happens because of higher fat content in liver (Gujjala et al., 2016; Tunsophon and Chootip, n.d.; Tuzcu et al., 2017a). Besides, some studies associate the liver damage to the high fatty acids content in the diet. However, other studies, in which the fat content is not modified also observed hepatic damage in the animals (El-Mahmoudy et al., 2013; Leontowicz et al., 2016; Sawale et al., 2016; Wang et al., 2014). Some studies found increased weight of heart and kidneys as well (Sawale et al., 2016; Xie et al., 2015).

The induction of hypercholesterolemia also decreases the activity of Superoxide dismutase (SOD) and Catalase (CAT) (important antioxidant enzymes) in the liver, decreasing also the antioxidant defense (Ben Gara et al., 2017; Harrabi et al., 2017; Tuzcu et al., 2017b). In addition, hypercholesterolemic diet can lower vitamin C levels in the animals body (Harrabi et al., 2017).

It can be observed in animals presenting hypercholesterolemia a decrease in myocardium function, increasing the chance of ischemia and reperfusion and enlarging the size of infarction. However, the mechanism involved in this alteration still not known (Katsarou et al., 2016; Wu et al., 2017; Xie et al., 2015).

Animal’s age may influence diet consumption and this directly affects weight gain throughout the experiment (Garcimarín et al., 2015) .

In this study we reviewed hypercholesterolemia models induced by diet. We could observe many protocols to induce hypercholesterolemia using animals of different ages and different diets. Our aim was to get together information, and not judge the best model, since they all achieved the goal that was to induce increased serum cholesterol.

References

Ampawong, S., Isarangkul, D., Aramwit, P., 2017. Sericin ameliorated dysmorphic mitochondria in high-cholesterol diet/streptozotocin rat by antioxidative property. Exp. Biol. Med. https://doi.org/10.1177/1535370216681553

Balzan, S., Hernandes, A., Reichert, C.L., Donaduzzi, C., Pires, V.A., Gasparotto, A., Cardozo, E.L., 2013. Lipid-lowering effects of standardized extracts of Ilex paraguariensis in high-fat-diet rats. Fitoterapia. https://doi.org/10.1016/j.fitote.2013.02.008

Ben Gara, A., Ben Abdallah Kolsi, R., Chaaben, R., Hammami, N., Kammoun, M., Paolo Patti, F., El Feki, A., Fki, L., Belghith, H., Belghith, K., 2017. Inhibition of key digestive enzymes related to hyperlipidemia and protection of liver-kidney functions by Cystoseira crinita sulphated polysaccharide in high-fat diet-fed rats. Biomed. Pharmacother. https://doi.org/10.1016/j.biopha.2016.11.059

Boudjeko, T., Ngomoyogoli, J.E.K., Woguia, A.L., Yanou, N.N., 2013. Partial characterization, antioxidative properties and hypolipidemic effects of oilseed cake of Allanblackia floribunda and Jatropha curcas. BMC Complement. Altern. Med. https://doi.org/10.1186/1472-6882-13-352

Bunnoy, A., Saenphet, K., Lumyong, S., Saenphet, S., Chomdej, S., 2015. Monascus purpureus-fermented Thai glutinous rice reduces blood and hepatic cholesterol and hepatic steatosis concentrations in diet-induced hypercholesterolemic rats. BMC Complement. Altern. Med. https://doi.org/10.1186/s12906-015-0624-5

Cardiologia, S.B. de, 2017. Atualização da diretriz brasileira de dislipidemias e prevenção da aterosclerose - 2017. Soc. Bras. Cardiol. 109. https://doi.org/10.5935/abc.20170121

Castro, L.C.V., Franceschini, S.D.C.C., Priore, S.E., Pelúzio, M.D.C.G., 2004. Nutrição e doenças cardiovasculares: Os marcadores de risco em adultos. Rev. Nutr. https://doi.org/10.1590/S1415-52732004000300010

Chijimatsu, T., Umeki, M., Kataoka, Y., Kobayashi, S., Yamada, K., Oda, H., Mochizuki, S., 2013. Lipid components prepared from a freshwater Clam (Corbicula fluminea) extract ameliorate hypercholesterolaemia in rats fed high-cholesterol diet. Food Chem.

https://doi.org/10.1016/j.foodchem.2012.08.070

Csont, T., Sárközy, M., Szcs, G., Szcs, C., Bárkányi, J., Bencsik, P., Gáspár, R., Földesi, I., Csonka, C., Kónya, C., Ferdinandy, P., 2013. Effect of a multivitamin preparation supplemented with phytosterol on serum lipids and infarct size in rats fed with normal and high cholesterol diet. Lipids Health Dis. https://doi.org/10.1186/1476-511X-12-138

de las Heras, N., Valero-Muñoz, M., Ballesteros, S., Gómez-Hernández, A., Martín-Fernández, B., Blanco-Rivero, J., Cachofeiro, V., Benito, M., Balfagón, G., Lahera, V., 2013. Factors involved in rosuvastatin induction of insulin sensitization in rats fed a high fat diet. Nutr. Metab. Cardiovasc. Dis. https://doi.org/10.1016/j.numecd.2012.11.009

De Las Heras, N., Valero-Muñoz, M., Martín-Fernández, B., Ballesteros, S., López-Farré, A., Ruiz-Roso, B., Lahera, V., De Las Heras, N., Valero-Muñoz, M., Martín-Fernández, B., Ballesteros, S., Lahera, V., Ruiz-Roso, B., n.d. Molecular factors involved in the hypolipidemic and insulin sensitizing effects of a ginger (Zingiber officinale Roscoe) extract in rats fed a high-fat diet.

Duncan, B.B., Chor, D., Aquino, E.M.L., Bensenor, I.M., Mill, J.G., Schmidt, M.I., Lotufo, P.A., Vigo, Á., Barreto, S.M., 2012. Doenças Crônicas Não Transmissíveis no Brasil: Prioridade para enfrentament e investigação. Rev. Saude Publica 46, 126–134. https://doi.org/10.1590/S0034-89102012000700017

El-Mahmoudy, A., Shousha, S., Abdel-Maksoud, H., Zaid, O.A., 2013. Effect of long-term administration of sildenafil on lipid profile and organ functions in hyperlipidemic rats. Acta Biomed.

El-Tantawy, W.H., Temraz, A., Hozaien, H.E., El-Gindi, O.D., Taha, K.F., 2015. Anti-hyperlipidemic activity of an extract from roots and rhizomes of Panicum repens L. on high cholesterol diet-induced hyperlipidemia in rats. Zeitschrift fur Naturforsch. - Sect. C J. Biosci. https://doi.org/10.1515/znc-2014-4147

El Rabey, H.A., Al-Seeni, M.N., Amer, H.M., 2013. Efficiency of barley bran and oat bran in ameliorating blood lipid profile and the adverse histological changes in hypercholesterolemic male rats. Biomed Res. Int. https://doi.org/10.1155/2013/263594

Fidèle, N., Joseph, B., Emmanuel, T., Théophile, D., 2017. Hypolipidemic, antioxidant and antiatherosclerogenic effect of aqueous extract leaves of Cassia. occidentalis Linn (Caesalpiniaceae) in diet-induced hypercholesterolemic rats. BMC Complement. Altern. Med. https://doi.org/10.1186/s12906-017-1566-x

Garcimartín, A., Santos-Opez, J.A., Bastida, S., Benedí, J., Anchez-Muniz, F.J. 2015. Silicon-Enriched Restructured Pork Affects the Lipoprotein Profile, VLDL Oxidation, and LDL Receptor Gene Expression in Aged Rats Fed an Atherogenic Diet 1–3. https://doi.org/10.3945/jn.115.213934

González-Peña, D., Checa, A., de Ancos, B., Wheelock, C.E., Sánchez-Moreno, C., 2017a. New insights into the effects of onion consumption on lipid mediators using a diet-induced model of hypercholesterolemia. Redox Biol. https://doi.org/10.1016/j.redox.2016.12.002

González-Peña, D., Giménez, L., de Ancos, B., Sánchez-Moreno, C., 2017b. Role of dietary onion in modifying the faecal bile acid content in rats fed a high-cholesterol diet. Food Funct. https://doi.org/10.1039/C7FO00412E

Gujjala, S., Putakala, M., Ramaswamy, R., Desireddy, S., 2016. Preventive effect of Caralluma fimbriata vs. Metformin against high-fat diet-induced alterations in lipid metabolism in Wistar rats. Biomed. Pharmacother. https://doi.org/10.1016/j.biopha.2016.09.029

Harrabi, B., Athmouni, K., Hamdaoui, L., Ben Mahmoud, L., Hakim, A., El Feki, A., Zeghal, K., Ghozzi, H., 2017. Polysaccharides extraction from Opuntia stricta and their protective effect against HepG2 cell death and hypolipidaemic effects on hyperlipidaemia rats induced by high-fat diet. Arch. Physiol. Biochem. https://doi.org/10.1080/13813455.2017.1307413

Hickman, T.B., Briefel, R.R., Carroll, M.D., Rifkind, B.M., Cleeman, J.I., Maurer, K.R., Johnson, C.L.P.H., 1998. Distributions and Trends of Serum Lipid Levels among United States Children and Adolescents Ages 4–19 Years: Data from the Third National Health and Nutrition Examination Survey 1. Prev. Med. (Baltim). 27, 879–890.

