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Decreased Myocardial Type 1 Collagen in Obese Rats is Associated with Increased of Leptin and Metalloproteinase-2 And -9 Activity

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Abstract

Background: Obesity is a risk factor for many medical complications. The adipocytes are influenced by several substances and secrete numerous peptides that act directly or indirectly on the cardiovascular system.

Objective: The purpose of this study was to test the hypothesis that the reduction in collagen type I is associated with increased activity of MMP-2/9 which in turn is linked to the elevation of leptin in obese rat's myocardium.

Methods: Thirty-day-old male Wistar rats were randomized into two groups: control and obese. The control group was fed a standard diet and the obese group was fed a high-fat diet for 30 weeks. Obesity was characterized by the adiposity index. The animal general characteristics and metabolic profile were evaluated. Myocardial type I collagen, leptin and Tissue Inhibitors of Metalloproteinases (TIMPs) protein expression; MMP-2 and -9 activities. The Pearson's correlation test was employed to determine the associations between variables.

Results: The obese animals had an increased adiposity index compared to control. Comorbidities associated with experimental obesity, such as glucose intolerance, hyperinsulinemia, hyperleptinemia and hypertension, were observed. Obesity promoted a reduction in type I collagen, TIMPs 1 and 2 protein levels, and increased leptin protein levels and activity of MMP-2 and -9 in the myocardium. There was a positive correlation between type I collagen and MMP-2 and MMP-9 and between leptin and both MMPs, all performed in the heart.

Conclusion: In conclusion, the hypothesis of this study was confirmed, as the reduction in collagen type I is associated with increased activity of MMP-2/9 which in turn is linked to the elevation of leptin in obese rats myocardium.

Keywords: Heart; Leptin; MMP-2 and 9; Obesity; Type I Collagen

Introduction

Obesity is a chronic metabolic disorder characterized by excessive accumulation of adipose tissue in relation to lean tissue. Currently, it is a global epidemic and a major public health problem that affects both developed and developing countries [1,2]. The adipocytes are influenced by several substances and secrete numerous peptides that act directly or indirectly on the cardiovascular system. Therefore, adipose tissue is not simply an energy reservoir, but also an active endocrine, paracrine and autocrine organ with multiple functions, including the ability to synthesize and release mediators that participate in many biological processes, including those that occur in the heart [3]. The heart is composed of myocytes, nerves, vessels and Extracellular Matrix (ECM). The main component of the ECM is collagen, predominantly type I and III, with type I being the most abundant, corresponding to approximately 80% of total myocardial collagen [4]. This protein, in a stable condition, contributes to the maintenance of cardiac architecture and function [5], produced by fibroblasts and degraded by the family of Matrix Metalloproteinases (MMPs) [6]. Several mechanisms act to ensure that the components of matrix degradation by MMPs are precisely controlled, including Tissue Inhibitor of Metalloproteinases (TIMPs) [7]. Collagen, in response to stimuli triggered by neuro-hormonal and/or mechanical agents, may change [5,8], due to increased synthesis and/or decreased degradation or vice versa.

Several studies have analyzed the expression of collagen type I and/or III in different tissues in experimental models of obesity [9-11]. There is limited information on the behavior of these types of collagen in the heart in obese animals; Carroll et al. showed increase in myocardial type I and III collagen in obese rabbits fed a high-fat diet for 12 weeks [12]. In contrast, Silva et al. [13] found decreased protein levels of myocardial type I collagen in obese Wistar rats fed an unsaturated high-fat diet for 30 weeks and found no changes in the levels of collagen type III, However, these authors did not study the mechanisms responsible for the decrease in myocardial type I collagen.

One of the possible factors involved is the increase of leptin hormone, secreted by the adipose tissue, which is involved in the myocardial type I collagen regulation [6,14-16]. Supporting such hypothesis, most studies, in vitro, have shown that leptin increases MMP-2 activity [6,15,16] and MMP-9 gene expression [6,17] involved in the degradation of type I collagen. On the other hand, Martínez-Martínez et al. [18] and Zibadi et al. [14] have found that leptin reduced the MMP-2 activity and MMP-9 gene expression, respectively, both *in vitro* research. The purpose of this study was to test the hypothesis, suggested by Silva et al. [13], that the reduction in type I collagen is associated with increased MMP-2/9 activity 103 which in turn is linked to the elevation of leptin in obese rat's myocardium.

