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Losartan reduces liver expression of plasminogen activator inhibitor-1 (PAI-1) in a high fat-induced rat nonalcoholic fatty liver disease model

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ABSTRACT

Objective: To evaluate the effect of losartan-an angiotensin II type 1 receptor (AT1R) antagonist- and telmisartan-an AT1R blocker with insulin-sensitizing properties-, on the hepatic expression of plasminogen activator inhibitor-1 (PAI-1) in a rat model of nonalcoholic fatty liver disease (NAFLD).
Methods: Rats were given a high-fat diet (HFD) for 8 weeks and after this period were randomly divided into 3 groups. For 12 weeks along with the same access to HFD, one group (9 rats) received losartan and another group received telmisartan (10 rats), both at 10 mg/kg intraperitoneally (ip) every 24 h. The third group (8 rats) received saline ip along with the HFD. Finally, a control group (6 rats) was fed with standard chow diet for 20 weeks.
Results: Fatty liver was reverted by both losartan and telmisartan. Both drugs had beneficial effects on insulin resistance, reaching statistical significance in telmisartan group. Expression of hepatic mRNA of PAI-1 showed a 42% decrease in losartan-treated rats in comparison with both HFD group and telmisartan-treated rats. To further evaluate this differential effect on *PAI-1* expression, we explored the effect of the drugs on liver expression of *TNFα*, *PEPCK-C* and *PPARα*, and no significant differences were observed.
Conclusion: These results indicate that AT1R blockers could be eligible drugs for reducing hepatic lipid accumulation in patients with NAFLD. However, only 12 weeks of losartan treatment strongly reduced hepatic PAI-1 gene expression. These differences could provide even more effective options for preventing fatty liver disease and its cardiovascular complications.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) affects approximately 15–25% of the general population worldwide [1]. Data from clinical studies indicate that NAFLD is an independent risk factor for the development of atherosclerosis and cardiovascular disease [2]. In this regard, increased carotid intima-media thickness and carotid plaques are frequently found in NAFLD patients [3]. Recent observations suggest that NAFLD is not merely a marker of cardiovascular disease but may be involved in its pathogenesis [2]. In fact, it is suggested that one potential mechanism by which NAFLD and cardiovascular disease are linked is via the release from the liver of plasminogen activator inhibitor-1 (PAI-1) – an inhibitor of fibrinolysis – along with other proatherogenic factors [4].

Supporting this hypothesis, two animal studies showed an overexpression of *PAI-1* in steatotic liver, suggesting that plasma PAI-1 levels are closely related with the production of PAI-1 in the liver [5]. In agreement with this observation, plasma PAI-1 concentrations are increased in patients with severe clinical forms of NAFLD [6].

Although association does not necessarily mean a causal effect relation, several lines of evidence based on biological plausibility support the potential role of PAI-1 on fatty liver disease. For instance, the *PAI-1* promoter region shows several response elements related with either metabolic or inflammatory pathways (such as tumor necrosis factor α (*TNFα*), transforming growth factor β (*TGFβ*), and very-low-density lipoprotein), as well as glucose and angiotensin II (AngII)-response sites [7]. In addition, recent experimental evidence showed that *TGFβ* and *PAI-1* are up-regulated

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in livers from animals that developed steatohepatitis after being exposed to an atherogenic-high fat diet [8].

Collectively, these results indicate that inhibition of *PAI-1* expression in hepatocytes might be a new potential pharmacological target in NAFLD. Inhibition of PAI-1 by selective antagonists is still under development, however, it has been shown that the blockade of the renin-angiotensin-system significantly reduces PAI-1 production [9].

Interestingly, few studies in animals and also uncontrolled clinical studies in patients with NAFLD [10–14] provided evidence about the potential efficacy of blocking the angiotensin II type 1 receptor (AT1R) in NAFLD. Also, current evidence suggests that the most promising treatments are the peroxisome proliferator activator receptor γ (PPARγ) agonists, acting to decrease hepatic lipid accumulation and attenuating the inflammatory response [15]. Nevertheless, there is no evidence about the effect of AT1R blockers on the hepatic expression of *PAI-1* in NAFLD. In addition, the effect of PPARγ activation on PAI-1 expression is controversial, as several reports indicate that PPARγ agonists increase the expression of PAI-1 in different tissues, such as human endothelial cells [16], adipocytes [17] and human proximal renal tubular cells [18].

Hence, based on the evidence described above and given that information about the effects of PPARγ agonists on liver PAI-1 expression is still uncertain, this study evaluated the effect on the hepatic expression of *PAI-1* of two angiotensin II receptor blockers (ARBs): losartan – a selective ARB- and telmisartan-an ARB considered also to act as a partial agonist of PPARγ – in a rat model of NAFLD. Additionally, we explored the efficacy of these drugs in reverting fatty liver disease and the effect of the two ARBs on metabolic parameters.

2. Material and methods

2.1. Animals

Twelve-week-old male Sprague–Dawley rats weighing 280 ± 20 g were purchased from the Research Animal Facility of School of Veterinary Medicine, University of Buenos Aires. All animals were housed individually with food and water freely available and were maintained at room temperature (23 + 1 °C) under a 12-h light/12-h dark cycle.