ıa Tabernero, M., Sarrí, B., Largo, C., Mar ınez- opez, S., Madrona, es, Luis Espartero, J., Bravo, L., Mateos, R., n.d. Comparative evaluation of the metabolic effects of hydroxytyrosol and its lipophilic derivatives (hydroxytyrosyl acetate and ethyl hydroxytyrosyl ether) in hypercholesterolemic rats. https://doi.org/10.1039/c3fo60677e

Kansal, S.K., Jyoti, U., Sharma, S., Kaura, A., Deshmukh, R., Goyal, S., 2015. Effect of zinc supplements in the attenuated cardioprotective effect of ischemic preconditioning in hyperlipidemic rat heart. Naunyn. Schmiedebergs. Arch. Pharmacol. https://doi.org/10.1007/s00210-015-1105-6

Katsarou, A.I., Kaliora, A.C., Chiou, A., Kalogeropoulos, N., Papalois, A., Agrogiannis, G., Andrikopoulos, N.K., 2016. Amelioration of oxidative and inflammatory status in hearts of cholesterol-fed rats supplemented with oils or oil-products with extra virgin olive oil components. Eur. J. Nutr. https://doi.org/10.1007/s00394-015-0947-5

Keys, A., Anderson, J.T., Grande, F., 1965. Serum cholesterol response to changes in the diet: II. The effect of cholesterol in the diet. Metabolism. 14, 759–65.

Leontowicz, M., Leontowicz, H., Jesion, I., Bielecki, W., Najman, K., Latocha, P., Park, Y.-S., Gorinstein, S., Maria, L., Hanna, L., Iwona, J., Wojciech, B., Katarzyna, N., Piotr, L., Yong-Seo, P., Shela, G., 2016. Kiwifruit Actinidia arguta supplementation protects aorta and liver in rats with induced hypercholesterolemia. Nutr. Res. https://doi.org/10.1016/j.nutres.2016.09.010

Li, M., Shu, X., Xu, H., Zhang, C., Yang, L., Zhang, L., Ji, G., 2016. Integrative analysis of metabolome and gut microbiota in diet-induced hyperlipidemic rats treated with berberine compounds. J. Transl. Med. https://doi.org/10.1186/s12967-016-0987-5

Otunola, G.A., Oloyede, O.B., Oladiji, A.T., Afolayan, A.J., 2014. Selected spices and their combination modulate hypercholesterolemia-induced oxidative stress in experimental rats. Biol. Res. https://doi.org/10.1186/0717-6287-47-5

Pandian, V., Aravindan, N., Subramanian, S., Somasundaran, S.T., 2014. Lipid-lowering effect of molluscan (Katelysia opima) glycosaminoglycan (GAG) in hypercholesterolemic induced rats. Biol. Chem. https://doi.org/10.1515/hsz-2013-0214

Ridker, P.M., Medical, H., 2016. From CRP to IL-6 to IL-1: Moving Upstream To Identify Novel Targets for Atheroprotection 118, 145–156. https://doi.org/10.1161/CIRCRESAHA.115.306656.From

Santos-López, J.A., Garcimartín, A., López-Oliva, M.E., Bautista-Vila, M., González-Muñoz, M.J., Bastida, S., Benedí, J., Sánchez-Muniz, F.J.,2017. Chia Oil–Enriched Restructured Pork Effects on

Oxidative and Inflammatory Status of Aged Rats Fed High Cholesterol/High Fat Diets. https://doi.org/10.1089/jmf.2016.0161

Santos, R.D., Gagliardi, a C., Xavier, H.T., Magnoni, C.D., Cassani, R., Lottenberg, a M., 2013. I Diretriz sobre o consumo de gorduras e saúde cardiovascular. Soc. Bras. Cardiol. 100, 1–40. https://doi.org/10.1590/S0066-782X2013000900001

Sawale, P.D., Pothuraju, R., Abdul Hussain, S., Kumar, A., Kapila, S., Patil, G.R., 2016. Hypolipidaemic and anti-oxidative potential of encapsulated herb (Terminalia arjuna) added vanilla chocolate milk in high cholesterol fed rats. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.7234

Schultz Moreira, A.R., Benedi, J., Bastida, S., Sánchez-Reus, I., Sánchez-Muniz, F.J., 2013. Nori- and Sea spaghetti-but not Wakame-restructured pork decrease the hypercholesterolemic and liver proapototic short-term effects of high-dietary cholesterol consumption. Nutr Hosp.Nutr Hosp 2828. https://doi.org/10.3305/nh.2013.28.5.6753

Shaodong, C., Haihong, Z., Manting, L., Guohui, L., Zhengxiao, Z., Ym, Z., 2013. Research of influence and mechanism of combining exercise with diet control on a model of lipid metabolism rat induced by high fat diet. Lipids Health Dis. https://doi.org/10.1186/1476-511X-12-21

Song, J.J., Tian, W.J., Kwok, L.Y., Wang, Y.L., Shang, Y.N., Menghe, B., Wang, J.G., 2017. Effects of microencapsulated Lactobacillus plantarum LIP-1 on the gut microbiota of hyperlipidaemic rats. Br. J. Nutr. https://doi.org/10.1017/S0007114517002380

Tunsophon, S., Chootip, K., n.d. Comparative effects of piperine and simvastatin in fat accumulation and antioxidative status in high fat-induced hyperlipidemic rats. Can. J. Physiol. Pharmacol. Downloaded from.

Tuzcu, Z., Orhan, C., Sahin, N., Juturu, V., Sahin, K., 2017b. Cinnamon Polyphenol Extract Inhibits Hyperlipidemia and Inflammation by Modulation of Transcription Factors in High-Fat Diet-Fed Rats. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2017/1583098

Walker, B.A.R.P., Arvidsson, U.B., 1953. Fat intake, serum cholesterol concentration, and atherosclerosis in the south african Bantu. Part I. Low fat intake and the age trens of serum cholesterol concentration in the south african Bantu 1358–1365.

Wang, Q., Du, Z., Zhang, H., Zhao, L., Sun, J., Zheng, X., Ren, F., 2015. Modulation of gut microbiota by polyphenols from adlay (*Coix lacryma-jobi* L. var. *ma-yuen* Stapf.) in rats fed a high-cholesterol diet. Int. J. Food Sci. Nutr. https://doi.org/10.3109/09637486.2015.1088941

Wang, Y.X., Li, Y., Sun, A.M., Wang, F.J., Yu, G.P., 2014. Hypolipidemic and antioxidative effects of aqueous enzymatic extract from rice bran in rats fed a high-fat and -cholesterol diet. Nutrients. https://doi.org/10.3390/nu6093696

Wihastuti, T.A., Sargowo, D., Tjokroprawiro, A., Permatasari, N., Widodo, M.A., Soeharto, S., 2014. Vasa vasorum anti-angiogenesis through H2O2, HIF-1α, NF-κB, and iNOS inhibition by mangosteen pericarp ethanolic extract (Garcinia mangostana Linn) in hypercholesterol-diet-given Rattus norvegicus Wistar strain. Vasc. Health Risk Manag. https://doi.org/10.2147/VHRM.S61736

Wilson, P.W.F., D'Agostino, R.B., Levy, D., Belanger, A.M., Silbershatz, H., Kannel, W.B., 1998. Prediction of Coronary Heart Disease Using Risk Factor Categories. Circulation. https://doi.org/10.1161/01.CIR.97.18.1837

WORLD HEALTH ORGANIZATION STUDY GROUP, 2013. Diet, nutrition and the prevention of chronic diseases.

Wu, Y., Tan, X., Tian, J., Liu, X., Wang, Y., Zhao, H., Yan, Z., Liu, H., Ma, X., 2017. PPARγ Agonist Ameliorates the Impaired Fluidity of the Myocardial Cell Membrane and Cardiac Injury in Hypercholesterolemic Rats. Cardiovasc. Toxicol. https://doi.org/10.1007/s12012-015-9352-9

Xie, Y., Zhang, H., Liu, H., Xiong, L., Gao, X., Jia, H., Lian, Z., Tong, N., Han, T., 2015. Hypocholesterolemic effects of Kluyveromyces marxianus M3 isolated from Tibetan mushrooms on diet-induced hypercholesterolemia in rat. Brazilian J. Microbiol. https://doi.org/10.1590/S1517-838246220131278

Yang, L., Han, G., Liu, Q.H., Wu, Q., He, H.J., Cheng, C.Z., Duan, Y.J., 2013. Rice protein exerts a hypocholesterolemic effect through regulating cholesterol metabolism-related gene expression and enzyme activity in adult rats fed a cholesterol-enriched diet. Int. J. Food Sci. Nutr. https://doi.org/10.3109/09637486.2013.804038

Yang, Y.H., Yang, J., Jiang, Q.H., 2013. Hypolipidemic effect of gypenosides in experimentally induced hypercholesterolemic rats. Lipids Health Dis. https://doi.org/10.1186/1476-511X-12-154