Materials and Methods

Animal and Experimental Protocol

After a 7-day period for acclimatization, 30-day-old male *Wistar* rats were randomly assigned to one of two groups: control (C; n=20) and obese (Ob; n=21). The C group was fed a standard rat chow (RC Focus 1765, Agroceres®, Rio Claro, SP, Brazil) containing 12.3% of kilocalories from fat, 57.9% from carbohydrates, and 29.8% from protein, whereas the Ob group were fed with one of four alternating high-fat diets (RC Focus 2413, 2414, 2415, and 2416, Agroceres®, Rio Claro, SP, Brazil) containing 49.2% of kilocalories from fat, 28.9% from carbohydrates, and 21.9% from protein. The four high-fat diets had the same nutritional composition, with the exception of flavoring additives, cheese, bacon, chocolate or vanilla. Each diet was changed daily, and the rats were maintained on their respective diets for 34 consecutive weeks. The high-fat diets were calorically rich compared to the standard diet (3.65 kcal/g vs. 2.95 146 kcal/g) due to the higher fat composition. The high-fat diet consisted of saturated and unsaturated fatty acids, which provided 20% and 80% of the fat-derived calories, respectively. Rats were housed in individual cages in an environmentally-controlled clean-air room at 23(±3) ˚C with a 12-hour light/dark cycle and 60(±5) % relative humidity. All experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Research Council (1996) [19] and were approved by the Botucatu Medical School Ethics Committee (UNESP, Botucatu, SP, Brazil)- (Protocol: 861-2011).

Animal General Characteristics, Metabolic and Endocrine Profiles

Animal general characteristics, metabolic and endocrine profiles included adiposity index, Final Body Weight (FBW), glucose tolerance, serum leptin and insulin concentrations. Since obesity is defined as an excessive amount of body fat in relation to lean mass [20], a criterion based on the adiposity index was used to determine obesity, according to data from earlier studies [21]. After fasting for 12 to 15 hours, animals were anesthetized (using intraperitoneal sodium pentobarbital 50 mg/kg), decapitated, and thoracotomized; the fat pads of adipose tissue were dissected and weighed. The adiposity index was calculated by the following formula: adiposity index = (body fat [BF]/FBW) × 100 [22].

Body fat was calculated as the sum the weight of the individual fat pads as follows: BF= epididymal fat + retroperitoneal fat + visceral fat. Serum triacylglycerol, total cholesterol, high and low-density lipoproteins, protein and albumin concentrations

were determined by specific kits (BIOCLIN®, Belo Horizonte, MG, Brasil).Since obesity can be accompanied by metabolic and endocrine disturbances [23], all animals underwent testing for glucose tolerance, leptinemia and insulinemia. After 15 and 30 weeks of treatment, glucose tolerance and insulin resistance were evaluated in all animals using a Glucose Tolerance Test (GTT). After a 4-to 6-hour fast, a blood sample was taken from the tip of the animal's tail and collected in a heparinized tube. The basal blood glucose concentration of each animal was immediately determined using a handheld glucometer (Accuchek Advantage; Roche Diagnostics Co., Indianapolis, IN, 177 USA). Subsequently, 2 g/kg of glucose (Sigma-Aldrich®, St Louis, MO, USA) was 178 given intraperitoneal and blood glucose concentrations were measured after 15, 30, 60, 90 and 120 minutes. Glucose intolerance was evaluated using the Area Under the Curve (AUC) for glucose.

