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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)

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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.

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

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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)

were passively rehydrated with approximately 300 μg of extracted proteins diluted in rehydration solution (8 M urea, 2 M thiourea, 4% (w/v) CHAPS, 65 mM DTT, 1 M Tris–HCl and trace bromophenol blue). First-dimensional isoelectric focusing was performed at 20 °C starting at 500 V with the voltage being gradually increased to 8000 V, totaling 45,000 Vh accumulations, using Ettan™ IPGphor™ 3 IEF power supply (GE Healthcare®) and IPGphor software (GE Healthcare®). After the electrofocusing procedure, the gel strips were equilibrated twice for 15 min shaking in 3 mL of equilibration buffer (6 M urea, 50 mM Tris–HCl pH 8.8, 30% (v/v) glycerol, 2% (w/v) SDS and 0.001% w/v bromophenol blue) containing 65 mM DTT or 135 mM IAA. In the second dimension SDS-PAGE, the equilibrated gel strips were placed on the top of 12% polyacrylamide gels, sealed with 0.1% (w/v) agarose and run at 50 mA/gel in SDS running buffer (25 mM Tris–HCl, 192 mM glycine and 0.1% (w/v) SDS) until the front dye reached the bottom of the gel (Ettan DALT six electrophoresis unit, GE Healthcare®). The gel was fixed with a sequence of steps (1–2% (v/v) orthophosphoric acid, 30% (v/v) ethanol; 2–2% (v/v) orthophosphoric acid and 3–2% (v/v) orthophosphoric acid, 18% (v/v) ethanol and 12% (w/v) ammonium sulfate). Finally, the gel was stained with Coomassie G-250 (0.08% (w/v)) for 5 days, when the background was destained with 20% ethanol (v/v) for image acquisition and analysis.

Image acquisition and analysis

The gels were scanned using an Amersham Image Scanner II (GE Healthcare®) and analyzed using ImageMaster 2D Platinum software (GE Healthcare®). These programs group images of the gels into classes containing biological triplicates of each condition. Initially, we evaluated the reproducibility of two-dimensional gels for each condition. The authenticity of each spot was evaluated by visual inspection and confirmed using the software, with the spots being excluded or added when necessary. The percentage volume of each spot was recorded and normalized according to the total intensity of the spots detected.

Tryptic digestion in polyacrylamide gel

The differentially expressed spots were excised from the gels and detained in a solution of 50% (v/v) acetonitrile/25 mM ammonium bicarbonate pH 8.0, with three washes of 15 min each, while shaking in a vortex mixer. The gels were then dehydrated in acetonitrile for 5 min and dried in a SpeedVac (Savant). The dehydrated fragments on the gels were digested at 37 °C overnight with trypsin (Promega cat. #V511) at 20 ng/mL in 25 mM ammonium bicarbonate. The resulting peptides were then extracted with 5% (v/v) formic acid for 30 min with constant stirring (vortex). Finally, the peptides were concentrated in a SpeedVac (Savant), to a final volume of 10 μL. The samples were passed through a Zip-Tip (C18 resin, p 1, Millipore Corporation, Bedford, MA) to remove salts. The column elution of peptides was performed in 50% (v/v) acetonitrile/trifluoro acetic acid (TFA) 0.1%. The samples were stored at −70 °C prior to further analysis by mass spectrometry.

Mass spectrometry

Time-of-flight mass spectrometry (TOF-MS) matrix-assisted laser desorption/ionization (MALDI) was conducted in a MALDI-TOF-TOF Autoflex III SmartBeam system (Bruker Daltonics®). Samples of peptides were mixed with twice the volume of a saturated solution of the matrix α-cyano-4-hydroxycinnamic acid (Sigma–Aldrich®) in 50% (v/v) acetonitrile/TFA 0.1% in a MALDI plate. MS mass spectra and MS/MS were acquired in reflector mode and internally calibrated with peptides cleaved by trypsin. Up to eight of the most intense ion signals were selected as precursors for the acquisition of MS/MS. The internal calibration mode MS was performed using a mixture of four peptides: des-Arg 1-Bradykinin (m/z = 904.468), angiotensin I (m/z = 1296.685), Glu1-fibrinopeptide B (m/z = 1570.677) and ACTH (18–39) (m/z = 2465.199). The spectrum MS/MS was externally calibrated using the fragmentation/mass known spectrum observed in MS/MS angiotensin I. The tandem mass spectrum was submitted to the non-redundant Swiss-Prot database using MASCOT software (http://www.matrixscience.com). The search parameters were as follows: no restrictions on molecular weight, all entries of origin, two wrong trypsin cleavages, variable modifications of methionine (oxidation), cysteine (carbamidomethylation), pyroglutamate and the formation of the N-terminal glutamine of peptides.