1. **Table A.1 – Summary of hypercholesterolemic diet-fed rat studies**

Author	Year	Initial age/ body weight	Diet composition	Diet duration	Serum Cholesterol diet group - comparison to control group
Chijimatsua et al. (Chijimatsu et al., 2013)	**2013**	**4 weeks ± 100 g**	**5g/kg cholesterol, 2.5g/kg sodium chloride**	**14 days**	**11.2 ± 0.6 mmol/l - increased**
Balzan et al. (Balzan et al., 2013)	**2013**	**8 weeks 250-300 g**	**2% cholesterol , 0.2% colic acid**	**60 days**	**127 ± 6.5 mg/dl - increased**
Shaodong et al. (Shaodong et al., 2013)	**2013**	**± 175g**	**1.5% cholesterol , 0.2% NaTDC, 5% sugar, 0,05% propiltiouracil**	**28 days**	**5.98 ± 1.07 mmol/l - increased**
El Rabey et al. (El Rabey et al., 2013)	**2013**	**140 – 160 g**	**1% cholesterol**	**8 weeks**	**114.26 ± 1.67 mg/dL - increased**

Pandian et al. (Pandian et al., 2014)	**2014**	**± 140 g**	**4% cholesterol, 1% cholic acid, 0.5% thiouracil**	**17 days**	**153.63 ± 11.72 mg/dl - increased**
Schultz Moreira et al. (Schultz Moreira et al., 2013)	**2013**	**90g**	**2.43% cholesterol, 0.49% cholic acid**	**1 week**	**2.23 ± 0.19 mmol/L - increased**
Yang et al. (Y. H. Yang et al., 2013)	**2013**	**180 - 200 g**	**3% cholesterol, 0.2% cholic acid, 0.5% propiltiouracil, 10% lard**	**4 weeks**	**19 ± 0.11 mmol/L - increased**
Tabernero et al. (ıa Tabernero et al., n.d.)	**2014**	**200 – 225 g**	**2% cholesterol , 0.4% cholic acid**	**8 weeks**	**Values not informed – serum cholesterol significantly increased**
Otunola et al. (Otunola et al., 2014)	**2014**	**± 168 g**	**1% cholesterol, 25% soy oil**	**4 weeks**	**Values not informed – serum cholesterol significantly increased**

Wihastuti et. al (Wihastuti et al., 2014)	**2014**	**8 weeks 150-200g**	**2% cholesterol, 0.2% cholic acid. 5% lard**	**3 months**	**Values not informed – serum cholesterol significantly increased**
Wang et al. (Wang et al., 2014)	**2014**	**190 - 230g**	**1% cholesterol, 0.2% bile salts, 10% lard, 10% yolk powder**	**42 days**	**2.65 ± 0.17 mmol/L - increased**
Bunnoy et al. (Bunnoy et al., 2015)	**2015**	**4 weeks 80-100g**	**12.5% palm oil, 12.5% lard, 5% cholesterol, 2% cholic acid**	**30 days**	**150.29 ± 20.51 mg/dl - increased**
Sawale at al. (Sawale et al., 2016)	**2016**	**4 - 6 weeks**	**2% cholesterol**	**60 days**	**194.2 ± 35.6 mg/dl - increased**
Katsarou et al.(Katsarou et al., 2016)	**2016**	**190 - 210g**	**2% cholesterol**	**9 weeks**	**88.4 (74.5–100.3) mg/dl - increased**
Garcimartín et al. (Garcimartín et al., 2015)	**2015**	**1 year 500g**	**16.3 g/kg cholesterol**	**8 weeks**	**3.3 ± 0.34 mmol/L - increased**

Wang et al. (Wang et al., 2015)	**2015**	**± 200 g**	**1% cholesterol**	**28 days**	**Values not informed – serum cholesterol significantly increased**
Wu et al. (Wu et al., 2017)	**2017**	**± 110 g**	**1% cholesterol, 10% yolk poder, 5% lard**	**10 weeks**	**Values not informed – serum cholesterol significantly increased**
Ampawong et al. (Ampawong et al., 2017)	**2017**	**8 weeks 200-300g**	**6% cholesterol**	**6 weeks**	**151.00 ± 10.74 mg/dL - increased**
Leontowicz et al. (Leontowicz et al., 2016)	**2016**	**± 114 g**	**1% cholesterol**	**42 days**	**2.25 ± 1.07 mmol/L - increased**
González-Peña et al. (González-Peña et al., 2017a)	**2017**	**8 weeks 245 ± 5g**	**2% cholesterol , 0.5% cholic acid**	**7 weeks**	**198.25 ± 10.61 mg/dL - increased**

Fidèle et al. (Fidèle et al., 2017)	**2017**	**± 178 g**	**1% cholesterol**	**4 weeks**	**156.09 ± 0.92 mg/dl - increased**
Santos-López et al. (Santos-López et al., 2017)	**2017**	**1 year ± 500g**	**1.26% cholesterol , 0.25% cholic acid**	**8 weeks**	**126.9 ± 13.2 mg/dl - increased**
González-Peña et al. (González-Peña et al., 2017b)	**2017**	**250g**	**2% cholesterol, 0.5% cholic acid**	**7 weeks**	**Values not informed – serum cholesterol significantly increased**
de las Heras et al. (de las Heras et al., 2013)	**2013**	**8 weeks 250 g**	**33.5% lard,1.5% soybean oil**	**7 weeks**	**53 ± 3.4 mg/dl - increased**
El-Mahmoudy et	**2013**	**6-8 weeks 180-210g**	**1% cholesterol, 2% coconut oil**	**60 days**	**168.55 ± 14.69 mg/dl - increased**

al. (El-Mahmoudy et al., 2013)					
Boudjeko et al. (Boudjeko et al., 2013)	**2013**	**180-210g**	**10g lard, 4g corn oil, 0,15g margarine**	**15 days**	**223.91 ± 20.4 mg/dl - increased**
Kansal et al. (Kansal et al., 2015)	**2015**	**200-300g**	**365g/kg cholesterol, 310g/kg yard**	**6 weeks**	**252.17 ± 14.72 mg/dl - increased**
El-Tantawy et al. (El-Tantawy et al., 2015)	**2015**	**150-200g**	**100g/kg corn oil**	**7 weeks**	**131 ± 9.6 mg/dl - increased**
Gujjala et al.(Gujjala et al., 2016)	**2016**	**170-190g**	**60% lard**	**90 days**	**194.2 ± 35.6 mg/dl increased**
Ben Gara et al.	**2017**	**± 180 g**	**10% sheep's fat, 0.1% cholic acid**	**6 weeks**	**1.14 ± 0.03 mmol/L - increased**

(Ben Gara et al., 2017)					
De Las Heras et al. (De Las Heras et al., n.d.)	**2016**	**8 weeks 250g**	**33.5% lard,1.5% soybean oil**	**5 weeks**	**46.0 ± 3.2 mg/dL - increased**
Harrabi et al. (Harrabi et al., 2017)	**2017**	**± 190 g**	**10g/kg cholesterol, 1g/kg cholic acid**	**2 months**	**3.21 ± 0.12 mmol/L - increased**
Tuzcu et al. (Tuzcu et al., 2017a)	**2017**	**± 180 g**	**400 g/kg Beef tallow**	**12 weeks**	**53.26 ± 1.90 mg/dL - increased**
Song et al. (Song et al., 2017)	**2017**	**4 weeks 120-150g**	**15% lard, 10% custard poder, 1.2% cholesterol, 0.3% sódum taurocholate**	**28 days**	**Values not informed – serum cholesterol significantly increased**
Csont et al. (Csont et al., 2013)	**2013**	**6 weeks 170-200g**	**2% cholesterol, 0.25% cholic acid**	**8 weeks**	**Values not informed – serum cholesterol significantly increased**

Li et al.(Li et al., 2016)	**2016**	**8 weeks 180-220g**	**10% lard, 20% sucrose, 2% cholesterol, 1% bile salt**	**8 weeks**	**Values not informed – serum cholesterol significantly increased**

5. MANUSCRITO EM INGLÊS

EFFECT OF CASSAVA FLOUR ON LIPIDIC AND REDOX PROFILE OF DISLIPIDEMIC WISTAR RATS (*RATTUS NORVEGICUS*)

(Manuscrito a ser submetido para a revista Plant Foods for Human Nutrition, fator de impacto: 2.465)

Effect of Cassava Flour on lipidic and redox profile of dislipidemic Wistar rats (*Rattus norvegicus*)

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ABSTRACT

One of the major risk factors for cardiovascular disease is high total cholesterol. It is known that some foods can reduce plasma cholesterol, such as oats. Cassava flour has a similar amount of fiber when compared to oats. In view of this, the objective of this study was to evaluate the hypocholesterolemic potential of cassava flour. Thirty Wistar (eight-week-old) rats were divided into 3 groups: control, high cholesterol diet (HC), high cholesterol diet + cassava flour (CF) and were treated for 8 weeks. The weight and food consumption of the animals were evaluated weekly. After euthanasia, analyzes of biochemical and oxidative stress profile were performed, besides histological analysis of the liver. Cassava flour was able to protect animals from lipoperoxidation, according to TBARS results and improve SOD activity and thiol content, however failed to improve the lipid profile and CAT in the animals. Cassava flour was possibly able to slow the progression of NASH (Non-Alcoholic Steatosis Hepatitis) according to liver histology.

INTRODUCTION

Cardiovascular diseases accounts for 17.3 million deaths per year worldwide and this number is estimated to double in the next 15 years [1]. It is now known that there is a strong relationship between cardiovascular diseases and genetic, environmental and lifestyle factors. When these factors coexist with risk factors for these diseases, the risk of coronary artery disease (CAD) increases exponentially [2]. The main risk factors associated with these diseases are hypertension, high total cholesterol, reduced HDL cholesterol, smoking, Diabetes Mellitus type 2 and age [3].