For hormonal analysis, trunk blood was collected in heparinized tubes and centrifuged at 3000g for 15 minutes at 4°C. Serum leptin and insulin concentrations were determined by Enzyme-Linked Immunosorbant Assay (ELISA), (Chemistry Analyzer BS-200, Mindray Medical International Limited, Shenzhen, China), using commercially available kits (EMD Millipore Corporation, Billerica, MA, USA) and the read by micro-plate reader (Spectra MAX, Molecular Devics, Sunnyvale, CA, USA).

Cardiovascular Profile

The cardiovascular profile of the animals was also assessed, using systolic blood pressure, cardiac tissue morphology, left ventricular collagen types I, TIMP 1 e 2 protein expression and MMP-2 and 9 activities.

Systolic Blood Pressure

At the end of the experiment, the systolic blood pressure was assessed by using the non-invasive tail-cuff method with a Narco BioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA) [24]. The average of two readings was recorded for each measurement.

Morphological Studies

The heart was removed and dissected at the time of euthanasia. The atrio, left and right ventricles weights as well as their respective relations with the final body weight were determined as indexes of cardiac remodeling.

Myocardial Collagen Types I, TIMP-1, TIMP-2 and Leptin Protein Levels

Left ventricular tissue was analyzed by Western Blot to quantify collagen types I and III protein levels. Briefly, ventricles isolated from control (C; n= 6 each group) and obese (Ob; n= 6 each group) rats were frozen with liquid nitrogen and homogenized in a buffer containing 10 mM Tris (pH 7.4), 100 Mm NaCl, 1 mM EDTA, 1 Mm EGTA, 1% Triton X-100, 10% glycerol, 0.1% Sodium Dodecyl Sulfate (SDS), and 0.5% 208 deoxycholate. The homogenate was centrifuged at 4°C for 20 minutes at 12000 rpm. The supernatant was collected and total protein content was determined by the Bradford Method (Bradford 1976). Samples were subjected to SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) in polyacrylamide gels (6% or 10% depending on protein 212 molecular weight). After electrophoresis, proteins were electro-transferred to nitrocellulose membrane (BioRad Biosciences; NJ, USC). Sample weights (50 μg) and transfers were monitored for equality and efficiency, respectively, with the use of 0.5% Ponceau S staining of the blot membrane. The blotted membrane was then blocked (using 5% nonfat dry milk, 10 mmol/L Tris-HCl [pH 7.6], 150 mmol/L NaCl, and 0.1% 217 Tween 20) for 2 hours at room temperature and incubated with specific antibodies overnight at 4°C. Binding of the primary antibody was detected with the use of peroxidase-conjugated secondary antibodies (anti-rabbit or anti-mouse depending on the protein at a 1:10000 dilution and incubated for 1.5 hours at room temperature), developed by enhanced chemiluminescence (Amersham Biosciences, NJ, USA) and detected by autoradiography. Quantification analysis of the blots was performed with use of Scion Image software (Scion, based on NIH Image). Mouse monoclonal antibodies to collagen types I (1:10000), TIMP 2 (1:1000), leptin (1:1000), and rabbit monoclonal antibodies to TIMP 1 (1:1000) and β-actin (1:1000) were obtained from Abcam (Cambridge, USA) and CELL SIGNALING (Danvers, USA), respectively. Targeted bands were normalized to the expression of cardiac β-actin.

Myocardial Metalloproteinases 2 and 9 Activities

Myocardial MMPs activities was determined as reported by Tyagi et al. [25]. In brief, left ventricular tissue from control (C; n= 6 each group) and obese (Ob; n= 6 each group) rats were homogenized in a buffer containing (Tris 50mM, pH 7,4, NaCl 0.2M, Triton-X 0,1% e CaCl2 10mM). The homogenate was centrifuged at 4°C for 20 minutes 233 at 12000 rpm. The supernatant was collected, and total protein content was determined by the Bradford Method (Bradford 1976). Samples were diluted in application sample buffer consisting of 0.5 M Tris, pH 6.8, 100% glycerol, and 0.05% bromophenol blue. The samples were loaded into the wells of 8% SDS-polyacrylamide containing 1% gelatin. Electrophoresis was carried out in a Bio-Rad apparatus at 80 V for 2 hours, until 238 the bromophenol blue reaches the bottom of the gel. The gel was removed and washed 2 times with 2.5% Triton-X-100 and then washed with 50 mM Tris pH 8.4. The gel was then incubated at 37°C overnight in activation solution consisting of 50 mM Tris pH 8.4, 5 mM CaCl2 and Zn Cl2. The staining was performed for 2 hours with 0.5% coomassie blue, and destaining was performed in 30% methanol and 10% acetic acid until clear bands over a dark background were observed. Staining and destaining were performed at room temperature on a rotatory shaker. The gels

