Angiotensin 1–7 and Hepatic Steatosis

Oral Formulation of Angiotensin-(1–7) Improves Lipid Metabolism and Prevents High-Fat Diet–Induced Hepatic Steatosis and Inflammation in Mice

John David Feltenberger,* João Marcus Oliveira Andrade,* Alanna Paraíso, Lucas Oliveira Barros, Aristides Batista Maia Filho, Ruben D.M. Sinisterra, Frederico B. Sousa, André Luiz Sena Guimarães, Alfredo Mauricio Batista de Paula, Maria José Campagnole-Santos, Mahboob Qureshi,
Robson Augusto Souza dos Santos, Sérgio Henrique Sousa Santos

Abstract—Angiotensin (Ang)-(1–7) has been described as an important tool on treating and preventing metabolic disorders. In this study, we aimed to evaluate the effect of an oral formulation of Ang-(1–7) included in hydroxypropylβ-cyclodextrin (HPβCD/Ang-[1–7]) on hepatic function, steatosis, and on liver inflammatory markers expression in mice treated with a high-fat diet. Male FVB/N mice were divided into 4 groups and fed for 60 days, with each group receiving 1 of the following diets: standard diet+HPβCD, standard diet+Ang-(1–7)/HPβCD, high-fat diet+HPβCD, or high-fat diet+Ang-[1–7]/HPβCD. Body weight, food intake, and blood parameters, such as total cholesterol, triglyceride, alaninetransaminases, and aspartate transaminases, were evaluated. Immunohistochemical analyses were performed for inflammatory markers tumor necrosis factor-α and interleukin-6. Expression of angiotensin converting enzyme, angiotensin-converting enzyme-2, interleukin-1β, tumor necrosis factor-α, interleukin-6, transforming growth factor-β, acetyl-CoA carboxylase, carbohydrate-responsive element–binding protein, peroxisome proliferator–activated receptor-γ, and sterol regulatory element–binding proteins-1c was evaluated by quantitative real-time polymerase chain reaction. The major findings of our study included reduced liver fat mass and weight, decreased plasma total cholesterol, triglyceride, and alaninetransaminase enzyme levels in the oral Ang-(1–7)–treated groups compared with the control groups. These results were accompanied by a significant reduction in tumor necrosis factor-α and interleukin-6 mRNA expression in the liver. Analyses of liver adipogenesis-related genes by quantitative real-time polymerase chain reaction showed that acetyl-CoA carboxylase, peroxisome proliferator–activated receptor-γ, and sterol regulatory element–binding proteins-1c mRNA expression were significantly suppressed. In conclusion, we observed that treatment with Ang-(1–7) improved metabolism and decreased proinflammatory profile and fat deposition in liver of mice. (Hypertension. 2013;62:324-330.) ● Online Data Supplement

Key Words: angiotensina-(1–7) ■ fatty liver ■ lipid metabolism ■ obesity ■ visceral steatosis, congenital

Obesity is characterized by an increase in white adipose tissue mass, which can result from an excess of food (energy) intake or altered energy expenditure.1 Obesity has been recently described as a systemic and local adipose proinflammatory state, and has been implicated in the development of common medically important complications, including hepatic steatosis, insulin resistance, and atherosclerosis.2–4 Classic markers of the obesity-induced inflammatory state include the augmented tissue and circulating levels of proinflammatory enzymes, procoagulant factors, cytokines, and chemokines.4,5

