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Proteomic white adipose tissue analysis of obese mice fed with a high-fat diet and treated with oral angiotensin-(1–7)

João Marcus Oliveira Andrade[a,f,1], Fernanda de Oliveira Lemos[b,1], Simone da Fonseca Pires[c], Ruben Dario Sinisterra Millán[d], Frederico Barros de Sousa[d], André Luiz Sena Guimarães[a], Mahboob Qureshi[g], John David Feltenberger[g], Alfredo Maurício Batista de Paula[a], Jaime Tolentino Miranda Neto[h], Miriam Teresa Paz Lopes[b], Hélida Monteiro de Andrade[c], Robson Augusto Souza Santos[e], Sérgio Henrique Sousa Santos[a,b,*]

[a] *Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros, Minas Gerais, Brazil*
[b] *Pharmacology Department, Biological Sciences Institute (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*
[c] *Parasitology Department, Biological Sciences Institute (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*
[d] *Department of Chemistry, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*
[e] *National Institute of Science and Technology (INCT-NanoBiofar), Physiology Department, Biological Sciences Institute (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil*
[f] *Nursing Department, Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros, Minas Gerais, Brazil*
[g] *Touro University Nevada College of Osteopathic Medicine, Las Vegas, NV, United States*
[h] *Physical Training School, Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros, Minas Gerais, Brazil*

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ABSTRACT

Angiotensin-(1–7) has been described as a new potential therapeutic tool for the treatment and prevention of metabolic disorders by regulating several pathways in visceral white adipose tissue (vWAT). The aim of this study was to access the proteins differentially regulated by Ang-(1–7) using proteomic analysis of visceral adipose tissue. Male mice were divided into three groups and fed for 60 days, with each group receiving one of the following diets: standard diet + HPβCD (ST), high fat diet + HPβCD (HFD) and high fat diet + Ang-(1–7)/HPβCD (HFD + Ang-(1–7)). Body weight, fat weight and food intake were measured. At the end of treatment, Ang-(1–7) induced a decrease in body and fat weight. Differential proteomic analysis using two-dimensional electrophoresis (2-DE) combined with mass spectrometry were performed. Results of protein mapping of mesenteric adipose tissue using 2-DE revealed the presence of about 450 spots in each gel ($n = 3$/treatment) with great reproducibility (>70%). Image analysis and further statistical analysis allowed the detection and identification of eight proteins whose expression was modulated in response to HFD when compared to ST. Among these, two proteins showed a sensitive response to Ang-(1–7) treatment (eno1 and aldehyde dehydrogenase). In addition, three proteins were expressed statistically different between HFD + Ang-(1–7) and HFD groups, and four proteins were modulated compared to standard diet. In conclusion, comparative proteomic analysis of a mice model of diet-induced obesity allowed us to outline possible pathways involved in the response to Ang-(1–7), suggesting that Ang-(1–7) may be a useful tool for the treatment of metabolic disorders.

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Introduction

Obesity is a serious and growing world health problem. Current epidemiological estimates suggest that 1.1 billion people worldwide are above their ideal weight [20], which is characterized by an increase in white adipose tissue mass as result of an excess of food (energy) intake or altered energy expenditure [14]. Obesity has been recently described as both a systemic and local adipose

* Corresponding author at: Pharmacology Department, Federal University of Minas Gerais (UFMG), Av. Antonio Carlos 6627 – ICB, Belo Horizonte 31270-901, Minas Gerais, Brazil. Tel.: +55 31 3409 2712; fax: +55 31 3409 2712.
E-mail address: sergiosousas@hotmail.com (S.H.S. Santos).
[1] These two authors are contributed equally to this study.

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proinflammatory state, and is recognized as a cause of health issues, such as insulin resistance, diabetes, hyperlipidemia, hypertension, and cardiovascular disease [23,31,42]. Classic markers of the obesity-induced inflammatory state include augmented circulating and tissue levels of proinflammatory enzymes, procoagulant factors, cytokines, and chemokines [15,42].

Visceral white adipose tissue (vWAT) has been identified as an important organ that directly and indirectly modulates inflammation and metabolism through pathways in various organs, such as the brain, liver, muscle and vascular endothelial cells [30]. Therefore, inhibition of excess vWAT could be an efficient strategy for the prevention of obesity and metabolic disorders.

The renin–angiotensin system (RAS) is now recognized as an important in the development of cardiovascular and metabolic disorders [25,28,29]. Angiotensin II (Ang II), a major effector of RAS, is known as a vasoconstrictor, however, a recent study has shown its role as a potential mediator in the activation of inflammatory mechanisms involved in obesity [5,36]. On the other hand, the angiotensin converting enzyme 2 (ACE2)/angiotensin-(1–7) Ang-(1–7)/Mas axis has been suggested as an important counter-regulatory arm in the RAS with the opposite effects of ACE/Ang II/AT1 [25,27]. Ang-(1–7) exerts an important antiobesity role through the Mas receptor [27–29].