were photographed, and the intensity of gelatinolytic action (clear bands) was analyzed in UVP, UV, and a White Darkhoni mage analyzer.

Statistical Analysis

All results were expressed as mean ± standard deviation and subjected to the Student's t- test for independent samples by Sigma Stat program. The normality test was the Kolmogorov Smirnov Normality Test. The associations between certain variables were performed by Pearson's correlation test. The level of significance considered was 5 % (α = 0.05).

Results

Animal General Characteristics

The animal general characteristics are displayed in (Table 1).

Groups			
Variables	**C (n=20)**	**Ob (n=21)**	***p value***
IBW (g)	151 ± 11	151 ± 11	0.290
FBW (g)	480 ± 51	534 ± 58	0.009
Epididymal (g)	9.3 ± 2.3	14.2 ± 3.4	4.0E-6
Epididyma l/100gFBW	1.9 ± 0.5	2.7 ± 0.6	1.6E-4
Retroperitoneal (g)	10.5 ± 3.3	21.7 ± 5.9	5.8E-9
Retroperitonea l/100gFBW	2.2 ± 0.7	4.1 ± 1.1	1.0E-7
Visceral (g)	6.3 ± 1.4	11.2 ± 4.2	1.6E-5
Viscera l/100gFBW	1.3 ± 0.3	2.1 ± 0.8	1.4E-4
BF (g)	26.1 ± 6.2	47.2 ± 12.3	3.2E-8
Adiposity index	5.6 ± 0.9	8.8 ± 1.6	3.3E-7
Food consumption (g/day)	22.8 ± 2.1	17.0 ± 2.3	2.0E-10
Caloric intake (kcal)	67.4 ± 6.3	62.1 ± 8.2	0.03
Feeding efficiency (%)	2.1 ± 0.2	2.7 ± 0.2	2.5E-10
Fasting glucose (g/dL)	0.09 ± 0.01	0.09 ± 0.01	0.82
Triglycerides (g/dL)	0.11 ± 0.04	0.10 ± 0.04	0.35
HDL (g/dL)	0.02 ± 0.002	0.03 ± 0.003	0.42
LDL (g/dL)	0.03 ± 0.005	0.03 ± 0.003	0.46
Protein (g/dL)	0.006 ± 0.0004	0.006 ± 0.0005	0.25
Albumin (g/dL)	0.003 ± 0.0001	0.003 ± 0.0002	0.28

Values are means ± SD. C: control; Ob: obese. IBW: initial body weight; FBW: final body weight; BF: body fat; HDL: high-density lipoprotein; LDL: low density lipoprotein. Student's t-test for independent samples.

Table 1: Animal general characteristics.

The final body weight, deposits of epididymal, retroperitoneal and visceral fat, total body fat and adiposity index were significantly higher in the Ob group compared to the C group ($p<0.05$). The animals in the Ob group ingested less amount of food and calories compared to C, however the feed efficiency was higher in Ob animals. There was no significant difference between groups in all serum measurements.

Metabolic and Endocrine Profiles

(Figure 1) shows that there was a significant increase in the glucose Area Under the Curve (AUC) and insulin and leptin levels in Ob animals compared to C animals.