Hepatosteatosis is the process describing the abnormal retention of lipids within a liver cell. It reflects an impairment of the normal processes of synthesis and elimination of triglyceride fat and is commonly associated to lose of hepatocyte function. There are several different causes of hepatosteatosis, including chronic alcohol consumption, B and C viral hepatitis, type 2 diabetes mellitus, obesity, and some metabolic disorders. In this study, we aimed to evaluate the effect of an oral formulation of Ang-(1–7) included in hydroxypropylβ-cyclodextrin (HPβCD/Ang-[1–7]) on hepatic function, steatosis, and on liver inflammatory markers expression in mice treated with a high-fat diet. Male FVB/N mice were divided into 4 groups and fed for 60 days, with each group receiving 1 of the following diets: standard diet+HPβCD, standard diet+Ang-(1–7)/HPβCD, high-fat diet+HPβCD, or high-fat diet+Ang-[1–7]/HPβCD. Body weight, food intake, and blood parameters, such as total cholesterol, triglyceride, alaninetransaminases, and aspartate transaminases, were evaluated. Immunohistochemical analyses were performed for inflammatory markers tumor necrosis factor-α and interleukin-6. Expression of angiotensin converting enzyme, angiotensin-converting enzyme-2, interleukin-1β, tumor necrosis factor-α, interleukin-6, transforming growth factor-β, acetyl-CoA carboxylase, carbohydrate-responsive element–binding protein, peroxisome proliferator–activated receptor-γ, and sterol regulatory element–binding proteins-1c was evaluated by quantitative real-time polymerase chain reaction. The major findings of our study included reduced liver fat mass and weight, decreased plasma total cholesterol, triglyceride, and alaninetransaminase enzyme levels in the oral Ang-(1–7)–treated groups compared with the control groups. These results were accompanied by a significant reduction in tumor necrosis factor-α and interleukin-6 mRNA expression in the liver. Analyses of liver adipogenesis-related genes by quantitative real-time polymerase chain reaction showed that acetyl-CoA carboxylase, peroxisome proliferator–activated receptor-γ, and sterol regulatory element–binding proteins-1c mRNA expression were significantly suppressed. In conclusion, we observed that treatment with Ang-(1–7) improved metabolism and decreased proinflammatory profile and fat deposition in liver of mice. (Hypertension. 2013;62:324-330.) ● Online Data Supplement

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From the Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Montes Claros, MG, Brazil (J.D.F., J.M.O.A., A.P., L.O.B., A.B.M.F., A.L.S.G, A.M.B.d.P., S.H.S.S.); Department of Pharmacology, Institute of Biological Sciences (S.H.S.S.) and National Institute of Science and Technology in Nanobiopharmaceutics (R.D.M.S., F.B.S., M.J.C.-S., R.A.S.d.S.), Universidade Federal de Minas Gerais, Minas Gerais, Belo Horizonte, Brazil; and Medical School, Touro University Nevada College of Medicine, Henderson, Las Vegas, NV (J.D.F., M.Q.). The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.111.00919/DC1.

*J.D.F. and J.M.O.A. contributed equally to this article.

Correspondence to Sérgio Henrique Sousa Santos, Department of Pharmacology, Universidade Federal de Minas Gerais, Av Antonio Carlos 6627-ICB, 31270-901, Belo Horizonte, MG, Brazil. E-mail sergiosousas@hotmail.com; or Robson Augusto dos Santos, Laboratório de Hipertensão, Departamento de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Av Antonio Carlos 6627-ICB, 31270-901, Belo Horizonte, MG, Brazil. E-mail robsouaat@gmail.com

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aberrations. Actually, fatty liver disease is considered the most prevalent form of hepatosteatosis associated with obesity and metabolic syndrome.

The renin-angiotensin system (RAS) is now recognized to play an important role in the development of cardiovascular and metabolic disorders. The RAS consists primarily of an enzymatic cascade in which angiotensinogen is converted to angiotensin (Ang) I and subsequently to Ang II by the actions of renin and ACE-convertase enzyme (ACE), respectively. Ang-(1–7) is formed mainly from Ang II by ACE-2 and indirectly from Ang I. The ACE-2/Ang-(1–7)/Mas axis has been suggested as an important counter-regulatory arm in the RAS with effects opposing those of ACE/Ang II/Ang II receptor, type I.

Recent studies showed an important participation of RAS in the nonalcoholic fatty liver disease (NAFLD) development and progression. The Ang II has been implicated as a major player in the altered hepatic lipid metabolism observed in NAFLD. Genetic disruption of several RAS components in rodent models results in a protection from high-fat diet (HFD)–induced obesity. In fact, knockout of renin, and liver-specific deletion of Ang II receptor type I also reduces hepatic steatosis in rodent models.

The role of Ang-(1–7) in metabolic regulation has been recently described. A recent study showed that Mas receptor deficiency in FVB/N mice induce dyslipidemia, lower glucose tolerance and insulin sensitivity, hyperinsulinemia, hyperleptinemia, decreased glucose uptake in white adipose cells, and an increase in adipose tissue mass. These observations suggest that chronic deficiency in Ang-(1–7)/Mas axis components can lead to a metabolic syndrome-like state. More recently, 2 different studies demonstrated that transgenic rats with high-circulating Ang-(1–7) plasma levels had improved lipid and glucose metabolism associated with decreased liver gluconeogenesis.