Recently, Rubio-Ruíz et al. found that the expression patterns of AT1 and AT2 receptors were significantly diminished and Mas expression increased with aging in the adipose tissue of rats with metabolic syndrome [24]. Additionally, Santos et al. [23] showed that Mas is expressed in adipose tissue and that Mas-deficient mice develop a metabolic syndrome-like state. In another study, Marcus et al. showed that chronic treatment with Ang-(1–7) for six months upregulated Mas expression in the epididymal fat of rats fed a high fructose diet [19].

Actually, the pharmacological effects of Ang-(1–7) were increased after the development of a new oral formulation characterized by a protected Ang-(1–7) molecule included in acyclic-oligosaccharides (cyclodextrin). This novel compound was denominated [hydroxypropylβ-cyclodextrin/Ang-(1–7) – HPβCD/Ang-[1–7)] [18]. Ang-(1–7), enclosed within this HPβCD, can be protected during the passage through the gastrointestinal tract after oral administration [11].

Recent studies indicated the important role of ACE2/Ang-(1–7)/Mas axis in metabolic functions in various organs and tissues. Santos et al. showed that Mas receptor deficiency in FVB/N mice induced dyslipidemia, lowered glucose tolerance and insulin sensitivity, decreased glucose uptake in white adipose cells, and an increase in adipose tissue mass [28]. Subsequent studies demonstrated improved lipid and glucose metabolism associated with decreased liver gluconeogenesis in transgenic rats with high-circulating Ang-(1–7) plasma levels [27]. In the last year, two new reports indicated an important role of the ACE2/Ang-(1–7)/Mas axis in the liver, suggesting that oral treatment with Ang-(1–7) induces improvement the status of steatohepatitis, as well as reduces adipogenesis-related markers [3,10,21,26].

Proteome analysis has recently been used in many studies of obesity, diabetes, aging and cancer [12,17,22,40]. The combination of MALDI-TOF/TOF Tandem Mass Spectrometry is a powerful method for the discovery of new biomarkers and pathways in a number of diseases. Nowadays, the profiling and discovery of novel biomarkers for diagnosis of disease-related obesity, as well as for accurate understanding of mechanisms and causes of metabolic disorders, are needed.

Thus, the aim of this study is to access the proteins differentially regulated by Ang-(1–7) using two-dimensional gel electrophoresis (2DE) associated with the approach of MALDI-TOF/TOF Tandem Mass Spectrometry in visceral white adipose tissue samples of the mice.

Methods and materials

Drug

Angiotensin-(1–7) associated with hydroxypropylβ-cyclodextrin [HPβCD]/Ang-(1–7). Was donated by Robson Santos (National Institute of Science and Technology – INCT-NanoBiofar). Daily dose (concentration of 100 μg/kg) [26].

Animals

The experiment was conducted with twenty-four male FVB/N mice (four weeks old) from the State University of Montes Claros (Montes Claros, Minas Gerais, Brazil). The mice were individually housed and placed in an air-conditioned room (22 ± 2 °C) with a 12 h light-dark cycle. After 7 days of the adaptation period, the mice were randomly divided into three groups ($n = 8$) and fed the following experimental diets for 8 weeks: G1: standard diet (ST); G2: high fat diet (HFD); G3: high fat diet plus angiotensin-(1–7) (HFD + Ang-(1–7)). The mice had free access to food and water during the experimental period.

All experimental procedures were approved by the Ethics Committee of the State University of Montes Claros for the care and use of the laboratory, and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual.

Diets

High-fat diet was prepared according to the protocols described previously [26], being composed of 24.0% carbohydrate, 15.0% protein and 61.0% fat, presenting a total of 5.28 kcal per 1 g of diet. Standard diet (Purina – Labina®), which was used for the regular maintenance of our mice, was composed of 66.0% carbohydrate, 23.0% protein and 11.0% fat presenting a total of 3.95 kcal per 1 g of diet [1,6]. All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, São Paulo, Brazil).

Measurements of body weight, food intake and tissue collection

The mice were individually housed and the food intake was measured daily during treatment in order to obtain food efficiency (food intake/body weight). Overnight fasted (12 h) mice were killed with Ketalar® (Pfizer Laboratório, São Paulo, Brazil) (Ketamine, 130 mg/kg) and Dorcipec® (Vallé S/A, Montes Claros, MG, Brazil) (0.3 mg/kg) after anesthesia. Samples of the mesenteric white adipose tissue were collected, weighed and immediately frozen in dry ice and stored at −80 °C for subsequent analysis.

Bidimensional electrophoresis

Two-dimensional gel electrophoresis (2-DE) using the IPG-DALT subsequent MALDI-TOF-TOF MS analyses were performed. Briefly, to extract total protein, frozen mesenteric mouse adipose tissue ($n = 8$) from ST, HFD, and HFD + Ang-(1–7) were homogenized overnight in extract buffer containing 8 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 65 mM dithiothreitol (DTT), 1 M Tris–HCl and 1% protease inhibitor cocktail (GE Healthcare®). Samples were centrifuged at 20,000 × *g* for 30 min, and the supernatants were collected. The protein content was determined using a 2-D Quant Kit (GE Healthcare®).

After removal of impurities with Cleanup kit (GE Healthcare), strips (Immobiline Dry Strip pH 3-10NL, 13 cm – GE Healthcare)

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