UNIVERSIDADE ESTADUAL DE MONTES CLAROS

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Avaliação Metabólica e Ecográfica dos Efeitos do Telmisartan no Tratamento da Doença Gordurosa Hepática não Alcoólica em Camundongos Alimentados com Dieta Normolipídica e Hiperlipídica

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Orientador: Prof. Dr. João Felício Rodrigues Neto

Coorientador: Prof. Dr. Sérgio Henrique Sousa Santos

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RESUMO

O Telmisartan é um anti-hipertensivo amplamente utilizado no tratamento da hipertensão leve a moderada, com excelente perfil de segurança. Nos últimos anos, evidências têm demosntrado efeitos promissores do Telmisartan no tratamento da doença gordurosa hepática não alcoólica (DGHNA), condição amplamente diagnosticada em humanos através de métodos de imagem não invasivos como a ultrassonografia (US). O objetivo do presente estudo foi investigar o papel do Telmisartan no tratamento da DGHNA em camundongos alimentados com uma dieta hiperlipídica através de parâmetros metabólicos e ecográficos. Camundongos machos FVB/N foram divididos em quatro grupos e alimentados por 60 dias com dieta normolipídica e hiperlipídica e em seguida foi associado ou não o tratamento com Telmisartan por gavagem por 28 dias. Durante o tratamento, foram mensurados o peso corporal e o consumo alimentar. Ao fim do tratamento, os animais foram submetidos aos testes de tolerância à glicose e de sensibilidade à insulina e ao exame ultrassonográfico do fígado e da gordura epididimal, com mesuração da ecogenicidade e do tamanho do fígado (eixo longitudinal) e da espessura do coxim adiposo epididimal. Após sacrifício por decapitação, os órgãos de interesse (fígado e gorduras epididimal e mesentérica) foram retirados e pesados. Amostras de sangue foram utilizadas para dosagens sérica e plasmática de colesterol total, colesterol HDL, triglicerídeos, alanina transaminase e aspartato aminotransferase. Análises histológicas do fígado também foram realizadas. Os resultados demonstram que o Telmisartan reduziu significativamente o peso do fígado e a infiltração histológica de gordura nos hepatócitos dos animais alimentados com dieta hiperlipídica, e esses achados puderam ser observados *in vivo* de maneira não invasiva através da medida das dimensões e da ecogenicidade do fígado pela US. Além disso, a medida do peso e da espessura do coxim de gordura epididimal pela US foi significativamente menor nos animais alimentados com dieta hiperlipídica e tratados com Telmisartan, evidenciando efeito benéfico da droga também neste parâmetro. O Telmisartan também reduziu o peso corporal, o peso do tecido adiposo mesentérico, a adiposidade, os níveis séricos da alanina aminotrasferase e melhorou o teste de tolerância à glicose e os níveis de colesterol HDL. Conclui-se que o tratamento oral com Telmisartan melhorou o metabolismo e a esteatose hepática em camundongos alimentados com dieta hiperlipídica, e que parâmetros ecográficos como a ecogenicidade hepática, a medida do eixo longitudinal do fígado e da espessura do coxim adiposo epididimal são eficazes como estimativas do peso do fígado e da gordura epididimal e do grau de infiltração gordurosa do fígado. Esses resultados reforçam o potencial do Telmisartan no tratamento da DGHNA e estimulam o uso da US em modelos murinos de esteatose hepática como ferramenta não invasiva para diagnóstico e acompanhamento longitudinal dos animais em detrimento de técnicas mais invasivas.

Palavras-chave: fígado gorduroso, ultrassom, terapêutica, camundongos

ABSTRACT

Telmisartan is an antihypertensive widely used in the treatment of mild to moderate hypertension, with an excellent safety profile. In recent years, evidence has shown promising effects of Telmisartan in the treatment of non-alcoholic fatty liver disease (NAFLD), a condition widely diagnosed in humans through non-invasive imaging methods such as ultrasonography (US). The aim of the present study was to investigate the role of Telmisartan in the treatment of NAFLD in mice fed with a high fat diet through metabolic and ultrasound parameters. Male FVB / N mice were divided into four groups and fed for 60 days with a normolipid and hyperlipidic diet, followed by treatment with Telmisartan by gavage for 28 days. During the treatment period, body weight and food consumption were measured. At the end of the treatment, the animals were submitted to glucose tolerance and insulin sensitivity tests and ultrasound examination of the liver and epididymal fat pad, with measurement of liver echogenicity and size (longitudinal axis) and of epididymal fat pad thickness. After sacrifice by decapitation, the organs of interest (liver, epididimal and mesenteric fats) were removed and weighed. Blood samples were used for serum and plasma levels of total cholesterol, HDL cholesterol, triglycerides, alanine transaminase and aspartate aminotransferase. Histological analyzes of the liver were also performed. The results demonstrate that Telmisartan significantly reduced liver weight and histological fat infiltration in hepatocytes of animals fed with a high fat diet, and these findings could be estimated in vivo noninvasively by measuring the size and echogenicity of the liver by US. In addition, the weight and the thickness of epididymal fat pad acessed by US was significantly lower in animals fed with a high fat diet and treated with Telmisartan, showing a beneficial effect of the drug in this parameter. Telmisartan also reduced body weight, mesenteric adipose tissue weight, adiposity, serum alanine aminotransferase levels, and improved glucose tolerance and HDL cholesterol. In conclusion, oral treatment with Telmisartan improved metabolism and hepatic steatosis in mice fed with a high fat diet, and echographic parameters such as liver echogenicity, liver longitudinal axis and epididymal fat pad thickness are effective in estimating liver and epididymal fat weight and the degree of fatty infiltration in the liver. These results reinforce the potential of Telmisartan in the treatment of NAFLD and stimulate the use of US in murine models of hepatic steatosis as a noninvasive tool for diagnosis and longitudinal monitoring of animals rather than more invasive techniques.

Keywords: fatty liver, ultrasonics, therapeutics, mice

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

SMet	Síndrome Metabólica
DGHNA	Doença Gordurosa Hepática não-alcoólica
EHNA	Esteatohepatite não-alcoólica
TEL	Telmisartan
RI	Resistência Insulínica
AGL	Ácidos Graxos Livres
SRA	Sistema Renina Angiotensina
AGT	Angiotensinogênio
Ang I	Angiotensina I
Ang II	Angiotensina II
AT1	Receptor tipo 1 da Angiotensina II
AT2	Receptor tipo 2 da Angiotensina II
Ang-(1-7)	Angiotensina-(1-7)
Mas	Receptor Mas
ECA	Enzima Conversora do Angiotensinogênio
BRA	Bloqueador dos receptores tipo I da angiotensina
iECA	Inibidores da Enzima Conversora do Angiotensinogênio
US	Ultrassonografia
TC	Tomografia Computadorizada
RM	Ressonância Magnética
TNF- α	Fator de Necrose Tumoral alfa
IL-6	Interleucina 6
PAI-1	Plasminogen Activator Inhibitor-1

SUMÁRIO

1 INTRODUÇÃO

Modificações na dieta e no estilo de vida levaram a aumentos globais na prevalência da Síndrome Metabólica (SMet), condição complexa que associa obesidade, intolerância à glicose, resistência à insulina, dislipidemia e hipertensão arterial [1-3]. A doença gordurosa hepática não alcoólica (DGHNA) é atualmente considerada uma manifestação hepática da SMet, devido à íntima associação entre as duas condições, que compartilham os mesmos fatores de risco [3-6], sendo uma das principais causas de doenças hepáticas crônicas, sobretudo em países ocidentais [7,8].

Um diagnóstico preciso da DGHNA requer técnicas de imagem ou histológicas [5,9], uma vez que a maioria dos pacientes é assintomática até os estágios tardios da doença [10] e os marcadores séricos, como as aminotransferases, são relativamente insensíveis e inespecíficos [11,12]. A ultrassonografia (US) é atualmente o teste mais utilizado para detecção da esteatose hepática, com as vantagens de ser seguro, não invasivo, sem radiação ionizante, amplamente disponível e de baixo custo [6,13-17].

O Telmisartan (TEL) é um bloqueador dos receptores tipo I da angiotensina (BRA) que é amplamente utilizado no tratamento da hipertensão arterial [18], sendo considerado uma droga de primeira linha no tratamento da hipertensão leve a moderada, com excelente perfil de segurança [19]. Nos últimos anos, o interesse nos efeitos metabólicos do TEL aumentou substancialmente, uma vez que ações benéficas no controle da SMet e do diabetes foram demonstradas [20-25]. No fígado, evidências também apontam benefícios do TEL, com efeitos promissores contra o acúmulo de lipídios e a inflamação em estudos animais [26-28].

2 OBJETIVOS

2.1 Objetivo geral

Analisar os efeitos do tratamento oral com Telmisartan em camundongos alimentados com dietas normolipídica e hiperlipídica sobre a doença gordurosa hepática não alcoólica através de parâmetros metabólicos e ecográficos.

2.2 Objetivos específicos

- Avaliar o peso corporal, a ingestão alimentar e o consumo energético entre os grupos em estudo;
- Analisar os efeitos do tratamento oral com Telmisartan sobre as dimensões e a ecogenicidade do fígado através de exame ultrassonográfico dos animais;
- Analisar os efeitos do tratamento oral com Telmisartan sobre a espessura do coxim de gorgura epididimal através de exame ultrassonográfico dos animais;
- Analisar os efeitos do tratamento oral com Telmisartan sobre o perfil glicêmico, por meio dos testes de sensibilidade insulínica e tolerância à glucose;
- Analisar os efeitos do tratamento oral com Telmisartan sobre o perfil lipídico, por meio da dosagem sérica do colesterol total, HDL e triglicerídeos;
- Analisar os efeitos do tratamento oral com Telmisartan sobre o peso do fígado e dos tecidos adiposos mesentérico e epididimal;
- Analisar os efeitos do tratamento oral com Telmisartan sobre marcadores séricos de dano hepatocelular através das transaminases e sobre a histologia hepática.

3 REVISÃO DE LITERATURA

3.1 Doença gordurosa hepática não alcoólica

3.1.1 Aspectos gerais

A DGHNA é hoje a patologia hepática mais comum no mundo [9,29,30], com previsão de ser a principal indicação de transplante hepático até o ano de 2030 [4]. Estimativas de sua prevalência global variam de 6,3% a 33,0% na população geral, com média de 20%, sendo mais elevada em países desenvolvidos [8,30,9]. A DGHNA é definida como um depósito lipídico nos hepatócitos correspondendo a mais de 5-10% do peso total do fígado, que não é resultado de uso abusivo de álcool (mulheres ≤20 g/d, homens ≤30 g/d). Para um diagnóstico acurado, técnicas de imagem ou histológicas são necessárias, e causas secundárias de esteatose hepatica devem ser excluídas, como drogas, virus da hepatite C, procedimentos cirúrgicos, nutrição parenteral total e erros inatos do metabolismo [5,9].

DGHNA é um termo que engloba todo o espectro da doença, que vai da esteatose simples à esteatohapatite não alcoólica (EHNA), que em sua forma mais severa pode levar à cirrose hepática e ao carcinoma hepatocelular, potencialmente fatais [4,6,9,31]. Histologicamente, a EHNA é caracterizada por esteatose hepática e sinais de inflamação intralobular, com degeneração e baloneamento dos hepatócitos. A prevalência estimada da EHNA é bem menor, variando de 3% a 5% [8,9]. Vinte por cento dos pacientes com EHNA desenvolvem cirrose, e 30%-40% desses pacientes morrem por causas relacionadas ao fígado [32].

3.1.2 Patogênese da doença gordurosa hepatica não alcoólica

Apesar da patogênese da DGHNA e sua progressão para EHNA não ser completamente elucidada, a resistência insulínica (RI), com ou sem todo o espectro da SMet, é sistematicamente apontada como o mecanismo central da esteatose hepatica em pacientes com DGHNA. Ainda, desequilíbrios entre mecanismos pró- e anti-oxidantes e entre citocinas pró- e anti-inflamatórias são componentes importantes na patogênese da DGHNA e em sua progressão para EHNA e cirrose. O papel de polimorfismos genéticos na predisposição ao desenvolvimento da esteatose, afetando vários passos do metabolismo normal de gordura e de carbohidratos, também está sob investigação [1,5,33].

3.1.2.1 Resistência à Insulina

Normalmente, a insulina age no músculo esquelético, nos adipócitos e no fígado para manter a homeostase da glicose e dos lipídios. No tecido adiposo, a insulina aumenta a captação de ácidos graxos livres (AGL), a sua conversão em triglicéridos e o seu armazenamento, juntamente com uma diminuição da lipólise. No músculo esquelético, estimula a captação de glicose. No fígado, promove o armazenamento de glicose como glicogênio, com inibição da glicogenólise e da gliconeogênese, além de reduzir a oxidação de ácidos graxos. O resultado final de todas essas ações é no sentido de se utilizar a glicose, reduzir a lipólise de AGL e promover o armazenamento de gordura na forma de triglicerídeos no tecido adiposo [5].

A resistência à insulina tem sido tradicionalmente definida como uma condição em que as células não respondem às ações normais da insulina. O corpo produz insulina, porém as células do tecido adiposo, músculo e fígado tornam-se resistentes e são incapazes de usá-la de maneira eficaz. No músculo esquelético, a RI provoca a redução da captação de glicose levando a hiperglicemia. No tecido adiposo, a RI compromete a ação anti-lipolítica da insulina, levando a uma liberação aumentada de AGL [5,34].

O defeito básico no desenvolvimento da esteatose hepática é o desequilíbrio entre a importação e a exportação de gordura para o fígado secundária à RI [5]. A RI e a consequente hiperinsulinemia parecem ser os principais fatores por trás das alterações nas vias hepáticas de captação, síntese, degradação e secreção de AGL, o que acaba por levar ao acúmulo de lípidos nos hepatócitos [35,36]. Concentrações plasmáticas elevadas de insulina, glicose e ácidos graxos promovem a captação hepática de ácidos graxos e de triglicéridos, a síntese lipídica *de-novo* e prejudicam a *b*-oxidação dos ácidos graxos por *feedback* negativo. O resultado é um deslocamento inapropriado de AGL para tecidos não adiposos como o fígado, o que, juntamente com citocinas inflamatórias e adipocinas específicas associadas à disfunção mitocondrial, inicia um círculo vicioso que contribui para o comprometimento da sinalização insulínica e RI hepáticas [5,37-39].

3.1.2.2 Teorias dos “dois insultos” e dos “múltiplos insultos paralelos”

Tradicionalmente, a teoria dos “dois insultos” foi desenvolvida para explicar a patogênese da DGHNA [40]: o insulto inicial ocorreria com o acúmulo de lipídios nos hepatócitos, promovendo RI, o que parece tornar o fígado mais suscetível a um segundo insulto, resultando em uma resposta inflamatória e progressão do dano hepático. O segundo golpe ocorreria, então, devido ao aumento do estresse oxidativo hepático, que por sua vez está associado ao aumento do metabolismo dos AGL, à redução da atividade antioxidante, ao aumento de citocinas pró-inflamatórias como o fator de necrose tumoral alfa (TNF-α) e dos níveis de endotoxinas e, especialmente, à disfunção mitocondrial e/ou ao estresse do retículo endoplasmático no fígado [35,36].

No entanto, a teoria dos "dois insultos" tem sido desafiada pelo reconhecimento de que numerosas vias são responsáveis pela patogênese da EHNA e sua progressão para fibrose, levando a uma coorte heterogênea de pacientes com diversas apresentações clínicas [41]. Com esta percepção, um novo modelo, mais realista, foi proposto: a teoria dos "múltiplos insultos paralelos" [41]. O principal conceito dessa teoria é que os diferentes eventos que determinam o dano hepático ocorrem de maneira paralela, não consecutivamente. Podemos considerar os seguintes elementos como os principais protagonistas patológicos: RI, estresse oxidativo, toxicidade dos tecidos adiposo e pancreático, metabolismo lipídico alterado, ácidos biliares, microbiota intestinal, endotoxinas bacterianas, sobrecarga sérica e hepática de ferro, imunidade inata e adaptativa e, finalmente, polimorfismos de genes envolvidos no armazenamento de lípidos, estresse oxidativo e fibrose hepática [5,33,42,43]. É importante ressaltar que todos os mecanismos de dano que estão envolvidos na patogênese da DGHNA aumentam duas condições que são centrais na progressão para EHNA: RI e inflamação crônica sistêmica [44].

3.1.3 Diagnóstico da doença gordurosa hepática não alcoólica

A DGHNA é geralmente subdiagnosticada, uma vez que a maioria dos pacientes permanece assintomática até estágios tardios da doença [10] e que marcadores séricos de hepatopatia, como as transaminases, são relativamente insensíveis e inespecíficos [11,12]. Desse modo, a biópsia hepática é atualmente o padrão-ouro para o diagnóstico e o estadiamento da DGHNA, e o único método confiável para diferenciar a EHNA da esteatose simples [16,45-49]. No

entanto, a biópsia é um procedimento invasivo e, portanto, inadequado para rastrear grande número de indivíduos ou para acompanhar o tratamento. Além disso, apresenta várias limitações, como potencial erro de amostragem, baixa reprodutibilidade, dificuldades em repetir o procedimento devido a preocupações éticas e a complicações como dor, sangramento, infecção e, em raros casos, a morte [17,46,47,50-52].

Por essas razões, vários métodos de imagem têm sido utilizados para avaliar pacientes com DGHNA, como a ultrassonografia (US), a tomografia computadorizada (TC) e a ressonância magnética (RM) [6,7,16,53], este último considerado o exame não invasivo de referência para avaliação da esteatose hepática [6,16,17,54,55]. No entanto, a RM é um método demorado e, assim como a TC, de alto custo. Além disso, a TC tem baixa sensibilidade para a detecção de esteatose leve e envolve exposição à radiação ionizante, o que torna essas ferramentas inadequadas para rastreamento em grande escala e para o seguimento longitudinal da doença [6,13,16,48]. Desse modo, a US representa atualmente uma excelente modalidade para avaliação da DGHNA, por ser um método seguro, não invasivo, amplamente disponível, acessível e que pode ser realizado repetidamente sem riscos para o paciente, sendo atualmente o teste mais utilizado para detecção da esteatose hepatica [6,13-17].

O diagnóstico ecográfico da esteatose é geralmente qualitativo, através da avaliação dos seguintes parâmetros: ecogenicidade hepática, contraste hepato-renal, penetração do eco nas porções profundas do fígado e definição dos vasos sanguíneos no parênquima hepático [13,15,16,48,56,57]. A esteatose aparece no US como um aumento difuso da ecogenicidade hepática, ou "fígado brilhante", devido ao aumento da reflexão dos ecos a partir do parênquima hepático, causado pelo acúmulo intracelular de vacúolos gordurosos [16]. Os órgãos circundantes, como o baço e o rim, podem ser usados como referência para detectar esse aumento da ecogenicidade do fígado [14], uma vez que o fígado gorduroso apresenta ecogenicidade maior que a do córtex renal e a do parênquima esplênico [6,16,58] (Figura 1). À medida que a infiltração gordurosa aumenta, a atenuação posterior do eco se torna mais significativa, prejudicando a visibilização das porções profundas do órgão e do diafragma. Observa-se, ainda, menor definição das paredes dos vasos intra-hepáticos, tanto pela menor penetração das ondas sonoras quanto pela pressão física exercida em pequenos vasos sangüíneos pelo baloneamento dos hepatócitos com gotículas de gordura [13,14]. Quando a esteatose é histologicamente superior a 20% e na ausência de doença hepática associada, essas características ultrassonográficas são capazes de diagnosticar a DGHNA com sensibilidade que varia de 81,8% a 100,0% e especificidade de até 98% [15,53,59,60], tendo boa correlação

com a RM [13,17]. De modo geral, a ecogenicidade do fígado aumenta com a gravidade da esteatose, o que permite que uma avaliação semiquantitativa do grau de infiltração gordurosa como se segue [6,14,48,53,59] (Figura 2):

- Normal: fígado apresentando ecogenicidade usual

- Leve (grau 1; Figura 2A): leve aumento difuso da ecogenicidade do fígado, visualização clara do diafragma e das paredes dos vasos intra-hepáticos;

- Moderado (grau 2; Figura 2B): moderado aumento difuso da ecogenicidade do fígado, obscurecendo as paredes dos vasos intra-hepáticos e o diafragma;

- Grave (grau 3; Figura 2C): aumento proeminente da ecogenicidade do fígado, com dificuldade de visualização dos vasos hepáticos e do diafragma.

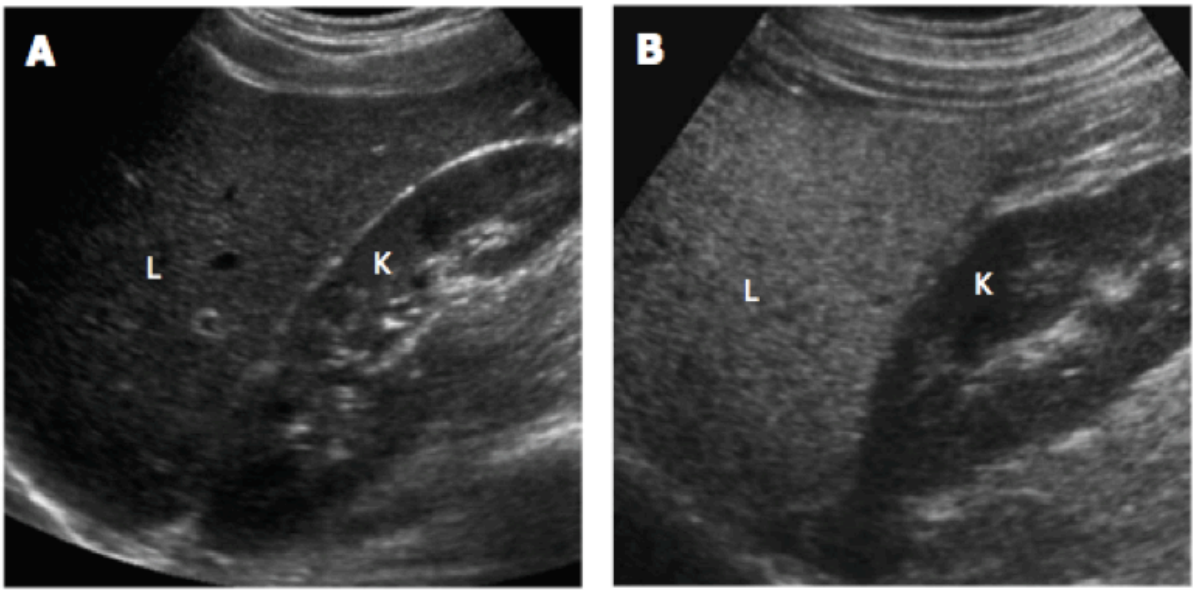

Figura 1. Avaliação ecográfica da esteatose hepática. A: US de um fígado normal, mostrando que a ecogenicidade do parênquima hepático (L) é similar à do córtex renal (K). B: US de um fígado esteatótico, mostrando ecogenicidade aumentada no parênquima hepático (L), que é claramente mais brilhante que o córtex renal (K). Adaptado de Lee & Park [16].