The pharmacological potential of Ang-(1–7) was significantly increased after the development of a new oral formulation characterized by a protected Ang-(1–7) molecule incorporated in acyclic-oligosaccharides (cyclodextrin). This novel compound was denominated hydroxypropyl-β-cyclodextrin [HPβCD]/Ang-(1–7). A pharmacokinetic test was conducted in rats to estimate the bioavailability of the compound, showing that oral formulation significantly increased plasma levels of Ang-(1–7) 12-fold over baseline with the observation for 6 hours after its administration (detailed Material is available in the online-only Data Supplement).

Importantly, the potential mechanism underlying the fat hepatoprotective effects of Ang-(1–7) included in HPβCD/Ang-(1–7) in vivo still remains unclear. Thus, the purpose of this study is to evaluate the effect of oral administration of Ang-(1–7) on hepatic functions and on the expression of liver inflammatory markers in mice consuming a HFD.

**Methods**

An expanded Methods section, detailing the techniques and procedures performed, is provided in online-only Data Supplement.

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**Animals**

The experiment was conducted with 32 male FVB/N mice divided into 4 groups (n=8) and fed the following experimental diets, respectively, for 8 weeks: standard diet (ST)+HPβCD, ST+Ang-(1–7)/HPβCD, HFD+HPβCD, and HFD Ang-(1–7)/HPβCD: 0.1 mg/kg (HFD+Ang-[1–7]). An additional experiment with 2 groups (ST with or without Ang-[1–7]/HPβCD, 0.1 mg/kg) was conducted to evaluate a possible consequence of this compound in nonobese mice (these data are provided in the online-only Data Supplement).

**Diets**

Obesity was induced in male FVB/N mice by HFD (24.55% of carbohydrate, 14.47% of protein, and 60.98% of fat, presenting a total of 5.28 kcal/g of diet). Control group was fed ST (50.30% of carbohydrate, 41.90% of protein, and 7.80% of fat with a total of 2.18 kcal/g of diet).

**Measurements of Body Weight, Food Intake, and Tissue Collection**

Food intake and body weight were measured twice a week during treatment. Tissues were collected and stored after euthanization.

**Determination of Blood Measurements**

Total serum cholesterol, triglycerides, high-density protein, and aspartate and alaninetransaminases were assayed using specific enzymatic kits.

**Reverse Transcription and Real-Time Polymerase Chain Reaction**

Expression of ACE, ACE-2, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, acetyl-CoA carboxylase, transforming growth factor-β, carbohydrate-responsive element–binding protein, peroxisome proliferator–activated receptor-γ and sterol regulatory element–binding protein-1c (SREBP-1c) was evaluated by quantitative real-time polymerase chain reaction.

**Histology and Oil Red O Staining**

Liver samples from mice were stained with hematoxylin and eosin and Oil Red, and evaluated under a conventional light microscope.

**Immunohistochemical Reactions**

Immunohistochemical reactions were performed for markers IL-6 and TNF-α.

**Statistical Analysis**

Data are expressed as the mean±SEM. The statistical significance of differences in mean values between mice groups was assessed by 1-way ANOVA followed by Bonferroni post-test.

**Results**

To examine the functional consequences of the oral treatment with Ang-(1–7), we studied the relationship between body and fat weight. Differences in body weight or food intake (g/body weight in grams) did not display differences between groups (Figure S1A and S1B in the online-only Data Supplement). However, analysis of the total fat mass (sum of adipose tissues: epididymal, mesenteric, and retroperitoneal) showed that HFD+Ang-(1–7) group exhibited a substantial decrease in fat mass (ST, 2.56±1.39; HFD, 3.80±1.27; HFD+Ang-[1–7], 2.07±0.59; 100 g/body weight in grams) in relation to HFD group (Figure S1C).
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206.9±54.18 versus HFD+Ang-[1–7], 140.6±21.62) compared with HFD group (Figure 1A and 1B). In addition, Ang-(1–7) treatment plasma high-density lipoprotein increase when compared with HFD group (ST, 28.20±4.77; HFD, 18.02±9.81; HFD+Ang-[1–7], 29.94±5.95; Figure 1C).