All animals received humane care, and the studies were conducted according to the regulations for the use and care of experimental animals. The institutional review board of animal care committee approved the study.

After acclimatization for 1 week, the rats were randomly divided into 2 experimental groups. One group included 6 rats that received standard chow diet (SCD) for 20 weeks, in an amount restricted to that spontaneously consumed at the beginning of the experiment (control group, SCD). The other group, including 27 animals, were allowed *ad libitum* access to high-fat solid diet (HFD) (40%, w/w bovine and porcine fat added to the standard chow as previously described [19]) for 8 weeks, and after this period rats were randomly divided in 3 groups and during the remaining 12 weeks animals were given the same *ad libitum* access to HFD. In addition, one group was given losartan 10 mg/kg intraperitoneally (ip) every 24 h (9 rats, HFD + L), and another group was given telmisartan 10 mg/kg ip every 24 h (10 rats, HFD + T). Losartan and telmisartan were generously donated by Laboratorio Roemmers (Buenos Aires, Argentina) and Laboratorios Bagó (Buenos Aires, Argentina), respectively. The third group (8 rats) received the same amount of vehicle (phosphate-buffered saline, 10 ml/kg) ip (HFD group).

In all the animals, food intake and body weight were monitored daily for the 20-week period. The variation of food intake during the treatment period for each rat was calculated by subtracting the average of 7 days food intake before to the pharmacological intervention from that obtained over the last week of the study.

Systolic arterial blood pressure (SABP) was measured once a week in all the acclimated animals by a plethysmographic tail-cuff method using a tail occlusor connected to a Hg manometer for calibration and a Sthatan transducer whose signal was digitalized by a A/D card inserted in a personal computer as previously described [19]. Each value corresponds to, at least, 3 independent measurements taken in a 5 min period.

At the completion of the study, animals were anesthetized with pentobarbital and sacrificed. Blood from individual rats was collected by cardiac puncture to determine plasma and serum levels of different parameters. Food was withdrawn from 8:00 a.m. to 4:00 p.m. before rats were sacrificed. Liver tissue was excised and weighed, and intraperitoneal and retroperitoneal fat were measured by direct weighting. Both liver and fat weights are expressed as liver/rat length (taken from nose to the tail origin) ratio (g/cm) to avoid the influence of body weight change.

Liver was quickly snap-frozen and stored in −76 °C until gene expression analysis. A portion of each liver was fixed in 10% formalin for histological analysis. Two additional samples of liver tissue (150 mg) were stored at −80 °C for quantifying liver lipids.

2.2. Biochemical measurements and liver PAI-1 level

Serum and sodium EDTA-plasma was obtained by centrifugation and stored at −80 °C until needed. Fasting glucose levels and serum alanine aminotransferase (ALT) were measured by an automatic biochemical analytical system (Architect, Abbott, Buenos Aires, Argentina). Plasma insulin levels were determined using a commercial quantitative ultra sensitive ELISA rat kit according as described in the manufacturer's instruction (CRYSTAL CHEM INC, Downers Grove, IL, USA). Insulin resistance was calculated by the Homeostasis model of assessment (HOMA) index [fasting plasma insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5].

Total PAI-1 levels in liver were measured in duplicate using an enzyme-linked immunoassay specific for rat PAI-1 according to the manufacturer's instructions (Rat PAI-1 Total Antigen Assay, Innovative Research, Novi, MI, USA). The sensitivity of the assay is = 0.032 ng/mL. All samples were tested blind to the experimental groups.

2.3. Histological analysis of liver tissue

Formalin-fixed liver tissue was processed, and 5-μm-thick paraffin sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome for histological analysis. In all the experimental groups, osmium tetroxide stain was also performed to estimate the degree of hepatic steatosis. Steatosis was evaluated blind to the experimental groups on both H&E and osmium-stained sections and was given a score from 0 to 4 as follows according the percentage of lipid-laden hepatocytes: 0, no steatosis; 1, fatty hepatocytes occupying less than 10% of the parenchyma; 2, between 10 and 30%; 3, between 30 and 60% and 4, fatty hepatocytes occupying more than 60% of the parenchyma, according of the scoring system described by Brunt et al. [20]. None of the livers showed either inflammation or fibrosis.

2.4. Measurement of liver triglyceride content

Hepatic lipids were purified as previously described by Cui et al. [21]. Triglyceride determination was performed blind to the experimental groups using an automatic biochemical analytical system (Architect, Abbott, Buenos Aires, Argentina), and results are expressed as micrograms of triglyceride per milligram of liver tissue (μg /mg liver).

2.5. RNA preparation and Real-Time RT-PCR for quantitative assessment of mRNA expression

Total RNA was prepared from rat livers with the use of the modified phenol extraction step method of Chomczynski and Sacchi [22], with an additional DNAse digestion.

For RT-PCR, 3 μg of total RNA was reverse-transcribed using random hexamers and Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Promega, WI, USA).