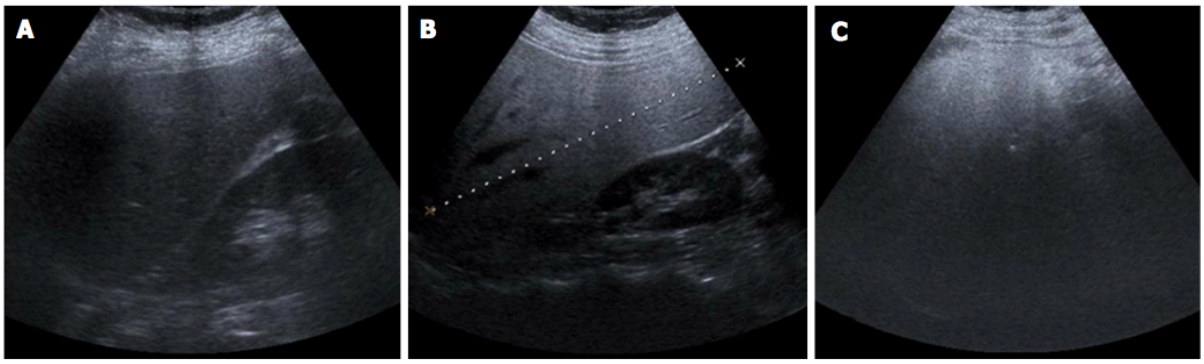

Figura 2. Graduação da esteatose hepática pelo US. A: Grau 1: infiltração gordurosa leve; B: Grau 2: infiltração gordurosa moderada; C: Grau 3: infiltração gordurosa acentuada. Adaptado de Koplay *et al*. [6].

No entanto, algumas limitações do método merecem ser descritas. É bem conhecido que a precisão diagnóstica da US diminui quando a infiltração gordurosa do fígado é inferior a 20-30% [6,13,15,58] e no contexto de doenças hepáticas crônicas, como a hepatite C [15,21,48]. Isso ocorre porque a fibrose hepática também pode aumentar a ecogenicidade do fígado [61], dificultando a distinsão entre esteatose e outros graus de DGHNA [48,53,62,63], limitação que também pode ser encontrada em outros métodos de imagem, como a TC e a RM [64]. Além disso, como a avaliação da ecogenicidade do fígado é subjetiva e qualitativa, o desempenho diagnóstico da US se torna altamente dependente das habilidades de leitura do operador [6,13-15,17] e pouco preciso para detecção de pequenas alterações na quantidade de gordura do fígado [14,16]. Fatores do paciente, como obesidade e meteorismo intestinal também podem interferir na qualidade da imagem [6].

Em modelos experimentais de doença hepatica em roedores, a US é um método viável, eficiente e de fácil realização, exigindo apenas leve sedação e depilação abdominal, sem necessidade de imobilização total ou de apneia para obtenção das imagens, consistindo portanto em um procedimento de baixo risco [56,65-67]. Modelos murinos de doença hepática difusa podem ser avaliados com precisão pela US tanto para fins diagnósticos como para acompanhamento longitudinal, alguns deles com correlação estatística dos achados ecográficos com a histologia [56,68,69]. Lessa *et al.* [56] demonstraram que a análise ecográfica qualitativa é sensível e específica o suficiente para diagnosticar doença hepática gordurosa em ratos e Fernández-Domínguez *et al.* [57] concluíram que a esteatose difusa foi facilmente diagnosticada em modelos de camundongos através da US.

Além da esteatose hepatica, outros parâmetros presentes no contexto da sindrome metabólica podem ser avaliados pela US, como a medida da espessura do coxim de gordura epididimal. Liao *et al.* [70] utilizaram com sucesso a medida ecográfica da espessura do coxim de gordura epididimal para acessar a quantidade desse depósito de tecido adiposo, reforçando a utilidade do método. Sabe-se que o aumento da gordura visceral está relacionado à hipertensão arterial, à dislipidemia e a um padrão metabólico prejudicado, mas também serve como um preditor independente de mortalidade em humanos [71].

3.1.4 Tratamento da doença gordurosa hepática não alcoólica

Embora muito progresso tenha sido feito na elucidação da epidemiologia, da história natural e da patogênese da DGHNA, opções limitadas de diretrizes clínicas baseadas em evidências estão disponíveis para o manejo dos pacientes. O tratamento farmacológico da DGHNA ainda está em evolução, sem terapia única que seja comprovadamente eficaz, especialmente em relação à modificação favoravel do curso da doença [5,33,36,39,44,72].

Uma vez que os fatores de risco cardiovascular e metabólico são altamente prevalentes entre os pacientes com DGHNA, o regime terapêutico ainda se baseia em intervenções gerais do estilo de vida, incluindo mudanças na dieta e aumento da atividade física, que devem ser recomendados em todos os casos [1,5,33,36,39]. No entanto, a maioria dos pacientes tem problemas em relação à adesão a longo prazo às intervenções de estilo de vida, de modo que a farmacoterapia se torna indispensável neste contexto [36,39,44].

A prioridade da farmacoterapia da DGHNA é prevenir a transformação da esteatose simples em esteatohepatite, além de melhorar a fisiopatologia da doença [9]. Dessa forma, em teoria, a resistência à insulina deveria ser o principal alvo terapêutico, juntamente com agentes com ação antioxidante, antiinflamatória e antifibrótica. Atualmente, a abordagem farmacológica de primeira linha para a DGHNA inclui um antioxidante (vitamina E) e uma droga que proporciona sensibilização à insulina (pioglitazona), uma vez que são os únicos medicamentos com grau suficiente de evidência em termos de eficácia, ambos apresentando um efeito positivo nas transaminases, no acúmulo de gordura e na inflamação. No entanto, a vitamina E não possui efeito comprovado sobre a fibrose e tãopouco sobre a morbimortalidade a longo prazo da doença, e a pioglitazona tem um impacto negativo sobre o

peso do paciente. Além disso, a segurança desses medicamentos continua incerta, uma vez que dados sobre sua utilização a longo prazo não estão disponíveis [5,33,39,44]. Outras drogas foram estudadas, como metformina, ácido ursodeoxicólico, estatinas, pentoxipilina e orlistat com resultados apenas parcialmente positivos. Entre os tratamentos emergentes, o Telmisartan é particularmente interessante, pois parece ter um impacto na resistência à insulina, na esteatose hepática, na inflamação e na fibrose, de acordo com estudos preliminares [33,39,44].

3.2 Sistema renina angiotensina

3.2.1 A visão atual do sistema renina angiotensina

Para uma melhor compreensão do papel do Telmisartan no tratamento da DGHNA, uma breve revisão sobre a importância do sistema renina-angiotensina (SRA) na síndrome metabólica e particularmente na DGHNA deve ser apresentada. O SRA é classicamente concebido como uma cascata hormonal única responsável pelo controle de funções cardiovasculares, renais e adrenais [73]. É iniciado principalmente pela expressão do Angiotensinogênio (AGT) em diferentes tecidos, uma proteína que é produzida por vários tipos de células, incluindo hepatócitos, adipócitos e células renais. No entanto, o fígado é considerado a principal fonte de AGT circulante na fisiologia normal [74,75]. A renina derivada do rim converte o AGT em angiotensina I (Ang I), um peptideo biologicamente inativo que é rapidamente hidrolisado pela enzima conversora da angiotensina (ECA) no octapeptideo angiotensina II (Ang II) [76]. A Ang II media as respostas biológicas através de dois receptores acoplados à proteína G, o receptor tipo 1 (AT1) e o receptor tipo 2 (AT2); no entanto, os principais efeitos da AngII são mediados pelo receptor AT1 [77,78]. O receptor AT1 está presente em abundância nos tecidos adultos, enquanto o receptor AT2 é expresso principalmente durante o desenvolvimento fetal e tem sua expressão estimulada em condições patológicas. A ativação da via ECA/AngII/AT1 usualmente desencadeia respostas fisiológicas nocivas, como reabsorção renal excessiva de sódio, contração anormal de células musculares lisas vasculares, secreção desproporcionalmente elevada de aldosterona e respostas cardiovasculares inapropriadas. Além disso, várias vias pró-inflamatórias, pró-oxidantes, pró-trombóticas e profibróticas são estimuladas pela ativação do receptor AT1 [79,80]. Aos

receptores AT2 geralmente são atribuídos efeitos opostos aos dos receptores AT1, de modo a contrabalanceá-los, tanto in vitro quanto in vivo [18].

Avanços recentes na biologia celular e molecular geraram mudanças substanciais na compreensão do SRA [73], que agora é encarado de maneira muito mais complexa. O conceito moderno do SRA inclui, além dos componentes clássicos, novas enzimas, péptidos, receptores e ações biológicas. Além disso, o SRA tecidual foi caracterizado em diferentes órgãos e sistemas, com interações significativas entre receptores, mediadores e vias metabólicas [38,73,81,82].

Uma das mudanças conceituais mais significativas foi a caracterização da Ang-(1-7), que através do receptor acoplado à proteína G Mas, tem ações opostas à da Ang II [38,83]. Várias vias enzimáticas podem estar envolvidas na formação da Ang-(1-7), mas ela é formada sobretudo diretamente da Ang II por uma nova enzima, homóloga à ECA, chamada ECA2 [38,73,76,84,85]. O eixo ECA2/Ang-(1-7)/Mas produz vasodilatação, bem como efeitos antiarrítmicos, antiproliferativos, anti-inflamatórios, antifibróticos e antitrombóticos [72,83,86-90].

Levando-se em consideração o papel oposto dos dois principais mediadores do SRA, Ang II e Ang-(1-7), uma nova visão do SRA foi proposta, acrescentando uma contra-regulação ao sistema. Neste modelo, o SRA pode ser concebido como um sistema de dupla função em que as ações vasoconstritoras/ proliferativas ou vasodilatadoras/ antiproliferativas são conduzidas principalmente pelo equilíbrio entre os dois braços do sistema, ECA/AngII/AT1 e ECA2/Ang-(1-7)/Mas [38,75,83-85,91-95] (Figura 3).

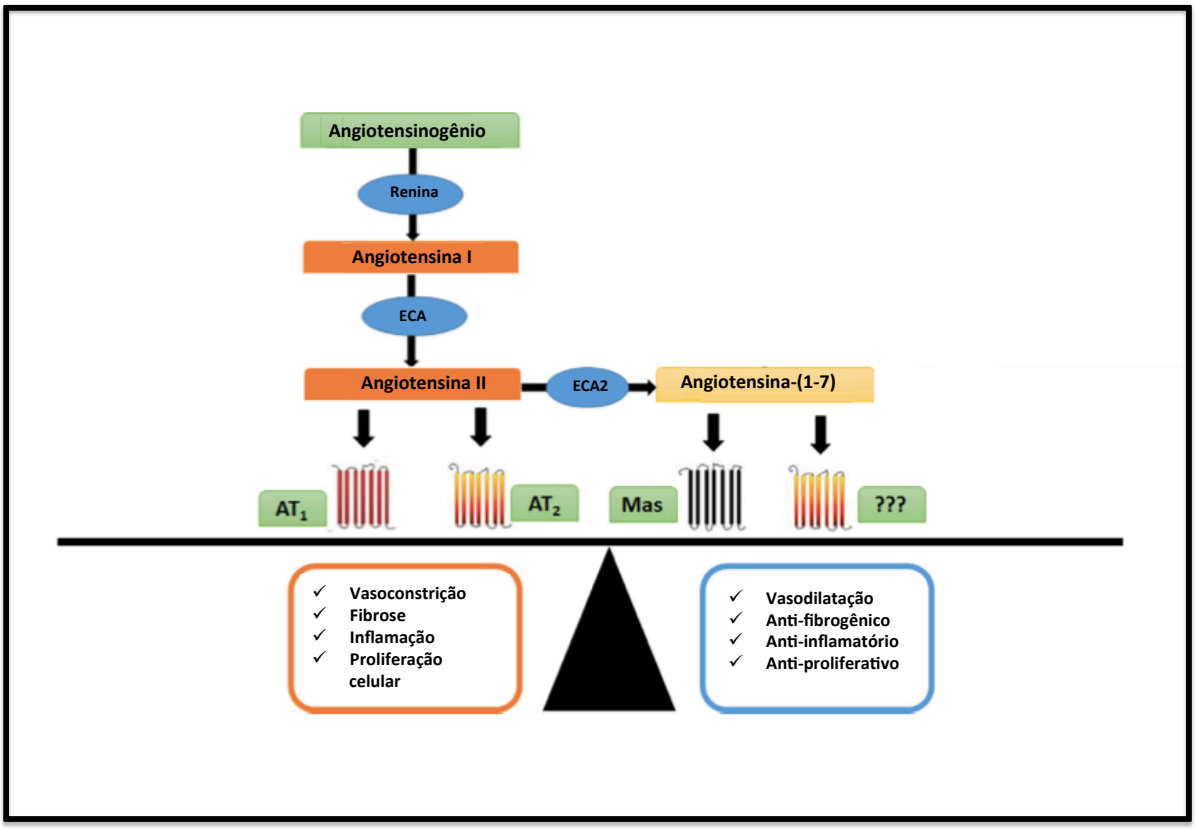

Figura 3. Visão atual do Sistema Renina Angiotensina. Atualmente compreende-se que o eixo ACE2/Ang-(1-7)/Mas é capaz de contrabalancear a maioria das ações deletérias do eixo ACE/Ang II/AT1, sobretudo em situações patológicas. ECA: Enzima Conversora do Angiotensinogênio; AT1: receptor tipo 1 da Angiotensina II; AT2: receptor tipo 2 da Angiotensina II; Mas: receptor Mas. Adaptado de Santos & Andrade [95].

3.2.2 O sistema renina-angiotensina e a doença gordurosa hepática não alcoólica

Diante de evidências do envolvimento do SRA no metabolismo de lipídios e de glicídios [83,89,93-96], seu papel na homeostase metabólica e na regulação da doença pode ser esperado [81,97,98]. Estudos recentes ressaltaram a importância do equilíbrio local entre os eixos ECA/Ang-II/AT1 e ECA2/Ang-(1-7)/Mas na proteção contra doenças metabólicas hepáticas (Macedo et al, 2014), com evidências de que a obesidade ativa o eixo ECA/AngII/AT1 do sistema [89,99,100]. Desse modo, a Ang II tem sido apontada como um dos principais atores na alteração do metabolismo hepático observada na DGHNA [101], influenciando negativamente a sinalização intracelular de insulina, com piora da RI. A Ang II também induz a geração de espécies reativas de oxigênio, iniciando e propagando a produção de mediadores pró-inflamatórios, incluindo TNF-α, interleucina-6 (IL-6) e inibidor do

ativador de Plasminogênio-1 (PAI-1), resultando em inflamação e comprometimento adicional da sinalização insulínica [20,81,102,103].

Por outro lado, efeitos benéficos do eixo ECA2/Ang-(1-7)/Mas sobre distúrbios metabólicos também foram demonstrados, com importante papel antiobesidade, melhorando a sensibilidade à insulina, a tolerância à glicose e a diabetes tipo 2, reduzindo a gordura corporal, aumentando a produção de adiponectina e revertendo a hiperleptinemia [75,83,89,90,94,104]. Feltenberger *et al.* [101] avaliaram pela primeira vez os efeitos da administração oral de Ang-(1-7) na esteatose hepatica induzida por dieta hiperlipídica, e observaram uma redução importante na massa gorda, no peso do fígado e na sua infiltração por gordura; além de diminuição do colesterol total circulante, dos triglicerídeos e de enzimas hepáticas; melhora do metabolismo lipídico e redução na expressão de citoquinas pró-inflamatórias. Estes efeitos foram associados à regulação benéfica da expressão dos genes do SRA. Outros estudos indicam um papel importante do eixo ECA2/Ang-(1-7)/Mas no fígado, sugerindo que o tratamento oral com Ang-(1-7) induz melhora da esteatohepatite, além de reduzir marcadores relacionados à adipogênese [38,96,101,105].

A consolidação do SRA sistêmico e local na patogênese da DGHNA criou considerável interesse nos efeitos metabólicos dos inibidores do SRA, uma vez que são drogas amplamente utilizadas, razoavelmente baratas e com excelente perfil de segurança [72]. Os inibidores da ECA (iECA) e os bloqueadores dos receptores da angiotensina (BRA) são utilizados para inibir o braço ACE/AngII/AT1 e estimular a atividade do eixo ACE2/Ang- (1-7)/Mas [20,106-108]. Ambos os medicamentos foram amplamente utilizados na insuficiência cardíaca congestiva, hipertensão, proteinúria e doença renal crônica [109] e também apresentam efeitos significativos na melhora do metabolismo de lipídios e de glicose [89,94,95,101,110]. Uma vez que o receptor AT1 tem um papel bem estabelecido na mediação da maioria das ações deletérias da Ang II no fígado, o antagonismo AT1 é considerado mais específico do que a inibição da ECA [75,111], com melhores resultados na sensibilidade à insulina. Uma possível explicação seria a ação inibitória dos iECA nos receptores AT1 e AT2, resultando na supressão dos efeitos contrarregulatórios do receptor AT2 nas ações do receptor AT1 [72].

Evidências apontam que os BRAs podem restaurar a sinalização de insulina intracelular e promover a redistribuição do excesso de gordura de sítios ectópicos para adipócitos maduros [3]. Além disso, os BRAs podem melhorar as transaminases, a esteatose hepática e a

inflamação preesntes na DGHNA [36,72]. No entanto, os diferentes tipos de BRAs parecem exercer efeitos disitintos em relação à seletividade do receptor, modo de ligação e metabolismo [3]. Neste contexto, o BRA Telmisartan tem um papel de destaque no tratamento e na prevenção da DGHNA.

3.3 Telmisartan: o mais promissor dos BRAs

Estudos recentes indicam que o TEL tem ações benéficas que limitam o desenvolvimento da síndrome metabólica e do diabetes, melhorando a resistência à insulina e protegendo contra o aparecimento e desenvolvimento de diabetes tipo 2 em pacientes hipertensos e com resistência insulínica [20-25]. De fato, parâmetros metabólicos, como peso corporal, deposição de gordura, tamanho das células adiposas e a resistência à insulina foram significativamente melhorados com o tratamento com TEL em estudos animais [3,20,24,26,28,112-115], freqüentemente com desempenho superior do TEL quando comparado a outros BRAs ou a outros bloqueadores do SRA [28,113,115].

Kudo *et al.* [27] demostraram pela primeira vez que o TEL diminui o tamanho dos adipócitos e aumenta a secreção de adiponectina sem afetar a ingestão de alimentos em um modelo murino de DGHNA, reduzindo o acúmulo de gordura visceral. Além disso, o TEL, mas não o BRA Valsartan, aumentou a expressão de genes relacionados ao metabolismo energético mitocondrial no músculo esquelético. Assim, além de um efeito de classe dos BRAs na modulação do tamanho de adipócitos, esses achados aumentam a possibilidade de que certas moléculas, como o TEL, possam ter um impacto particularmente forte no volume das células de gordura e no acumúmulo de gordura, além de efeitos específicos no metabolismo energético, com efeitos protetores sobre a obesidade visceral induzida pela dieta [113].

No fígado, o TEL apresentou efeitos anti-oxidantes e melhorou marcadores de fibrose hepática em diferentes contextos patológicos [104], como em modelos de colangite esclerosante primária [22], de ligação de ducto biliar [116] e de fibrose induzida por estreptozotocina [117] ou por dieta de metionina e colina [118].

Como mencionado anteriormente, as alterações patológicas da DGHNA podem ao menos em parte resultar da ativação do braço inflamatório do SRA [28], sobretudo na vigência da

resistência à insulina. Uma vez que o TEL não apenas bloqueia o SRA mas também reduz a resistência à insulina [20-25], seus efeitos na histologia do fígado devem ser esperados. De fato, evidências morfológicas de melhora da esteatohepatite não alcoólica foram encontradas em estudos animais com TEL [26-28,119], reduzindo a inflamação e a fibrose através da supressão da infiltração de macrófagos no fígado [27,28]. O tratamento com TEL atenuou a esteatose, com redução dos triglicerídeos hepáticos, além de atenuar a fibrogênese, com redução da expressão de colágeno tipo I [27]. Enjoji *et al.* [3] demonstraram que o TEL melhorou significativamente a resistência à insulina e a lesão hepática em pacientes com DGHNA e com hepatite C crônica, efeito mais evidente do que com outros BRAs, sugerindo que esse medicamento pode ser usado como agente protetor do fígado nestas condições.

Apesar de todas as evidências acima, atualmente o tratamento de pacientes com DGHNA com Telmisartan e com outros BRAs só é formalmente recomendado em pacientes com indicação bem estabelecida de terapia anti-hipertensiva [36,44].

4 PRODUTOS

4.1 Artigo 1: "*Role of Angiotensin type I Receptor Blocker Telmisartan in the treatment of Non-alcoholic fatty liver disease: a Brief Review"*, formatado segundo as normas para publicação no periódico Hypertension Research (Fator de Impacto: 3,508; Qualis Interdisciplinar: B1). ***Status: submetido para publicação.***

4.2 Produto 2: "*Telmisartan attenuates hepatic steatosis in high-fat fed mice: non-invasive evidence by ultrasound imaging",* formatado segundo as normas para publicação no periódico Journal of the American Society of Hypertension (Fator de Impacto: 3,263).

4.1 PRODUTO 1

Role of Angiotensin type I Receptor Blocker Telmisartan in the treatment of Non-alcoholic fatty liver disease: a Brief Review

Short title: Telmisartan in the NAFLD treatment

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ABSTRACT

Non-alcoholic fatty liver disease is currently being considered an important component of the metabolic syndrome (MetS). This condition spectrum includes from simple hepatic steatosis to non-alcoholic steatohepatitis, being correlated to liver-related deaths and predicted to be the most frequent indication for liver transplantation by 2030. Insulin resistance is directly correlated to the central mechanisms of hepatic steatosis in NAFLD patients, which is strongly correlated to the unbalance of the renin-angiotensin system that is involved in lipid and glucose metabolism. Among the emerging treatment approaches for NAFLD, the antihypertensive telmisartan, seems a potential candidate, as it has positive effects on liver, lipid and glycemic metabolism, especially through its action on the renin-angiotensin system, by blocking the ACE/AngII/AT1 axis and increasing ACE2/Ang(1-7)/MAS axis activation. However, treatment with this drug is only recommended for patients with established indication of anti-hypertensive therapy. Thus, increasing the need for large-randomized controlled trials aimed to elucidate the telmisartan effects on liver disease, especially NAFLD. In this perspective, the present review aims to provide a brief examination of the NAFLD/NASH pathogenesis news and the role of Telmisartan on preventing liver disorders, improving the discussion on potential therapies.

Key-words: Non-alcoholic fatty liver disease; Obesity; Renin-angiotensin system; Insulin resistance; Telmisartan.

INTRODUCTION

Diet and lifestyle changes have led to worldwide increases in the prevalence of metabolic syndrome, a complex disorder where obesity, glucose intolerance, insulin resistance, dyslipidemia, and hypertension are associated (1-3). Non-alcoholic fatty liver disease (NAFLD) is now considered a hepatic component of the metabolic syndrome (MetS) because of the close association between the two conditions that share the same risk factors (3-6).

NAFLD is currently the most common liver disorder (7-9), with a prediction to be the most frequent indication for liver transplantation by 2030 (4). The global estimated prevalence of NAFLD ranges from 6.3% to 33% in the general population with a median prevalence of 20%, being higher in developed countries (8-10). NAFLD is defined as a lipodystrophy with lipid-deposit accumulation in the hepatocytes accounting for more than 5–10% of the total hepatic weight, which is not due to excessive alcohol use (women ≤20 g/d, men ≤30 g/d). An accurate diagnosis requires imaging or histological techniques, and secondary causes of hepatic steatosis should be excluded such as drugs, hepatitis C virus, surgical procedures, total parenteral nutrition, and various innate metabolism disorders (5, 9).

NAFLD is a term that encompasses the entire spectrum of this disease, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which in its most severe form which can lead to life-threatening hepatic cirrhosis and hepatocellular carcinoma (4, 6, 9, 11). Histologically, NASH is characterized by hepatic steatosis and signs of intralobular inflammation, with ballooning degeneration of hepatocytes. The estimated prevalence of NASH is much lower and ranges from 3% to 5% (9, 10). Twenty percent of NASH patients are reported to develop cirrhosis, and 30%-40% of patients with NASH cirrhosis experience liver-related death (12).

Among the emerging treatments of NAFLD, the antihypertensive drug Telmisartan is of particularly interest, as it seems to have positive hepatic and extra hepatic impacts, according to preliminary studies (13-16).