Hepatic function analyses showed that alaninetransaminases serum levels were lower in ST and HFD+Ang-(1–7) when compared with HFD group (ST, 33.09±6.78; HFD, 54.66±11.71; HFD+Ang-[1–7], 32.81±4.53). Serum aspartate aminotransferase levels were lower in HFD+Ang-(1–7) when compared with the ST group (ST, 31.80±6.76; HFD, 48.14±7.51; HFD+Ang-[1–7], 39.67±6.59; Figure 1D and 1E).

In addition, analysis showed that HFD+Ang-(1–7) group has a substantial decrease in the total liver weight in HDF+Ang-(1–7) (ST, 0.035±0.0027; HFD, 0.046±0.0059; HFD+Ang-[1–7], 0.039±0.0039) in relation to HFD (Figure 2A). Hence, we performed a histological analysis to examine the effect of Ang-(1–7) on the development of fatty liver. Large hepatic lipid droplets were diffusely present in the livers of the HFD group mice compared with the other groups (Figure 2B).

Analyses of mRNA expression of adipogenesis-related genes showed that acetyl-CoA carboxylase, peroxisome proliferator–activated receptor–γ, and SREBP-1c mRNA expression in the liver were significantly suppressed in HFD+Ang-(1–7) group in comparison with the HFD group (Figure 3A and 3C). However, the expression of carbohydrate-responsive element–binding protein did not display differences among groups (Figure S2A).

Analysis of the expression of RAS components showed decreased levels of ACE in HFD+Ang-(1–7) mice (HFD, 1.19±0.35 versus HFD+Ang-[1–7], 0.65±0.19; Figure 3D). In addition, significantly increased expression of ACE-2 (HFD, 0.86±0.07 versus HFD+Ang-[1–7], 1.32±0.35) in HFD+Ang-[1–7] mice was observed (Figure 3E).

The mRNA expression of proinflammatory cytokines by quantitative real-time polymerase chain reaction in liver showed a significant decrease of IL-6 (ST, 0.68±0.081; HFD, 1.07±0.17; HFD+Ang-[1–7], 0.75±0.18) and TNF-α (ST, 1.01±0.4; HFD, 1.52±0.17; HFD+Ang-[1–7], 0.73±0.15) in HFD+Ang-(1–7) group (Figure 4A and 4B). The expression of the transforming growth factor–β and IL-1β did not differ among the groups (Figure S2B and S2C).

Immunohistochemical reactions showed decreased expression of IL-6 and TNF-α in ST and HFD+Ang-(1–7) mice in relation to HFD mice (Figure 4C and 4D).

Treatment of nonobese mice with Ang-(1–7)/HPβCD (0.1 mg/kg) did not alter body weight, body fat, serum lipid levels, glycemia, and hepatic enzymes (Figures S3 and S4).

Discussion

In this study, we evaluated for the first time the effects of oral Ang-(1–7) administration in high-fat–induced steatosis, liver metabolism, and inflammation. We 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 proinflammatory cytokines. These effects were associated with beneficial regulation of the RAS genes expression.

Figure 1. Plasmatic parameters. A, Total cholesterol. B, Triglycerides. C, High-density lipoprotein. D, Alanine transaminase. E, Aspartate transaminase (AST). Control mice (white bars), mice fed a high-fat diet (HFD; gray bars), or mice fed an angiotensin (Ang)-(1–7)–supplemented HFD (HFD+Ang-[1–7]; black bars) for 8 weeks. *P<0.05, **P<0.01 between HFD vs HFD+Ang-(1–7) and standard diet (ST).
Despite unchanged food intake and body weight, mice exhibited a significant decrease in fat mass. Previous studies have demonstrated the prejudicial effects of HFD on fat and metabolic performance. The absence of alterations in food intake indicates that the difference in fat mass was not induced by decreased appetite and was probably caused by changes in metabolic regulation. Previous studies also showed a beneficial metabolic role of Ang-(1–7) in body fat mass. The RAS exerts significant effects on body weight in rodents. An important human study showed reduced white adipose tissue angiotensinogen levels by –27%, renin by –43%, and ACE activity by –12% after reduction of 600 kcal in daily caloric intake. Another study showed that Mas-knockout mice have a substantial increase in fat mass in relation to wild-type FVB/N mice with similar diameter of the adipocytes among groups, indicating that the increase in adipose tissue mass was not a result of hypertrophy in adipocytes but probably hyperplasia.