CAD is characterized by obstruction or narrowing of the coronary arteries, leading to insufficient blood supply to the heart. One of the major modifiable risk factors for CAD is high cholesterol [4, 5]. Its control, mainly of the LDL-cholesterol (low density lipoprotein) can modify the cardiovascular outcomes like infarct and death positively [5].

Studies show a relationship between overproduction of reactive species and atherosclerosis [6, 7]. Increased oxidative stress associated with decreased antioxidant defenses can lead to metabolic disorders and changes in cellular signaling [8].

It is known that lifestyle modification and improvement of eating habits are very important for the control of high cholesterol. One of the changes that has a big influence is an adequate consumption of fiber in the diet [3].

The *Association of Official Analytical Chemists* (AOAC), defines dietary fiber as the nondigestible portion of the plant food, which resists intestinal digestion and absorption, but with complete or partial fermentation in the large intestine [9]. Dietary fibers include polysaccharides, oligosaccharides, lignin, and other substances that promote beneficial physiological effects to humans such as laxatives, attenuation of blood cholesterol and/or attenuation of glycemia [10].

The decrease in cholesterol in relation to the consumption of fibers is already well known and presents significant results [11]. Examples of foods that are associated with a significant amount of dietary fiber are oats and cassava flour. In oats, total dietary fiber ranges from 9.62 to 13.86% [12].

Oats and cassava flour have a similar composition with respect to the total fiber content, according to a study by Montagnac [13]. Cassava flour is widely consumed by Brazilians and has a reduced cost when compared to oats. It is also a food that does not contain gluten and can be consumed by individuals who have celiac disease, while several brands of oats may contain traces of this protein.

The aim of this study was to evaluate the effects of cassava flour on lipid and metabolic profile in dyslipidemic rats, in order to identify a dietary alternative for dislipidemia.

MATERIALS AND METHODS

The study was approved by the Comissão de Ética no Uso de Animais (CEUA) of Universidade Federal de Ciências da Saúde de Porto Alegre – UFCSPA (approval number #534/17).

Experimental design

Thirty male Wistar rats 8 weeks old (weighing 200 ± 50 g) were obtained from the Central Animal House of UFCSPA. The animals were maintained on 12 h light/12 h dark cycles at a constant room temperature (20-24° C) with free access to water and food.

After 1 week of acclimatization, the animals were randomly allocated into three groups, with 10 rats in each group: (i) normal control group: animals fed with normal chow throughout the experimental period of 8 weeks; (ii) high cholesterol (HC) group: rats fed with high cholesterol diet throughout the experimental period of 8 weeks; high cholesterol + cassava flour (CF) group: rats fed with high cholesterol enriched with cassava flour diet throughout the experimental period of 8 weeks. The composition of diets (control, HC and CF) is shown in Table 1. The amount of cassava flour chosen was based on studies of a similar food (oats) in the same species and with the same study time [12, 13] .

Table 1. Composition of nutrients from high cholesterol diet compared to standard diet and cassava flour diet

	Standard diet	*High cholesterol diet (HC)*	*High cholesterol + cassava flour diet (CF)*

Protein (%)	22	18.8	18.8
Carbohydrates (%)	55	40.9	40,9
Fat (%)	4.5	40.3	40.3
Cholesterol (%)	0	1	1
Colic acid (%)	0	0.25	0.25
Cassava Flour (g/kg)	0	0	50

At the end of the treatment, animals were kept in 4h fasting and sacrificed by decapitation without anestesia. Samples from blood and liver and were collected and kept at -80°C for further analysis or fixed in formalin.

Plasma preparation

Blood was centrifuged at 8000 rpm for 15 min within 30 min of collection into citrate buffer containing tubes. Plasma was separated and transferred to Eppendorff tubes for storage at −20 °C before analysis.

Tissue preparation

Liver was homogenized in sodium phosphate buffer pH 7.4 containing KCl (1:10, w/v). The homogenates were centrifuged at 3,500 rpm for 10 min at 4º C. Immediately the supernatant was separated and used for biochemical determinations.

Liver of the animals was, also, fixed in 10% formalin solution. The tissue was processed and embedded in paraffin, cuts of 4μm were prepared and stained with hematoxylin-eosin; the stained areas were analyzed under an optical microscope with an increase of x200.

Weight gain

The animals were weighted three times a week, so the weight gain could be quantified. At the end of the experimental time, the naso-anal length was measured, and the Lee Index was calculated.

Biochemical profile evaluation

Plasma levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, triacylglycerols, glucose, urea, creatinine, proteins, AST (aspartate aminotransferase), ALT (alanine aminotransferase) were measured according to fabricant instructions (Bioclin / Quibasa © 2012).

Redox profile determination

The redox profile was determined on liver homogenate.

Thiobarbituric acid-reactive substances (TBARS): Samples were mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and then heated in boiling water bath for 60 min. TBARs were determined by the absorbance at 535 nm and reported as nmol TBARs/mg of protein [14].

Sulfhydryls: This method is based on the 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) reduction by thiols that produces a yellow solution which absorption is measured spectrophotometrically at 412 nm [15]. Results were reported as nmol TNB/mg of protein.

Catalase (CAT) assay: activity was assayed according to Aebi [16], based on the decomposition of H_2O_2 monitored spectrophotometrically at 240 nm, at ambient temperature. One CAT unity is defined as 1 mmol of hydrogen peroxide consumed per minute and the specific activity was reported as units/mg of protein.

Superoxide dismutase (SOD) assay: SOD activity was measured spectrophotometrically at 480nm to Misra and Fridovich [17]. This method is based on the inhibition of adrenaline autoxidation in alkaline medium by the enzyme. Results were reported as units/mg of protein.

Protein determination

Protein was determined spectrophotometrically at 650 nm by the Lowry and colleagues [18]. This method uses bovine serum albumin as standard.

Histological study

Sections of 5 µm thickness of liver tissue of 3 animals per group were stained with Hematoxylin-Eosin and examined under an Olympus CX41 light microscope x400.

Statistical analysis

Statistical analysis was performed using the GraphPad Prisma 6. Data were presented as means ± SD. Determinations were obtained from ten animals per group, normality was analyzed by Bartlett's test and the differences were examined using one-way analysis of variance (ANOVA) followed by a Tukey post hoc test. Values of $p < 0.05$ were considered significant.

RESULTS

Was observed a higher weight gain in the cassava flour group (CF) when compared to the control group ($p<0.05$). Moreover, no statistically significant difference was observed in the weight gain of the high cholesterol (HC) group when compared to the control group (figure 1). The Lee index did not present significant differences between the three groups. Throughout the 8 weeks of study, the animals gained weight longitudinally, and began to present differences in this parameter in the three groups from the third week (figure 2).

In figure 3 it can be noted that the caloric consumption of the HC and CF groups were significantly higher than the control group ($p<0.05$).

Figure 4 shows the amount of epididymal white adipose tissue in the three studied groups. The HC and CF groups had more adipose tissue than the control group at the end of the study ($p<0.05$).

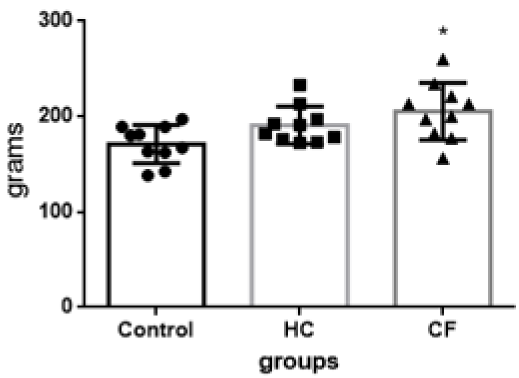

Figure 1 –Weight gain of rats treated with different diets. All values are expressed as mean ± SD (n=10). * different when compared to control group ($p<0.05$, ANOVA followed by Tukey Test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

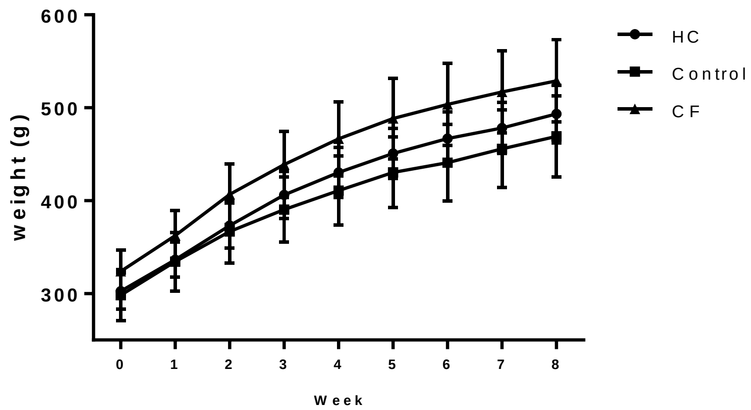

Figure 2 – Weight gain among study time. Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