Real-time was performed for quantitative assessment of mRNA expression on a iCycler thermocycler (BioRad Hercules, CA), using SYBR-Green (Invitrogen, Buenos Aires, Argentina) as a fluorescent dye. Primer sequences, and resulting PCR product lengths are shown in the Supplementary Appendix 1. All the real-time PCR reactions were run in triplicate and all samples were tested blind to the experimental groups. Relative expression of target gene mRNA was normalized to the amount of a housekeeping gene (β actin mRNA) to conduct between group comparisons. A fluorescence threshold cycle value (Ct) was calculated for each sample according to as previously reported [23]. Levels of mRNA were expressed as the ratio of the estimated amount for the target gene relative to β actin mRNA levels using the Ct values for a previously estimated efficiency of 2.

2.6. Statistical analysis

Quantitative data are expressed as mean ± SE. Pair-wise mean differences were evaluated by Tukey's test after ANOVA on untransformed or log transformed variables or the non-parametric Newman–Keuls according to the distribution and homoscedasticity of the variables. For testing steatosis gradation (as a categorical response variable) differences, we used a model with ordinal multinomial distribution and probit as a link function with animal length and adipose tissue as continuous predictor variables. Data were also adjusted for body weight when applicable. Statistical adjustment for multiple comparisons was not performed when the groups were compared regarding metabolic findings as it was shown that multiple testing is not an issue when traits under study are physiologically related (in our study all the phenotypes are strongly related to each other, and each test does not represent an independent opportunity for a type I error) [24]. The same applied to the comparisons regarding gene expressions because they were sequentially planned post hoc.

Statistical significance was defined as $p < 0.05$. We used the Statistica program package, StatSoft (Tulsa, OK, USA) to perform these analyses.

3. Experimental results

3.1. Effects of losartan and telmisartan on biochemical parameters

As shown in Fig. 1, upper left panel and upper right panel, at the end of the experiment, treatment with telmisartan significantly decreased plasma glucose (mg/dL) (160 ± 6) and insulin levels (uU/L) (44 ± 28) in comparison with HFD group (199 ± 6 and 187 ± 30, respectively), and also in comparison with the control SCD group (186 ± 7 and 96 ± 35, respectively) and the HFD + L group (177 ± 6 and 102 ± 28, respectively). In contrast, the HFD + L group showed a significant decrease in plasma glucose levels but not insulin levels in comparison with the HFD group (Fig. 1, upper left panel and upper right panel). HOMA index was significantly lower in telmisartan-treated group (18 ± 15) compared with both the HFD group (96 ± 16) and the HFD + L group (47 ± 15), which was similar to that of the SCD group (45 ± 19) (Fig. 1, lower left panel).

ALT levels (UI/L) in both HFD + L (28 ± 3) and HFD + T (29 ± 2) groups, were lower than the HFD group (39 ± 3) (Fig. 1, Lower right panel).