Several studies have documented the possible role of decreased liver fatty acid oxidation in the development and...
progression of NAFLD and hypothesized that impaired mitochondrial function may be involved.29–31 In summary, these studies showed that decreased hepatic mitochondrial fatty acid oxidation was associated with increased hepatic triacylglycerol accumulation. Supporting these data, HFD-induced obesity in mice was associated with increased hepatic peroxisome proliferator–activated receptor-γ, carnitine palmitoyltransferase-1, and acetyl-CoA carboxylase, and these changes were associated with decreased serum triacylglycerol.32 Furthermore, ACE knockout mice exhibit increased whole-body energy expenditure and increased liver expression of genes involved in fatty acid oxidation,17 supporting a direct role of Ang II in regulating fatty acid oxidation. Our study corroborate these findings once we showed that administration of Ang-(1–7) can lead to a decrease in the expression of genes related to hepatic mitochondrial fatty acid oxidation, with acetyl-CoA carboxylase and peroxisome proliferator–activated receptor-γ.

Diet-induced obesity is largely caused by disorders of fat metabolism, resulting in a massive accumulation of fat in several tissues. Lipid and energy metabolism are regulated by a complex network of signaling processes, and we, therefore, investigated mRNA expression of key genes regulating lipid metabolism. Decreased total cholesterol and triglyceride levels were shown in rats with increased circulating Ang-(1–7).12 A possible explanation would be decreased levels of mRNA expression in genes encoding lipogenic proteins, such as SREBP-1c. The lipogenic transcription factor, SREBP-1c, plays a crucial role in regulating fatty acid synthesis, and SREBP-1c–responsive major genes encode a rate-limiting enzyme in de novo fatty acid biosynthesis.33–36 SREBP-1c is activator of the complete program of cholesterol and fatty acid synthesis in the liver. Thus, Ang-(1–7) can have an effect on de novo lipogenesis and lipid metabolism in liver.

Several studies reported that TNF-α, IL-6, and IL-1β are increased in obesity and this increase is correlated with numerous metabolic disorders.13 In patients with obesity, adipose and liver tissues are characterized by high-intensity inflammation and increased secretion of cytokines.17 TNF-α, IL-6, and IL-1β may be the most pernicious because they alter adipose tissue function, influencing adipogenesis, and are involved in the metabolic complications of obesity with NAFLD.38

TNF-α is a cytokine that plays a critical role in systemic and local inflammation that is produced by activated macrophages and other cell types under various pathophysiological states. With the association of obesity and NAFLD in the metabolic syndrome, it is not surprising that increased serum and liver TNF-α expression is observed in patients39 and rodent models of NAFLD.40,41 Several data suggested the involvement of TNF-α and IL-6 in the metabolic syndrome and progression of NAFLD, perhaps, in part through increased mitochondrial dysfunction and

![Figure 4. Effects of 8-week angiotensin (Ang)-(1–7) treatment on gene expression inflammatory markers by real-time polymerase chain reaction and immunohistochemistry in mice fed a high-fat diet (HFD). A and C, Interleukin (IL)-6. B and D, Tumor necrosis factor (TNF)-α. Control mice (white bars), mice fed a HFD (gray bars), or mice fed an Ang-(1–7)–supplemented HFD (HFD+Ang-(1–7); black bars) for 8 weeks. *P<0.05, **P<0.01 between HFD vs HFD+Ang-(1–7) and standard diet (ST).](http://hyper.ahajournals.org/)

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activation of fibrogenesis.42,43 TNF-α also can contribute to the development of insulin resistance through disruption of the insulin signaling cascade at the level of insulin receptor substrate-1 pathway by Ang II.44 Ang II–infused rats presented increased IL-6 expression in the liver with associated increase in monocyte recruitment and overall inflammation.45 A recent study showed that Ang-(1–7) was able to reduce inflammatory markers in rats with diabetic nephropathy.46 These data were supported by a study that revealed different molecular approaches for Ang-(1–7)–modulated inflammatory responses in mouse peritoneal macrophages, decreasing IL-6 and TNF-α.47 A more recent work has demonstrated that high-circulating Ang-(1–7) exerts significant anti-inflammatory effects by decreasing the expression of inflammatory markers in adipose tissue of high-fat–treated rats.33 A limitation of this study was not to have evaluated the direct effect of HFD on tissue Ang-(1–7) levels. However, our main focus was to evaluate the ability of oral administration of Ang-(1–7) preventing inflammation and liver steatosis and metabolism.