In this perspective, the present review aims to provide a brief examination of the NAFLD/NASH pathogenesis news and the role of Telmisartan on preventing liver disordes, improving the discussion on potential therapies.

NAFLD pathogenesis and Insulin Resistance

Although the pathogenesis of NAFLD development and progression towards NASH is somewhat unclear, insulin resistance (IR), with or without full-blown MetS, is reportedly related as the central mechanism of hepatic steatosis in patients with NAFLD, which in turn develops in the setting of an inappropriate diet, sedentary lifestyle, obesity, and advancing age. Also, imbalances between pro- and antioxidant mechanisms and also pro- and anti-inflammatory cytokines are important components for NAFLD pathogenesis. The role of genetic variations in predisposing to the steatosisdevelopment by affecting various steps in the normal fat and carbohydrates metabolism is also under investigation (1, 5, 14).

Normally, insulin acts on skeletal muscle, adipocytes, and liver for maintaining glucose and lipid homeostasis. Insulin increases the uptake of free fatty acid (FFA), conversion to triglycerides and storage along with a decreased lipolysis in the adipose tissue; stimulates glucose uptake by skeletal muscles; storage of glucose as glycogen in the liver with inhibition of glycogenolysis and gluconeogenesis and reduces oxidation of fatty acids in the liver. The net result of all these actions is to utilize glucose, reduce lipolysis of FFA, and promote storage of fats as triglycerides in the adipose tissue (5).

Insulin resistance has traditionally been defined as a condition in which cells fail to respond to

the normal actions of insulin. The body produces insulin, but the cells in the adipose, muscle, and liver become resistant and are unable to use it as effectively. In the skeletal muscle, IR causes reduced glucose uptake leading to hyperglycemia. In the adipose tissue, IR impairs the anti-lipolytic action of insulin leading to increased release of FFA (5, 17).

The basic defect in the development of hepatic steatosis is the fat imbalance between import and export to and from the liver secondary to IR (5). Insulin resistance and subsequent hyperinsulinemia seem to be the major factors behind the alterations in the hepatic pathways of uptake, synthesis, degradation, and secretion of free fatty acids which ultimately leads to accumulation of lipids in the hepatocytes (18, 19). Elevated plasma concentrations of insulin, glucose, and fatty acids promote hepatic fatty acid and triglyceride uptake, de-novo lipid synthesis and impair b-oxidation of fatty acids by negative feedback. The result is an inappropriate shifting of FFA to nonadipose tissue, such as the liver, and this, together with inflammatory cytokines and specific adipokines associated with mitochondrial dysfunction initiates a vicious circle that contributes to impaired insulin signaling and hepatic IR (5, 15, 20, 21).

The “two-hit” and the “multiple parallel hits” hypothesis

Traditionally, the two-hit hypothesis was established to explain the pathogenesis of NAFLD (22): the initial insult occurs with the accumulation of lipids in hepatocytes promoting IR. These changes seem to make the liver susceptible to a second insult, resulting in an inflammatory response and progression to liver damage. The second hit occurs due to increased hepatic oxidative stress which is associated with increased free fatty acid metabolism, diminished antioxidant activity, increased proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and endotoxin levels, and especially mitochondrial dysfunction and/or endoplasmic reticulum stress in the liver (18, 19).

The “two-hit” hypothesis has been challenged by the recognition that numerous complex pathways are responsible for the pathogenesis of NASH and progression to fibrosis, leading to a heterogeneous patient cohort with diverse clinical presentations (23). With this insight, a new and more realistic model was proposed: the "multi-parallel hits" hypothesis (24). The main concept of this theory is that different events occur that in turn determine liver damage, but these events take place in parallel, not consecutively. We can consider the following elements as the main pathological protagonists: IR, oxidative stress, adipose and pancreas tissues toxicity, altered lipid metabolism, bile acids, gut microbiota, bacterial endotoxins, serum and liver iron overload, innate and adaptive immunity, and finally the polymorphisms of the genes involved in lipid accumulation, oxidative stress, and hepatic fibrosis (5, 14, 25-27). It is important to underline that all the mechanisms of damage that are involved in the pathogenesis of NAFLD enhance two conditions that are central in the progression of NASH: IR and systemic chronic inflammation (13).

NAFLD treatment

While much progress has been made in elucidating the epidemiology, natural history, and pathogenesis of NAFLD/NASH, there remains no effective therapy, with limited options of evidence-based clinical guidelines for patient management. Pharmacological treatment of patients with NAFLD is still evolving, with no single therapy that has clearly been proved effective, especially, in modifying the course of the disease (5, 13-15, 19, 28).

Since cardiovascular and metabolic risk factors are highly prevalent among NAFLD/NASH patients, the backbone of treatment regimens for these patients still comprises general lifestyle interventions, including dietary changes and increased physical activity that should be recommended in all patients (1, 5, 14-16, 19). However, most patients may experience problems regarding long-term adherence to lifestyle interventions due to their attitudes or

physical inability so that pharmacotherapy becomes indispensable in this context (13, 15, 19).

The priority for NAFLD pharmacotherapy is to prevent transformation of NAFLD into NASH and to improve the pathophysiology of the disease (9). Therefore, in theory, insulin resistance should be the main target, together with antioxidative, antiinflammatory, and antifibrotic agents. Currently, the first-line pharmacological approaches for NASH are the antioxidant vitamin E and the insulin sensitizer pioglitazone, as they are the unique drugs that have provided a sufficient degree of evidence in terms of efficacy, both showing a positive effect on transaminases, fat accumulation, and inflammation. However, vitamin E has no proven effect on fibrosis and on long-term morbidity and mortality and pioglitazone has a negative impact on weight. In addition, the safety of this drugs remains uncertain as data about its long-term use are not available (5, 13-16). Other drugs have been studied such as metformin, ursodeoxycholic acid, statins, pentoxifylline, and orlistat, but with only partially positive results. Among the emerging treatments, Telmisartan is particularly interesting, as it seems to have an impact on insulin resistance, liver steatosis, inflammation, and fibrosis, according to preliminary studies (13-16). However, the lack of large randomized clinical trials precludes the use of this drug as a consolidated option treatment, being formally recommended only in NALFD patients with an established indication of anti-hypertensive therapy (13, 19).

In the present review, we have focused on available data of the antihypertensive drug Telmisartan on treating and preventing fatty liver disease.

Renin-angiotensin system: the current view

Telmisartan is an angiotensin (Ang) type I receptor blocker (ARB) that has been widely used for the treatment of hypertension and hypertension-related cardiovascular end-organ damage (29), being considered a first-line drug in mild to moderate hypertension, with an excellent safety profile (30). Among the ARBs, Telmisartan is emerging as the most promising drug for

the treatment of NAFLD in terms of both safety and efficacy (13). The importance of renin–angiotensin system (RAS) in metabolic syndrome and particularly in NAFLD should be presented for a better comprehension of the role of Telmisartan in the treatment of this condition.

The renin–angiotensin system is classically conceived as a single hormonal cascade responsible for controlling cardiovascular, renal and adrenal functions (31). It is primarily initiated by the expression of Angiotensinogen (AGT) at different tissues, a protein that is produced by several cell types, including hepatocytes, adipocytes and kidney cells. However, the liver is considered the primary source of circulating AGT in normal physiology (32, 33). Kidney-derived renin converts angiotensinogen into angiotensin I (Ang I), a biologically inactive peptide that is rapidly hydrolyzed by angiotensin-converting enzyme (ACE) to the octapeptide angiotensin II (Ang II) (34). Ang II mediates biological responses through two G-protein-coupled receptors, the Ang II receptor type 1 (AT1R) and Ang II receptor type 2 (AT2R); however, the main described effects are AT1R mediated (35, 36). AT1R is in abundance in adult tissues whereas AT2R is mainly expressed during fetal development and is up-regulated in pathologic conditions. The activation of ACE-Ang II-AT1R frequently contributes to physiopathological changes such as excessive renal sodium reabsorption, abnormal vascular smooth muscle cell contraction, disproportionately high aldosterone secretion and inappropriate cardiovascular responses. In addition, several proinflammatory, pro-oxidant, prothrombotic and profibrotic pathways are stimulated by AT1R activation (37, 38). AT2R is generally reported to mediate effects opposing and counterbalancing those mediated by AT1R *in vitro* as well as *in vivo*(29).

Advances in cellular and molecular biology, as well as physiological and pharmacological approaches, have generated a substantial change in our understanding of the RAS (31). It is now clear that the circulating and tissue RAS are far more complex than previously

anticipated. The modern concept of the RAS includes, in addition to the classical components, novel enzymes, peptides, receptors and biological actions. Additionally, tissue RAS has been characterized in different organs and systems, in which significant interactions between receptors, mediators, and metabolic pathways have been discovered (21, 31, 39, 40).

One of the most significant conceptual changes of this hormonal system is the characterization of Ang-(1–7), that through the G protein-coupled receptor Mas, has opposing actions to Ang II (41, 42). Several enzymatic routes may be involved in Ang-(1-7) formation, but it is mainly formed directly from Ang II by a new enzyme, homolog to the ACE, called ACE2 (31, 34, 43-46). The ACE2-angiotensin-(1-7)-Mas axis can produce NO-dependent vasodilation as well as antiarrhythmic, antiproliferative, anti-inflammatory, antifibrotic and antithrombotic effects (28, 41, 42, 47-50).

Taking into account the opposite role of the two main mediators of RAS, Ang II and Ang-(1–7), a number of research groups have proposed a new view of the RAS, adding a counter-regulation in the system. In this model, the RAS can be envisioned as a dual function system in which the vasoconstrictor/proliferative or vasodilator/antiproliferative actions are primarily driven by the balance between both arms of the RAS, ACE-Ang II-AT1R and ACE2-Ang-(1-7)- Mas. Thus, it is now accepted that the ACE2/Ang-(1–7)/Mas axis is able to counteract most of the deleterious actions of the ACE/Ang II/AT1R axis, especially in pathological conditions (32, 42-44, 46, 51-54).

Renin-angiotensin system and NAFLD

The function of the RAS in metabolic homeostasis and disease regulation has been the subject of considerable interest in the last decade (39, 55, 56). There is an increasing body of evidence showing the RAS involvement in metabolic regulation, playing an important role in lipid and glucose metabolism (42, 49, 53, 57, 58). Recent studies have pointed out the local

balance importance between ACE/Ang-II/AT1R and ACE2/Ang-(1-7)/Mas arms to avoid liver metabolic diseases (59).

Results from experimental animals and humans suggest that obesity activates the RAS arm composed of ACE/Ang II/AT1R (49, 60, 61) (Figure 1). Ang II has been implicated as a major player in the altered hepatic lipid metabolism observed in NAFLD (62), influencing intracellular insulin signaling by several mechanisms which may result in worsening insulin resistance, the main pathophysiological element of NAFLD (39, 63-65). Ang II also induces the generation of reactive oxygen species (ROS), initiating and propagating the production of pro-inflammatory mediators, including TNF-α, interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1), resulting in inflammation and additional impairment of insulin signaling. Kanno et al and Wei et al showed that an increased expression of Ang II induces non-alcoholic fatty liver disease and modulates inflammatory cell recruitment into the liver during liver injury(66, 67). Accordingly, liver injuries in AT1-knockout mice present reduced inflammation and fibrosis (68, 69). Paizis et al demonstrated that AT1R genes are up-regulated in areas of active hepatic fibrogenesis, meaning that chronic injury up-regulates RAS in local tissues, which seems to contribute to the vicious cycle of steatosis-necroinflammation-fibrosis (13, 70).

On the other hand, increasing evidence has also shown the beneficial effects of the Ang-(1-7)/Mas axis on liver pathology and on metabolic disorders, exerting an important anti-obesity roleby improving insulin sensitivity, glucose tolerance and type 2 diabetes, reducing body fat, increasing adiponectin production and reverting hyperleptinemia(32, 42, 49, 50, 57, 59). Feltenberger et al evaluated for the first time the effects of a mouse model of oral Ang-(1–7) administration in high-fat–induced steatosis, liver metabolism, and inflammation and observed an important reduction in fat mass, liver weight, and hepatic steatosis associated

with decreased circulating total cholesterol, triglyceride, and alaninetransaminase enzyme; improved lipid metabolism; and decreased expression of proinflammatory cytokines(62). These effects were associated with beneficial regulation of the RAS genes expression. Other reports indicated an important role of the ACE2/Ang-(1–7)/Mas axis in the liver, suggesting that oral treatment with Ang-(1–7) improves the status of steatohepatitis, as well as reduces adipogenesis-related markers (46, 58, 62)**.** Lastly, in humans with liver disease, both ACE2 gene expression and plasma Ang-(1-7) are increased when compared with healthy livers, confirming that the key regulator of Ang-(1-7) production in the alternative axis of the RAS is up-regulated in response to hepatic injury (71). All these observations confirmed the protective role of ACE2/Ang(1-7)/MAS axis activation and might represent a promising strategy for treatment targeting hepatic disorders (59).

NAFLD and RAS blockers

The established role of both circulating and local RAS in the pathogenesis of NAFLD and NASH produced considerable interest on the effect of RAS inhibitors since they are widely used, reasonably inexpensive, and with excellent safety profile (28). ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs), are used to inhibit the ACE/Ang II/AT1 arm and stimulate the activity of the ACE2/Ang- (1–7)/Mas axis (64, 72). Both medications have been broadly used in congestive heart failure, hypertension, proteinuria and chronic kidney disease (73, 74) and also present significant effects on improving lipid and glucose metabolism (21, 49, 57, 62, 75). Since the AT1R has a well-established role in mediating most of the deleterious actions of Ang II in the liver, the AT1R antagonism is considered more specific than ACE inhibition (32, 76). Evidence indicates that treatment with ARBs results in greater improvement in insulin sensitivity and a larger reduction in the risk of new onset diabetes mellitus. One possible explanation could be the inhibitory action of ACE-I on both AT1R and AT2R, resulting in suppression of the counterbalancing effects of AT2R on the actions of

AT1R (28).

Also, there is substantial evidence to suggest that Ang-(1-7) is involved in the beneficial actions of RAS inhibitors (77). It is important to mention that after chronic administration of AT1R blockers, Ang-(1-7) levels increases several times (78, 79), suggesting that this heptapeptide may contribute to RAS blockade metabolic effects (31, 64, 72).

The evidence is accumulating to show that ARBs can restore impaired intracellular insulin signaling and promote redistribution of excess fat from those ectopic sites to mature adipocytes, resulting in improved insulin sensitivity (3). Also, ARBs may improve transaminases, hepatic steatosis and inflammation in the NAFLD setting (19, 28). The effect of ARBs on hepatic fibrosis in different animal models is also well described in the literature. ARBs inhibit stellate cell activity in obese mice, leading to decreased hepatic fibrosis (59, 80). The evidence in humans shows that subjects with NAFLD submitted to ARB treatment commonly have decreased liver fibrosis markers (69, 81). These results suggest that liver fibrosis is mediated via the renin-angiotensin system ACE/Ang II/AT1R axis, and demonstrate a beneficial role of angiotensin receptor blockers (59).

The benefits for each type of ARB deservers further evaluation, since it appears that not all ARB types have the same effects concerning receptor selectivity, binding mode, and metabolism (3). In this context, the ARB Telmisartan has a prominent role in treating and preventing NAFLD.

Telmisartan: the most promising ARB

New evidences indicate that Telmisartan has beneficial actions limiting MS development and improving diabetes, insulin resistance and protecting patients with hypertension (64, 82-86). Indeed, metabolic parameters such as body weight, fat accumulation, fat cell size and insulin

resistance have been consistently ameliorated with Telmisartan treatment in animal studies (3, 64, 85, 87-92), frequently with a superior performance of Telmisartan when compared to other ARBs or to other RAS blockers (88, 91, 93, 94).

Kudo et al showed for the first time that Telmisartan decreases the adipocyte size and upregulates the adiponectin secretion without affecting food intake in a murine NASH model, reducing the accumulation of visceral fat (95). Moreover, Telmisartan, but not the ARB valsartan, increased the expression of both nuclear-encoded and mitochondrial-encoded genes in skeletal muscle known to play important roles in mitochondrial energy metabolism. Thus, in addition to a class effect of ARBs in modulating adipocyte size, these findings raise the possibility that certain molecules, like Telmisartan, may have a particularly strong impact on fat cell volume and fat accumulation, as well as distinctive effects on energy metabolism, which may help to protect against dietary-induced visceral obesity and weight gain (88)**.** The efficacy of TEL in reducing visceral fat mass may be relevant for patients since an increase in visceral fat is related to hypertension, dyslipidemia and an impaired metabolic pattern, but also serves as an independent predictor of mortality in men (96).

In the liver, Telmisartan has been proved to positively affect hepatic fibrosis markers in different pathological contexts (59). The anti-oxidative effects of Telmisartan have been studied (97), and its beneficial effects on hepatocytes can be partially based on the drug's anti oxidative properties (59). Telmisartan reduces hepatic injuries resulting from type I diabetes mellitus (83); and when associated with Propranolol, reduces several liver fibrosis signals, such as hydroxyproline, bile duct proliferation procollagen-**α**1, endothelin-1 and metalloproteinases on a Primary Sclerosing Cholangitis (PSC)-like mouse model (98). Telmisartan also prevented liver fibrosis in a rat bile duct ligation model (99). In mice on a high-fat diet that had received a low dose of streptozotocin (STZ) 2 days after birth, treatment with Telmisartan reduced hepatic inflammation and fibrosis (100). Similarly, Telmisartan

reduced liver fibrosis that had been induced in rats by a short- or long- term methionine- and choline-deficient diet (101), and also prevented the occurrence of hepatocellular carcinoma (102).

As mentioned earlier in this article, pathological changes in NAFLD may at least partly result from activation of the inflammatory arm of RAS (92), especially in the context of insulin resistance. Once Telmisartan not only blocks RAS, but also has been consistently proven to reduce insulin resistance (64, 82-86), its effects on liver histology should be expected. Indeed, morphological evidence of amelioration of non-alcoholic steatohepatitis was found in several animal studies with Telmisartan(89, 92, 95, 103), reducing inflammation and fibrosis by suppressing macrophage infiltration into the liver (92, 95). Telmisartan treatment attenuated liver steatosis with decreased hepatic triglycerides, besides attenuating liver fibrogenesis, with decreased type I collagen and transforming growth factor-**β**1 (TGF-**β**1) mRNA expressions (95). Enjoji*et al.* demonstrated that Telmisartan significantly improved insulin resistance and attenuated liver injury in NAFLD and in chronic hepatitis C patients, an effect that was more evident than with other ARBs, suggesting that this drug may be used as a liver-protecting agent in these conditions(3). In a recent 1-year randomized control trial, Alam et al observed that Telmisartan significantly improved the overall histology of NASH patients, reducing inflammation and fibrosis markers, independent of weight reduction(30). Also, Telmisartan was similarly effective in hypertensive and non-hypertensive NASH patients and had very minimum side effects during this period of treatment.

Given the superiority of Telmisartan in treating and reversing metabolic and hepatic parameters of Mets, the possibility that its effects may go beyond just blockade of the type 1 angiotensin II receptor has been raised (88). To test this theory, Rong et al demonstrated for the first time that Telmisartan ameliorated diet-induced obesity, insulin resistance and fatty liver in AT1R knock-out mice on a high-fat diet, suggesting that this drug may exert

additional AT1R-independent beneficial effects on metabolism(85).

Concerning AT1R-independent effects, it is important to emphasize the relationship between Telmisartan and peroxisome proliferator-activated receptor gamma (PPAR-γ) activation. Telmisartan works as a partial agonist of PPAR-γ(59, 85, 89, 95, 104), a property that does not appear to be shared by other ARBs (92, 105). Nuclear PPARγ receptor is a transcription factor regulating many genes related to adipogenesis, lipid metabolism and insulin sensitivity (89). Telmisartan influences the expression of PPAR-γ target genes involved in carbohydrate and lipid metabolism; reducing glucose, insulin, and triglyceride levels in rats fed a high-fat, high-carbohydrate diet (87). PPAR-γ increases insulin sensitivity, high-density lipoprotein levels, and decreases inflammation, oxidative stress, cell proliferation, migration and fatty acid and triglyceride levels, but without causing the fluid collection associated with full agonists of PPAR-γ, such as pioglitazone or rosiglitazone (35, 59, 104, 106, 107). Figure 2 illustrates these two Telmisartanaction mechanisms in the treatment of NAFLD.

The concept of an agent with dual PPAR-γ agonist and AT1 receptor antagonist actions is indeed promising, particularly from the standpoint of synergistic metabolic actions. However, some limitations should be noted (108). PPAR-γ effects are elicited only by micromolar concentrations of Telmisartan, whereas low nanomolar concentrations are sufficient to block AT1 receptors (109). Thus, Telmisartan is >1000-fold more potent as an AT1 receptor antagonist than as a PPAR-γ agonist (108). According to this, most of the effect of Telmisartan on metabolism is likely to be mediated by blockade of AT1 receptors, together with compensatory overstimulation of AT2 receptors and activation of ECA2/Ang (1-7)/Mas axis, as angiotensin II levels rise during chronic AT1 blockade.

Furthermore, structural differences between ARBs result in differences in their pharmacological and pharmacokinetic properties and subsequently in their binding affinity to

the Ang II receptor. The effectiveness of Telmisartan might be adequately explained by its greatest affinity for the AT1R among the ARBs, longer half-life and greater lipophilicity, resulting in greater in vivo blockade of the AT1R by Telmisartan(13, 28, 88, 108). However, the synergy between PPAR-γ activation and angiotensin receptor blockade is clear from preclinical data (108).

As another possible PPAR-γ-independent mechanism, Miesel et al investigated HPA axis activity after AT1R blockade(91). HPA hyper-reactivity has been verified in rats and patients with diabetes (110) and AT1R identified as regulators of stress reactions (111). Moreover, the AngII-stimulated hyper-reactivity in the HPA axis was found to account for the reduction in glucose utilization in obese Zucker rats (112), revealing a functionally relevant crosstalk between AngII, the HPA axis, and metabolic functions. They demonstrated that HPA reactivity was reduced after AT1R blockade in rats with diet-induced metabolic syndrome. The simultaneous decrease in ACTH indicates a pituitary mechanism, requiring that peripherally administered TEL penetrate the blood–brain barrier. This was verified by measuring TEL concentrations in the cerebrospinal fluid and indirectly by the ability of TEL to antagonize the central effects of AngII(113). All these mechanisms could explain the differences between ARBs on the effects on insulin resistance, transaminase levels and liver histology and the superiority of Telmisartan in treating NAFLD and in preventing it's progression to hepatic cirrhosis.

Conclusion

In conclusion, blocking RAS in order to inhibit the ACE/Ang II/AT1R axis and increasing ACE2/Ang(1-7)/MAS axis activation is an important strategy to treat NAFLD. Between RAS blockers, Telmisartan is the most promising drug, not only for its favorable pharmacokinetic proprieties and safety but also because it seems to exert additional AT1-independent benefits

on metabolism, related to a partial PPAR-γ agonism and/or to a positive central action on HPA axis. However, large multicenter randomized-controlled trials are needed to consolidate these findings. Currently, treatment with Telmisartan and with other RAS blockers can only be formally recommended in NALFD patients with an established indication of anti-hypertensive therapy.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic medicine : a journal of the British Diabetic Association.* 2006;23(5):469-480.

2. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365(9468):1415-1428.

3. Enjoji M, Kotoh K, Kato M, et al. Therapeutic effect of ARBs on insulin resistance and liver injury in patients with NAFLD and chronic hepatitis C: a pilot study. *International journal of molecular medicine.* 2008;22(4):521-527.

4. Byrne CD, Targher G. NAFLD: a multisystem disease. *Journal of hepatology.* 2015;62(1 Suppl):S47-64.

5. Duseja A, Singh SP, Saraswat VA, et al. Non-alcoholic Fatty Liver Disease and Metabolic Syndrome-Position Paper of the Indian National Association for the Study of the Liver, Endocrine Society of India, Indian College of Cardiology and Indian Society of Gastroenterology. *Journal of clinical and experimental hepatology.* 2015;5(1):51-68.