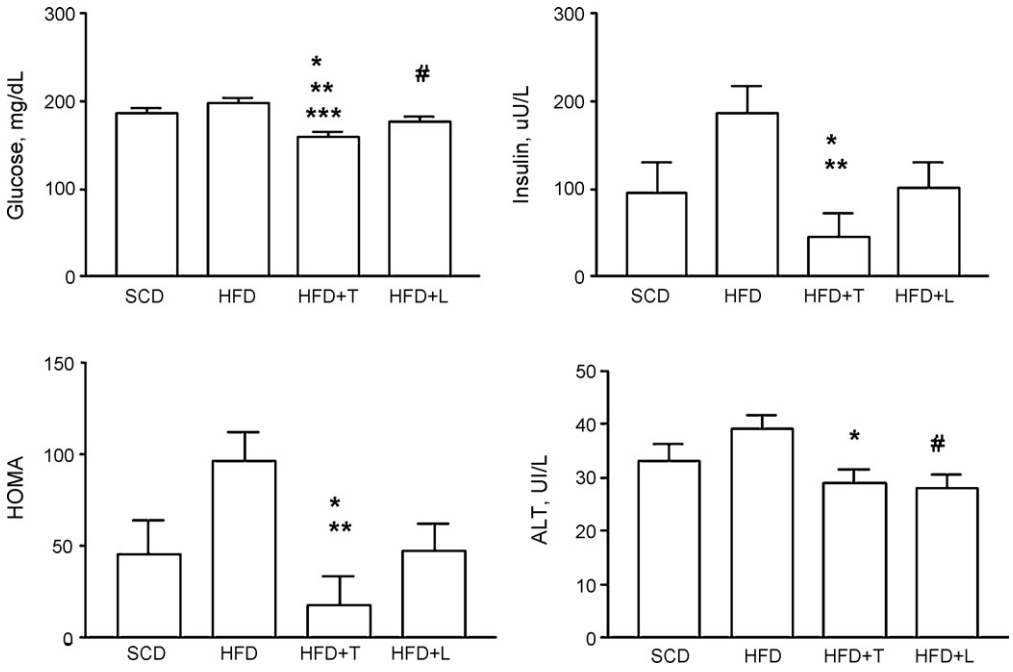

Fig. 1. Effect of angiotensin II receptor blockers on biochemical and metabolic parameters. Bar plots of plasma glucose levels (Upper left panel) ($^{*}p < 0.00004$ vs. HFD, $^{**}p < 0.009$ vs. SCD, $^{***}p < 0.049$ vs. HFD + L, $^{\#}p < 0.013$ vs. HFD), insulin levels (Upper right panel) ($^{*}p < 0.002$ vs. HFD, $^{**}p < 0.04$ vs. HFD + L), HOMA index (Lower left panel) ($^{*}p < 0.0009$ vs. HFD, $^{**}p < 0.03$ vs. HFD + L), and ALT levels (Lower right panel) ($^{*}p < 0.01$ vs. HFD, $^{\#}p < 0.009$ vs. HFD); pair-wise comparisons were determined in each experimental group at the end of the experiment. Data are presented as mean ± SE. SCD: standard chow diet, HFD: high fat diet, HFD + T: high fat diet plus telmisartan, HFD + L: high fat diet plus losartan groups.

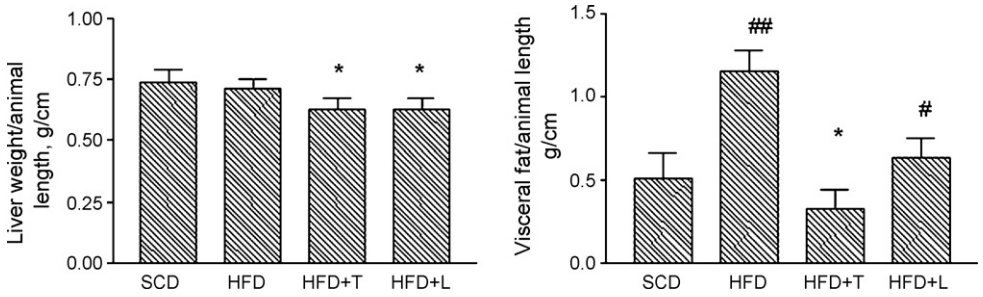

Fig. 2. Bar plots of organ fat accumulation Liver weight at the end of the experiment normalized by animal length (g/cm) (Left panel) $^{*}p < 0.05$ vs. HFD and SCD. Visceral fat tissue at the end of the experiment normalized by animal length (g/cm) (Right panel) $^{*}p < 0.000006$ vs. HFD, $^{\#}p < 0.03$ vs. HFD, $^{\#\#}p < 0.01$ vs. SCD. Data are presented as mean ± SE. SCD: standard chow diet, HFD: high fat diet, HFD + T: high fat diet plus telmisartan, HFD + L: high fat diet plus losartan groups.