Statistical analysis

Data were expressed as the mean ± SEM. The statistical significance of differences in mean values between mice groups were assessed by one-way and the Bonferroni post test. Significance level was set at $P < 0.05$. Student's t-test was used to compare the percentage spot volume for each condition, and those that showed significant differences between the two classes were selected for identification by mass spectrometry.

Results

Effect of Ang-(1–7) on body and VAT weights

Ang-(1–7) treatment decreased body weight in HFD + Ang-(1–7) mice when compared with HFD during the period of treatment (Table 1). We did not observe significant alterations between groups when the food efficiency was evaluated (food intake/body weight) (Table 1). Analysis of mesenteric visceral adipose tissue (ST: 0.0129 ± 0.0039 g/g BW; HFD: 0.0198 ± 0.0031; HFD + Ang-(1–7): 0.0151 ± 0.0034) demonstrated a reduced fat composition in HFD + Ang-(1–7) (Table 1).

Proteomic analysis of vWAT

Results of protein mapping of mesenteric adipose tissue using 2-DE revealed that about 450 spots were observed in each gel (n = 3/treatment) with a great reproducibility (>70%) (Figs. 1 and 2). Image analysis and further statistical analysis allowed detection and identification of eight spots whose expression was modulated

Table 1
Effects of Ang-(1–7) on body and mesenteric WAT weight in mice fed with a high-fat diet.

Group	Final weight (g)	Initial weight (g)	Final weight (g) - Initial weight (g)	Food intake (g/day/BW)	Mesenteric fat weight (g/BW)
ST	32.12	21.77	10.35 ± 2.01**	0.172 ± 0.012	0.0052 ± 0.0031**
HFD	37.89	23.04	14.85 ± 1.87	0.181 ± 0.015	0.0073 ± 0.0054
HFD + Ang-(1–7)	34.67	23.15	11.52 ± 1.58*	0.179 ± 0.014	0.0058 ± 0.0028*

Standard diet group (ST). High-fat diet group (HFD). High-fat diet plus Ang-1–7 [HFD + Ang-(1–7)].
* $P < 0.05$ in comparison to the HFD group.
** $P < 0.01$ in comparison to the HFD group.
Data are mean ± SEM (n = 8 per group).