6. Koplay M, Sivri M, Erdogan H, Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. *World journal of hepatology.* 2015;7(5):769-776.

7. Law K, Brunt EM. Nonalcoholic fatty liver disease. *Clinics in liver disease.* 2010;14(4):591-604.

8. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology.* 2011;140(1):124-131.

9. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology.* 2012;55(6):2005-2023.

10. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary pharmacology & therapeutics.* 2011;34(3):274-285.

11. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology.* 2006;43(2 Suppl 1):S99-S112.

12. McCullough AJ. Pathophysiology of nonalcoholic steatohepatitis. *Journal of clinical gastroenterology.* 2006;40 Suppl 1:S17-29.

13. Gitto S, Vitale G, Villa E, Andreone P. Treatment of nonalcoholic steatohepatitis in adults: present and future. *Gastroenterology research and practice.* 2015;2015:732870.

14. Milic S, Mikolasevic I, Krznaric-Zrnic I, et al. Nonalcoholic steatohepatitis: emerging targeted therapies to optimize treatment options. *Drug design, development and therapy.* 2015;9:4835-4845.

15. Takahashi Y, Sugimoto K, Inui H, Fukusato T. Current pharmacological therapies for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World journal of gastroenterology.* 2015;21(13):3777-3785.

16. Nakajima K. Multidisciplinary pharmacotherapeutic options for nonalcoholic Fatty liver disease. *International journal of hepatology.* 2012;2012:950693.

17. Gallagher EJ, Leroith D, Karnieli E. Insulin resistance in obesity as the underlying cause for the metabolic syndrome. *The Mount Sinai journal of medicine, New York.* 2010;77(5):511-523.

18. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology.* 2001;120(5):1183-1192.

19. Baran B, Akyuz F. Non-alcoholic fatty liver disease: what has changed in the treatment since the beginning? *World journal of gastroenterology.* 2014;20(39):14219-14229.

20. Chaldakov GN, Stankulov IS, Hristova M, Ghenev PI. Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Current pharmaceutical design.* 2003;9(12):1023-1031.

21. Santos SH, Andrade JM. Angiotensin 1-7: a peptide for preventing and treating metabolic syndrome. *Peptides.* 2014;59:34-41.

22. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology.* 1998;114(4):842-845.

23. Younossi ZM, Reyes MJ, Mishra A, Mehta R, Henry L. Systematic review with meta-analysis: non-alcoholic steatohepatitis - a case for personalised treatment based on pathogenic targets. *Alimentary pharmacology & therapeutics.* 2014;39(1):3-14.

24. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology.* 2010;52(5):1836-1846.

25. Baskol G, Baskol M, Kocer D. Oxidative stress and antioxidant defenses in serum of patients with non-alcoholic steatohepatitis. *Clinical biochemistry.* 2007;40(11):776-780.

26. Nelson JE, Wilson L, Brunt EM, et al. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology.* 2011;53(2):448-457.

27. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology.* 2014;146(6):1513-1524.

28. Paschos P, Tziomalos K. Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. *World journal of hepatology.* 2012;4(12):327-331.

29. de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacological reviews.* 2000;52(3):415-472.

30. Alam S, Kabir J, Mustafa G, Gupta U, Hasan SK, Alam AK. Effect of telmisartan on histological activity and fibrosis of non-alcoholic steatohepatitis: A 1-year randomized control trial. *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association.* 2016;22(1):69-76.

31. Pereira RM, dos Santos RA, da Costa Dias FL, Teixeira MM, Simoes e Silva AC. Renin-angiotensin system in the pathogenesis of liver fibrosis. *World journal of gastroenterology.* 2009;15(21):2579-2586.

32. Santos SH, Simoes e Silva AC. The therapeutic role of Renin-Angiotensin System blockers in obesity- related renal disorders. *Current clinical pharmacology.* 2014;9(1):2-9.

33. Santos RA, Ferreira AJ. Angiotensin-(1-7) and the renin-angiotensin system. *Current opinion in nephrology and hypertension.* 2007;16(2):122-128.

34. Giacchetti G, Sechi LA, Rilli S, Carey RM. The renin-angiotensin-aldosterone system, glucose metabolism and diabetes. *Trends in endocrinology and metabolism: TEM.* 2005;16(3):120-126.

35. Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. *Journal of hypertension.* 2004;22(12):2253-2261.

36. Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM. Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacology & therapeutics.* 2010;128(1):119-128.

37. Tamura K, Tanaka Y, Tsurumi Y, et al. The role of angiotensin AT1 receptor-associated protein in renin-angiotensin system regulation and function. *Current hypertension reports.* 2007;9(2):121-127.

38. Iwai M, Horiuchi M. Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-Mas receptor axis. *Hypertension research : official journal of the Japanese Society of Hypertension.* 2009;32(7):533-536.

39. Putnam K, Shoemaker R, Yiannikouris F, Cassis LA. The renin-angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. *American journal of physiology. Heart and circulatory physiology.* 2012;302(6):H1219-1230.

40. Herichova I, Szantoova K. Renin-angiotensin system: upgrade of recent knowledge and perspectives. *Endocrine regulations.* 2013;47(1):39-52.

41. Santos RA, Simoes e Silva AC, Maric C, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100(14):8258-8263.

42. Santos RA, Ferreira AJ, Simoes ESAC. Recent advances in the angiotensin-converting enzyme 2-angiotensin(1-7)-Mas axis. *Experimental physiology.* 2008;93(5):519-527.

43. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation research.* 2000;87(5):E1-9.

44. Seki E, De Minicis S, Osterreicher CH, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nature medicine.* 2007;13(11):1324-1332.

45. Yoshiji H, Noguchi R, Ikenaka Y, et al. Losartan, an angiotensin-II type 1 receptor blocker, attenuates the liver fibrosis development of non-alcoholic steatohepatitis in the rat. *BMC research notes.* 2009;2:70.

46. Santos RA, Ferreira AJ, Verano-Braga T, Bader M. Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin-angiotensin system. *The Journal of endocrinology.* 2013;216(2):R1-R17.

47. Ferreira AJ, Santos RA. Cardiovascular actions of angiotensin-(1-7). *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas.* 2005;38(4):499-507.

48. Fyhrquist F, Saijonmaa O. Renin-angiotensin system revisited. *Journal of internal medicine.* 2008;264(3):224-236.

49. Santos SH, Fernandes LR, Pereira CS, et al. Increased circulating angiotensin-(1-7) protects white adipose tissue against development of a proinflammatory state stimulated by a high-fat diet. *Regulatory peptides.* 2012;178(1-3):64-70.

50. Mario EG, Santos SH, Ferreira AV, Bader M, Santos RA, Botion LM. Angiotensin-(1-7) Mas-receptor deficiency decreases peroxisome proliferator-activated receptor gamma expression in adipocytes. *Peptides.* 2012;33(1):174-177.

51. Wei HS, Li DG, Lu HM, et al. Effects of AT1 receptor antagonist, losartan, on rat hepatic fibrosis induced by CCl(4). *World journal of gastroenterology.* 2000;6(4):540-545.

52. Seki E, Park E, Fujimoto J. Toll-like receptor signaling in liver regeneration, fibrosis and carcinogenesis. *Hepatology research : the official journal of the Japan Society of Hepatology.* 2011;41(7):597-610.

53. Silva AR, Aguilar EC, Alvarez-Leite JI, et al. Mas receptor deficiency is associated with worsening of lipid profile and severe hepatic steatosis in ApoE-knockout mice. *American journal of physiology. Regulatory, integrative and comparative physiology.* 2013;305(11):R1323-1330.

54. Villela DC, Passos-Silva DG, Santos RA. Alamandine: a new member of the angiotensin family. *Current opinion in nephrology and hypertension.* 2014;23(2):130-134.

55. de Kloet AD, Krause EG, Woods SC. The renin angiotensin system and the metabolic syndrome. *Physiology & behavior.* 2010;100(5):525-534.

56. Kalupahana NS, Moustaid-Moussa N. The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. *Obesity reviews : an official journal of the International Association for the Study of Obesity.* 2012;13(2):136-149.

57. Santos SH, Braga JF, Mario EG, et al. Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1-7). *Arteriosclerosis, thrombosis, and vascular biology.* 2010;30(5):953-961.

58. Bilman V, Mares-Guia L, Nadu AP, et al. Decreased hepatic gluconeogenesis in transgenic rats with increased circulating angiotensin-(1-7). *Peptides.* 2012;37(2):247-251.

59. Moreira de Macedo S, Guimaraes TA, Feltenberger JD, Sousa Santos SH. The role of renin-angiotensin system modulation on treatment and prevention of liver diseases. *Peptides.* 2014;62:189-196.

60. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *American journal of physiology. Regulatory, integrative and comparative physiology.* 2004;287(4):R943-949.

61. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. *Current hypertension reports.* 2008;10(2):93-98.

62. Feltenberger JD, Andrade JM, Paraiso A, et al. Oral formulation of angiotensin-(1-7) improves lipid metabolism and prevents high-fat diet-induced hepatic steatosis and inflammation in mice. *Hypertension.* 2013;62(2):324-330.

63. Marrero MB, Fulton D, Stepp D, Stern DM. Angiotensin II-induced insulin resistance and protein tyrosine phosphatases. *Arteriosclerosis, thrombosis, and vascular biology.* 2004;24(11):2009-2013.

64. Prasad A, Quyyumi AA. Renin-angiotensin system and angiotensin receptor blockers in the metabolic syndrome. *Circulation.* 2004;110(11):1507-1512.

65. Wei Y, Sowers JR, Nistala R, et al. Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *The Journal of biological chemistry.* 2006;281(46):35137-35146.

66. Kanno K, Tazuma S, Nishioka T, Hyogo H, Chayama K. Angiotensin II participates in hepatic inflammation and fibrosis through MCP-1 expression. *Digestive diseases and sciences.* 2005;50(5):942-948.
67. Wei Y, Clark SE, Morris EM, et al. Angiotensin II-induced non-alcoholic fatty liver disease is mediated by oxidative stress in transgenic TG(mRen2)27(Ren2) rats. *Journal of hepatology.* 2008;49(3):417-428.
68. Kanno K, Tazuma S, Chayama K. AT1A-deficient mice show less severe progression of liver fibrosis induced by CCl(4). *Biochemical and biophysical research communications.* 2003;308(1):177-183.
69. Yokohama S, Tokusashi Y, Nakamura K, et al. Inhibitory effect of angiotensin II receptor antagonist on hepatic stellate cell activation in non-alcoholic steatohepatitis. *World journal of gastroenterology.* 2006;12(2):322-326.
70. Paizis G, Gilbert RE, Cooper ME, et al. Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. *Journal of hepatology.* 2001;35(3):376-385.
71. Liu J, Gong H, Zhang ZT, Wang Y. Effect of angiotensin II and angiotensin II type 1 receptor antagonist on the proliferation, contraction and collagen synthesis in rat hepatic stellate cells. *Chinese medical journal.* 2008;121(2):161-165.
72. Kyvelou SM, Vyssoulis GP, Karpanou EA, et al. Effects of antihypertensive treatment with angiotensin II receptor blockers on lipid profile: an open multi-drug comparison trial. *Hellenic journal of cardiology : HJC = Hellenike kardiologike epitheorese.* 2006;47(1):21-28.
73. Alfie J, Aparicio LS, Waisman GD. Current strategies to achieve further cardiac and renal protection through enhanced renin-angiotensin-aldosterone system inhibition. *Reviews on recent clinical trials.* 2011;6(2):134-146.
74. Meune C, Wahbi K, Duboc D, Weber S. Meta-analysis of Renin-Angiotensin-aldosterone blockade for heart failure in presence of preserved left ventricular function. *Journal of cardiovascular pharmacology and therapeutics.* 2011;16(3-4):368-375.
75. Santos RA, Ferreira AJ, Nadu AP, et al. Expression of an angiotensin-(1-7)-producing fusion protein produces cardioprotective effects in rats. *Physiological genomics.* 2004;17(3):292-299.
76. Carey RM, Padia SH. Angiotensin AT2 receptors: control of renal sodium excretion and blood pressure. *Trends in endocrinology and metabolism: TEM.* 2008;19(3):84-87.
77. Ferrario CM, Averill DB, Brosnihan KB, et al. Vasopeptidase inhibition and Ang-(1-7) in the spontaneously hypertensive rat. *Kidney international.* 2002;62(4):1349-1357.
78. Campbell DJ. The renin-angiotensin and the kallikrein-kinin systems. *The international journal of biochemistry & cell biology.* 2003;35(6):784-791.
79. Simoes e Silva AC, Diniz JS, Pereira RM, Pinheiro SV, Santos RA. Circulating renin Angiotensin system in childhood chronic renal failure: marked increase of Angiotensin-(1-7) in end-stage renal disease. *Pediatric research.* 2006;60(6):734-739.
80. Hirose A, Ono M, Saibara T, et al. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. *Hepatology.* 2007;45(6):1375-1381.
81. Yokohama S, Yoneda M, Haneda M, et al. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology.* 2004;40(5):1222-1225.

82. Investigators O, Yusuf S, Teo KK, et al. Telmisartan, ramipril, or both in patients at high risk for vascular events. *The New England journal of medicine.* 2008;358(15):1547-1559.

83. Halici Z, Bilen H, Albayrak F, et al. Does telmisartan prevent hepatic fibrosis in rats with alloxan-induced diabetes? *European journal of pharmacology.* 2009;614(1-3):146-152.

84. Guo J, Friedman SL. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. *Fibrogenesis & tissue repair.* 2010;3:21.

85. Rong X, Li Y, Ebihara K, et al. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. *Diabetologia.* 2010;53(8):1727-1731.

86. Guimaraes GG, Santos SH, Oliveira ML, et al. Exercise induces renin-angiotensin system unbalance and high collagen expression in the heart of Mas-deficient mice. *Peptides.* 2012;38(1):54-61.

87. Benson SC, Pershadsingh HA, Ho CI, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension.* 2004;43(5):993-1002.

88. Sugimoto K, Qi NR, Kazdova L, Pravenec M, Ogihara T, Kurtz TW. Telmisartan but not valsartan increases caloric expenditure and protects against weight gain and hepatic steatosis. *Hypertension.* 2006;47(5):1003-1009.

89. Clemenz M, Frost N, Schupp M, et al. Liver-specific peroxisome proliferator-activated receptor alpha target gene regulation by the angiotensin type 1 receptor blocker telmisartan. *Diabetes.* 2008;57(5):1405-1413.

90. Souza-Mello V, Gregorio BM, Cardoso-de-Lemos FS, de Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. *Clinical science.* 2010;119(6):239-250.

91. Miesel A, Muller-Fielitz H, Johren O, Vogt FM, Raasch W. Double blockade of angiotensin II (AT(1))-receptors and ACE does not improve weight gain and glucose homeostasis better than single-drug treatments in obese rats. *British journal of pharmacology.* 2012;165(8):2721-2735.

92. Michel MC, Brunner HR, Foster C, Huo Y. Angiotensin II type 1 receptor antagonists in animal models of vascular, cardiac, metabolic and renal disease. *Pharmacology & therapeutics.* 2016;164:1-81.

93. Miura Y, Yamamoto N, Tsunekawa S, et al. Replacement of valsartan and candesartan by telmisartan in hypertensive patients with type 2 diabetes: metabolic and antiatherogenic consequences. *Diabetes care.* 2005;28(3):757-758.

94. Vitale C, Mercuro G, Castiglioni C, et al. Metabolic effect of telmisartan and losartan in hypertensive patients with metabolic syndrome. *Cardiovascular diabetology.* 2005;4:6.

95. Kudo H, Yata Y, Takahara T, et al. Telmisartan attenuates progression of steatohepatitis in mice: role of hepatic macrophage infiltration and effects on adipose tissue. *Liver international : official journal of the International Association for the Study of the Liver.* 2009;29(7):988-996.

96. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity.* 2006;14(2):336-341.

97. Cianchetti S, Del Fiorentino A, Colognato R, Di Stefano R, Franzoni F, Pedrinelli R. Anti-inflammatory and anti-oxidant properties of telmisartan in cultured human umbilical vein endothelial cells. *Atherosclerosis.* 2008;198(1):22-28.
98. Marui N, Offermann MK, Swerlick R, et al. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *The Journal of clinical investigation.* 1993;92(4):1866-1874.
99. Yi ET, Liu RX, Wen Y, Yin CH. Telmisartan attenuates hepatic fibrosis in bile duct-ligated rats. *Acta pharmacologica Sinica.* 2012;33(12):1518-1524.
100. Cynis H, Kehlen A, Haegele M, et al. Inhibition of Glutaminyl Cyclases alleviates CCL2-mediated inflammation of non-alcoholic fatty liver disease in mice. *International journal of experimental pathology.* 2013;94(3):217-225.
101. Nakagami H, Kiomy Osako M, Nakagami F, et al. Prevention and regression of non-alcoholic steatohepatitis (NASH) in a rat model by metabosartan, telmisartan. *International journal of molecular medicine.* 2010;26(4):477-481.
102. Tamaki Y, Nakade Y, Yamauchi T, et al. Angiotensin II type 1 receptor antagonist prevents hepatic carcinoma in rats with nonalcoholic steatohepatitis. *Journal of gastroenterology.* 2013;48(4):491-503.
103. Rosselli MS, Burgueno AL, Carabelli J, Schuman M, Pirola CJ, Sookoian S. Losartan reduces liver expression of plasminogen activator inhibitor-1 (PAI-1) in a high fat-induced rat nonalcoholic fatty liver disease model. *Atherosclerosis.* 2009;206(1):119-126.
104. Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. *Circulation.* 2004;109(17):2054-2057.
105. Kurtz TW. Treating the metabolic syndrome: telmisartan as a peroxisome proliferator-activated receptor-gamma activator. *Acta diabetologica.* 2005;42 Suppl 1:S9-16.
106. Clasen R, Schupp M, Foryst-Ludwig A, et al. PPARgamma-activating angiotensin type-1 receptor blockers induce adiponectin. *Hypertension.* 2005;46(1):137-143.
107. Pershadsingh HA. Treating the metabolic syndrome using angiotensin receptor antagonists that selectively modulate peroxisome proliferator-activated receptor-gamma. *The international journal of biochemistry & cell biology.* 2006;38(5-6):766-781.
108. Ernsberger P, Koletsky RJ. Metabolic actions of angiotensin receptor antagonists: PPAR-gamma agonist actions or a class effect? *Current opinion in pharmacology.* 2007;7(2):140-145.
109. Kakuta H, Sudoh K, Sasamata M, Yamagishi S. Telmisartan has the strongest binding affinity to angiotensin II type 1 receptor: comparison with other angiotensin II type 1 receptor blockers. *International journal of clinical pharmacology research.* 2005;25(1):41-46.
110. Johren O, Dendorfer A, Dominiak P, Raasch W. Gene expression of mineralocorticoid and glucocorticoid receptors in the limbic system is related to type-2 like diabetes in leptin-resistant rats. *Brain research.* 2007;1184:160-167.
111. Aguilera G, Kiss A, Luo X. Increased expression of type 1 angiotensin II receptors in the hypothalamic paraventricular nucleus following stress and glucocorticoid administration. *Journal of neuroendocrinology.* 1995;7(10):775-783.
112. Muller-Fielitz H, Hubel N, Mildner M, Vogt FM, Barkhausen J, Raasch W. Chronic blockade of angiotensin AT(1) receptors improves cardinal symptoms of

metabolic syndrome in diet-induced obesity in rats. *British journal of pharmacology.* 2014;171(3):746-760.

113. Gohlke P, Weiss S, Jansen A, et al. AT1 receptor antagonist telmisartan administered peripherally inhibits central responses to angiotensin II in conscious rats. *The Journal of pharmacology and experimental therapeutics.* 2001;298(1):62-70.

FIGURES

Figure 1.

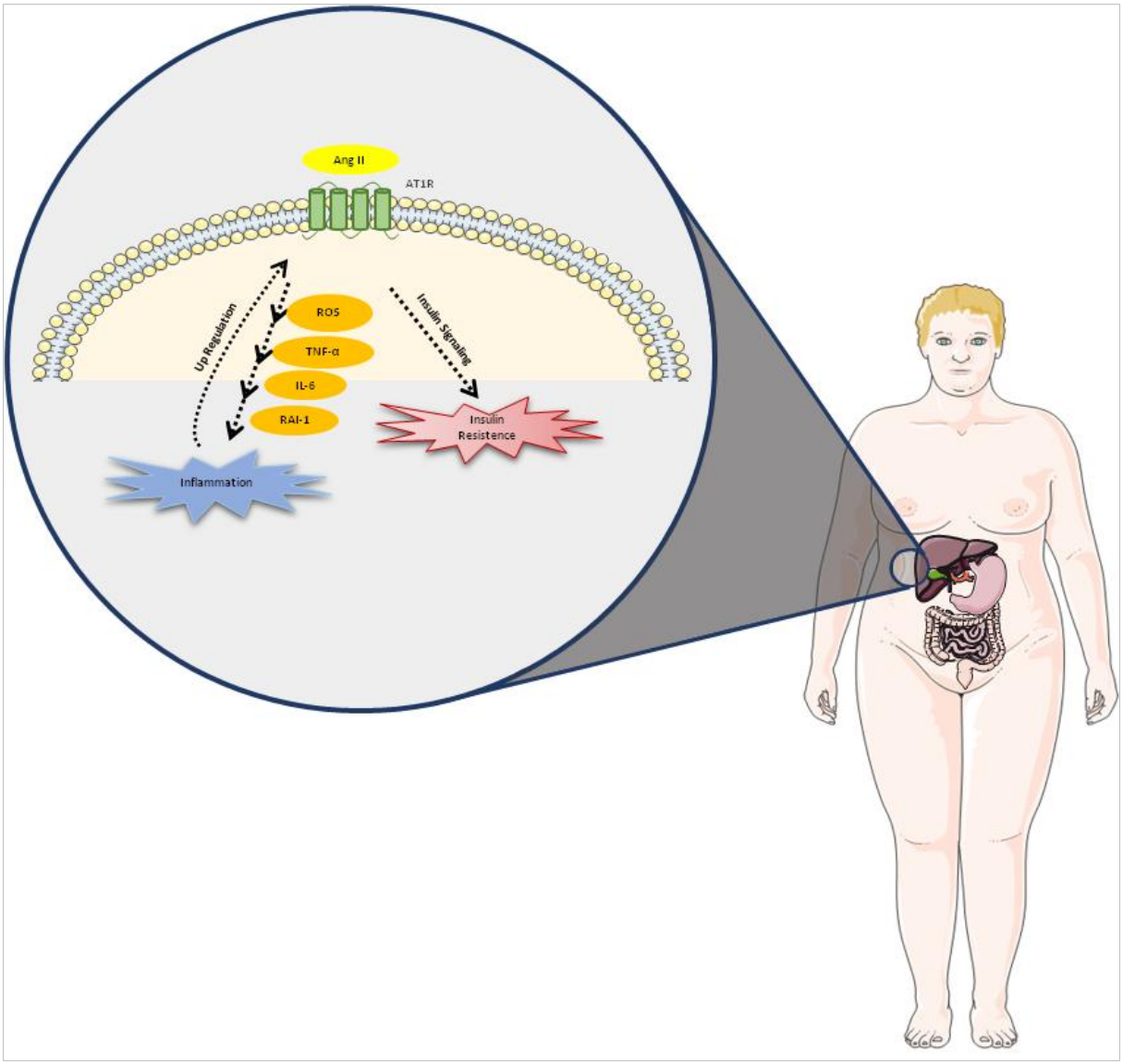

Fig. 1. Ang II as a major player in NAFLD. Ang II impairs intracellular insulin signaling, resulting in worsening of insulin resistance, the main pathophysiological element of NAFLD. Ang II also induces generation of reactive oxygen species (ROS), initiating and propagating the production of pro-inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1), resulting in inflammation, additional impairment of insulin signaling and up-regulation of AT1R genes, contributing to a vicious cycle of steatosis-necroinflammation-fibrosis.