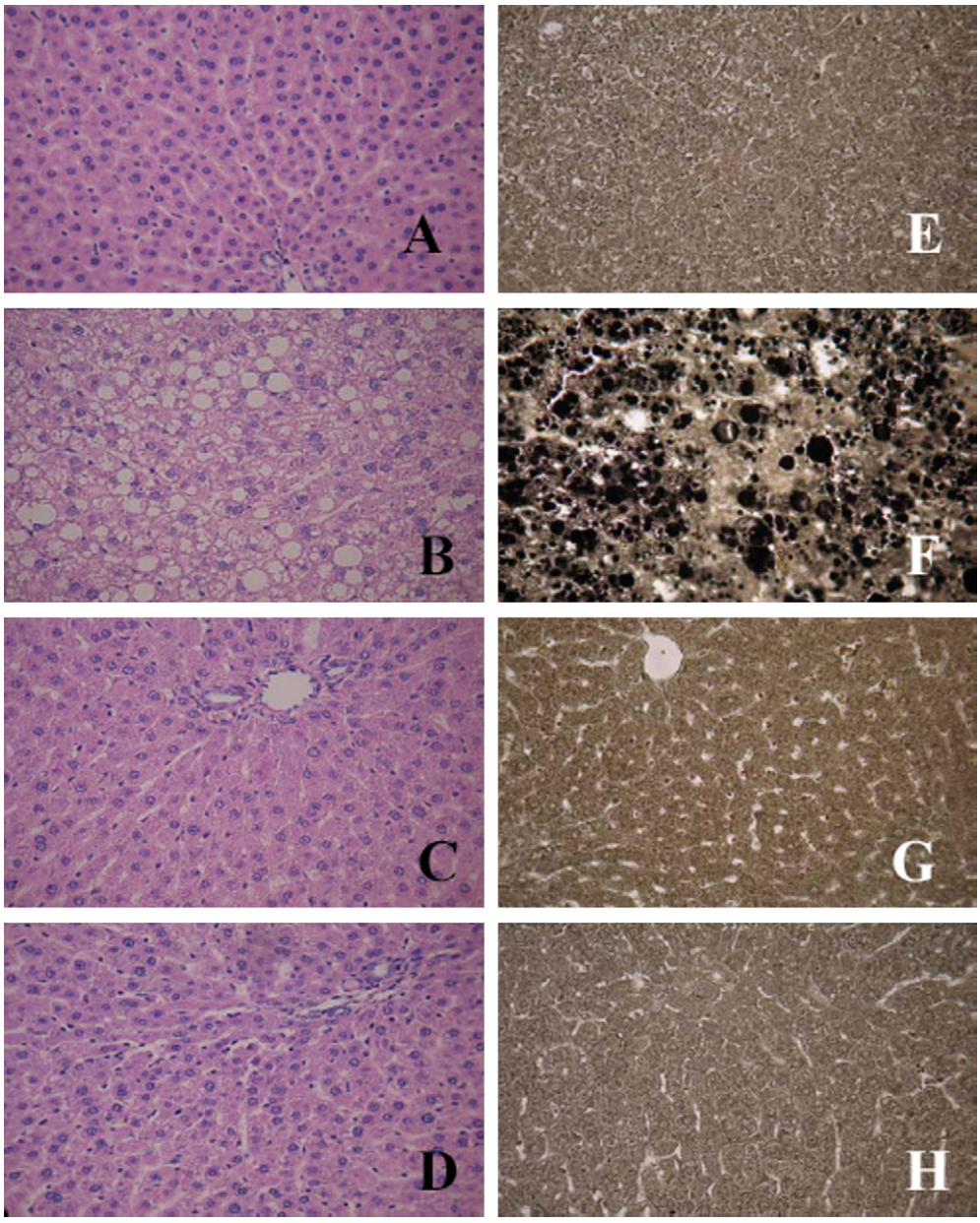

Fig. 3. Liver histology of a representative animal from each experimental group. Hematoxylin & Eosin (Panels A, B, C and D) and Osmium tetroxide (Panels E, F, G and H) staining of livers sections at the end of the experiment of a representative rat from each experimental group as described in Section 2. Severe panlobular hepatic steatosis (micro and macrovesicular) can be observed in high fat diet group in the HE staining (Panel B) or in the osmium tetroxide staining as lipid globules in black (Panel F). Liver slices from the treated groups (Losartan, panel C and G, and Telmisartan, panel D and H) were histologically comparable to those from control rats (panel A and E). Original magnification: 40×. Panel A and E: standard chow diet, Panel B and F: high fat diet, Panel C and G: high fat diet plus losartan and Panel D and H: high fat diet plus telmisartan.

3.2. Effects of losartan and telmisartan on body weight gain, food intake and organ fat accumulation

As expected, HFD rats gained weight throughout the study. However, the weight gain (g) during the pharmacological treatment period in rats treated with either losartan (-47 ± 13) or telmisartan (-79 ± 13) was significantly lower than in HFD rats (34 ± 14) or in SCD controls (11 ± 17), (Supplementary Appendix 2, Left panel) and in fact, they showed a significant decrease in body weight, being this effect even more important in the telmisartan-treated group.

In comparison with the absolute daily food intake (g/day) at the beginning of the pharmacological treatment period, food consumption at the end of the experiment was lower in the HFD + T rats (-10.4 ± 2.0) compared with both the HFD rats (1.4 ± 2.2) and the HFD + L rats (-0.9 ± 2.0) (Supplementary Appendix 2, right panel). Similar results were observed when food intake was adjusted by body weight and length of the animal (data not shown).

Effects on liver weight and visceral fat weight normalized by animal length (g/cm) are presented in Fig. 2, left and right panel, respectively. In both losartan (0.63 ± 0.04) and telmisartan-treated rats (0.64 ± 0.04), relative liver weight was significantly decreased compared with both control rats (0.75 ± 0.05) and HFD-rats (0.71 ± 0.0). Visceral fat content was significantly decreased in both HFD + L (0.64 ± 0.13) and HFL + T (0.33 ± 0.12) groups compared with HFD rats (1.15 ± 0.13).

3.3. Effects of losartan and telmisartan on Blood pressure

Even though HFD did not elevate SABP (119 ± 3 mmHg vs. 120 ± 3 mmHg), treatment with either telmisartan or losartan lowered SABP in conscious rats. This decrease in SABP was approximately 37 mmHg for HFD + L (81 ± 3 mmHg vs. 117 ± 3 mmHg) and HFD + T (76 ± 3 mmHg vs. 114 ± 3 mmHg) groups showing the efficacy of the pharmacological administration.

3.4. Effects of losartan and telmisartan on fatty liver disease: Liver histology and liver triglyceride content

Fig. 3 (Hematoxylin & Eosin staining and Osmium tetroxide staining) shows the analysis of hepatic histology of a representative animal from each experimental group. After 20 weeks, HFD-rats showed severe hepatic microvesicular and macrovesicular fat. Rats treated with either losartan or telmisartan completely eliminated this hepatic steatosis. The quantitative evaluation of the liver histology of all rats in each group is shown in Fig. 4, upper panel. Ordinal multinomial regression analysis indicated that this significant effect was independent of animal length and visceral fat depot.