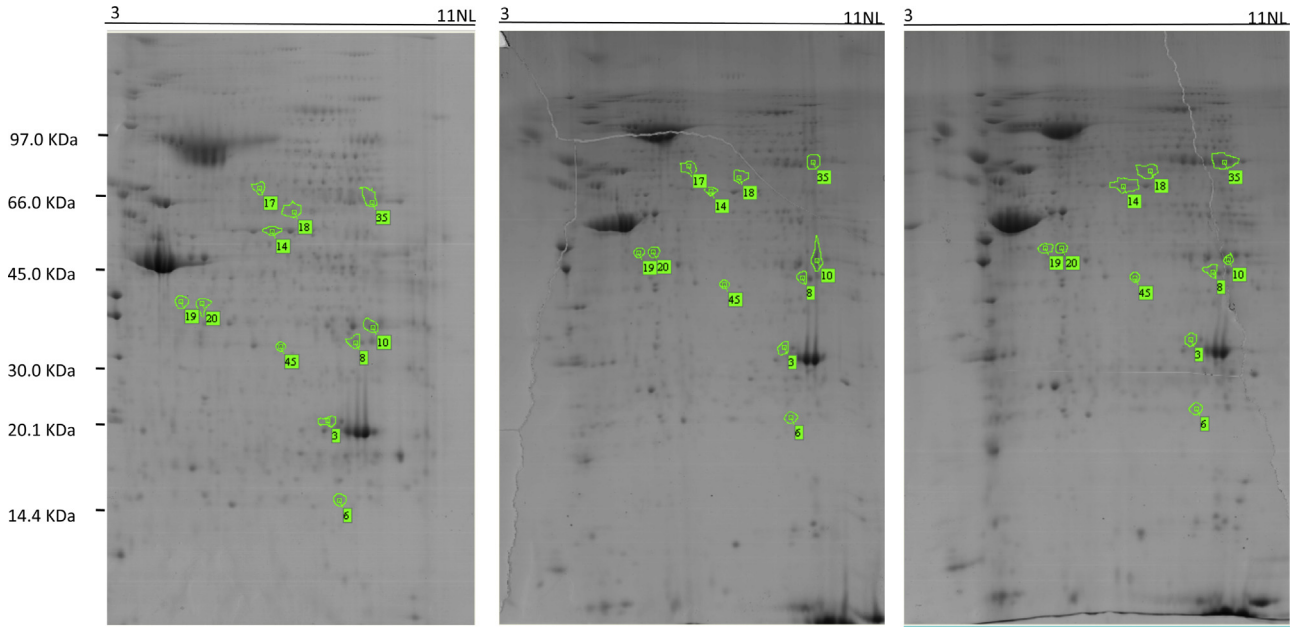

Fig. 1. Representative 2-DE gel images of Coomassie-stained proteins of VAT. Arrows indicate differentially regulated proteins. (A) Standard diet group (ST). (B) High-fat diet group (HFD). (C) High-fat diet plus Ang-1–7 [HFD + Ang-(1–7)]. IEF was performed with strips of 13 cm, pH 3–10 non-linear. The second dimension was performed on 12% polyacrylamide gel.

in response to HFD when compared to ST. Among those, two proteins showed a sensitive response to Ang-(1–7) treatment. Beside this, three proteins were expressed statistically different between Ang-(1–7), HFD and four proteins modulated by standard diet (Fig. 1).

In particular, protein levels of protein disulfide-isomerase A3 precursor, alpha-actin, carbonic anhydrase 2 and unnamed protein product were up-regulated after HFD feeding (Table 2), when compared to ST, while eno1 protein, aldehyde dehydrogenase, mitochondrial precursor, ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, isoform CRA e and cytosolic malate dehydrogenase were down-regulated.

To identify the effects of Ang-(1–7) in HFD-induced obese mice, we characterized the statistical difference between HFD and HFD + Ang-(1–7). Consequently, there were five differential proteins between these groups. Among them, expression levels of carbonic anhydrase 2, eno1 protein and aldehyde dehydrogenase mitochondrial precursor proteins, in HFD + Ang-(1–7) group were significantly increased and monoglyceride lipase, annexin A2 and cytosolic malate dehydrogenase were decreased compared with the HFD group ($P < 0.05$). The incremental difference of Eno1 protein and aldehyde dehydrogenase proteins was the most significant compared with other proteins (Table 2) because these same proteins were increased in the ST group in relation to the HFD group.