Figure 2.

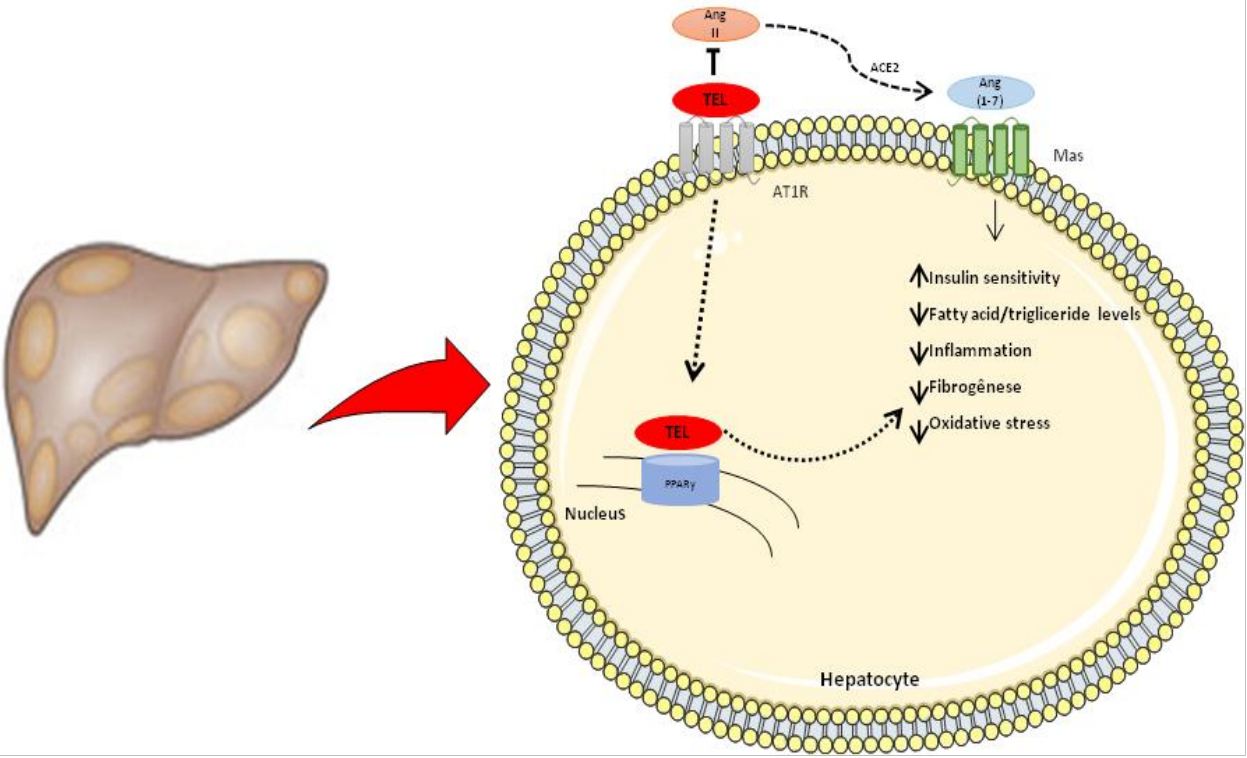

Fig. 2. Liver-protecting actions of Telmisartan in NAFLD.Telmisartan blocks AT1R, inhibiting the ACE/Ang II/AT1R axis and stimulates the Ang-(1-7)/Mas axis of RAS, improving insulin sensitivity, lipid metabolism and decreasing expression of proinflammatory cytokines, with suppression of macrophage infiltration into the liver. The result is morphological improvement in hepatic steatosis and in fibrogenic markers. Telmisartan also has anti oxidative properties and works as a partial agonist of the nuclear receptor PPAR-γ, anaction that contributes toincrease insulin sensitivity and decrease inflammation, oxidative stress, cell proliferation, and fatty acid/triglyceride levels.

4.2 PRODUTO 2

Telmisartan attenuates hepatic steatosis in high-fat fed mice: non-invasive evidence by ultrasound imaging

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Abstract: *Objective:* Telmisartan (TEL) is an angiotensin type I receptor blocker (ARB) widely used as an antihypertensive drug. In the last years, increasing evidence of its metabolic benefits appointed this drug as the most promising ARB for treatment of nonalcoholic fatty liver disease (NAFLD), a condition widely diagnosed in humans with ultrasound imaging (US) tests. The aim of the present study was to investigate the role of Telmisartan in treating NAFLD in mice fed with a high-fat diet and to access this result through the non-invasive technique of US. *Methods and procedures*: Twenty-four male mice were divided into four groups and fed for 60 d with a standard diet (ST), standard diet plus TEL (ST + TEL 5 mg/kg/d by gavage for 4 weeks), high-fat diet (HFD) or high-fat diet plus TEL (HFD + TEL 5 mg/kg/d by gavage for 4 weeks). Body weight, food intake, and serum total cholesterol, triacylglycerol, insulin, alanine transaminase, and aspartate aminotransferase were evaluated. Liver histology was analyzed. US imaging was performed to access liver echogenicity and size and epididymal fat pad thickness. *Results*: The major finding of the present study was that TEL reduced hepatic steatosis in the HFD + TEL group, and that this result was demonstrated non-invasively through US measurements of liver longitudinal axis and echogenicity. Also, US measurement of epididymal fat pad thickness showed that TEL reduced the amount of this adipose tissue, outcome confirmed by its weight. TEL also reduced body weight, mesenteric adipose tissue, transaminases, and improved glucose tolerance test and HDL cholesterol. *Conclusions*: We observed that treatment with Telmisartan improved metabolism and decreased NAFLD in mice with obesity-inducible diet, and that these results can be demonstrated non-invasively by ultrasound analysis. These data

reinforce Telmisartan's potential as a therapeutic candidate of NAFLD and stimulate the use of US in hepatic steatosis murine experimental models.

Introduction

Diet and lifestyle changes have led to worldwide increases in the prevalence of metabolic syndrome, a complex disorder where obesity, glucose intolerance, insulin resistance, dyslipidemia, and hypertension are associated [1-3]. Non-alcoholic fatty liver disease (NAFLD) is now considered a hepatic component of metabolic syndrome because of the close association between the two conditions, that share the same risk factors [3-6].

NAFLD is one of the most common causes of chronic liver diseases in Western countries, occurring in approximately 30% of the general population [7-8]. NAFLD is defined as a lipid-deposit accumulation in the hepatocytes, which is not due to excessive alcohol use (women ≤20 g/d, men ≤30 g/d). Accumulation of fat within hepatocytes causes oxidative stress, which can lead to liver injury, inflammation, and fibrosis. The aggressive subset of NAFLD, nonalcoholic steatohepatitis (NASH), accounts for a large and increasing number of patients who have cirrhosis, liver failure, and even hepatocellular carcinoma [9].

An accurate diagnosis requires imaging or histological techniques [5,10], once most patients are asymptomatic until late stages of disease [11], and serum markers such as aminotransferases are relatively insensitive and nonspecific [12,13]. Ultrasound (US) is currently the most widely used test for detecting hepatic steatosis, with the advantages of being safe, non-invasive, non-radiation, widely available and cost effective [9,14-18].

Telmisartan (TEL) is an angiotensin type I receptor blocker (ARB) that has been widely used for the treatment of hypertension and hypertension-related cardiovascular end-organ damage [19], being considered a first-line drug in mild to moderate hypertension, with an excellent safety profile [20]. In the last years, interest in metabolic effects of TEL has substantially increased, once beneficial effects in limiting the development of metabolic syndrome and diabetes has been demonstrated [21-26]. In the liver, evidence also shows metabolic benefits of this ARB, with promising protective effects against lipid accumulation and inflammation in animal studies [27-29].

Thus in this study we aimed to investigate whether Telmisartan treatment could improve NAFLD in high-fat fed mice, and we explored the non-invasive technique of US to diagnose this disease. Positive results would reinforce Telmisartan's potential as a therapeutic candidate of NAFLD and stimulate the use of US in hepatic steatosis murine experimental models.

Materials and methods

Animals

The experiment was conducted with 24 male FVB/N mice (4 wk old), which were randomly divided into four groups (n = 6) and fed with the following respective experimental diets for 60 d: Standard diet (ST); Standard diet plus Telmisartan (ST + TEL 5 mg/kg/d by gavage for 4 weeks); high-fat diet (HFD); high-fat diet plus Telmisartan (HFD + TEL 5 mg/kg/d by gavage for 4 weeks). Metabolic effects of TEL have been demonstrated especially when it was given at doses ≥ 5 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$ [30-37]. According to previous studies, the FVB/N strain is considered a preferable model for evaluation of metabolic disorders [38,39].

Diets

The standard diet (Labina, Purina, St. Louis, MO, USA) used for regular maintenance of the mice is composed of 50.30% carbohydrate, 41.90% protein, and 7.80% fat, with a total of 2.18 kcal per 1 g of diet. The high-fat diet was composed 24.55% of carbohydrate, 14.47% of protein, and 60.98% of fat, presenting a total of 5.28 kcal per 1 g of diet. All the high-fat diet components were purchased from Rhoster LTDA (São Paulo, Brazil).

Measurements of body weight, food intake, and tissue collection

The mice were individually housed and the food intake was measured twice per week during treatment to determine food efficiency (food intake/body weight). Overnight-fasted mice were sacrificed with ketamine (130 mg/kg) and xylazine (0.3 mg/kg) after anesthesia; sample tissues were collected, weighted, and immediately frozen in liquid nitrogen and stored at – 80 ^{o}C for posterior analysis.

Ultrasound Imaging

Animals were studied in supine position, in the day before sacrifice, by the same trained radiologist, using a Medison® ultrasound equipment (Seoul, South Korea), with a multifrequency linear transducer (7.0 to 12 MHz). All imaging were performed in fundamental brightness mode (B-mode), with optimization of the gain and the time gain compensation settings, which were kept constant throughout the experiment. The acoustic focus was placed in the center of the target organs (liver and epididymal fat pad), with measurement of liver echogenicity, size (longitudinal axis) and epididymal fat pad thickness [40-42]. Liver echogenicity was analyzed using the public domain Java image processing program Image-J (Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). A region of interest (ROI) was determined arbitrarily in the constant area of ultrasound images of the liver parenchyma, covering a wide area and excluding large vessels as far as possible. The average intensity in the ROI was measured and relative values were compared [14,43].

Determination of blood measurements

Serum was obtained after centrifugation (3200 rpm for 10 min at 4_C). Total serum cholesterol, high-density lipoprotein (HDL), triacylglycerol, insulin, and aspartate and alanine

transaminases (AST and ALT) were assayed using enzymatic kits (Wiener Laboratories, Rosario, Argentina).

Hematoxylin and eosin staining

Liver samples were fixed in formaldehyde solution (10%) and embedded in paraffin serially sectioned at 5 mm, stained with hematoxylin and eosin (HE), and evaluated under a conventional light microscope using an Olympus BX50 microscope (Tokyo, Japan).

Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0, GraphPad Software Inc., San Diego, CA, USA) and analyzed with confidence 95% ($P < 0.05$). Data are expressed as the mean ± SD. The statistical significance of differences in mean values among mice groups was assessed by one-way ANOVA followed by Bonferroni post-test.

Results

Mice fed with HFD had a higher energy intake than those fed with STD diet, and treatment with Telmisartan did not reduce their energy intake (ST: 2,69 ± 0.07; ST + TEL: 2,40 ± 0.05; HFD: 3,20 ± 0.13; HFD + TEL: 3,25 ± 0.15) (Fig. 1A,B). ST animals had lower body weight when comparing with HFD animals during all the experiment, and treatment with Telmisartan significantly decreased body weight in the HFD group but not in the ST group (ST: 281.4 ± 5.51; ST + TEL: 317.5 ± 11.25; HFD: 373.6 ± 11.25; HFD + TEL: 326.6 ± 15.12) (Fig. 1C,D).

Epididymal fat pad weight was higher in HFD group when comparing with ST group, and treatment with Telmisartan significantly reduced this weight in the HFD + TEL group (ST: 0,007 ± 0.001; ST + TEL: 0.01 ± 0.0006; HFD: 0.02 ± 0.002; HFD + TEL: 0.013 ± 0.001) (Fig. 2C). US measurements of epididymal fat pad thickness shows the same results, with higher measures in HFD animals than in ST animals, and with a significant reduction in epididymal fat pad thickness in the HFD + TEL group in relation to the HFD group (ST: 0.667 ± 0.0667; ST + TEL: 0.733 ± 0.066; HFD: 0.967 ± 0.033; HFD + TEL: 0.600 ± 0.058) (Fig. 2A,B).

Mesenteric adipose tissue also had significantly reduced weight in HFD + TEL group when comparing with the HFD group (ST: 0.004 ± 0.001; ST + TEL: 0.005 ± 0.0004; HFD: 0.08 ± 0.02; HFD + TEL: 0.004 ± 0.0004) (Fig 2D). Total adiposity was higher in HFD group in comparison with ST groups and was significantly reduced in HFD + TEL group when comparing with the HFD group (ST: 0.013 ± 0.001; ST + TEL: 0.016 ± 0.001; HFD: 0.031 ± 0.002; HFD + TEL: 0.018 ± 0.001 (Fig 2E).

A low glucose tolerance was observed in HFD mice when compared with STD animals, and this parameter was significantly improved in HFD + TEL group (ST: 27314 ± 3393; ST + TEL: 32355 ± 1214; HFD: 49454 ± 2439; HFD + TEL: 39802 ± 1401) (Fig. 3A). Although insulin sensitivity test showed this same tendency, no statistical significance was observed (Fig. 3B).

The HFD + TEL group had higher concentrations of HDL cholesterol than ST and HFD groups (ST: 64.08 ± 4.40; ST + TEL: 56.20 ± 4.02; HFD: 65.98 ± 2.94; HFD + TEL: 83.33 ± 3.44) (Fig. 4B,C). Triacylglycerols did not significantly differ between the groups (ST: 111.8 ± 6.29; ST + TEL: 125.7 ± 10.49; HFD: 128.3 ± 9.304; HFD + TEL: 142.0 ± 13.53) (Fig. 4A). Regarding parameters related to liver damage, values of ALT (ST: 33.0 ± 2.1; ST + TEL: 38.67 ± 2.40; HFD: 42 ± 1.79; HFD + TEL: 20.75 ± 3.35; P = 46.56) were significantly reduced in the HFD + TEL group (Fig. 4D,E).

In addition, analysis indicated that the HFD group had a substantial increase in total liver weight in relation to ST mice, and that HFD + TEL significantly reduced this weight (ST: 1.833 ± 0.105; ST + TEL: 1.889 ± 0.07; HFD: 2.233 ± 0.012; HFD + TEL: 1.405 ± 0.065) (Fig. 5D). US examination of liver size demonstrated higher measurements of liver longitudinal axis in HFD when comparing with ST animals, with a substantial decrease in HFD + TEL group in relation to HFD group (ST: 1.467 ± 0.088; ST + TEL: 2.000 ± 0.200; HFD: 2.233 ± 0.088; HFD + TEL: 1.467 ± 0.120) (Fig. 5A,B). US analysis of liver ecogenicity showed elevated mean gray values in HFD group, with a significant reduction of this parameter after Telmisartan treatment in the HFD + TEL group when comparing with HFD animals (ST: 78.68 ± 3.894; ST + TEL: 97.1 ± 5.556; HFD: 130.9 ± 0.012; HFD + TEL: 94.27 ± 9.199) (Fig. 5A,C). This result was subjectively observed by the radiologist during the exam by an increase in the brightness of the hepatic parenchyma in the HFD group, and a decrease in this parameter in the HFD + TEL group (Fig. 5A). The liver histologic examinations in mice fed the HFD indicated prominent steatosis (Fig. 5F). Hence, we performed a histologic analysis to examine the effect of Telmisartan on the development of fatty liver. Large hepatic lipid droplets were diffusely present in the livers of the HFD group mice compared with the ST group, and a significantly decrease in this aspect was observed in the HFD + TEL group (ST: 0.159 ± 0.049; ST + TEL: 1.253 ± 0.388; HFD: 2.080 ± 0.659; HFD + TEL: 0.642 ± 0.050) (Fig. 5E,F).

Discussion

To our knowledge, this is the first study that analyses the efficacy of the ARB Telmisartan in the treatment of NAFLD in high-fat fed mice by using the non-invasive and cost-effective technique of ultrasound imaging. While much progress has been made in elucidating the epidemiology, natural history, and pathogenesis of NAFLD/NASH, there remains no effective therapy, with limited options of evidence-based clinical guidelines for patient management. Pharmacological treatment of patients with NAFLD is still evolving, with no single therapy that has clearly been proved effective, especially, in favorably modifying the course of the disease [5,44-48].

In this study, we demonstrated that Telmisartan effectively protects against NAFLD and ameliorates several metabolic parameters in high-fat–fed mice. This is in coherence with previous studies that appoint this drug as the most promising ARB for treatment of NAFLD in terms of both safety and efficacy [46]. A central point to explain these results involves pathological changes in NAFLD, that may at least in part result from activation of the inflammatory arm of renin-angiotensin-system (RAS), especially in the context of insulin resistance [29]. The basic defect in the development of hepatic steatosis is the imbalance between import and export of fat to and from the liver secondary to insulin resistance [5,45,49]. Also, imbalances between pro- and antioxidant mechanisms and between pro- and anti-inflammatory cytokines are important components of NAFLD pathogenesis and disease progression toward NASH and fibrosis [1,5,47]. Here we demonstrated that HFD mice had a worse glucose profile when compared with STD animals, with a low glucose tolerance, and that this parameter was significantly improved by Telmisartan treatment. Concerning insulin profile, we observed the same tendency in the insulin sensitivity test, but no statistical significance was observed, what might be explained by the relatively short duration of the experiment.

The renin angiotensin system (RAS) is currently considered a dual function system with opposite actions, so that the ACE2/Ang-(1–7)/Mas axis is able to counteract most of the deleterious actions of the ACE/Ang II/AT1R axis, especially in pathological conditions [38,50-56]. Evidence suggests that obesity activates the RAS arm composed of ACE/Ang II/AT1R [57-59], and Angiotensin II (Ang II) has been implicated as a major player in the altered hepatic lipid metabolism observed in NAFLD [39], influencing intracellular insulin signaling by several mechanisms which may result in worsening of insulin resistance [21,60-62]. Ang II also induces generation of reactive oxygen species (ROS), initiating and propagating the production of pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1), resulting in inflammation and additional impairment of insulin signaling [63-66] contributing to the vicious cycle of steatosis-necroinflammation-fibrosis [46]. On the other hand, increasing evidence has shown the beneficial effects of the ACE2/Ang-(1-7)/Mas axis on liver pathology and on metabolic disorders, exerting an important anti-obesity role by improving insulin sensitivity, glucose tolerance and type 2 diabetes, reducing body fat, increasing adiponectin production and reverting hyperleptinemia [38,39,53,55,59,67-71].

Concerning AT1R-independent effects, it is important to emphasize the relationship between Telmisartan and peroxisome proliferator-activated receptor gamma (PPAR-**γ**) activation. Telmisartan works as a partial agonist of PPAR- **γ** [25,27,28,70,72], a property that does not appear to be shared by other ARBs [29,73]. Nuclear PPARγ receptor is a transcription factor regulating many genes related to adipogenesis, lipid metabolism and insulin sensitivity [27]. Parcial agonists of PPAR- **γ** like Telmisartan increases insulin sensitivity, high-density lipoprotein levels, and decreases inflammation, oxidative stress, cell proliferation, migration and fatty acid and triglyceride levels, but without causing the fluid collection associated with full agonists of PPAR-γ, such as pioglitazone or rosiglitazone [70,72,74-76].

Once Telmisartan not only modulates RAS in order to inhibit its deleterious arm and to activate its protective one [21,77-80], but also works as a parcial agonists of PPAR- γ [25,27,28,70,72] and has been consistently proven to reduce insulin resistance [21-26], its effects in liver histology should be expected. We demonstrated a significant improvement on liver size and on hepatic lipid droplets infiltration in the HFD group treated with Telmisartan, in concordance with previous studies [27-29]. These morphological findings were positively correlated with the US exam of the liver the day before sacrifice. Ultrasound was effective in demonstrating a higher liver size in HFD group and a substantial decrease in this parameter when the HFD animals were treated with Telmisartan. Also, US analysis of liver echogenicity showed elevated mean gray values in HFD group, with a significant reduction of this parameter after Telmisartan treatment in HFD + TEL group. As in humans, hepatic steatosis in rodents is classically described on US as a diffuse increase in hepatic echogenicity, or "bright liver", due to increased reflection of US from the liver parenchyma, which is caused by intracellular accumulation of fat vacuoles [15,16,18,40]. Another parameters, such as reduced visualization of the diaphragm and of small peripheral vessels, with no changes in liver surface, can also be utilized [40].

Although liver biopsy is still considered the gold standard for diagnosing and staging of NAFLD [16,81-85] it is an invasive procedure with some limitations and complications such as potential sampling error, low reproducibility, pain, bleeding, infection and, in rare instances, death [9,82,83,86-88]. For this reason, various imaging methods have been utilized to evaluate NAFLD, such as US, computed tomography (CT), and magnetic resonance imaging (MRI) [7,16,18,89], the latter considered the noninvasive standard of reference for evaluation of hepatic steatosis [9,16,18,90,91]. However, MRI is expensive and time-consuming and CT, besides being expensive and having low sensitivity for mild hepatic steatosis, involves radiation exposure, making these tools inappropriate and not cost-effective for large-scale NAFLD screening and for longitudinal follow-up [14,16,18,84]. From these perspectives, US represents an excellent examination modality that is safe, non-invasive, non-radiation, widely available, cost effective, and can be performed repeatedly with no biological risk, being currently the most widely used test for detecting hepatic steatosis in humans [9,14-16-18].

In experimental murine liver disease models, US is feasible, efficient, and easy to be done, requiring only slight sedation and abdominal shaving, not demanding total immobility or apnea while obtaining the images, therefore, consisting in a low risk procedure [40,92-94]. Murine models of diffuse liver disease can be accurately evaluated with US both for diagnostic purposes and for follow-up, some of them with statistical correlation of US findings with histology [40,95]. Lessa *et al.* [40] demonstrated that qualitative ultrasound analysis is sensitive and specific enough to diagnose fatty liver disease in rats and Fernández-Domínguez *et al.* [41] concluded that diffuse steatosis was easily diagnosed in mice models with US evaluation. The ability to accurately measure important features of liver disease in rodents with noninvasive methods such as US is of tremendous value [9], once it can reliably substitute histological diagnosis in NAFLD models in several circunstances, allowing the reduction of the number of animals in research [40]. Also, it provides a non- invasive tool to

long-term follow-up, which is usually needed in pre-clinical drug or cell-based therapy [40,41].

However, it is well-recognized that the diagnostic accuracy of US diminishes with less than 20-30% of hepatic steatosis [14,17,18,89,96] and in the setting of chronic liver diseases, such as chronic hepatitis C [18,82,84,97]. This happens because hepatic fibrosis may also increase liver echogenicity [98] making it impossible for US to distinguish between NASH and other degrees of NAFLD [14,84,89-100] limitation also shared by other imaging methods such as CT and RMI. Also, evaluation of liver echogenicity is usually made in a subjective and qualitative manner [9] what makes the diagnostic performance of US highly dependent on the operator reading skills [14-16,17,18] and not accurate for detecting small changes in liver fat content [15,16].