Biochemical analysis of hepatic triglycerides content showed that the amount of triglycerides was increased significantly in the model group (HFD: 16.6 ± 1.6 μg/mg liver) in comparison with SCD controls (9.5 ± 1.6 μg/mg liver) and also with the HFD-L (9.4 ± 1.2 μg/mg liver) and the HFD-T (7.3 ± 0.5 μg/mg liver) groups, Fig. 4, lower panel. Then, treatment with losartan and telmisartan significantly improved triglycerides concentration, and this data correlated with the histological data. Results remain significant when liver triglycerides content was adjusted by animal body weight ($p < 0.0002$).

3.5. Effect of losartan and telmisartan on PAI-1 mRNA expression

Real time PCR analysis of the hepatic PAI-1 gene expression showed 42% and 50% decrease of *PAI-1* mRNA abundance in the HFD + L group in comparison with that of HFD rats and HFD + T rats, respectively (Fig. 5, upper left panel). Normalization using a second reference gene (GAPDH-Glyceraldehyde-3-phosphate dehydrogenase) showed the same significant decrease in the HFD + L group in comparison with both the HFD and HFD + T groups (data not shown).

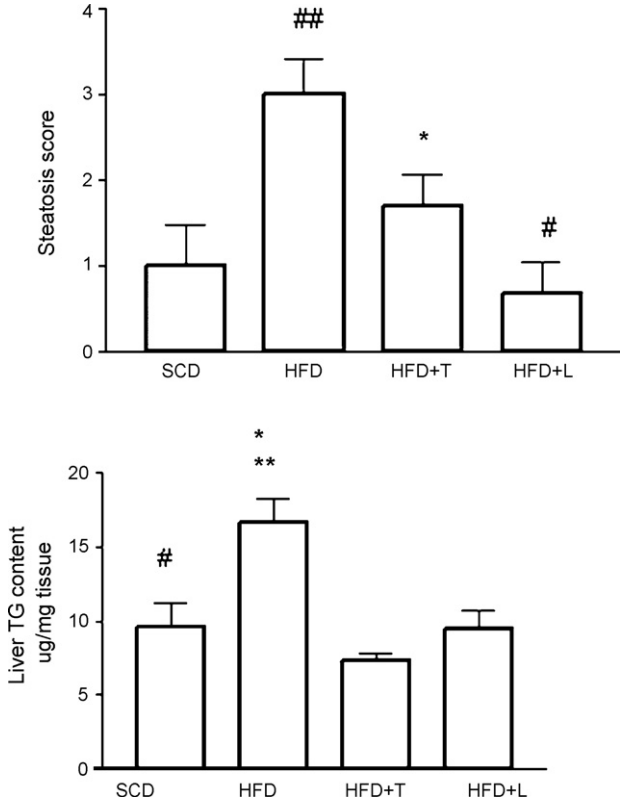

Fig. 4. Liver histology score and hepatic triglyceride content. Quantitative evaluation of steatosis scores from Hematoxylin & Eosin and osmium tetroxide stain of liver sections at the end of the experiment in all rats from each experimental group (Upper panel). Steatosis was given a score from 0 to 4 as described in Section 2. $^{*}p < 0.04$ vs. HFD, $^{\#}p < 0.02$ vs. HFD, $^{\#\#}p < 0.005$ vs. SCD. Data are presented as mean ± SE. For testing steatosis gradation (as a categorical response variable) differences, we used a model with ordinal multinomial distribution and probit as a link function with animal length and adipose tissue as continuous predictor variables. Liver triglycerides content at the end of the experiment (Lower panel). Results (μg/mg liver) are expressed as mean ± SE. $^{\#}p < 0.001$ vs. HFD, $^{*}p < 0.006$ vs. HFD + L, $^{**}p < 0.00001$ vs. HFD + T. SCD: standard chow diet, HFD: high fat diet, HFD + T: high fat diet plus telmisartan, HFD + L: high fat diet plus losartan groups.

Additionally, measurement of total PAI-1 levels in liver tissue (free, latent, and complexed PAI-1, expressed in pg/mg proteins) showed that the HFD + L group had significantly lower levels (6.33 ± 0.90) compared to the HFD (9.5 ± 0.83, $p < 0.05$) and the HFD + T (11.22 ± 0.83, $p < 0.01$) groups. Liver PAI-1 levels in the control SCD group was 8.08 ± 0.90.

3.6. Effect of losartan and telmisartan on TNFα mRNA expression

Previous evidence showed that TNFα and the local renin-angiotensin system coordinately stimulate PAI-1 production in hepatocytes [25]. To determine whether *TNFα* mRNA was influenced by the pharmacological treatment, we explored the liver expression of this gene in all the experimental groups.

TNFα mRNA abundance seemed to be higher in the HFD group, with borderline significance (Fig. 5, upper right panel). Although the expression of hepatic *TNFα* was not significantly modified either by losartan or telmisartan, when contrasting with the model (HFD) group, even though with a marginal statistical significance, the losartan-treated group seemed to show a reduced expression of hepatic TNFα compared with the HFD rats.