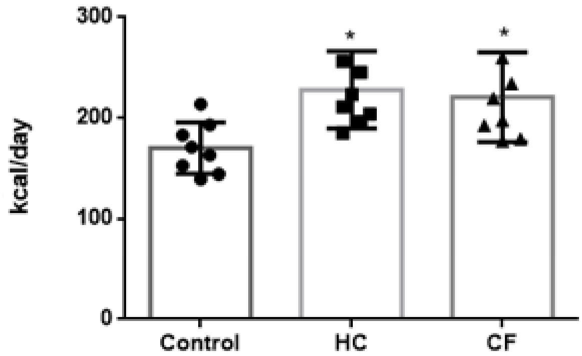

Figure 3 – Caloric consumption of rats treated with different diets. All values are expressed as mean ± SD (n=10). * different when compared to control group ($p<0.05$, ANOVA followed by Tukey Test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

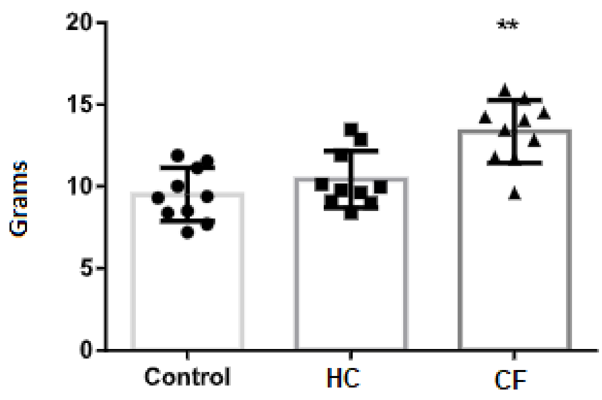

Figure 4 –Epididymal white adipose tissue of rats treated with different diets. All values are expressed as mean ± SD (n=10). * different when compared to control group ($p<0.01$, ANOVA

followed by Tukey Test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

As we can observe in Table 1, the intake of the high cholesterol diet significantly increased the content of total cholesterol and LDL cholesterol in the diet group compared to the control group ($p<0.0001$). It is also possible to observe that the diet containing cassava flour was not able to prevent the increase of total cholesterol and LDL in the supplemented group ($p<0.0001$). Regarding triglycerides, it was observed that the treated groups had lower levels than the control group ($P<0.0001$). The HDL-cholesterol and glycemia did not present significant difference.
Still in table 1, data of urea, creatinine, AST and ALT were analyzed. It can be observed a significant increase of AST on HC and CF groups ($p=0.001$) and ALT only on CF group ($p=0.026$). Urea was decreased in the treated groups when compared to the control group ($p <0.0001$), and creatinine was not different among the groups ($p=0.081$).

Table 1 – Plasma biochemical profile of Wistar rats supplemented or not with cassava flour.

Parameters	**Groups**		
	Control	***HC***	***CF***
Triglycerides (mg/dL)	124.2 ± 40.0	43.1 ± 13.2*	70.8 ± 20.7*
Total cholesterol (mg/dL)	63.5 ± 10,3	124.0 ± 19.7*	125.3 ± 16.2*
HDL - cholesterol (mg/dL)	16.1 ±6.5	12.9 ± 4.7	12.7 ± 3.2
LDL – cholesterol (mg/dL)	24.0 ± 11.4	100.3 ± 20.2*	101.1 ± 20.5*
Glycemia(mg/dL)	187.1 ± 26.0	178.3 ± 23.8	174.6 ± 31.3

Urea (mg/dL)	39.8 ± 9.5	17.4 ± 4.9*	20.70 ± 4.9*
Creatinine (mg/dL)	0.45 ± 0.17	0.29 ± 0.11	0.44 ± 0.16
AST (mg/dL)	112.8 ± 14.0	164.5 ± 70.6*	179.1 ± 33.0*
ALT (mg/dL)	49.9 ± 25.6	80.8 ± 36.5	88.0 ± 24.7*

All values are expressed as mean ± SD (n = 7-10). * Difference when compared to control group ($p<0.05$, ANOVA followed by Tukey Test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

As regards liver redox status, the sulfhydryl content analyzed in the liver of the animals presented differences between the control group and the HC and CF groups, which had this parameter significantly increased ($p<0.0001$) (figure 5).

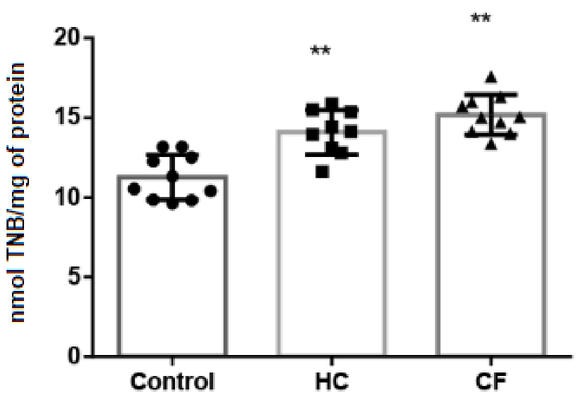

Figure 5 –Sulfhydryl content in liver of rats treated with different diets. All values are expressed as mean ± SD (n = 10). ** different when compared to control ($p <0.01$, ANOVA followed by Tukey's test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

TBARS, a marker of lipoperoxidation, was higher in the liver of the HC group comparing to the control, and cassava flour was able to prevent this alteration ($p<0.0001$) (figure 6).

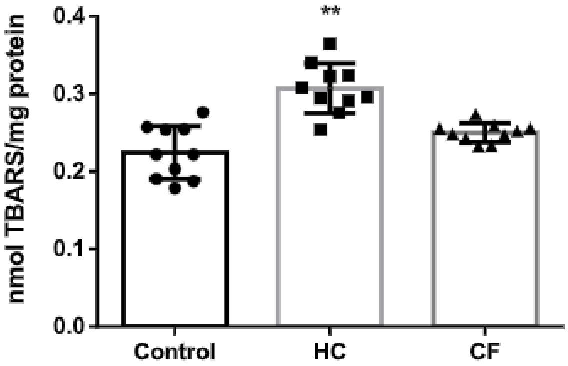

Figure 6 –TBARS in liver of rats treated with different diets. All values are expressed as mean ± SD (n = 10). * significant difference between control ($p < 0.01$, ANOVA followed by Tukey's test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

SOD activity was also higher in the liver of the HC group compared to the control, and cassava flour was able to prevent the increase ($p<0.0001$) (figure 7).

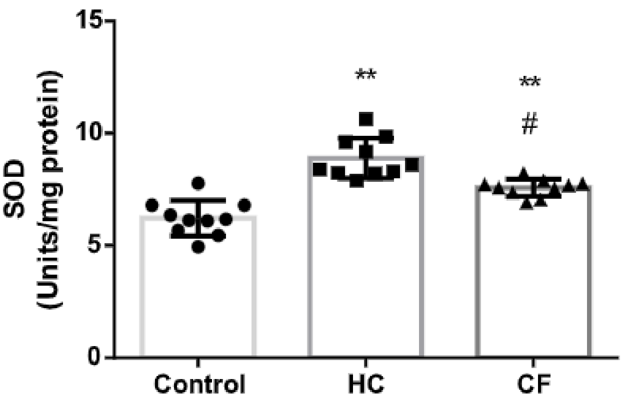

Figure 7 –SOD content in liver of rats treated with different diets. All values are expressed as mean ± SD (n = 10). ** different when compared to control, # different when compared to HC ($p < 0.0001$, ANOVA followed by Tukey's test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

CAT activity was lower in the liver of the HC group compared to the control, and even lower in the CF group ($p<0.0001$) (figure 6).

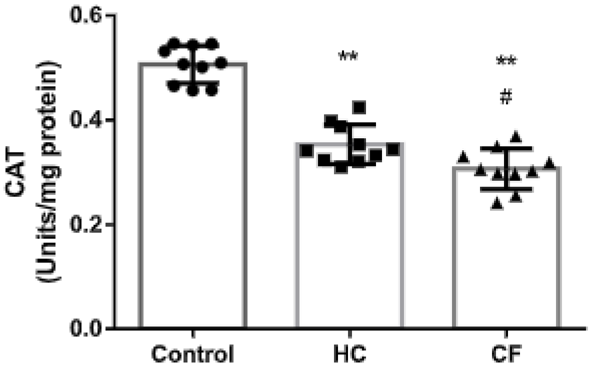

Figure 8 –CAT content in liver of rats treated with different diets. All values are expressed as mean ± SD (n = 10). ** different when compared to control # different when compared to HC (p <0.0001, ANOVA followed by Tukey's test). Control: standard diet; HC: high cholesterol diet; CF: high cholesterol plus cassava flour diet.

In Figure 9, it is possible to notice the liver damage in the HC and CF groups. We can see the portal triad in the three groups. The image of the control group shows a healthy liver. Figure 9B shows hepatocytes with intense macro and microvesicular steatosis, besides balonization and an apparent thickening in the Disse space. In the Figure 9C were also found hepatocytes with intense predominantly macrovesicular steatosis, but with less balonization and less thickening in the Disse space.