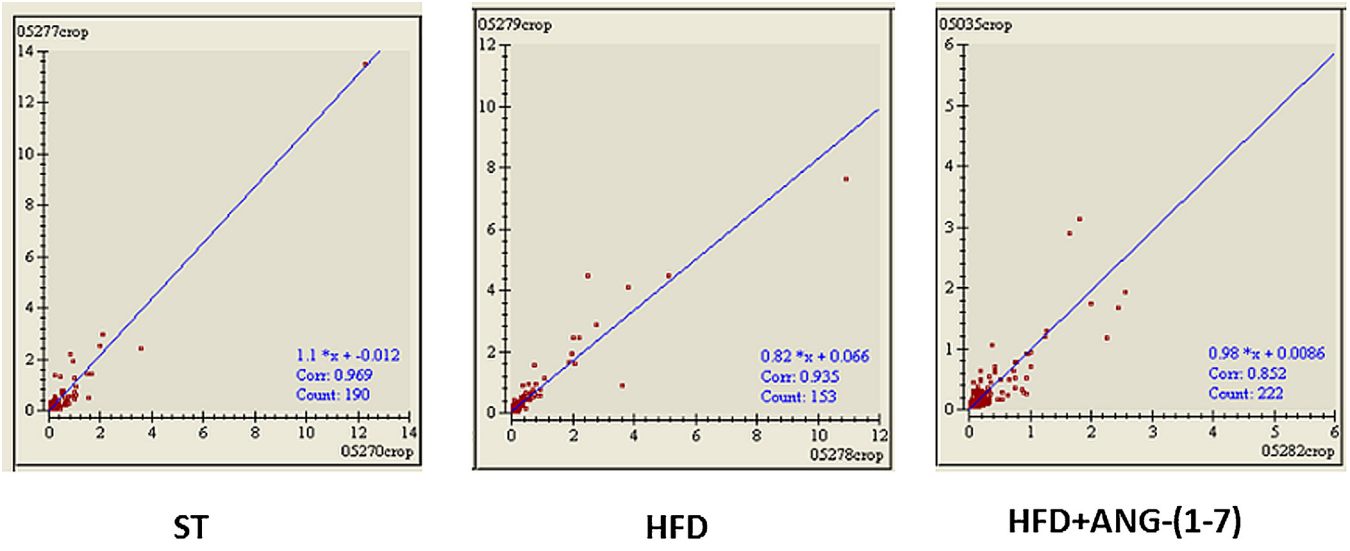

Fig. 2. The reproducibility of the gels of each experimental condition. Scatter plots show the high correlation between the volume percentage of the spots in the gels. (A) Standard diet group (ST). (B) High-fat diet group (HFD). (C) High-fat diet plus Ang-1–7 [HFD + Ang-(1–7)]. Correlation index (Corr.) = 1 indicates complete correlation between the gels.

Table 2
List of Identified proteins in visceral white adipose tissue of mice fed with standard diet (ST), high fat diet (HFD) and HFD + Ang-(1–7).

Spot ID	Protein	GI	p*I*	Mass Da	Coverage %	Mascot score	Abundance ($P < 0.05$)
3	Carbonicanhydrase 2	gi|33243954	6.52	29,138	11	102	HFD > HFD + Ang-(1–7) ST > HFD + Ang-(1–7)
6	Peroxiredoxin-1	gi|6754976	8.26	22,390	16	214	ST > HFD + Ang-(1–7)
8	Monoglyceride lipase isoform b Monoglyceride lipase isoform a	gi|6754690 gi|261878516	6.67 7.21	33,708 35,633	21 20	261 260	HFD > HFD + Ang-(1–7) ST > HFD + Ang-(1–7)
10	Annexin A2	gi|6996913	7.55	38,937	9	204	HFD > HFD + Ang-(1–7)
14	Eno1 protein	gi|34784434	5.86	40,099	17	219	ST > HFD HFD + Ang-(1–7) > HFD
17	Proteindisulfide-isomerase A3 precursor	gi|112293264	5.88	57,099	6	89	HFD > ST
18	Aldehydedehydrogenase, mitochondrial precursor	gi|6753036	7.53	57,015	5	122	ST > HFD HFD + Ang-(1–7) > HFD ST > HFD + Ang-(1–7)
19	Alpha-actin (aa 40–375)	gi|49864	5.45	38,016	6	97	HFD > ST
20	Unnamed protein product	gi|74191399	5.29	41,994	14	209	HFD > ST HFD + Ang-(1–7) > ST
35	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, isoform CRAe	gi|148677501	8.24	54,675	8	84	ST > HFD
45	cytosolicmalatedehydrogenase, partial	gi|387129	6.16	36,625	10	209	ST > HFD ST > HFD + Ang-(1–7)

GI: NCBInr database accession number. Coverage: percent of identified sequence to the complete sequence of the known protein. For each protein, the relative intensity was averaged and expressed as a mean (SEM of three separate experiments).

Three proteins (peroxiredoxin-1, monoglyceride lipase and aldehyde dehydrogenase, mitochondrial precursor) were down-regulated in the HFD + Ang-(1–7) when compared to ST group (Table 2). Only unnamed protein product was up-regulated in HFD + Ang-(1–7) (Table 2). However, protein disulfide-isomerase A3 precursor and alpha-actin, which were increased in ST group when compared with HFD, did not show differences between ST and HFD + Ang-(1–7) group. In the same way, the expression of ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1, isoform CRA e and cytosolic malate dehydrogenase were modified when compared between ST and HFD + Ang-(1–7), unlike when observed between HFD and ST groups.

Discussion

The present study analyzed the antiobesity effect of Ang-(1–7) on HFD-fed mice through proteomic analysis of visceral WAT. The most striking results from our proteomic study showed global changes in causative proteins associated with obesity; three of these proteins were examined for the first time for their regulatory actions in regard to obesity: carbonic anhydrase 2, monoglyceride lipase and annexin A2.

In our study, we observed an increase in carbonic anhydrases (CAs) expression in HFD group in comparison with Ang-(1–7) mice. CAs are ubiquitous metalloenzymes and include at least five distinct CA families (a–e). The primary function of CA is to catalyze the reversible hydration of carbon dioxide to form bicarbonate and protons. In mammals, 16 isozymes have been identified showing a difference in catalytic activity, subcellular localization and tissue distribution [33].