In conclusion, this study shows that oral treatment with Ang-(1–7) offers a protective effect against the proinflammatory response profile in the liver and improves lipid metabolism in an obese mouse model induced by a HFD. In mice treatments, Ang-(1–7) reduced fat mass, liver weight, total cholesterol, triglycerides, alaninetransaminase enzymes, and improved lipid metabolism decreasing expression proinflammatory cytokines in HFD-fed mice. These effects were associated with beneficial regulation of RAS genes with increased ACE-2 and decreased ACE expression. These results support the hypothesis of the use oral Ang-(1–7) as a novel therapeutic agent for the prevention and treatment of obesity-related disorders and fat-liver diseases.

Perspectives
The oral formulation of Ang-(1–7) completely prevented fat deposition in liver and improved metabolic profile. Thus, our current findings confirm and extend previous data demonstrating the great participation of Ang-(1–7) in the lipid and glucose metabolism. Altogether, these results suggest that oral formulation of Ang-(1–7) could be considered as a putative new and innovative therapeutic drug for the treatment of liver steatosis and obesity-associated diseases.

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Disclosures
R.A. Souza dos Santos has helped for the availability of Ang-(1–7) used in the study. The other authors report no conflicts.

References


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### Novelty and Significance

**What Is New?**

- This study shows for the first time that an oral formulation of angiotensin-(1–7) is able to prevent fat-liver steatosis in high-fat–treated animals.

**What Is Relevant?**

- The present results are extremely relevant once obesity, hypertension, and diabetes mellitus are the 3 main findings in metabolic syndrome, which is the most common clinical state associated with fat-liver steatosis.

**Summary**

We showed that oral Angiotensin-(1–7) produced an important reduction in total body fat mass, liver weight, and hepatic steatosis, associated with decreased circulating total cholesterol, triglyceride, and alanine transaminase enzyme, and improved lipid metabolism with decreased expression of proinflammatory cytokines. These effects were associated with beneficial regulation of the renin-angiotensin system genes expression.
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ORAL FORMULATION OF ANGIOTENSIN-(1-7) IMPROVES LIPID METABOLISM AND PREVENTS HIGH-FAT-DIET INDUCED HEPATIC STEATOSIS AND INFLAMMATION IN MICE

Short title: Oral Ang-(1-7) prevented liver steatosis

*John David Feltenberger¹,³, *João Marcus Oliveira Andrade¹, Alanna Paraíso¹, Lucas Oliveira Barros¹, Aristides Batista Maia Filho¹, Ruben D.M. Sinisterra⁴(PhD), Frederico B. Sousa⁴, André Luiz Sena Guimarães¹ (PhD), Alfredo Maurício Batista de Paula¹ (PhD), Maria José Campagnole-Santos⁴ (PhD), Mahboob Qureshi³ (MD, PhD), Robson Augusto dos Santos⁴ (MD, PhD), Sérgio Henrique Sousa Santos¹,²,#(PhD).

¹Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Montes Claros, MG, Brazil.
²Institute of Biological Sciences. Department of Pharmacology; Universidade Federal de Minas Gerais, Minas Gerais, Brazil.
³Touro University Nevada College of Medicine, Henderson, Las Vegas, NV, USA.
⁴National Institute of Science and Technology in Nanobiopharmaceutics; Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

# To whom correspondence may be addressed

* Equally contributed to this work
Footnotes

Correspondence may be addressed to:

Dr. Sérgio H S Santos
Pharmacology Department
Universidade Federal de Minas Gerais
AvAntonio Carlos 6627-ICB – 31270-901, Belo Horizonte, MG, Brazil
FAX/Phone: (55-31)3409-2695/2724 - Email: sergiosousas@hotmail.com

and

Dr. Robson Santos
Laboratório de Hipertensão, Departamento de Fisiologia e Biofísica
Universidade Federal de Minas Gerais
AvAntonio Carlos 6627-ICB
31270-901, Belo Horizonte, MG, Brazil
FAX/Phone: (55-31)3499-2924/2956
Email: robsonsant@gmail.com
Supplemental Introduction