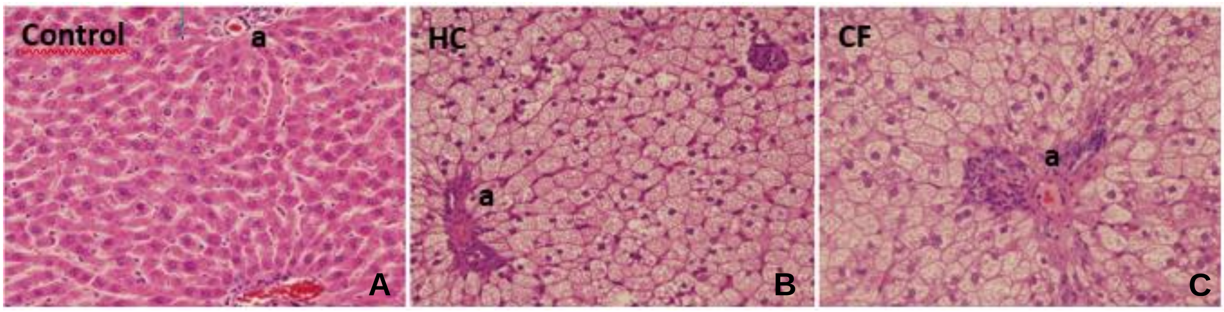

Figure 9 - Histological images in liver of rats treated with different diets. a = portal triad. (A) Control: standard diet; (B) HC: high cholesterol diet; (C) CF: high cholesterol plus cassava flour diet.

DISCUSSION

Cardiovascular diseases are one of the leading causes of death in developed and developing countries [19], and its major modifiable risk factors are inadequate diet, sedentary lifestyle and smoking [2]. Poor diet, high in fat and cholesterol, can lead to development of atherosclerotic plaques, which in turn obstruct the arteries of the heart, leading to CAD [5]. Among dietary modifications that can be made to prevent CAD is the increase in fiber intake [20]. Cassava flour is rich in fiber and does not contain gluten, however , it is a poorly studied food [21]. In this sense, our goal was to evaluate the effect of cassava flour supplementation on metabolic parameters in dyslipidemic rats induced by increased cholesterol diet.

The CF and HC groups consumed more calories when compared to the control group, and therefore had a greater weight gain. The highest calorie intake may have been due to the taste of the diet. There is evidence that the flavor influences the amount of food consumed directly and, consequently, the weight gain of the animals [22].

Our results showed that the total and LDL cholesterol could not be improved with consumption of the CF diet. The control group and the treated groups had a large difference in these two parameters, and this could be influenced by the difference in calories between the control diet and the other two diets.

According to Souza, the fiber content of cassava flour is satisfactory [23] and similar to that of oats [12], therefore we expected to find a hypocholesterolemic effect, since oats demonstrated a hypocholesterolemic effect [24, 25]. When the soluble fiber and beta-glucans content of the two foods is compared, it can be seen that oats have higher amounts [23, 26]. This may have been the reason for the effect of cassava flour on total cholesterol and LDL cholesterol levels not having been as expected in the study.

A study that used oats as intervention tested at various concentrations and found a significant difference in cholesterol reduction between groups with different amounts of added oats, being 50, 100 and 150 g/kg of diet [27]. With this in view, it is also possible that the amount of cassava flour added to the diet was not sufficient to cause a change since only 50g / kg cassava flour diet was added to the CF group.

The parameters of glycemia and HDL were not modified. This result of HDL can be explained by its direct relation with physical exercise, and in the present study the animals were not trained. Studies that evaluated this parameter and that trained the animals were able to observe a significant modification [28].

The consumption of high fat diets leads to an accumulation of triglycerides in the liver if it is not used as a substrate for the organism [29]. This is not in agreement with the findings of the present study, where the plasma triglyceride levels of the control group were higher than those of the treated groups. However, when analyzing the livers of the animals, it was noticed that the HC and CF groups had damages in this organ, with a clear hepatic steatosis. Furthermore, hepatic steatosis may be related to the increase in plasma levels of ALT and AST observed in the HC and CF groups. In addition, studies show a relationship between a diet high in cholesterol and fat and the increase of these two parameters [30, 31].

We also observed a lower urea level in plasma of animals treated with high cholesterol diet. It is possible that a lower intake of proteins associated to an energy-rich diet decrease the formation of urea in the liver, since amino acids are used for protein synthesis and are not degraded as energy source [32, 33]. Allied to this, the hepatic damage observed in the groups CF and HC can lead to a decrease in activity of urea cycle reducing urea synthesis [33, 34]. Creatinine plasma levels is not affected by liver function, once it is synthetized from creatine in the muscle [35], which explains that we did not observe differences among the groups.

Regarding the redox parameters, the cassava flour was able to protect the animals from lipoperoxidation, according to liver TBARS results. A study that evaluated the same parameter using oats as a treatment, instead of cassava flour, also obtained positive results [36], which indicates a similarity between the two foods, and can be explained by the similar fiber content, even if the type of fiber differs. Still on the TBARS in the liver, it can be said that the inflammation is smaller in the CF group, which is according to the findings of the histological evaluation of the organ.

Sulfhydryls are characterized as SH- thiol groups that act as antioxidants in the body, such as glutathione and when present in proteins and other compounds can be oxidized in the presence of free radicals [37]. Karaman and cols demonstrated that rats submitted to a high fat diet presented a recover of glutathione levels at 90 days, but not at 30 or 180 days, as well as an improve in glutathione reductase[38]. In our study, the sulfhydryl content in liver of treated animals showed an increase which it is possible a compensatory response of antioxidant system stimulated by the consumption of fat and cholesterol. In our study the animals were treated by 60 days, then it is possible that we were observing a similar effect of Karaman e cols results.

The increase of SOD activity may indicate a compensatory effect against free radicals increase in HC group, similar to observed on thiol content. It is possible that the high fat/high cholesterol diet cause an increase in free radicals leading to an unbalance and stimulating antioxidant defenses. In other words, the increase in free radicals content give rise to SOD activity as a protection mechanism [39, 40]. The cassava flour was able to partially prevent the increase of this enzyme activity, probably because in this group there is less free radicals formation. This is in accordance to TBARS results which shows less oxidative damage in cassava flour group. On the other hand, our results shown a reduction in catalase activity in treated groups. These results may be explained by the unbalance caused by excessive fat ingestion in HC and CF groups. Still, cassava flour treatment was not able to prevent this decrease in CAT activity. In physiological situations, there is a basal amount of catalase, which creates a redox balance between oxidants and antioxidants in the body [41]. Our CAT results are in agreement with literature, once it has been already established that there is an inverse relationship between catalase activity and damage caused by high fat and high cholesterol diet consumption [42].

On the histological evaluation of the liver, it is possible to verify that both HC and CF group present NASH (nonalcoholic steatosis hepatitis), but in the CF group this condition presents in a more initial stage. For NASH to be confirmed, it would be necessary to confirm the existence of fibrosis and the thickening of the space of Disse by specific histological techniques. It can be said that cassava flour prevented further liver damage from these blades. A study by Xie et al. also observed hepatic damage in the liver of animals treated with high cholesterol diet [43].

CONCLUSION

Cassava flour was able to protect animals submitted to a high fat/high cholesterol diet from oxidative stress once prevent lipid peroxidation and improve SOD activity and thiol content. However, it failed to improve the lipid profile and CAT activity in the animals. Besides, cassava flour was possibly able to slow the progression of NASH according to liver histology. Nevertheless, further studies are needed to complement the findings of the present study, possible using greater amounts of cassava flour on diet.

REFERENCES

1. WHO (2013) 65th World Health Assembly closes with new global health measures

2. Castro LCV, Franceschini SDCC, Priore SE, Pelúzio MDCG (2004) Nutrição e doenças cardiovasculares: Os marcadores de risco em adultos. Rev. Nutr.

3. Cardiologia SB de (2017) Atualização da Diretriz Brasileira de Dislimidemias e prevenção da Aterosclerose

4. Lewington S, Whitlock G, Clarke R, et al (2007) Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths. Lancet 370:1829–1839

5. Baigent C, Blackwell L, Emberson J, et al (2010) Efficacy and safety of more intensive lowering of LDL cholesterol : a meta-analysis of data from 170 000 participants in 26 randomised trials. Lancet 376:1670–1681. https://doi.org/10.1016/S0140-6736(10)61350-5

6. Demir B, Demir E, J GAJ, et al (2014) The Association between the Epicardial Adipose Tissue Thickness and Oxidative Stress Parameters in Isolated Metabolic Syndrome Patients : A Multimarker Approach. 2014:

7. Youn J, Siu KL, Lob H, et al Role of Vascular Oxidative Stress in Obesity and Metabolic Syndrome

8. Roberts CK, Sindhu KK (2009) Oxidative stress and metabolic syndrome. Life Sci 84:705–712. https://doi.org/10.1016/j.lfs.2009.02.026

9. Mello VD d., Laaksonen DE (2009) Fibras na dieta: tendências atuais e benefícios à saúde na síndrome metabólica e no diabetes melito tipo 2. Arq Bras Endocrinol Metabol 53:1–10. https://doi.org/10.1590/S0004-27302009000500004

10. Almaraz RS, Fuentes MM, Milla SP, et al (2015) Indicaciones de diferentes tipos de fibra en distintas patologías. Nutr Hosp. https://doi.org/10.3305/nh.2015.31.6.9023

11. Brug J, Tak NI, te Velde SJ, et al (2008) Taste preferences, liking and other factors related to fruit and vegetable intakes among schoolchildren: results from observational studies. Br J Nutr. https://doi.org/10.1017/S0007114508892458

12. Gutkosky LC, Trombetta C (1999) Avaliação dos teores de fibra alimentar e de beta-glicanas em cultivares de aveia (Avena sativa L). Ciência e Tecnol Aliment 19:387–390.