CAs regulate many functions in homeostasis, such as pH regulation, ion transport, bone resorption and secretion of gastric, cerebrospinal fluid and pancreatic juices [34]. The most important function of CA is related to the respiration and transport of CO_2/bicarbonate in various metabolizing tissues. This enzyme is also involved in electrolyte secretion, CO_2 and pH homeostasis, CO_2 fixation and biosynthetic reactions such as gluconeogenesis and ureagenesis [32]. Additionally, many CAs, like CA 2, have been drug targets for a long time for the treatment of obesity [7,8,35]. Thus, CA inhibitors may provide a novel therapy for obesity.

Recently, the role of CAs was indicated in diabetes [9,39,41]. Studies have been shown CAs to play a major role in diabetic microangiopathy and diabetic cardiomyopathy [39]. Torella et al. showed that CA 2 is activated by high glucose levels and directly induces cardiomyocyte hypertrophy and death *in vitro*. CA 2 was shown to be a direct target for repression by microRNA-23b, which was downregulated in myocardial samples from diabetes mellitus type 2 patients. MicroRNA-23b is regulated by p38 mitogen-activated protein kinase, and it modulates high-glucose CA 2-dependent effects on cardiomyocyte survival *in vitro* [39].

Other important protein involved in metabolic regulation is monoglyceride lipase (MGL), which was down-regulated in the HFD + Ang-(1–7) group. Studies showed increases in monoglyceride lipase (MGL) expression and activity in mice fed with a high fat diet, thus suggesting a potential role for MGL in dietary lipid assimilation [4]. Another study showed that monoglyceride lipase influences energy metabolism through at least two mechanisms. First, it hydrolyzes monoacylglycerols (MG) into fatty acids and glycerol, which can be used for energy production or synthetic reactions. Second, MGL degrades 2-arachidonoyl glycerol (2-AG), the most abundant endogenous ligand of cannabinoid receptors (CBR). Activation of CBR affects energy homeostasis by central orexigenic stimuli by promoting lipid storage and reduction of energy expenditure [38]. These results demonstrate that MGL deficiency in mice reduces lipolysis and VLDL synthesis and attenuates high fat diet-induced insulin resistance [38].

The last protein analyzed, is a member of annexin family. Annexins are calcium-dependent phospholipid binding proteins that are more divergent in function than structure [13]. This protein family is comprised of at least 20 members that share a highly conserved and tightly packed α-helical core domain, as well as calcium and phospholipid binding domains [13].

Annexin A2 is unique among the annexins and it is known to function largely in a heterotetramer form, composed of two 36-kDa subunits of annexin A2 (p36) complexed with two 11-kDa S100A10 protein subunits (p11). This protein is highly expressed on the surface of macrophages, where it enjoys promiscuous binding with several proteins, thus participating in many facets of macrophage biology. Additionally, it was also demonstrated that annexin 2 plays a crucial role in the phagocytosis of apoptotic

lymphocytes, an event at the end of an immune response, which results in the reduction of inflammation by the release of immunosuppressive cytokines [37]. However, evidence is beginning to accumulate regarding to the role of soluble, extracellular annexins in inflammation and the immune response, which may not be that simple.

Recently, it was shown that annexin 2 acts by activating ERK MAP kinases in stromal cells [16]. MAP kinases are key regulators of macrophage activation [2]. It is reasonable to assume that annexin 2 might stimulate this pathway, and thus, cells shedding annexin 2 could capitalize on this additional function as an inflammatory mediator. Additionally, Swisher et al. showed that annexin 2 activates macrophages, stimulating MAP kinase and NF-κB signaling pathways and induces the production of three key cytokines involved in the establishment of inflammation: TNF-α, IL-1β, and IL-6. These important but potentially damaging inflammatory mediators taper off quickly [37].

Conclusion

In conclusion, comparative proteome analysis of the mice model of diet-induced obesity allowed us to outline possible pathways involved in the response to Ang-(1–7). Proteins identified here are involved in cellular functions that include lipid metabolism, redox processes, and signal and energy transduction. Some of these have already been linked to obesity and its associated disorders; suggesting that the newly identified proteins might also have importance in obesity, and thus require further investigation. These changes provide valuable new molecular insights into the mechanism of the antiobesity effects of Ang-(1–7). Thus, we believe that the findings presented in this paper will lead to new insights into the understanding of this pathology as well as potential treatments.

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