The pharmacological potential of Ang-(1-7) was significantly increased after the development of a new oral formulation characterized by a protected Ang-(1-7) molecule incorporated in acyclic-oligosaccharides (cyclodextrin). This novel compound was denominated [hydroxypropylβ-cyclodextrin/Ang-(1-7) - HPβCD/Ang-(1-7)] (20). It has been described that Ang-(1-7) incorporated into this hydroxypropylβ-cyclodextrin (HPβCD) cavity, can be protected during the passage through the gastrointestinal tract after oral administration (21). A pharmacokinetic test was conducted in rats to estimate the bioavailability of the heptapeptide in the circulation after oral administration of aqueous solution. The experiment tested the vehicle, free Ang-(1-7) peptide and the inclusion compound of Ang-(1-7) with HPβCD. Oral administration of HPβCD/Ang-(1-7) significantly increased plasma levels of Ang-(1-7), with a maximum increase of approximately 12-fold over baseline seen at 6 hours after administration. In contrast, the maximum increase in plasma Ang1-7 levels seen with administration of Ang-(1-7) without HPβCD was only 1.5 fold over baseline. Thus, the bioavailability of Ang-(1-7) delivered by HPβCD/Ang-(1-7) was increased after administration and continued to increase for approximately 6 hours (22). The same study revealed that oral administration of HPβCD/Ang-(1-7) (30 μg/kg) significantly reduced the deleterious effects induced by myocardial infarction on systolic and diastolic tension, perfusion pressure, and heart rate (22).

Supplemental Methods

Animals - The experiment was conducted with twenty-four male FVB/N mice (four weeks old) from the State University of Montes Claros - Unimontes (Montes Claros, Minas Gerais, Brazil), and took place in accordance with the institution’s guide for the care and use of laboratory animals, with the approval of our internal animal ethics committee. The mice were individually housed and placed in an air-conditioned room (22 ± 2°C) with a 12 h light-dark cycle. After a 7 day adaptation period, the mice were randomly divided into three groups (n=8) and fed the following respective experimental diets for 8 weeks: High Fat Diet (HFD) plus HPβCD, Standard Diet (ST) plus HPβCD; High Fat Diet plus Angiotensin-(1-7)/HPβCD – 0,1mg/ kilogram (HFD+Ang-(1-7). The mice had free access to food and water during the experimental period.

Diets - Obesity was induced in male FVB/N mice by feeding a high-fat diet for 8 weeks. The diets were prepared according to the standards of the Association of Official Analytical Chemists as described previously (1,2).Diet macronutrients were weighed and mixed homogeneously to form a soft dough. The diet was stored separately in a
refrigerator in sealed plastic boxes. Standard diet (Purina - Labina®) used for regular maintenance of the mice is composed 50.30% of carbohydrate, 41.90% of protein and 7.80% of fat with a total of 2.18 kcal per 1g of diet. 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 1g of diet (2). All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, SP, Brazil).

**Measurements of body weight, food intake, and tissue collection** - The mice were individually housed and food intake was measured twice a week during treatment to obtain food efficiency (food intake/ body weight). Overnight fasted mice were killed by decapitation and samples of blood, adipose tissues (epidymal, mesenteric and retroperitoneal sites) and liver were collected, weighed, immediately frozen in dry ice and stored at -80ºC for subsequent analysis.

**Determination of Blood Measurements** - Serum was obtained after centrifugation (3200 rpm for 10 minutes at 4ºC). Total serum cholesterol, triglycerides, high-density protein (HDL) and aspartate and alanine transaminases were assayed using enzymatic kits (Wiener®, Argentina). Measurements were made in Wiener BT-3000 plus Chemistry Analyzer (Wiener®, Argentina).

**Reverse transcription and Real Time PCR** - Total RNA from the liver was prepared using TRIzol reagent (Invitrogen Corp.®, San Diego, California, USA), treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®) using random hexamer primers. Levels of ACE, ACE2, IL-1β, TNF-α, IL-6, ACC, TGF-β, ChREBP, PPAR-γ and SREBP-1c mRNA were determined by real-time PCR using SYBR Green reagent (Applied Biosystems®, USA) in a PlusOne platform (Applied Biosystems). Gene expression was normalized to the endogenous GAPDH. The relative comparative CT method of Livak and Schmittgen (3) was applied to compare gene expression levels between groups, using the equation $2^{-\Delta\Delta CT}$.