We also demonstrated that Telmisartan reduced the adiposity and the weights of mesenteric and of epididymal adipose tissues in HFD + TEL group wen comparing to the HDF group. Importantly, the amount of the epididymal adipose deposit was well demonstrated non-invasively by US measurements of epidydymal fat pad thickness. Liao *et al.* [42] have already utilized echographic measurements of epididymal fat pad thickness to successfully access the amount of this adipose tissue deposit, reinforcing that this technique can also be used to estimate this morphological parameter. Kudo *et al.* [28] showed for the first time that Telmisartan decreases the adipocyte size and upregulates the adiponectin secretion without affecting food intake in a murine NASH model, reducing the accumulation of visceral fat. Moreover, Telmisartan, but not the ARB Valsartan, increased the expression of both nuclear-encoded and mitochondrial-encoded genes in skeletal muscle known to play important roles in mitochondrial energy metabolism. Thus, in addition to a class effect of ARBs in modulating adipocyte size, these findings raise the possibility that certain molecules, like Telmisartan, may have a particularly strong impact on fat cell volume and fat accumulation, as well as distinctive effects on energy metabolism, which may help to protect against dietary-induced visceral obesity and weight gain [32]**.** The efficacy of TEL in reducing visceral fat mass may be relevant for patients since an increase in visceral fat is related to hypertension, dyslipidemia and an impaired metabolic pattern, but also serves as an independent predictor of mortality in men [101].

Conclusion

In conclusion, the present study indicates that oral treatment with Telmisartan offers a protective effect against NAFLD and improves lipid and glucose metabolism in high-fat–fed mice. We also demonstrated that ultrasound is a non-invasive tool that can be reliable used to diagnose hepatic steatosis and to estimate the amount of visceral fat mass by measuring the thickness of epididymal fat pad, contributing to the refinement of experimental technique in murine studies against more invasive procedures. However, if the distinction between simple steatosis and steatohepatitis or fibrosis is needed, or if small alterations in steatosis severity are an important parameter, histologic analysis is still necessary.

Competing interests

The authors declare no conflict of interest.

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References

1. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome-a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; 23:469–80.

2. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415–28.

3. Enjoji M, Kotoh K, Kato M, Higuchi N, Kohjima M, Nakashima M, Nakamuta M. Therapeutic effect of ARBs on insulin resistance and liver injury in patients with NAFLD and chronic hepatitis C: A pilot study. *Int J Mol Medicine* 2008;22: 521-527.

4. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol.* 2015; 62(1 Suppl):S47–S64.

5. Duseja A, Singhy S, Saraswatz VA, Acharyax SK, Chawla YK, Chowdhuryk S, Dhiman RK, Jayakumar RV, *et al.* Non-alcoholic Fatty Liver Disease and Metabolic Syndrome-Position Paper of the Indian National Association for the Study of the Liver, Endocrine Society of India, Indian College of Cardiology and Indian Society of Gastroenterology. *J Clin Exp Hepatol* 2015;5:51–68.

6. Lupşor-Platon M, Stefănescu H, Mureşan D, et al. Noninvasive assessment of liver steatosis using ultrasound methods. *Med Ultrason* 2014; 16:236–245. [PubMed: 25110765].

7. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005; 288: E462-E468 [PMID: 15339742 DOI: 10.1152/ajpendo.00064.2004].

8. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; 34: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x].

9. Kramer H, Pickhardt PJ, Kliewer MA, Hernando D, Chen GH, Zagzebski JA, Reeder SB. Accuracy of Liver Fat Quantification With Advanced CT, MRI, and Ultrasound Techniques: Prospective Comparison With MR Spectroscopy. *AJR Am J Roentgenol* 2017; January; 208(1): 92–100. doi:10.2214/AJR.16.16565.

10. Chalasani N, Younossi Z, Lavine JE *et al.* The diagnosis and management of non-

alcoholic fatty liver disease: practice guideline by the American Association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. *Hepatology 2012;* 55, no. 6, pp. 2005–2023, 2012.

11. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; 54: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452].

12. Schwimmer JB, Dunn W, Norman GJ, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010; 138:1357–1364. [PubMed: 20064512].

13. Rehm JL, Wolfgram PM, Hernando D, Eickhoff JC, Allen DB, Reeder SB. Proton density fat- fraction is an accurate biomarker of hepatic steatosis in adolescent girls and young women. *Eur Radiol* 2015; 25:2921–2930. [PubMed: 25916386].

14. Kuroda H, Kakisaka K, Kamiyama N, Oikawa T, Onodera M, Sawara K, Oikawa K, Endo R, Takikawa Y, Suzuki K. Non-invasive determination of hepatic steatosis by acoustic structure quantification from ultrasound echo amplitude. *World J Gastroenterol* 2012; 18(29): 3889-3895 ISSN 1007-9327 (print) ISSN 2219-2840.

15. Ho MC, Lee YH, Jeng YM, Chen CN, Chang KJ, Tsui PH. Relationship between Ultrasound Backscattered Statistics and the Concentration of Fatty Droplets in Livers: An Animal Study. *PLoS One* 2013; 8(5): e63543. Published online 2013 May 21. doi: 10.1371/journal.pone.0063543.

16. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20 (23): 7392-7402. Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i23/7392.htm. DOI: http://dx.doi.org/10.3748/wjg.v20. i23.7392.

17. Khov N, Sharma A, Riley T. Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol*; 20(22): 6821-6825. DOI:10.3748/wjg.v20.i22.6821.

18. Koplay M, Sivri M, Erdogan H, Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. *World J Hepatol* 2015; 7(5): 769-776 ISSN 1948-5182 (online). DOI: 10.4254/wjh.v7.i5.769.

19. De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 2000; 52: 415-472.

20. Alam S, Kabir J, Mustafa G, Gupta U, Hasan S, Alam A. Effect of telmisartan on histological activity and fibrosis of non-alcoholic steatohepatitis: A 1-year randomized control trial. Saudi J *Gastroenterol* 2016;22:69-76.

21. Prasad A, Quyyumi AA. Renin–angiotensin system and angiotensin receptor blockers in the metabolic syndrome. *Circulation* 2004;110:1507–12.

22. Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H *et al*. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008;358: 1547–1559.

23. Halici Z, Bilen H, Albayrak F, Uyanik A, Cetinkaya R, Suleyman H, *et al*. Does telmisartan prevent hepatic fibrosis in rats with alloxan-induced diabetes? *Eur J Pharmacol* 2009;614:146–52.

24. Guo J, Friedman SL. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. *Fibrog Tissue Repair* 2010;3:21.

25. Rong, X., Li, Y., Ebihara, K., Zhao, M., Naowaboot, J., Kusakabe, T., et al. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. *Diabetologia* 2010;53, 1727–1731.

26. Guimaraes GG, Santos SH, Oliveira ML, Pimenta-Velloso EP, Motta DF, Martins AS, et al. Exercise induces renin-angiotensin system unbalance and high collagen expression in the heart of Mas-deficient mice. *Peptides* 2012;38:54–61.

27. Clemenz M, Frost N, Schupp NM *et al*. Liver-specific peroxisome proliferator-activated receptor α target gene regulation by the angiotensin type 1 receptor blocker telmisartan. *Diabetes 2008;*57:5, pp. 1405–1413.

28. Kudo H, Yata Y, Takahara T, Kawai K, Nakayama Y, Kanayama M, *et al*. Telmisartan attenuates progression of steatohepatitis in mice: Role of hepatic macrophage infiltration and effects on adipose tissues. *Liver Int* 2009;29, 988–996.

29. Michel M, Brunner HR, Foster C, Huo Y. Angiotensin II type 1 receptor antagonists in animal models of vascular, cardiac, metabolic and renal disease. *Pharmacology & Therapeutics* 2016;164:1–81.

30. Wienen W, Richard S, Champeroux P, Audeval-Gerard C. Comparative antihypertensive and renoprotective effects of telmisartan and lisinopril after long-term treatment in hypertensive diabetic rats. *J Renin Angiotensin Aldosterone Syst* 2001;2: 31–36.

31. Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M *et al*. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004;43: 993–1002.

32. Sugimoto K, Qi NR, Kazdova L, Pravenec M, Ogihara T, Kurtz TW. Telmisartan but not valsartan increases caloric expenditure and protects against weight gain and hepatic steatosis. *Hypertension* 2006;47: 1003–1009.

33. Sugimoto K, Kazdova L, Qi NR, Hyakukoku M, Kren V, Simakova M *et al*. Telmisartan increases fatty acid oxidation in skeletal muscle through a peroxisome proliferator-activated receptor-gamma dependent pathway. *J Hypertens* 2008;26: 1209–1215.

34. Kamari Y, Harari A, Shaish A, Peleg E, Sharabi Y, Harats D *et al*. Effect of telmisartan, angiotensin II receptor antagonist, on metabolic profile in fructose-induced hypertensive, hyperinsulinemic, hyperlipidemic rats. *Hypertens Res* 2008;31: 135–140.

35. Souza-Mello V, Gregório BM, Cardoso-de-Lemos FS, Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. *Clinical Science* 2010; 119: 239–250 doi:10.1042/CS20100061.

36. Younis F, Stern N, Limor R, Oron Y, Zangen S, Rosenthal T. Telmisartan ameliorates hyperglycemia and metabolic profile in nonobese Cohen-Rosenthal diabetic hypertensive rats via peroxisome proliferator activator receptor-gamma activation. *Metabolism* 2010;59: 1200–1209.

37. Miesel A, Müller-Fielitz H, Jöhren O, Vogt FM, Raasch W. Double blockade of angiotensin II (AT1)-receptors and ACE does not improve weight gain and glucose homeostasis better than single-drug treatments In obese rats. *British Journal of Pharmacology* 2012;165: 2721–2735.

38. Santos SH, Fernandes LR, Mario EG, Ferreira AV, Porto LC, Alvarez-Leite JI, *et al*. Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. *Diabetes* 2008;57:340–7.

39. Feltenberger JD, Andrade JM, Paraiso A, Barros LO, Filho AB, Sinisterra RD, *et al*. Oral formulation of angiotensin-(1-7) improves lipid metabolism and prevents high-fat diet-induced hepatic steatosis and inflammation in mice. *Hypertension* 2013;62:324–30.

40. Lessa AS, Paredes BD, Dias JV, Carvalho AB, Quintanilha LF, Takiya CM, *et al*. Ultrasound imaging in an experimental model of fatty liver disease and cirrhosis in rats. *BMC Veterinary Research* 2010; 6:6 http://www.biomedcentral.com/1746-6148/6/6.

41. Fernández-Domínguez I, Echevarria-Uraga JJ, Gómez N, Luka Z, Wagner C, Lu SC, *et al*. High-frequency ultrasound imaging for longitudinal evaluation of non-alcoholic fatty liver disease progression in mice. *Ultrasound Med Biol* 2011;37(7): 1161–1169. doi:10.1016/j.ultrasmedbio.2011.04.012.

42. Liao AH, Ma WC, Wu MF. Evaluation of ultrasound combined with chitosan for the control of weight and local fat in mice. *Ultrasound in Med. & Biol* 2013;39:10, pp. 1794–1803. http://dx.doi.org/10.1016/j.ultrasmedbio.2013.04.025.

43. Miyata Y, Miyahara T, Moriyasu F. Decreased accumulation of ultrasound contrast in the liver of nonalcoholic steatohepatitis rat model. *World J Gastroenterol* 2011; 17(37): 4191-4198 ISSN 1007-9327 (print) ISSN 2219-2840 (online).

44. Paschos P, Tziomalos K. Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. *World J Hepatol* 2012; 4: 327-331 [PMID: 23355909 DOI: 10.4254/wjh.v4.i12.327].

45. Baran B, Akyüz F. Non-alcoholic fatty liver disease: What has changed in the treatment since the beginning? *World J Gastroenterol* 2014; 20(39): 14219-14229. DOI: 10.3748/wjg.v20.i39.14219.

46. Gitto, G. Vitale, E. Villa, and P. Andreone. Treatment of Nonalcoholic Steatohepatitis in

Adults: Present and Future. *Gastroenterology Research and Practice* 2015; Article ID 732870, 14 pages. doi:10.1155/2015/732870.

47. Milic S, Mikolasevic I, Krznaric-Zrnic I, Stanic M, Poropat G, Stimac D, Vlahovic-Palcevski V, Orlic L. Nonalcoholic steatohepatitis: emerging targeted therapies to optimize treatment options. *Drug Design, Development and Therapy* 2015;9:4835–4845.

48. Takahashi Y, Sugimoto K, Inui H, Fukusato T. Current pharmacological therapies for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; 21(13): 3777-3785. DOI: 10.3748/wjg.v21.i13.3777.

49. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; 120: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256].

50. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, *et al*. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000;87:E1–9.

51. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007;13:1324–32.

52. Seki E, Park E, Fujimoto J. Toll-like receptor signaling in liver regeneration, fibrosis and carcinogenesis. *Hepatol Res* 2011;41:597–610.

53. Santos SH, Andrade JM, Fernandes LR, Sinisterra RD, Sousa FB, Feltenberger JD, *et al*. Oral angiotensin-(1–7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-kappaB in rats fed with high-fat diet. *Peptides* 2013;46:47–52.

54. Silva AR, Aguilar EC, Alvarez-Leite JI, da Silva RF, Arantes RM, Bader M, *et al*. Mas receptor deficiency is associated with worsening of lipid profile and severe hepatic steatosis in ApoE-knockout mice. *Am J Physiol Regul Integr Comp Physiol* 2013;305:R1323–30.

55. Santos SH, Simoes e Silva AC. The therapeutic role of Renin-Angiotensin System blockers in obesity-related renal disorders. *Curr Clin Pharmacol* 2014;9:2–9.

56. Villela DC, Passos-Silva DG, Santos RA. Alamandine: a new member of the angiotensin family. *Curr Opin Nephrol Hypertens* 2014;23(2):130–4.

57. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R943–9.

58. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. *Curr Hypertens Rep* 2008;10:93–8.

59. Santos SH, Mario BJ, Pôrto EG, Rodrigues-Machado Mda LC, Murari G, Botion A, et al. Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1-7). *Arterioscler Thromb Vasc Biol* 2012;30: 953–61.

60. Marrero MB, Fulton D, Stepp D, Stern DM. Angiotensin II-induced insulin resistance and protein tyrosine phosphatases. *Arterioscler Thromb Vasc Biol* 2004; 24: 2009-13.

61. Wei Y, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, Manrique C, Stump CS. Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *J Biol Chem* 2006; 281: 35137-35146 [PMID: 16982630 DOI: 10.1074/jbc.M601320200].

62. Putnam K, Shoemaker R, Yiannikouris F, Cassis LA. The renin–angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. *Am J Physiol Heart Circ Physiol* 2012; 302:H1219–30.

63. Kanno K, Tazuma S, Chayama K. AT1A-deficient mice show less severe pro- gression of liver fibrosis induced by CCl(4). *Biochem Biophys Res Commun* 2003; 308:177–83.

64. Kanno K, Tazuma S, Nishioka T, Hyogo H, Chayama K. Angiotensin II participates in hepatic inflammation and fibrosis through MCP-1 expression. *Dig Dis Sci* 2005; 50:942–8.

65. Yokohama S, Tokusashi Y, Nakamura K, Tamaki Y, Okamoto S, Okada M, et al. Inhibitory effect of angiotensin II receptor antagonist on hepatic stellate cell activation in non-alcoholic steatohepatitis. *World J Gastroenterol* 2006;12:322–6.

66. Wei Y, Clark SE, Morris EM, Thyfault JP, Uptergrove GM, Whaley-Connell AT, et al. Angiotensin II-induced non-alcoholic fatty liver disease is mediated by oxidative stress in transgenic TG(mRen2)27(Ren2) rats. *J Hepatol* 2008;49:417–28.

67. Santos SH, Braga JF, Mario EG, Porto LC, Rodrigues-Machado Mda G, Murari A, *et al*. Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1–7). *Arterioscler Thromb Vasc Biol* 2010;30:953–61.

68. Bilman V, Mares-Guia L, Nadu AP, Bader M, Campagnole-Santos MJ, Santos RA, *et al*. Decreased hepatic gluconeogenesis in transgenic rats with increased circulating angiotensin-(1-7). *Peptides* 2012;37:247–51.

69. Mario EG SS, Ferreira AV, Bader M, Santos RA, Botion LM. Angiotensin-(1-7) Mas-receptor deficiency decreases peroxisome proliferator-activated receptor gamma expression in adipocytes. *Peptides* 2012;33:174–7.

70. Macedo SM, Guimaraes TA, Feltenberger JD, Santos SHS. The role of renin-angiotensin system modulation on treatment and prevention of liver diseases. *Peptides* 2014;62:189–196.

71. Oliveira Andrade JM, Paraiso AF, Garcia ZM, Ferreira AV, Sinisterra RD, Sousa FB, et al. Cross talk between angiotensin-(1–7)/Mas axis and sirtuins in adipose tissue and metabolism of high-fat feed mice. *Peptides* 2014;55:158–65.

72. Schupp M, Janke J, Clasen R, Unger T, Kintscher U, "Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-γ activity," *Circulation* 2004; vol. 109, no. 17, pp. 2054–2057, 2004.

73. Kurtz TW. Treating the metabolic syndrome: telmisartan as a peroxisome proliferator-activated receptor-gamma activator. *Acta Diabetol* 2005; 42 Suppl1: p.S9-16.

74. Kurtz, T.W. and M. Pravenec, Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. *J Hypertens* 2004;22(12): p. 2253-61.

75. Clasen R. *et al*. PPARgamma-activating angiotensin type-1 receptor blockers induce adiponectin. *Hypertension* 2005; 46(1): p. 137-43.

76. Pershadsingh HA. Treating the metabolic syndrome using angiotensin receptor antagonists that selectively modulate peroxisome proliferator-activated receptor-gamma. *Int J Biochem Cell Biol* 2006; **38**(5-6): p. 766-81.

77. Campbell DJ. The renin-angiotensin and the kallikrein-kinin systems. *Int J Biochem Cell Biol* 200;. **35**(6): p. 784-91.

78. Kyvelou SM *et al*. Effects of antihypertensive treatment with angiotensin II receptor blockers on lipid profile: an open multi-drug comparison trial. *Hellenic J Cardiol* 2006; 47(1): p. 21-8.

79. Simoes e Silva AC, *et al*. Circulating renin Angiotensin system in childhood chronic renal failure: marked increase of Angiotensin-(1-7) in end-stage renal disease. Pediatr Res 2006; 60(6): p. 734-9.

80. Pereira RM, *et al*. Renin-angiotensin system in the pathogenesis of liver fibrosis. *World J Gastroenterol* 2009; 15(21): p. 2579-86.

81. Bravo AA**,** Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; 344**:** 495-500

82. Chen CH**,** Lin ST, Yang CC, Yeh YH, Kuo CL, Nien CK. The accuracy of sonography in predicting steatosis and fibrosis in chronic hepatitis C. *Dig Dis Sci* 2008; 53: 1699-1706 [PMID: 17939048 DOI: 10.1007/s10620-007-0048-2].

83. Baršić N**,** Lerotić I, Smirčić-Duvnjak L, Tomašić V, Duvnjak M. Overview and developments in noninvasive diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; 18: 3945-3954 [PMID: 22912545 DOI: 10.3748/wjg.v18. i30.3945].

84. Cheung CL, Lam KSL, Wong ICK, Cheung BMY. Non-invasive score identifies ultrasonography- diagnosed non-alcoholic fatty liver disease and predicts mortality in the USA. *BMC Medicine* 2014; 12:154 http://www.biomedcentral.com/1741-7015/12/154.

85. Polyzos SA, Mantzoros CS. Necessity for timely noninvasive diagnosis of nonalcoholic fatty liver disease. *Metabolism* 2014; 63: 161-167 [PMID: 24290839 DOI: 10.1016/j.metabol.2013.10.010].

86. Wai CT, Tan LH, Kaur M, Da Costa M, Quak SH, Tan KC. Pitfalls in interpreting liver biopsy results: the story of the blind men and the elephant. *Liver Transpl* 2002; 8: 1200-1201 [PMID: 12474163 DOI: 10.1053/jlts.2002.36843].

87. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; 128: 1898-1906 [PMID: 15940625 DOI: 10.1053/j.gastro.2005.03.084].

88. Tsuchiya H, Ebata Y, Sakabe T, Hama S, Kogure K, Shiota G. High-fat, high-fructose diet induces hepatic iron overload via a hepcidin-independent mechanism prior to the onset of liver steatosis and insulin resistance in mice. *Metabolism* 2013; 62: 62-69 [PMID: 22854109 DOI: 10.1016/j.metabol.2012.06.008].

89. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; 123: 745-750.

90. Mehta SR, Thomas EL, Patel N, et al. Proton magnetic resonance spectroscopy and ultrasound for hepatic fat quantification. Hepatol Res. 2010; 40:399–406. [PubMed: 20236356].

91. Heger M, Marsman HA, Bezemer R, Cloos MA, van Golen RF, van Gulik TM. Non-invasive quantification of triglyceride content in steatotic rat livers by (1)H-MRS: when water meets (too much) fat. *Acad Radiol* 2011; 18:1582–1592. [PubMed: 22055799].

92. Mai W, Barraud L, Lefrancois L, Scoazec JY, Guerret S, Vray D, Merle P, Vitvitski-Trepo L, Trepo C, Janier MF. Ultrasound detection of spontaneous hepato-cellular carcinomas in X/myc bitransgenic mice. *Liver Int* 2004; 24:651–657. [PubMed: 15566518].

93. Schmitz V, Tirado-Ledo L, Tiemann K, Raskopf E, Heinicke T, Ziske C, Gonzalez-Carmona MA, Rabe C, *et al*. Establishment of an orthotopic tumour model for hepatocellular carcinoma and non-invasive in vivo tumour imaging by high resolution ultrasound in mice. *J Hepatol* 2004; 40:787–791. [PubMed: 15094226].

94. Graham KC, Wirtzfeld LA, MacKenzie LT, Postenka CO, Groom AC, MacDonald IC, Fenster A, Lacefield JC, Chambers AF. Three-dimensional high-frequency ultrasound imaging for longitudinal evaluation of liver metastases in preclinical models. *Cancer Res* 2005; 65:5231– 5237. [PubMed: 15958568].

95. Lee GP, Jeong WI, Jeong DH, Do SH, Kim TH, Jeong KS: Diagnostic evaluation of carbon tetrachloride-induced rat hepatic cirrhosis model. *Anticancer Res* 2005; 25:1029-1038.

96. Chen CL**,** Cheng YF, Yu CY, Ou HY, Tsang LL, Huang TL, Chen TY, Concejero A, *et al.* Living donor liver transplantation: the Asian perspective. *Transplantation* 2014; 97 Suppl 8: S3 [PMID: 24849827 DOI: 10.1097/TP.0000000000000060].

97. Perez NE, Siddiqui FA, Mutchnick MG, Dhar R, Tobi M, Ullah N, Saksouk FA, Wheeler DE, Ehrinpreis MN. Ultra- sound diagnosis of fatty liver in patients with chronic liver disease: a retrospective observational study. *J Clin Gastroenterol* 2007; 41: 624-629 [PMID: 17577120 DOI: 10.1097/01. mcg.0000225680.45088.01].

98. Hepburn MJ, Vos JA, Fillman EP, Lawitz EJ. The accuracy of the report of hepatic steatosis on ultrasonography in patients infected with hepatitis C in a clinical setting: a retrospective observational study. *BMC Gastroenterol* 2005; 5: 14 [PMID: 15829009 DOI: 10.1186/1471-230X-5-14].

99. Mathiesen UL, Franzén LE, Aselius H, Resjö M, Jacobsson L, Foberg U, Frydén A, Bodemar G. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis* 2002; 34: 516-522 [PMID: 12236486].

100. Mishra P**,** Younossi ZM. Abdominal ultrasound for diagnosis of nonalcoholic fatty liver disease (NAFLD). *Am J Gastroenterol* 2007; 102: 2716-2717 [PMID: 18042105 DOI: 10.1111/ j.1572-0241.2007.01520.x].