https://doi.org/10.1590/S0101-20611999000300016

13. Montagnac JA, Davis CR, Tanumihardjo SA (2009) Nutritional Value of Cassava for Use as a Staple Food and Recent Advances for Improvement. Compr Rev Food Sci Food Saf 8:181–194. https://doi.org/10.1111/j.1541-4337.2009.00077.x

14. Yagi, K; Ohkawa, H; Ohishi N (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–8

15. Aksenov MY, Markesbery WR (2001) Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett. https://doi.org/10.1016/S0304-3940(01)01636-6

16. H. Aebi (1984) Catalase invitro. Methods Enzym 6:105–121

17. Misra HP, Fridovich I (1972) The Role of Superoxide Anion in the Epinephrine and a Simple Assay for Superoxide Dismutase * Autoxidation of. J Biol Chem 247:3170–1175

18. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin. J Biol Chem 193:265–275. https://doi.org/10.1016/0304-3894(92)87011-4

19. Dehghan M, Mente A, Zhang X, et al (2017) Articles Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. https://doi.org/10.1016/S0140-6736(17)32252-3

20. Bohm N, Kulicke WM (1999) Rheological studies of barley (1-3), (1-4)-b-glucan in concentrated solution: Mechanistic and kinetic investigation of the gel formation. Carbohydr Res. https://doi.org/10.1016/S0008-6215(99)00036-1

21. Montagnac JA, Davis CR, Tanumihardjo SA (2009) Nutritional Value of Cassava for Use as a Staple Food and Recent Advances for Improvement. Compr Rev Food Sci Food Saf 8:181–194. https://doi.org/10.1111/j.1541-4337.2009.00077.x

22. Oliva L, Aranda T, Caviola G, et al (2017) In rats fed high-energy diets, taste, rather than fat content, is the key factor increasing food intake: a comparison of a cafeteria and a lipid-supplemented standard diet. PeerJ 5:e3697. https://doi.org/10.7717/peerj.3697

23. Souza ML, Menezes HC de (2004) Processamento de amêndoa e torta de castanha-do-Brasil e farinha de mandioca: parâmetros de qualidade. Ciência e Tecnol Aliment

24:120–128. https://doi.org/10.1590/S0101-20612004000100022

24. Gu J, Jing L, Ma X, et al (2015) GC-TOF-MS-based serum metabolomic investigations of naked oat bran supplementation in high-fat-diet-induced dyslipidemic rats. J Nutr Biochem. https://doi.org/10.1016/j.jnutbio.2015.07.019

25. Keys A, Anderson JT, Grande F (1965) Serum cholesterol response to changes in the diet: II. The effect of cholesterol in the diet. Metabolism 14:759–65

26. Zhou X, Lin W, Tong L, et al (2016) Hypolipidaemic effects of oat flakes and β-glucans derived from four Chinese naked oat (Avena nuda) cultivars in Wistar-Lewis rats. J Sci Food Agric. https://doi.org/10.1002/jsfa.7135

27. Silva MAM da, Barcelos M de FP, Sousa RV de, et al (2003) Efeito das fibras dos farelos de trigo e aveia sobre o perfil lipídico no sangue de ratos (Rattus norvegicus) wistar. Ciência e Agrotecnologia 27:1321–1329. https://doi.org/10.1590/S1413-70542003000600017

28. Wang Y, Xu D (2017) Effects of aerobic exercise on lipids and lipoproteins. Lipids Health Dis 16:1–8. https://doi.org/10.1186/s12944-017-0515-5

29. Moura LP de, Dalia RA, Araújo MB de, et al (2012) Alterações bioquímicas e hepáticas em ratos submetidos à uma dieta hiperlipídica/hiperenergética. Rev Nutr, Campinas 25:0–1. https://doi.org/10.1590/S1415-52732012000600001

30. Ulla A, Alam MA, Sikder B, et al (2017) Supplementation of Syzygium cumini seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats. BMC Complement Altern Med. https://doi.org/10.1186/s12906-017-1799-8

31. Zouari R, Hamden K, Feki A El, et al (2016) Protective and curative effects of Bacillus subtilis SPB1 biosurfactant on high-fat-high-fructose diet induced hyperlipidemia, hypertriglyceridemia and deterioration of liver function in rats. Biomed Pharmacother. https://doi.org/10.1016/j.biopha.2016.09.023

32. Matos SL, De Paula H, Pedrosa ML, et al (2005) Dietary models for inducing hypercholesterolemia in rats. Brazilian Arch Biol Technol. https://doi.org/10.1590/S1516-89132005000200006

33. Oliva L, Alemany M, Remesar X (2019) The Food Energy / Protein Ratio Regulates the Rat Urea Cycle but Not Total Nitrogen Losses. 1–12. https://doi.org/10.3390/nu11020316

34. Sabater D, Agnelli S, Arriarán S, et al (2014) Altered Nitrogen Balance and Decreased Urea Excretion in Male Rats Fed Cafeteria Diet Are Related to Arginine Availability. 2014:

35. Nelson, David L.; Cox MM (2014) Princípios de bioquímica de Lehninger, 6ª edição. Porto Alegre

36. Al-Malki AaL (2013) Oat attenuation of hyperglycemia-induced retinal oxidative stress and NF- B activation in streptozotocin-induced diabetic rats. Evidence-based Complement Altern Med 2013:. https://doi.org/10.1155/2013/983923

37. Halliwell, B; Gutteridge J. (2007) Free radicals in biology and medicine. Oxford University Press, New York

38. Karaman YK, Novgorodtseva TP, Yan VI (2013) Effects on Alimentary High-Fat Diet on Thiol Disul fi de Homeostasis in Rats. 155:752–756

39. Ferreira ALA, Matsubara LS (1997) Radicais Livres: Conceitos E Mecanismo De Lesão. Rev Ass Med Bras 43:61–69

40. Séfora-Sousa M, De Angelis-Pereira MC (2013) Mecanismos moleculares de ação anti-inflamatória e antioxidante de polifenóis de uvas e vinho tinto na aterosclerose. Rev Bras Plantas Med 15:617–626. https://doi.org/10.1590/S1516-05722013000400020

41. Barroso MV, Graça-Reis A, Cattani-Cavalieri I, et al (2019) Mate tea reduces high fat diet-induced liver and metabolic disorders in mice. Biomed Pharmacother 109:1547–1555. https://doi.org/10.1016/j.biopha.2018.11.007

42. Piao L, Choi J, Kwon G, Ha H (2017) Endogenous catalase delays high-fat diet-induced liver injury in mice. Korean J Physiol Pharmacol 21:317–325. https://doi.org/10.4196/kjpp.2017.21.3.317

43. Xie Y, Zhang H, Liu H, et al (2015) Hypocholesterolemic effects of Kluyveromyces marxianus M3 isolated from Tibetan mushrooms on diet-induced hypercholesterolemia in rat. Brazilian J Microbiol. https://doi.org/10.1590/S1517-838246220131

6. CONCLUSÃO

A farinha de mandioca protegeu do estresse oxidativo os animais submetidos a uma dieta rica em colesterol, uma vez que preveniu a peroxidação lipídica e aumentou a atividade de SOD. No entanto, falhou na melhora do perfil lipídico e da atividade da CAT nos animais. Além disso, a farinha de mandioca possivelmente retardou a progressão de esteatose hepática não alcoólica nos animais de acordo com a histologia de fígado. Nesse sentido, mais estudos são necessários para complementar os achados do presente estudo, possivelmente utilizando maior quantidade de farinha de mandioca na dieta e comparando esse alimento à aveia.

7. ANEXOS

7.1 Carta de aprovação CEUA

UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 215/17

2) DATA DO PARECER: 08/11/2017 **Parecer 534/17**

3) TÍTULO DO PROJETO:

Efeito da farinha de mandioca sobre o perfil lipídico no sangue de ratos ...

4) PESQUISADOR RESPONSÁVEL:

Alethea Barschak

5) RESUMO DO PROJETO:

Consta resumo adequado no projeto

6) OBJETIVOS DO PROJETO:

Avaliar e comparar os efeitos da farinha de mandioca e do farelo de aveia sobre o perfil lipídico e metabólico em ratos dislipidêmicos

7) FINALIDADE DO PROJETO: [ ] Ensino [x] Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título	[x] Adequado	[ ] Comentários
Introdução	[x] Adequada	[ ] Comentários
Objetivos	[x] Adequados	[ ] Comentários
Relevância e Justificativa	[x] Adequados	[ ] Comentários
Materiais e Métodos	[x] Adequados	[ ] Comentários
Cronograma para execução da pesquisa	[x] Adequado	[ ] Comentários
Orçamento e fonte financiadora	[x] Adequados	[ ] Comentários
Referências Bibliográficas	[x] Adequadas	[ ] Comentários

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:

[x] Sim [ ] Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de desestresse: B [ ] C [ ] D [x] E [ ]

Justifique:

Espécie: Rattus norvegicus – linhagem Wistar Kyoto

Número Amostral: 87

Redução Amostral: [ ] Sim [X] Não

Justifique:

Substituição de Metodologia: [ ] Sim [x] Não

Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia: [X] Sim [ ] Não

Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Acomodação e manutenção dos animais: [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Anestesia dos animais (se aplicável): [X] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Eutanásia dos animais (se aplicável): [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Local de Realização (Biotério/Laboratório): UFCSPA, Laboratório de Fisiologia Comportamental e Metabólica. Consentimento do responsável pelo laboratório.