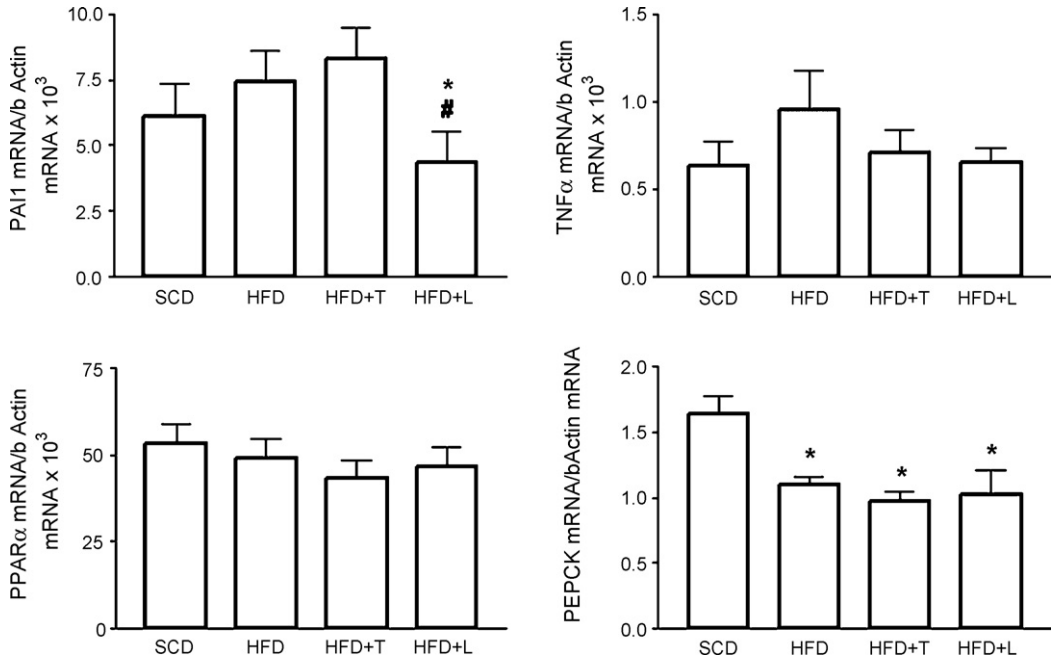

Fig. 5. Expression of liver *PAI-1*, *TNFα*, *PEPCK-C* and *PPARα* mRNA analyzed by quantitative real-time PCR in each experimental group. Each bar represents mean ± SE of values from 6, 8, 10 and 9 animals in SCD: standard chow diet, HFD: high fat diet, HFD + T: high fat diet plus telmisartan, HFD + L: high fat diet plus losartan, respectively. In each sample, gene expression were normalized to the expression of β actin and multiplied by 1000, except for *PEPCK-C*. In *PAI-1* panel, $^{*}p < 0.02$ vs. HFD, and $^{\#}p < 0.01$ vs. HFD + T. In *PEPCK-C* panel, $^{*}p < 0.01$ vs. SCD control group.

3.7. Effect of losartan and telmisartan on PEPCK-C mRNA expression

Because phosphoenolpyruvate carboxykinase (*PEPCK-C*) is a key mediator of the effects of PPARγ ligands on fatty acid metabolism and insulin sensitivity, liver expression of this gene was further evaluated in all experimental groups. We found a significant decrease in liver expression of *PEPCK-C* in the HFD, HFD + L and HFD-T groups in comparison with the SCD controls (Fig. 5, lower right panel), indicating that the HFD *per se* decreased *PEPCK-C* expression.

3.8. Effect of losartan and telmisartan on PPARα mRNA expression

We explored the effects of the two drugs on liver expression of the peroxisome proliferator-activated receptor-alpha (*PPARα*). We chose *PPARα* because this transcription factor is involved in the control of the expression of genes encoding fatty acid oxidation enzymes, mitochondrial fatty acid oxidation, and is also implicated in interference with atherogenic and inflammatory processes with a potential effect on *PAI-1* expression. However, as shown in Fig. 5, lower left panel, liver expression of *PPARα* was similar in all the experimental groups, and no significant differences were observed.

4. Discussion

Both human and animal studies showed that NAFLD is associated with increased PAI-1 levels [5,6].

In this study we evaluated the effect of two ARBs on the hepatic expression of PAI-1 in a rat model of NAFLD induced by HFD, as previous studies showed that activation of the renin-angiotensin system mediated by the AT1R is associated with PAI-1 production.

We found for the first time that losartan, but not telmisartan, significantly reduced both liver *PAI-1* gene expression and total PAI-1 protein levels in liver tissue. We also observed that both losartan and telmisartan were effective in reversing hepatic steatosis and hepatic lipid accumulation.

Our experimental observations deserve several comments. The first one is about the effect of the AT1R antagonism on liver *PAI-1* gene expression. As far to our knowledge, the data we are reporting here, which are in line with the previous observation of Brown and coworkers in knockout mice (AT (1a)−/−) [26], show for the first time that losartan is able to decrease liver *PAI-1* mRNA.

In addition, we further extended the knowledge about a differential therapeutic effect of two ARBs, as telmisartan seems not to influence liver-*PAI-1* expression.