**Histology and oil red O staining**
Liver samples were fixed in 10% neutral-buffered formalin at 4ºC overnight, dehydrated through a graded alcohol series, xylene and paraffin, and then embedded in paraffin. Sections of 5 μm were prepared for H&E. For Oil Red O staining, liver tissues, which were frozen, were cut at 5 μm, mounted on slides and allowed to dry for 1-2 hrs. The sections were fixed with 10% formalin for 10 min and then the slides were rinsed with PBS (PH 7.4). After air dry, the slides were placed in 100% propylene glycol for 2 min, and stained in 0.5% Oil Red O solution in propylene glycol for 30 min. The slides were transferred to an 85% propylene glycol solution for 1 min., rinsed in distilled water for 2 changes, and processed for hematoxylin counter staining. Images (×40 objective lenses) were captured with Evolution LC Color light camera (MediaCybernetics®, USA).

**Immunohistochemical reactions** - For immunohistochemical reactions, 3 μm-thick sections were mounted on organosilane-coated slides. The primary mouse monoclonal antibodies against anti-IL-6 (clone sc-28343, Santa Cruz®, California, EUA) and TNF-α (clone ab66579, ABCAM®, Cambridge, UK) cytokines were detected with the aid of an LSAB™ kit (product # K0690; Dako®, Glostrup, Denmark) employing chromogen
diaminobenzidine for color development. Finally, slides were counterstained with Mayer's hematoxylin and were mounted. Negative controls were obtained by substituting normal whole rabbit serum (product # X0902; Dako®, Glostrup, Denmark) for the primary antibodies. Positive controls were applied according to manufacturer's instructions. Only cells that presented brown cytoplasmic staining were considered positive.

**Statistical analysis** - All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, California, USA) and analyzed with confidence 95% (p<0.05). Data are expressed as the mean ± SEM. The statistical significance of differences in mean values between mice groups was assessed by One-Way ANOVA followed by Bonferroni post-test.

**REFERENCES**


**Figure S1**

![Figure S1](image_url)

**Figure S1.** Effect of Ang-(1-7) on the body weight, total fat wt. and food intake in HFD-fed mice (A-C). (A) Body weight (n=8). (B) Food intake (g/body weight). (C)
White adipose tissue total weight (g/100g body weight). Control mice (white bars), mice fed a high fat diet (gray bars), or mice fed an Ang-(1-7)-supplemented high fat diet (HFD+Ang-(1-7); black bars) for 8 weeks. *P <0.05 between HFD when compared between and HFD+Ang-(1-7).

**Figure S2**

(A) CHREBP

(B) IL-1β

(C) TGF-β

Figure S2. Effects of 8-week Ang-(1-7) treatment on gene expression levels of lipid metabolism of the liver and renin-angiotensin system components in mice fed a high fat diet (A-C). (A) ChREBP (B) IL-1β. (C) TGF-β. Control mice (white bars), mice fed a high fat diet (gray bars), or mice fed an Ang-(1-7)-supplemented high fat diet (HFD+Ang-(1-7); black bars) for 8 weeks.
Figure S3

Figure S3. Food intake, body weight, body fat and liver weight (A-D). (A) Body weight (n=8). (B) Food intake (g/body weight). (C) Epididymal white adipose tissue weight (g/100g body weight). (D) Mesenteric white adipose tissue weight (g/100g body weight). (E) Retroperitoneal white adipose tissue weight (g/100g body weight). (F) Liver tissue total weight (g/100g body weight). Control mice (white bars), or mice fed an Ang-(1-7)-supplemented standard diet (ST+ANG-(1-7); black bars) for 6 weeks. There was no statistically significant difference between groups.
Figure S4

Figure S4. Plasmatic parameters (A-F). (A) Glucose. (B) Triglycerides. (C) Total cholesterol. (D) HDL. (E) ALT. (F) AST. Control mice (white bars), or mice fed an Ang-(1-7)-supplemented standard diet (ST+Ang-(1-7); black bars) for 6 weeks. There was no statistically significant difference between groups.