101. Kuk JL, *et al*. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity (Silver Spring)* 2006; 14(2): p. 336-41.

Figures

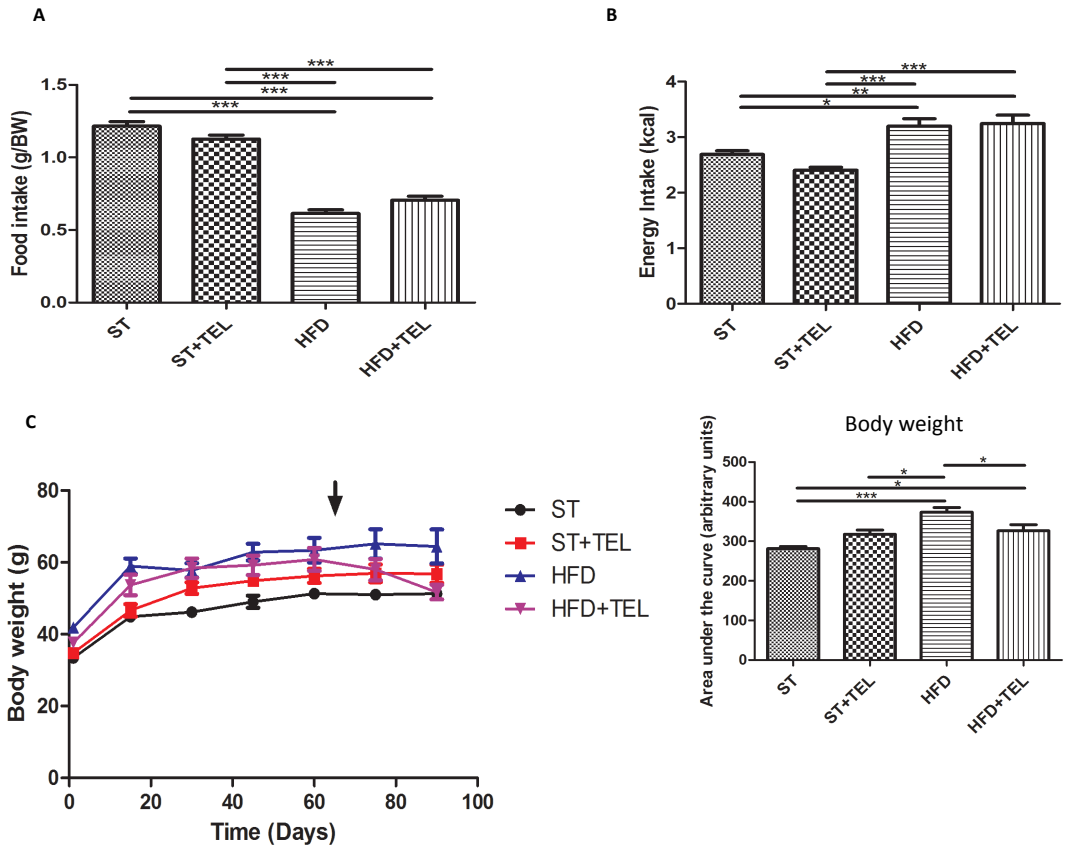

Figure 1. Food intake, energy intake and body weight in mice treated with Telmisartan and fed with standard and high fat diet (ST, ST+TEL, HFD, HFD+TEL). Food intake (A), energy intake (B) and body weight (C). Data are presented as mean ± SEM; * $p<0.05$, ** $p<0,01$; *** $p<0,001$ versus group indicated.

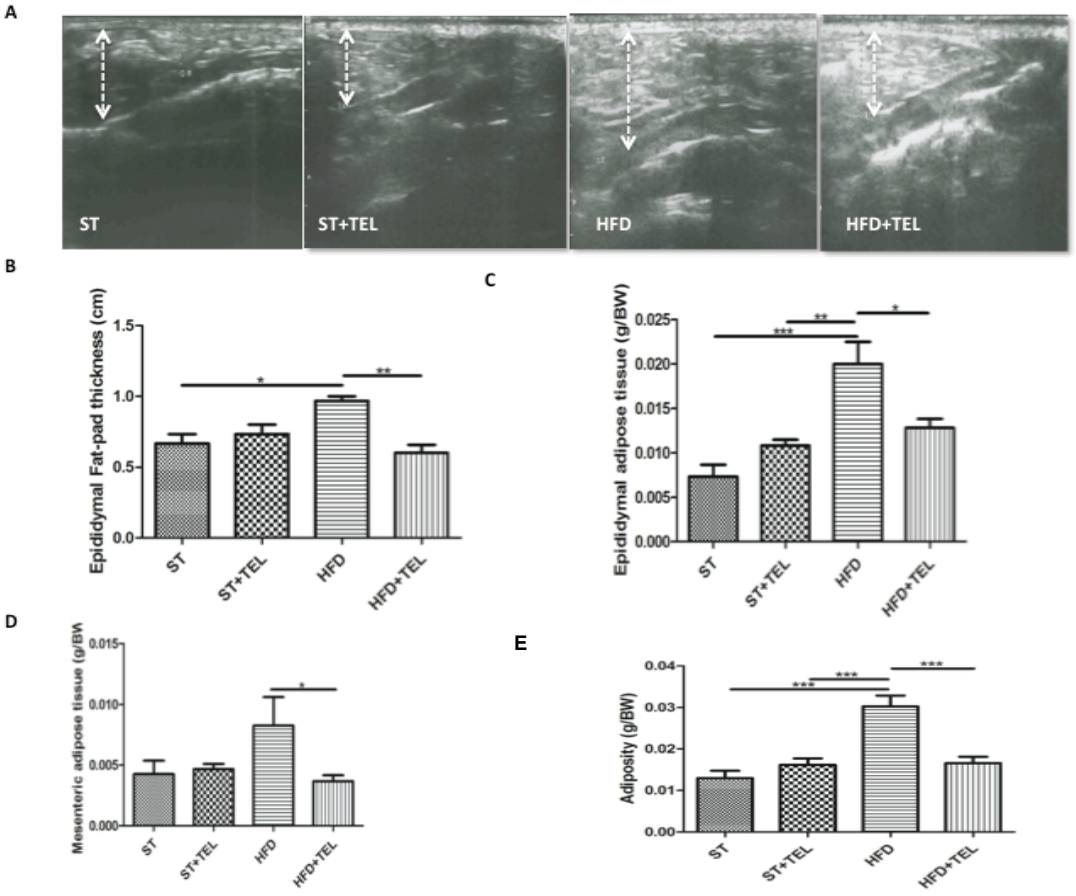

Figure 2. Adiposity, epididymal and mesenteric adipose tissues in mice treated with Telmisartan and fed with standard and high fat diet (ST, ST+TEL, HFD, HFD+TEL). Epididymal US images (A), epididymal fat pad thickness acessed by US (B), epididymal adipose tissue weight (C), mesenteric adipose tissue weight (D) and adipisity (E). Arrows in A indicate the epididymal fat pad. Data are presented as mean ± SEM; * $p<0.05$, ** $p<0,01$; *** $p<0,001$ versus group indicated.

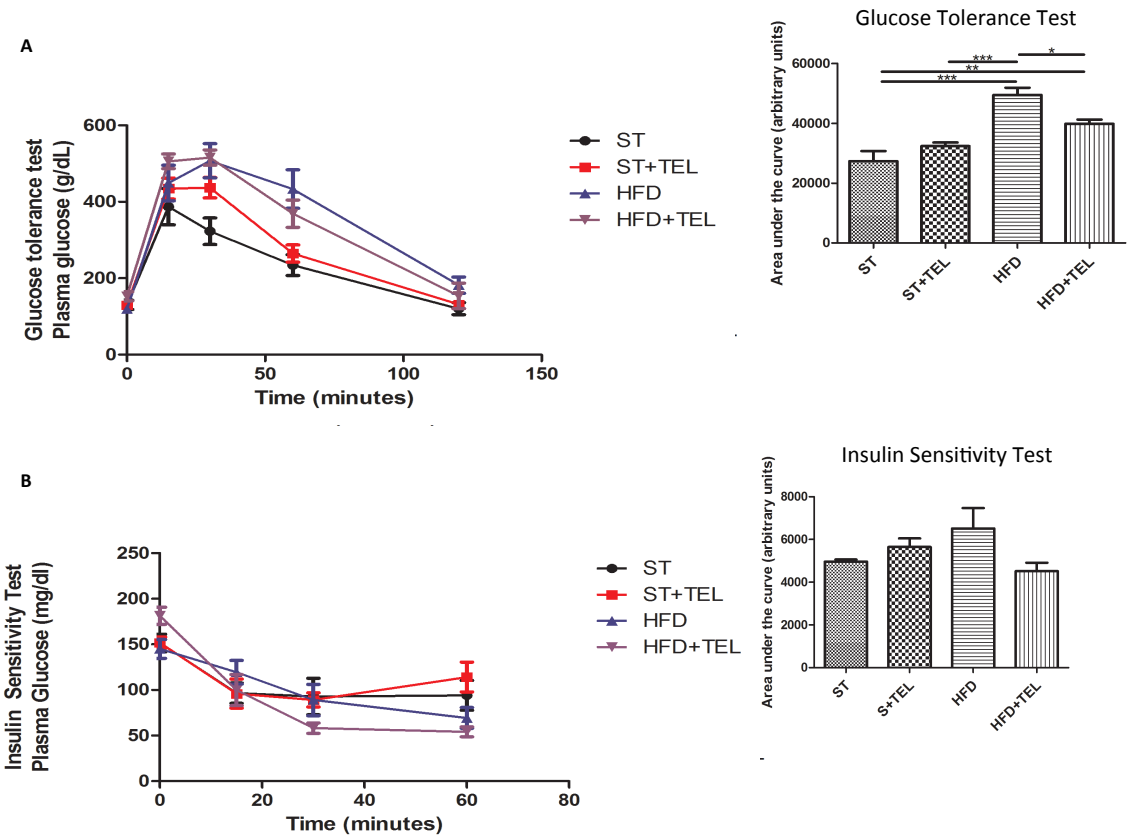

Figure 3. Insulin sensitivity and glucose tolerance tests in mice treated with Telmisartan and fed with standard and high fat diet (ST, ST+TEL, HFD, HFD+TEL). Glucose tolerance test (A) and Insulin sensitivity test (B). Data are presented as mean ± SEM; * $p<0.05$, ** $p<0,01$, *** $p<0,001$ versus group indicated.

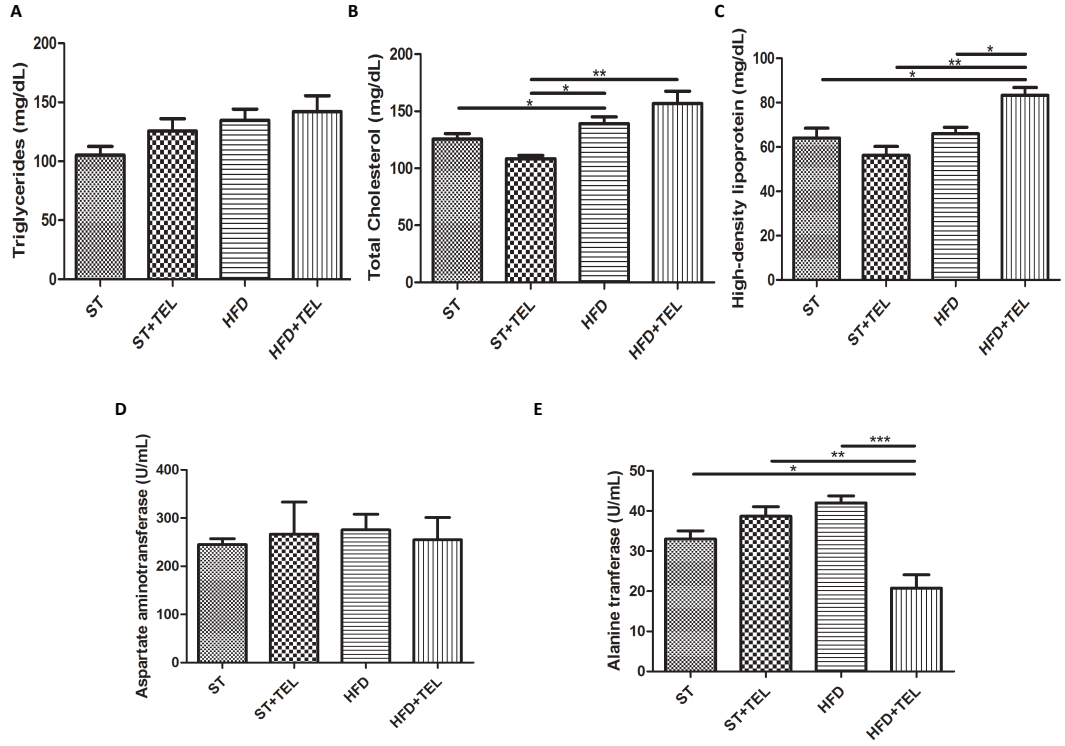

Figure 4 – Lipid parameters and hepatics enzims in mice treated with Telmisartan and fed with standard and high fat diet (ST, ST+TEL, HFD, HFD+TEL). Triglycerides (A), Total Cholesterol (B), High-density lipoprotein (C), Aspartate aminotransferase (D) and Alanine transferase (E). Data are presented as mean ± SEM; * $p<0.05$, ** $p<0,01$, *** $p<0,001$ versus group indicated.

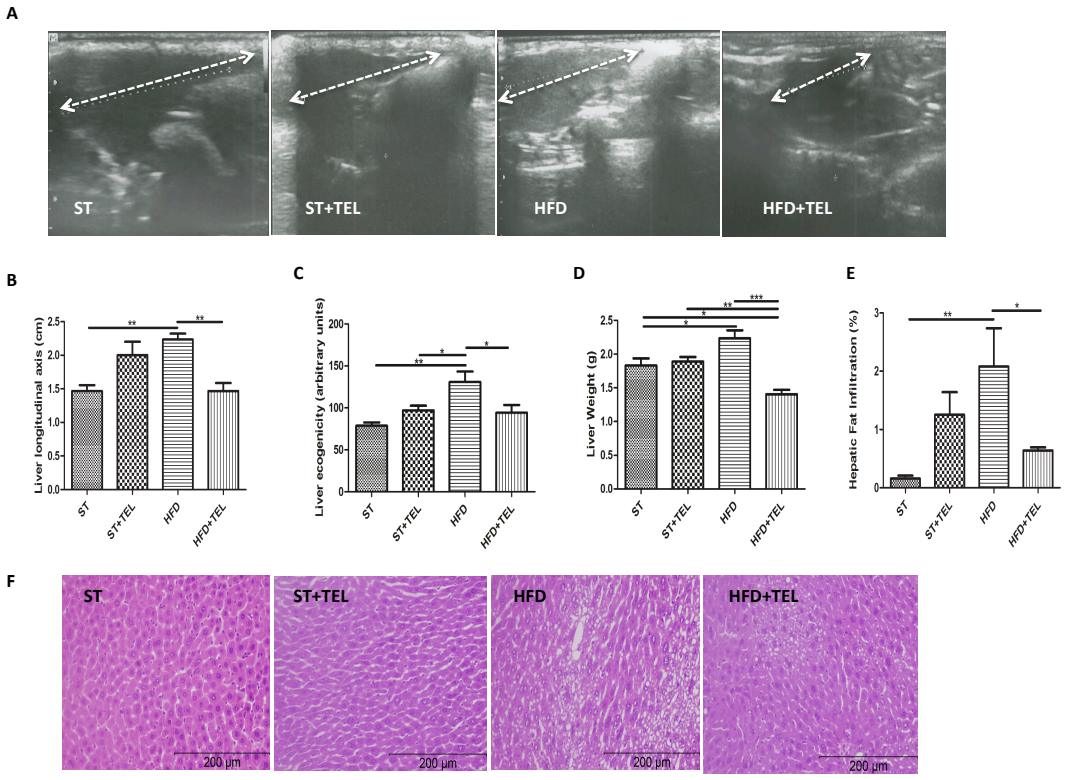

Figure 5. Liver ultrassonography (US), weight and hematoxylin/eosin staining in mice treated with Telmisartan and fed with standard and high fat diet (ST, ST+TEL, HFD, HFD+TEL). Liver US images (A), longitudinal axis measured by US (B), ecogenicity (C), weight (D), fat infiltration (E) and hematoxylin/eosin staining (F). Arrows in A indicate the liver. Data are presented as mean ± SEM; * $p<0.05$, ** $p<0,01$; *** $p<0,001$ versus group indicated.

5 CONSIDERAÇÕES FINAIS

Os resultados do presente estudo mostraram que o tratamento oral com o BRA Telmisartan reduziu a infiltração gordurosa no fígado de camundongos alimentados com dieta hiperlipídica e melhorou parâmetros metabólicos relacionados ao metabolismo de carboidratos e de gorduras. Ainda, demonstramos que a US é um método de imagem não invasivo que pode ser utilizado *in vivo* para detecção de alterações morfológicas no fígado de modelos murinos de DGHNA, com detecção inclusive de melhora de parâmetros ecográficos após o tratamento medicamentoso. Da mesma maneira, a US pôde estimar o aumento do depósito de gordura epididimal nos camundongos alimentados com dieta hiperlipídica, e a redução desse parâmetro após o tratamento.

Dentre as principais limitações deste estudo destacamos que nem sempre os dados de estudos metabólicos podem ser extrapolados entre espécies, limitação intrínseca a estudos experimentais em animais. As razões podem incluir uma fisiopatologia espécie-dependente de determinadas condições metabólicas, ou mesmo um resposta diferente ao tratamento medicamentoso proposto em algumas espécies [28]. Exemplificando esta situação, é sabido que coelhos podem ser mais sensíveis aos efeitos hipotensivos dos BRAs que outras espécies, uma vez que doses tipicamente usadas em ratos podem causar hipotensão profunda ou até mesmo a morte nestes animais [120]. Por outro lado, algumas espécies podem ser menos responsivas a alguns BRAs uma vez que os receptors AT1 têm menor afinidade por essas moléculas, como é o caso do uso de BRAs como losartan ou valsartan em cachorros [121].

Ainda como limitação do presente estudo destaca-se o fato de os exames ultrasonográficos terem sido realizados em aparelhos não dedicados ao uso veterinário, com transdutores lineares próprios para uso em humanos, e portanto com frequências de pulsação abaixo das normalmente utilizadas para estudos experimentais em animais de pequeno porte [56,57], o que reduz a resolução especial e a nitidez da imagem.

A capacidade de medir com precisão características morfológicas do fígado e do tecido adiposo em roedores através de métodos não invasivos e em tempo real como a US é de enorme valor, uma vez que pode substituir de forma confiável o diagnóstico histológico em modelos de DGHNA em várias circunstâncias, contribuindo para o refinamento da técnica experimental, permitindo inclusive a redução do número de animais em pesquisas. Além disso, fornece uma ferramenta não invasiva para o acompanhamento a longo prazo da doença

e para o acompanhamento do tratamento nos diversos modelos experimentais, geralmente necessários em fases pré-clínicas de testes terapêuticos. Ressalta-se, no entanto, a limitação da US na distinção entre esteatose simples, esteatohepatite e fibrose e na detecção de pequenas alterações no grau de infiltração gordurosa do fígado, situações nas quais a análise histológica é necessária.

Esses resultados reforçam o potencial do Telmisartan no tratamento da DGHNA e estimulam o uso da US em modelos murinos experimentais de tratamento de esteatose hepática como ferramenta não invasiva para diagnóstico e acompanhamento longitudinal dos animais em detrimento de técnicas mais invasivas.

REFERÊNCIAS

1. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome- a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006;23:469–80.

2. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365:1415–28.

3. Enjoji M, Kotoh K, Kato M, Higuchi N, Kohjima M, Nakashima M, Nakamuta M. Therapeutic effect of ARBs on insulin resistance and liver injury in patients with NAFLD and chronic hepatitis C: A pilot study. *Int J Mol Medicine* 2008;22: 521-527.

4. Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol.* 2015; 62(1 Suppl):S47–S64.

5. Duseja A, Singhy S, Saraswatz VA, Acharyax SK, Chawla YK, Chowdhuryk S, Dhiman RK, Jayakumar RV, *et al*. Non-alcoholic Fatty Liver Disease and Metabolic Syndrome—Position Paper of the Indian National Association for the Study of the Liver, Endocrine Society of India, Indian College of Cardiology and Indian Society of Gastroenterology. *J Clin Exp Hepatol* 2015;5:51–68.

6. Koplay M, Sivri M, Erdogan H, Nayman A. Importance of imaging and recent developments in diagnosis of nonalcoholic fatty liver disease. *World J Hepatol* 2015;7(5): 769-776 ISSN 1948-5182 (online). DOI: 10.4254/wjh.v7.i5.769.

7. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005; 288: E462-E468 [PMID: 15339742 DOI: 10.1152/ajpendo.00064.2004].

8. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; 34: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x].

9. Chalasani N, Younossi Z, Lavine JE et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. *Hepatology* 2012;55:6:2005–2023.

10. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a metaanalysis. *Hepatology* 2011; 54: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452].

11. Schwimmer JB, Dunn W, Norman GJ, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. *Gastroenterology* 2010; 138:1357–1364. [PubMed: 20064512].

12. Rehm JL, Wolfgram PM, Hernando D, Eickhoff JC, Allen DB, Reeder SB. Proton density

fat- fraction is an accurate biomarker of hepatic steatosis in adolescent girls and young women. *Eur Radiol* 2015; 25:2921–2930. [PubMed: 25916386].

13. Kuroda H, Kakisaka K, Kamiyama N, Oikawa T, Onodera M, Sawara K, Oikawa K, Endo R, *et al.* Non-invasive determination of hepatic steatosis by acoustic structure quantification from ultrasound echo amplitude. *World J Gastroenterol* 2012;18(29):3889-3895. ISSN 1007-9327 (print) ISSN 2219-2840.

14. Ho MC, Lee YH, Jeng YM, Chen CN, Chang KJ, Tsui PH. Relationship between Ultrasound Backscattered Statistics and the Concentration of Fatty Droplets in Livers: An Animal Study. *PLoS One* 2013;8(5):e63543. Published online 2013 May 21. doi: 10.1371/journal.pone.0063543.

15. Khov N, Sharma A, Riley T. Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014;20(22):6821-6825. DOI:10.3748/wjg.v20.i22.6821.

16. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; 20(23): 7392-7402. Available from: URL: http://www.wjgnet.com/1007-9327/full/ v20/i23/7392.htm DOI: http://dx.doi.org/10.3748/wjg.v20. i23.7392.

17. Kramer H, Pickhardt PJ, Kliewer MA, Hernando D, Chen GH, Zagzebski JA, Reeder SB. Accuracy of Liver Fat Quantification With Advanced CT, MRI, and Ultrasound Techniques: Prospective Comparison With MR Spectroscopy. *Am J Roentgenol* 2017;208(1): 92–100. doi:10.2214/AJR.16.16565.

18. De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev* 2000; 52: 415-472.

19. Alam S, Kabir J, Mustafa G, Gupta U, Hasan S, Alam A. Effect of telmisartan on histological activity and fibrosis of non-alcoholic steatohepatitis: A 1-year randomized control trial. *Saudi J Gastroenterol* 2016;22:69-76.

20. Prasad A, Quyyumi AA. Renin–angiotensin system and angiotensin receptor blockers in the metabolic syndrome. *Circulation* 2004;110:1507–12.

21. Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H *et al*. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008;358: 1547–1559.

22. Halici Z, Bilen H, Albayrak F, Uyanik A, Cetinkaya R, Suleyman H, *et al.* Does telmisartan prevent hepatic fibrosis in rats with alloxan-induced diabetes? *Eur J Pharmacol* 2009;614:146–52.

23. Guo J, Friedman SL. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. *Fibrog Tissue Repair* 2010;3:21.

24. Rong X, Li Y, Ebihara K, Zhao M, Naowaboot J, Kusakabe T, *et al*. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. *Diabetologia* 2010;53, 1727–1731.