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade

12) RECOMENDAÇÃO:

[X] Aprovado

[ ] Com Pendência

[ ] Não aprovado

Comentários gerais sobre o projeto:

Os questionamentos foram devidamente respondidos e as pendências atendidas.

7.2. Normas da revista Archives of Physiology and Biochemistry (Referente ao capítulo 2)

About the Journal

Archives of Physiology and Biochemistry is an international, peer-reviewed journal publishing high-quality, original research. Please see the journal's Aims & Scope for information about its focus and peer-review policy.

Please note that this journal only publishes manuscripts in English.

Archives of Physiology and Biochemistry accepts the following types of article: Original papers, Reviews, Editorials.

Peer Review and Ethics

Taylor & Francis is committed to peer-review integrity and upholding the highest standards of review. Once your paper has been assessed for suitability by the editor, it will then be single blind peer reviewed by independent, anonymous expert referees. Find out more about what to expect during peer review and read our guidance on publishing ethics.

Preparing Your Paper

Structure

Your paper should be compiled in the following order: title page; abstract; keywords; main text introduction, materials and methods, results, discussion; acknowledgments; declaration of interest statement; references; appendices (as appropriate); table(s) with caption(s) (on individual pages); figures; figure captions (as a list).

Word Limits

Please include a word count for your paper. There are no word limits for papers in this journal.

Style Guidelines

Please refer to these quick style guidelines when preparing your paper, rather than any published articles or a sample copy.

Please use British (-ise) spelling style consistently throughout your manuscript.

Please use double quotation marks, except where “a quotation is ‘within’ a quotation”. Please note that long quotations should be indented without quotation marks.

General Style Authors are asked to take into account the diverse audience of the journal. Please avoid the use of terms that might be meaningful only to a local or national audience, or provide a clear explanation where this is unavoidable. However, papers that reflect the particularities of a social and cultural system are acceptable. Some specific points on style follow:

Authors should write in clear, concise UK English. Language and grammar should be consistent with Fowler's English Usage; spelling and meaning of words should conform to Webster's Dictionary. If English is not your native language please ensure the manuscript has been reviewed by a native speaker. Please note: extensive rewriting of the text will not be undertaken by the editorial staff.

Latin terminology, including microbiological and species nomenclature, should be italicised.

Use standard convention for human and animal genes and proteins: italics for genes and regular font for proteins, and upper case for human products and lower case for animal products.

“US” is preferred to “American”, “USA” to “United States”, and “UK” to “United Kingdom”.

Punctuation of common abbreviations should adhere to the following conventions: “e.g.”; “i.e.”; “cf.”. Note that such abbreviations should not generally be followed by a comma or a (double) point/period.

Upper case characters in headings and references should be used sparingly, e.g. only the first word of paper titles, subheadings and any proper nouns begin upper case; similarly for the titles of papers from journals in the references and elsewhere.

Apostrophes should be used sparingly. Thus, decades should be referred to as follows: “The 1980s [not the 1980‟s] saw …”. Possessives associated with acronyms (e.g. APU), should be written as follows: “The APU‟s findings that …” but note that the plural is “APUs”.

All acronyms for national agencies, examinations, etc., should be spelled out the first time they are introduced in text or references. Thereafter the acronym can be used if appropriate, e.g. “The work of the Assessment of Performance Unit (APU) in the early 1980s …” and subsequently, “The APU studies of achievement …”, in a reference “(Department of Education and Science [DES] 1989a)”.

Brief biographical details of significant national figures should be outlined in the text unless it is quite clear that the person concerned would be known internationally. Some suggested editorial comments in a typical text are indicated in the following with square brackets: “From the time of H. E. Armstrong [in the 19th century] to the curriculum development work associated with the Nuffield Foundation [in the 1960s], there has been a shift from constructivism to heurism in the design of [British] science courses”.

The preferred local (national) usage for ethnic and other minorities should be used in all papers. For the USA, “African-American”, “Hispanic” and “Native American” are used, e.g. “The African-American presidential candidate, Jesse Jackson …”; for the UK, “Afro-Caribbean” (not “West Indian”), etc.

Material to be emphasised by italicisation in the printed version should be italicised in the typescript rather than underlined. Please use such emphasis sparingly.

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7.3 Normas da revista Plant Foods for Human Nutrition (Referente ao capítulo 3)

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16 double-spaced page requirement for original ms; 30 pages for review article; 4 pages for short communication. Title page, references, tables and figures should be included in the manuscript body.

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References

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1. Negotiation research spans many disciplines [3].
2. This result was later contradicted by Becker and Seligman [5].
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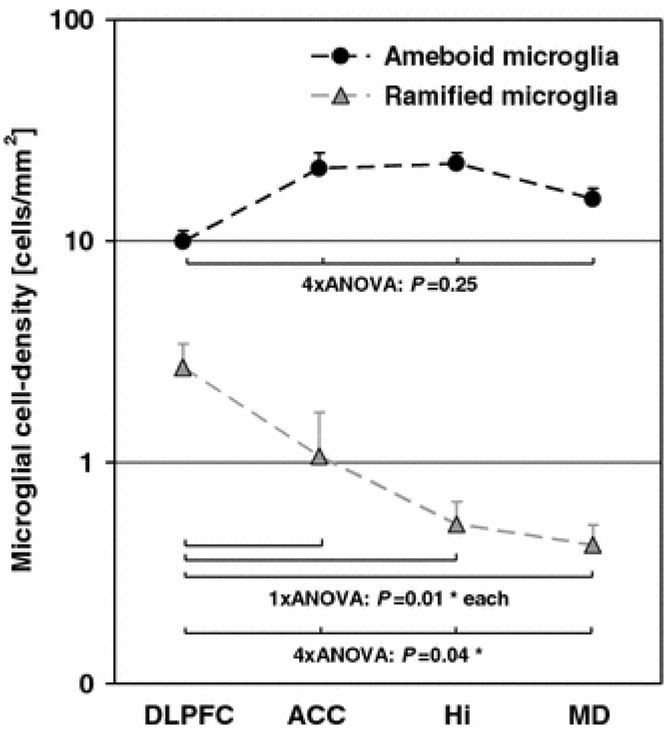

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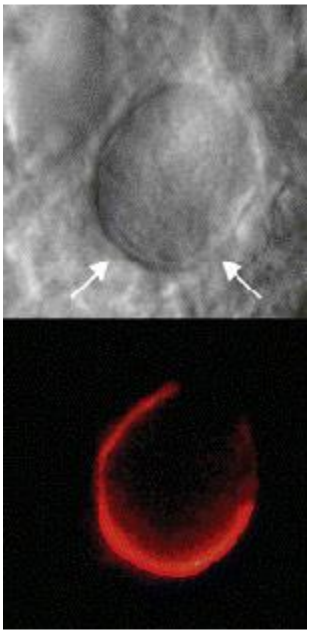

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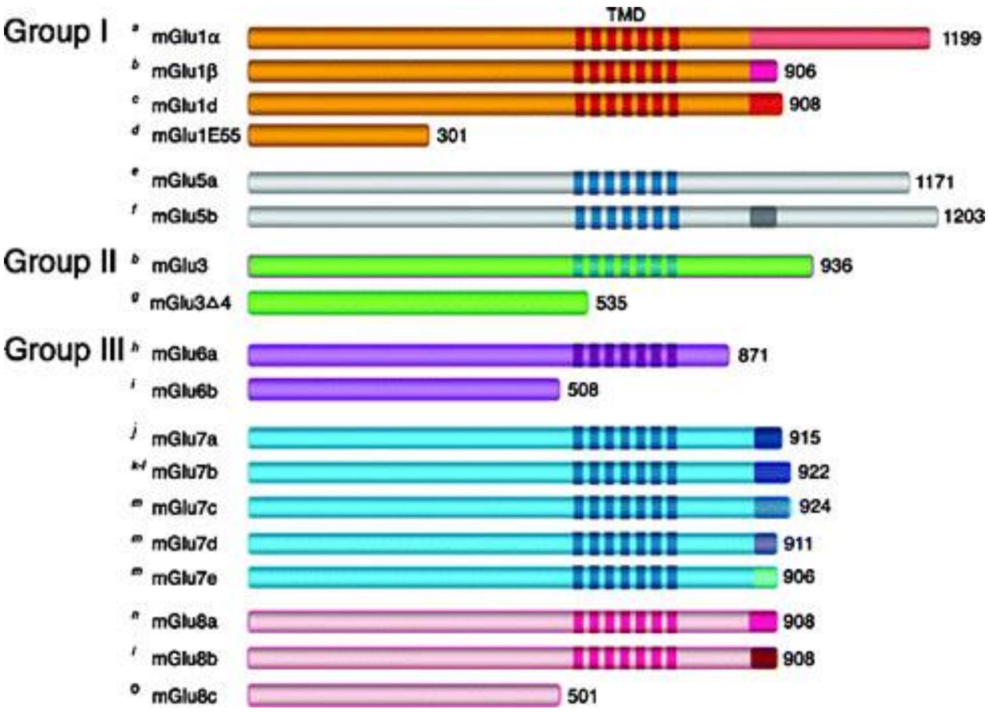

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