The open question arises as to what extent this functional dissimilarity between losartan and telmisartan may be explained by differences in the chemical structural characteristic of both ARBs that can even influence, for instance, their binding affinity to the AT1R suggesting the possibility that many effects of some ARBs are specific to their structure and are not shared among all AT1R blockers [27,28]. For example, it was shown that losartan and telmisartan both produce a rightward shift of the AngII dose-response curve, the maximal response is unaffected by surmountable antagonists, such as losartan, whereas is reduced by insurmountable antagonists (such as telmisartan), leading to a nonparallel displacement of the AngII response curve (meaning that there is a slow dissociation of the antagonist from the AT1 receptor and that AngII is not the best competitor for telmisartan at the receptor level) [29]. Considering that AngII stimulates *PAI-1* expression, these observations may explain, at least in part, the difference that we observed between both drugs regarding liver *PAI-1* expression. Although interesting, further exploration of the different mechanisms of the two drugs on PAI-1 is beyond the scope of this study.

On the other hand, in an effort to understand the molecular mechanisms related with our findings, we evaluated mRNA liver expression of several candidate genes.

As a previous report showed that *TNFα* is involved in the PAI-1 production from hepatocytes [25], we evaluated the effect of the drugs on liver *TNFα* expression and we did not observe significant differences either in losartan or in telmisartan-treated groups in comparison with the model group. The lack of histological signs of liver injury and inflammation observed in our experimental

model must be kept in mind when interpreting these results. It is noteworthy to mention that other studies support our observation. For instance, Buettner et al. have systematically compared the metabolic and molecular effects of different HFDs with varying fatty acid compositions and observed that hepatic steatosis was induced by all type of HFD but no signs of inflammation or fibrosis were detected in any studied group [30]. Even more important, liver histologies and hepatic gene expression profiles in the mentioned models were dependent on the HFD composition [30]. On the contrary, Cai et al. by using a transgenic approach to activate NF-κB selectively in hepatocytes observed that lipid accumulation in the liver leads to sub-acute hepatic inflammation through NF-κB activation and downstream cytokine production [31]. Thus, HFD-induced phenotype varies distinctly among different studies and the role of inflammatory response triggered by HFD is still controversial at least when the studied phenotype is fatty liver.

Based on the main difference between losartan and telmisartan regarding the molecular target to which each drug is aimed at, we evaluated different molecular pathways associated with two peroxisome proliferator-activated receptors (PPARs). Firstly, we assessed the effect of the two drugs on liver expression of *PEPCK-C*, a key downstream gene product of PPARγ activation. Interestingly, both losartan and telmisartan decreased liver mRNA expression of *PEPCK-C*, an effect that may explain the plasma glucose-lowering activity of the AT1 receptor antagonism [32,33]. The same decrease was observed in the HFD group, hence, we cannot reject the hypothesis that the HFD *per se* is associated with a downregulation of the *PEPCK-C* liver expression. Moreover, regulatory elements in the *PEPCK* gene promoter are responsible for the tissue-specific and developmental, hormonal and dietary regulation of gene expression [34]. In addition, our observation about a decrease of liver *PEPCK-C* mRNA in relation to HFD was already reported by others [35].

As a final approach to evaluate the differing effects of losartan and telmisartan on liver *PAI-1* expression we quantified *PPARα* mRNA, as telmisartan seemed to increase *PPARα* expression in liver tissue [36]. In our model, we did not observe significant changes in hepatic *PPARα* mRNA in any of the treated groups, however, we cannot rule out the effects of the drugs on other *PPARα* target genes.

A second major observation of our study is that liver lipotoxicity triggered by HFD was reverted by both losartan and telmisartan. The effect of telmisartan on protecting against hepatic steatosis was previously reported in a HFD-rat model [12]. We may speculate that some other important mechanisms are contributing to this effect of the ARB. For instance, losartan selectively inhibited oxidative stress via downregulation of NADPH oxidase [37].

A final and probably most intriguing potential implication of the current study concerns the observation that losartan and telmisartan significantly reduced weight gain in the treated groups. This observation is of substantial interest, because there is increasing evidence that treatment with other PPARγ ligands, such as thiazolidinediones, results in weight gain [38]. Although in our study this effect seems to be due to a decrease in food intake, Sugimoto et al. observed that telmisartan increases caloric expenditure [39]. Certainly, the mechanisms by which ARBs protect against weight gain deserve further investigation.

Owing to the critical role that the renin-angiotensin system plays in both the pathogenesis of insulin resistance and liver fibrosis, it is not unexpected that ARBs are being suggested as a potential preventive therapy for NAFLD. Consequently, in patients with metabolic syndrome, the clinical benefits of ARBs extend beyond blood pressure reduction to encompass tissue-protective effects in different target organs, which results in reduction of cardiovascular mortality and morbidity [38].

Our experimental results indicate that only losartan significantly reduced the HFD-increased hepatic *PAI-1* mRNA abundance. These differences could be important in choosing effective options for preventing hepatic lipid accumulation in NAFLD.

Conflict of interest

No conflicts of interest exist. Nothing to disclosure.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2009.01.026.

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