25. Guimaraes GG, Santos SH, Oliveira ML, Pimenta-Velloso EP, Motta DF, Martins AS, et al. Exercise induces renin-angiotensin system unbalance and high collagen expression in the heart of Mas-deficient mice. *Peptides* 2012;38:54–61.

26. Clemenz M, Frost N, Schupp NM et al.Liver-specific peroxisome proliferator-activated receptor α target gene regulation by the angiotensin type 1 receptor blocker telmisartan. *Diabetes* 2008;57:5:1405–1413.

27. Kudo H, Yata Y, Takahara T, Kawai K, Nakayama Y, Kanayama M, *et al*. Telmisartan attenuates progression of steatohepatitis in mice: Role of hepatic macrophage infiltration and effects on adipose tissues. *Liver Int* 2009;29, 988–996.

28. Michel M, Brunner HR, Foster C, Huo Y. Angiotensin II type 1 receptor antagonists in animal models of vascular, cardiac, metabolic and renal disease. *Pharmacology & Therapeutics* 2016;164:1–81.

29. Law K, Brunt EM. Nonalcoholic fatty liver disease. *Clinics in Liver Disease* 2010;14:4:591–604.

30. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle- aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; 140: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038].

31. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; 43: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973].

32. McCullough AJ. Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; 40 Suppl 1: S17-S29 [PMID: 16540762 DOI: 10.1097/01.mcg.0000168645.86658.22].

33. Milic S, Mikolasevic I, Krznaric-Zrnic I, Stanic M, Poropat G, Stimac D, Vlahovic-Palcevski V, Orlic L. Nonalcoholic steatohepatitis: emerging targeted therapies to optimize treatment options. *Drug Design, Development and Therapy* 2015:9 4835–4845.

34. Gallagher EJ, Leroith D, Karnieli E. Insulin resistance in obesity as the underlying cause for the metabolic syndrome. *Mt Sinai J Med* 2010;77:511–23.

35. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; 120: 1183-1192 [PMID: 11266382 DOI: 10.1053/gast.2001.23256].

36. Baran B, Akyüz F. Non-alcoholic fatty liver disease: What has changed in the treatment since the beginning? *World J Gastroenterol* 2014;20(39):14219-14229. DOI: 10.3748/wjg.v20.i39.14219.

37. Chaldakov GN, Stankulov IS, Hristova M, Ghenev PA. Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Curr Pharm Des* 2003;9:1023–1031.

38. Santos SH, Andrade JM, Fernandes LR, Sinisterra RD, Sousa FB, Feltenberger JD, *et al*. Oral angiotensin-(1–7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-kappaB in rats fed with high-fat diet. *Peptides* 2013;46:47–52.

39. Takahashi Y, Sugimoto K, Inui H, Fukusato T. Current pharmacological therapies for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; 21(13): 3777-3785. DOI: 10.3748/wjg.v21.i13.3777.

40. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; 114: 842-845 [PMID: 9547102].

41. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 2010;52:5:1836–1846, 2010.

42. Baskol G, Baskol M, Kocer D. Oxidative stress and antioxidant defenses in serum of patients with non-alcoholic steatohepatitis. *Clin Biochem* 2007; 40: 776-780 [PMID: 17477913 DOI: 10.1016/j.clinbiochem.2007.02.006].

43. Nelson JE, Wilson L, Brunt EM, Yeh MM, Kleiner DE, Unalp-Arida A, Kowdley KV. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology* 2011; 53: 448-457 [PMID: 21274866 DOI: 10.1002/hep.24038].

44. Gitto G, Vitale E, Villa P, Andreone. Treatment of Nonalcoholic Steatohepatitis in Adults: Present and Future. *Gastroenterology Research and Practice* 2015;2015, 14 pages. Article ID 732870, doi:10.1155/2015/732870.

45. Bravo A A**,** Sheth S G, Chopra S. Liver biopsy. *N Engl J Med* 2001; 344**:** 495-500.

46. Chen CH**,** Lin ST, Yang CC, Yeh YH, Kuo CL, Nien CK. The accuracy of sonography in predicting steatosis and fibrosis in chronic hepatitis C. *Dig Dis Sci* 2008; 53: 1699-1706 [PMID: 17939048 DOI: 10.1007/s10620-007-0048-2].

47. Baršić N**,** Lerotić I, Smirčić-Duvnjak L, Tomašić V, Duvnjak M. Overview and developments in noninvasive diagnosis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; 18: 3945-3954 [PMID: 22912545 DOI: 10.3748/wjg.v18. i30.3945].

48. Cheung CL, Lam KSL, Wong ICK, Cheung BMY. Non-invasive score identifies ultrasonography- diagnosed non-alcoholic fatty liver disease and predicts mortality in the USA. *BMC Medicine* 2014, 12:154 http://www.biomedcentral.com/1741-7015/12/154.

49. Polyzos SA, Mantzoros CS. Necessity for timely noninvasive diagnosis of nonalcoholic fatty liver disease. *Metabolism* 2014;63:161-167 [PMID: 24290839 DOI: 10.1016/j.metabol.2013.10.010].

50. Wai CT, Tan LH, Kaur M, Da Costa M, Quak SH, Tan KC. Pitfalls in interpreting liver biopsy results: the story of the blind men and the elephant. *Liver Transpl* 2002; 8: 1200-1201 [PMID: 12474163 DOI: 10.1053/jlts.2002.36843].

51. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F,

et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; 128: 1898-1906 [PMID: 15940625 DOI: 10.1053/j.gastro.2005.03.084].

52. Tsuchiya H, Ebata Y, Sakabe T, Hama S, Kogure K, Shiota G. High-fat, high-fructose diet induces hepatic iron overload via a hepcidin-independent mechanism prior to the onset of liver steatosis and insulin resistance in mice. *Metabolism* 2013; 62: 62-69 [PMID: 22854109 DOI: 10.1016/j.metabol.2012.06.008].

53. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, *et al*. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; 123: 745-750.

54. Mehta SR, Thomas EL, Patel N, et al. Proton magnetic resonance spectroscopy and ultrasound for hepatic fat quantification. *Hepatol Res* 2010; 40:399–406. [PubMed: 20236356].

55. Heger M, Marsman HA, Bezemer R, Cloos MA, van Golen RF, van Gulik TM. Non-invasive quantification of triglyceride content in steatotic rat livers by (1)H-MRS: when water meets (too much) fat. *Acad Radiol* 2011; 18:1582–1592. [PubMed: 22055799].

56. Lessa AS, Paredes BD, Dias JV, Carvalho AB, Quintanilha LF, Takiya CM, Tura BR, Rezende GFM, *et al*. Ultrasound imaging in an experimental model of fatty liver disease and cirrhosis in rats. *BMC Veterinary Research* 2010,6:6 http://www.biomedcentral.com/1746-6148/6/6.

57. Fernández-Domínguez I, Echevarria-Uraga JJ, Gómez N, Luka Z, Wagner C, Lu SC, Mato JM, Martínez-Chantar ML, *et al*. High-frequency ultrasound imaging for longitudinal evaluation of non-alcoholic fatty liver disease progression in mice. *Ultrasound Med Biol* 2011; 37(7): 1161–1169. doi:10.1016/j.ultrasmedbio.2011.04.012.

58. Chen CL**,** Cheng YF, Yu CY, Ou HY, Tsang LL, Huang TL, Chen TY, Concejero A, *et al.* Living donor liver transplantation: the Asian perspective. *Transplantation* 2014; 97 Suppl 8: S3 [PMID: 24849827 DOI: 10.1097/TP.0000000000000060].

59. Lee SS, Park SH, Kim HJ, Kim SY, Kim MY, Kim DY, Suh DJ, Kim KM, *et al*. Non-invasive assessment of hepatic steatosis: prospective comparison of the accuracy of imaging examinations. *J Hepatol* 2010;52:579-585 [PMID: 20185194 DOI: 10.1016/j.jhep.2010.01.008].

60. Singh D, Das CJ, Baruah MP. Imaging of non alcoholic fatty liver disease: A road less travelled. *Indian J Endocrinol Metab* 2013; 17: 990-995 [PMID: 24381873 DOI: 10.4103/2230-8210.122606].

61. Hepburn MJ, Vos JA, Fillman EP, Lawitz EJ. The accuracy of the report of hepatic steatosis on ultrasonography in pa- tients infected with hepatitis C in a clinical setting: a retrospective observational study. *BMC Gastroenterol* 2005; 5: 14 [PMID: 15829009 DOI: 10.1186/1471-230X-5-14].

62. Mathiesen UL, Franzén LE, Aselius H, Resjö M, Jacobsson L, Foberg U, Frydén A, Bodemar G. Increased liver echogenicity at ultrasound examination reflects degree of

steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. *Dig Liver Dis* 2002; 34: 516-522 [PMID: 12236486].

63. Mishra P**,** Younossi ZM. Abdominal ultrasound for diagno- sis of nonalcoholic fatty liver disease (NAFLD). *Am J Gastroenterol* 2007; 102**:** 2716-2717 [PMID: 18042105 DOI: 10.1111/ j.1572-0241.2007.01520.x].

64. Charatcharoenwitthaya P, Lindor KD. Role of radiologic modalities in the management of non-alcoholic steatohepatitis. *Clin Liver Dis* 2007; 11**:** 37-54, viii [PMID: 17544971 DOI: 10.1016/j.cld.2007.02.014].

65. Mai W, Barraud L, Lefrancois L, Scoazec JY, Guerret S, Vray D, Merle P, Vitvitski-Trepo L, Trepo C, Janier MF. Ultrasound detection of spontaneous hepato-cellular carcinomas in X/myc bitransgenic mice. *Liver Int* 2004; 24:651–657. [PubMed: 15566518].

66. Schmitz V, Tirado-Ledo L, Tiemann K, Raskopf E, Heinicke T, Ziske C, Gonzalez-Carmona MA, Rabe C, *et al*. Establishment of an orthotopic tumour model for hepatocellular carcinoma and non-invasive in vivo tumour imaging by high resolution ultrasound in mice. *J Hepatol* 2004; 40:787–791. [PubMed: 15094226].

67. Graham KC, Wirtzfeld LA, MacKenzie LT, Postenka CO, Groom AC, MacDonald IC, Fenster A, Lacefield JC, *et al*. Three-dimensional high-frequency ultrasound imaging for longitudinal evaluation of liver metastases in preclinical models. *Cancer Res* 2005; 65:5231–5237. [PubMed: 15958568].

68. Layer G, Zuna I, Lorenz A, Zerban H, Haberkorn U, Bannasch P, Van Kaick G, Räth U: Computerized ultrasound B-scan texture analysis of experimental diffuse parenchymal liver disease: correlation with histopathology and tissue composition. *J Clin Ultrasound* 1991, 19:193-201.

69. Matsuhashi T, Yamada N, Shinzawa H, Takahashi T: An evaluation of hepatic ultrasound speed in injury models in rats: correlation with tissue constituents. *J Ultrasound Med* 1996, 15:563-570.

70. Liao AH, Ma WC, Wu MF. Evaluation of ultrasound combined with chitosan for the control of weight and local fat in mice. *Ultrasound in Med. & Biol* 2013;39:10:1794–1803. http://dx.doi.org/10.1016/j.ultrasmedbio.2013.04.025.

71. Kuk JL, *et al*. Visceral fat is an independent predictor of all-cause mortality in men. *Obesity (Silver Spring)* 2006;14(2): 336-41.

72. Paschos P, Tziomalos K. Nonalcoholic fatty liver disease and the renin-angiotensin system: Implications for treatment. *World J Hepatol* 2012; 4: 327-331 [PMID: 23355909 DOI: 10.4254/wjh.v4.i12.327]

73. Pereira RM, *et al*. Renin-angiotensin system in the pathogenesis of liver fibrosis. *World J Gastroenterol* 2009;15(21): 2579-86.

74. Santos RA, Ferreira AJ. Angiotensin-(1-7) and the renin- angiotensin system. *Curr Opin Nephrol Hypertens* 2007; 16: 122-8.

75. Santos SH, Simoes e Silva AC. The therapeutic role of Renin-Angiotensin System blockers in obesity-related renal disorders. *Curr Clin Pharmacol* 2014;9:2–9.

76. Giacchetti G, Sechi LA, Rilli S, Carey RM. The renin-angiotensin- aldosterone system, glucose metabolism and diabetes. *Trends Endocrinol Metab* 2005;16:120–126.

77. Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. *J Hypertens* 2004;22(12): p. 2253-61.

78. Kuba K, Imai Y, Ohto-Nakanishi T, *et al*. Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. *Pharmacol Ther* 2010;128(1):119–28.

79. Tamura K, Tanaka Y, Tsurumi Y, *et al.* The role of angiotensin AT1 receptor-associated protein in renin-angiotensin system regulation and function. *Curr Hypertens Rep* 2007; 9: 121-7.

80. Iwai M, Horiuchi M. Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2- angiotensin-(1-7)-Mas receptor axis. *Hypertens Res* 2009; 32: 533-6.

81. Putnam K, Shoemaker R, Yiannikouris F, Cassis LA. The renin–angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome. *Am J Physiol Heart Circ Physiol* 2012;302:H1219–30.

82. Herichova I, Szantoova K. Renin–angiotensin system: upgrade of recent knowledge and perspectives. *Endocr Regul* 2013;47:39–52.

83. Santos SH, Fernandes LR, Mario EG, Ferreira AV, Porto LC, Alvarez-Leite JI, *et al*. Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. *Diabetes* 2008;57:340–7.

84. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, *et al*. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000;87:E1–9.

85. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, *et al.* TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007;13:1324–32.

86. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, *et al*. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci USA* 2003; 100: 8258-8263.

87. Ferreira AJ, Santos RAS.Cardiovascularactionsofangiotensin-(1-7). *Braz J Med Biol Res* 2005;38:499–507.

88. Fyhrquist F, Saijonmaa O. Renin-angiotensin system revisited. *J Intern Med* 2008; 264: 224-236.

89. Santos SH, Mario BJ, Pôrto EG, Rodrigues-Machado Mda LC, Murari G, Botion A, *et al*. Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1-7). *Arterioscler Thromb Vasc Biol* 2012;30: 953–61.

90. Mario EG SS, Ferreira AV, Bader M, Santos RA, Botion LM. Angiotensin-(1-7) Mas-receptor deficiency decreases peroxisome proliferator-activated receptor gamma expression in adipocytes. *Peptides* 2012;33:174–7.

91. Wei HS, Li DG, Lu HM, Zhan YT, Wang ZR, Huang X, et al. Effects of AT1 receptor antagonist, losartan, on rat hepatic fibrosis induced by CCl(4). *World J Gastroenterol* 2000;6:540–5.

92. Seki E, Park E, Fujimoto J. Toll-like receptor signaling in liver regeneration, fibrosis and carcinogenesis. *Hepatol Res* 2011;41:597–610.

93. Silva AR, Aguilar EC, Alvarez-Leite JI, da Silva RF, Arantes RM, Bader M, *et al*. Mas receptor deficiency is associated with worsening of lipid profile and severe hepatic steatosis in ApoE-knockout mice. *Am J Physiol Regul Integr Comp Physiol* 2013;305:R1323–30.

94. Santos SH, Braga JF, Mario EG, Porto LC, Rodrigues-Machado Mda G, Murari A, *et al.* Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1–7). *Arterioscler Thromb Vasc Biol* 2010;30:953–61.

95. Santos SHS, Andrade JMO. Angiotensin 1–7: A peptide for preventing and treating metabolic syndrome. *Peptides* 59 (2014) 34–41.

96. Bilman V, Mares-Guia L, Nadu AP, Bader M, Campagnole-Santos MJ, Santos RA, et al. Decreased hepatic gluconeogenesis in transgenic rats with increased circulating angiotensin-(1-7). *Peptides* 2012;37:247–51.

97. de Kloet AD, Krause EG, Woods SC. The renin angiotensin system and the metabolic syndrome. *Physiol Behav* 2010;100:525–34.

98. Kalupahana NS, Moustaid-Moussa N. The renin–angiotensin system: a link between obesity, inflammation and insulin resistance. *Obes Rev* 2012;13:136–49.

99. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol* 2004;287:R943–9.

100. Cassis LA, Police SB, Yiannikouris F, Thatcher SE. Local adipose tissue renin-angiotensin system. *Curr Hypertens Rep* 2008;10:93–8.

101. Feltenberger JD, Andrade JM, Paraiso A, Barros LO, Filho AB, Sinisterra RD, *et al*. Oral formulation of angiotensin-(1-7) improves lipid metabolism and prevents high-fat diet-induced hepatic steatosis and inflammation in mice. *Hypertension* 2013;62:324–30.

102. Marrero MB, Fulton D, Stepp D, Stern DM. Angiotensin II- induced insulin resistance and protein tyrosine phosphatases. *Arterioscler Thromb Vasc Biol* 2004; 24: 2009-13.

103. Wei Y, Sowers JR, Nistala R, Gong H, Uptergrove GM, Clark SE, Morris EM, Szary N, *et al.* Angiotensin II-induced NADPH oxidase activation impairs insulin signaling in skeletal muscle cells. *J Biol Chem* 2006; 281: 35137-35146 [PMID: 16982630 DOI: 10.1074/jbc.M601320200].

104. Macedo SM, Guimaraes TA, Feltenberger JD, Santos SHS. The role of renin-angiotensin system modulation on treatment and prevention of liver diseases. *Peptides* 62 (2014) 189–196.

105. Oliveira Andrade JM, Paraiso AF, Garcia ZM, Ferreira AV, Sinisterra RD, Sousa FB, et al. Cross talk between angiotensin-(1–7)/Mas axis and sirtuins in adipose tissue and metabolism of high-fat feed mice. *Peptides* 2014;55:158–65.

106. Kyvelou SM, *et al*. Effects of antihypertensive treatment with angiotensin II receptor blockers on lipid profile: an open multi-drug comparison trial. *Hellenic J Cardiol* 2006; 47(1): p. 21-8.

107. Campbell DJ. The renin-angiotensin and the kallikrein-kinin systems. *Int J Biochem Cell Biol* 2003; 35(6): p. 784-91.

108. Simoes e Silva AC, *et al*. Circulating renin Angiotensin system in childhood chronic renal failure: marked increase of Angiotensin-(1-7) in end-stage renal disease. *Pediatr Res* 2006;60:6:734-739.

109. Meune C, Wahbi K, Duboc D, Weber S. Meta-analysis of renin–angiotensin- aldosterone blockade for heart failure in presence of preserved left ventricular function. *J Cardiovasc Pharmacol Ther* 2011;16:368–75.

110. Santos RA, Ferreira AJ, Nadu AP, Braga AN, de Almeida AP, Campagnole- Santos MJ, *et al*. Expression of an angiotensin-(1–7)-producing fusion protein produces cardioprotective effects in rats. *Physiol Genomics* 2004;17:292–9.

111. Carey RM, Padia SH. Angiotensin AT2 receptors: control of renal sodium excretion and blood pressure. *Trends Endocrinol Metab* 2008; 19: 84-87.

112. Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M *et al*. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004;43: 993–1002.

113. Sugimoto K, Qi NR, Kazdova L, Pravenec M, Ogihara T, Kurtz TW. Telmisartan but not valsartan increases caloric expenditure and protects against weight gain and hepatic steatosis. *Hypertension* 2006;47: 1003–1009.

114. Souza-Mello V, Gregório BM, Cardoso-de-Lemos FS, Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin

alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. *Clinical Science* 2010;119**:**239–250 doi:10.1042/CS20100061.

115. Miesel A, Müller-Fielitz H, Jöhren O, Vogt FM, Raasch W. Double blockade of angiotensin II (AT1)-receptors and ACE does not improve weight gain and glucose homeostasis better than single-drug treatments In obese rats. *British Journal of Pharmacology* 2012;165 2721–2735.

116. Yi E, Liu R, Wen Y, Yin C. Telmisartan attenuates hepatic fibrosis in bile duct- ligated rats. *Acta Pharmacol Sin* 2012;33:1518–1524.

117. Cynis H, Kehlen A, Haegele M, Hoffmann T, Heiser U, Masato F, *et al*. Inhibi- tion of glutaminyl cyclases alleviates CCl2-mediated inflammation of non-alcoholic fatty liver disease in mice. *Int J Exp Pathol* 2013;94, 217–225.

118. Nakagami H, Osako MK, Nakagami F, Shimosato T, Minobe N, Moritani T, *et al*. Prevention and regression of non-alcoholic steatohepatitis (NASH) in a rat model by metabosartan, telmisartan. *Int J Mol Med* 2010;26, 477–481.

119. Rosselli MS, *et al*. Losartan reduces liver expression of plasminogen activator inhibitor-1 (PAI-1) in a high fat-induced rat nonalcoholic fatty liver disease model. *Atherosclerosis* 2009;206:1:119-126.

120. Sato K, Chatani F, Ito K, Kito G. Characterization of the high sensitivity of rabbits to the effects of TCV-116, an angiotensin II receptor antagonist. *Fundam Appl Toxicol* 1997;35, 49–54.

121. de Gasparo M, Whitebread S. Binding of valsartan to mammalian angiotensin AT1 receptors. *Regul Pept* 1995;59, 303–311.

ANEXO

ANEXO A – Parecer do Comitê de Ética e Bem Estar Animal/UNIMONTES

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
COMITÊ DE ÉTICA EM EXPERIMENTAÇÃO E BEM-ESTAR ANIMAL Unimontes

PARECER CONSUBSTANCIADO

Montes Claros, 29 de fevereiro de 2012.

Processo N. °22
Título do Projeto: **Avaliação do Perfil Metabólico e de marcadores associados à inflamação em camundongos da linhagem FVB/N submetidos à dieta hiperlipídica e tratados com inibidores da ciclooxigenase II (COX-2) e Resveratrol**
Orientador: **Prof. Sérgio Henrique Sousa Santos**

Histórico

A síndrome metabólica (SM) é um transtorno complexo representado por um conjunto de fatores de risco cardiovasculares relacionados à deposição central de gordura e à resistência à ação da insulina (5). Entre esses fatores de risco, incluem-se a dislipidemia, a obesidade centrípeta, a alteração na homeostase glicêmica e a hipertensão arterial sistêmica. A prevalência de SM na população em geral é de aproximadamente 24% (6), chegando a mais de 80% entre os pacientes com *Diabetes Mellitus* (DM) tipo 2. A SM é um importante fator de risco de mortalidade precoce em indivíduos não-diabéticos e em pacientes com DM tipo 2. Entretanto, o papel da SM como entidade independente e associada a um maior risco para o desenvolvimento de eventos cardiovasculares tem sido recentemente questionado. A obesidade está associada a uma resposta inflamatória crônica, caracterizada pela produção anormal de adipocinas e a ativação de alguns vias de sinalização pró-inflamatórias, resultando na indução de vários marcadores de inflamatórios. Diversos componentes, como o resveratrol e os inibidores da COX-2 têm apresentado efeitos positivos na resposta inflamatória em modelos de animais obesos, demonstrando-se viáveis na modulação de entidades patológicas envolvidas na síndrome metabólica.

Mérito

Será avaliado o perfil lipídico e glicêmico de camundongos da linhagem FVB/N submetidos à dieta hiperlipídica e tratados com resveratrol e inibidores da COX-2. Os resultados do presente projeto permitirão conhecer o efeito da administração do resveratrol e inibidores da COX-2 em modelos animais de síndrome metabólica, sendo de fundamental importância como testes pré-clínicos de avaliação de efeitos adversos, abrindo a perspectiva de desenvolvimento de novos medicamentos para combater as doenças cardiometabólicas que mais acometem e matam a população mundial.

Parecer

A Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes analisou o processo **022** e entende que o mesmo está completo e dentro das normas da Comissão e das Resoluções do Conselho Nacional de Controle e Experimentação Animal. Sendo assim, somos pela **APROVAÇÃO** do projeto de pesquisa.

Prof. Orlando Raphael Lopasso Júnior
Presidente da Comissão de Ética em Experimentação e Bem-Estar Animal da UNIMONTES