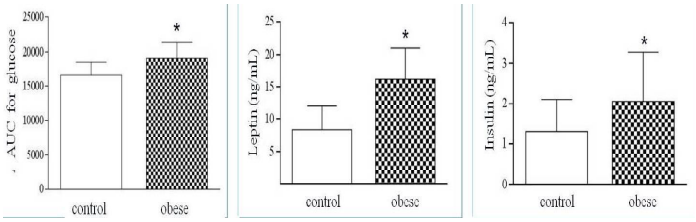

Figure 1: Area Under Curve (AUC) of intraperitoneal glucose tolerance test**,** leptin and insulin in control and obese rats. Data are presented as mean ± SD; Student's t-test. * p=0,0006; # p=8,5E-6; & p=0,002.

Cardiovascular Profile

Cardiac Morphological Profile and Systolic Blood Pressure: (Table 2) shows that systolic blood pressure was significantly higher in Ob animals compared to C animals.

Groups			
Variables	**C (n=20)**	**Ob (n=21)**	***p value***
Heart (g)	1.10 ± 0.10	1.17 ± 0.13	0.06
LV/FBW. 10^{-3}	1.72 ± 0.11	1.71 ± 0.12	0.44
RV/FBW. 10^{-3}	0.48 ± 0.09	0.47 ± 0.05	0.64
AT/FBW. 10^{-3}	0.20 ± 0.03	0.18 ± 0.03	0.14
SBP	127 ± 11	134 ± 12	0.04
Values are means ± SD. C: control; Ob: obese. LV: left ventricle weight; RV: right ventricle weight; AT: atrio; FBW: final body weight; LVW/FBW; RVW/FBW; AT/FBW ratio; SBP: systolic blood pressure; 10^{-3} = 0,001. Student's t-test for independent samples.			

Table 2: Cardiac morphological profile and systolic blood pressure

Myocardial Collagen Type I, TIMP-1, TIMP-2 and Leptin Protein Levels: (Figure 2) shows that obesity promoted a significant reduction in collagen type I and TIMP-1 and -2 protein levels, however there was a significant increase in cardiac leptin protein in the Ob group compared with the C group.

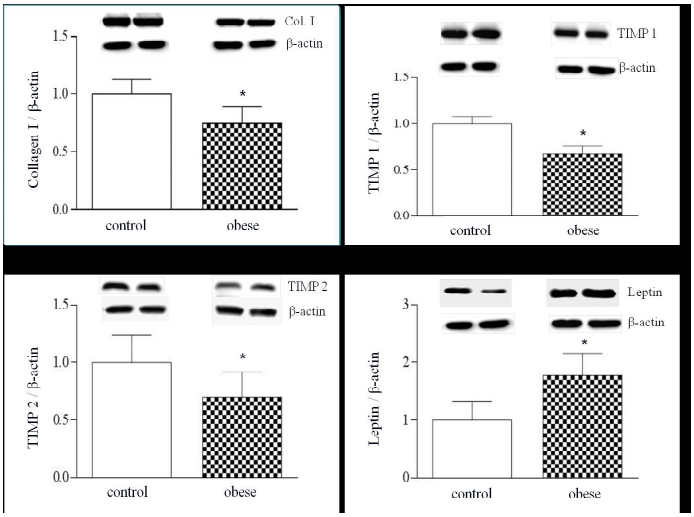

Figure 2. Western bolt analysis of collagen type I**,** TIMP-1 and -2 and leptin protein expression in the heart of control and obese rats. Group control, n=06; group obese, n=06. Blots were scanned. Histography presentation of collagen type I/β-actin, TIMP-1/β-actin, TIMP-2/β-actin and leptin/β-actin ratios. Data are present as mean ± SD from six different animals per group; Student's t-test for independent samples. * p=0,002; # p=1,7E-5; & p=0,033; ** p=0,003.

Myocardial MMP-2 and -9 activities: (Figure 3) shows in electrophoresis gel identification of two bands of degradation corresponding to MMP-2: inactive MMP-2 (pro-MMP-2) with a molecular weight of approximately 72 kDa and active MMP-2 with a molecular weight of approximately 64 kDa. Between the two bands mentioned, it was possible to identify the band of MMP-2 intermediate degradation. Only the active form of MMP-9 was visualized with a molecular weight of approximately 92 kDa. There was a significant increase of both MMPs in the Ob animals.

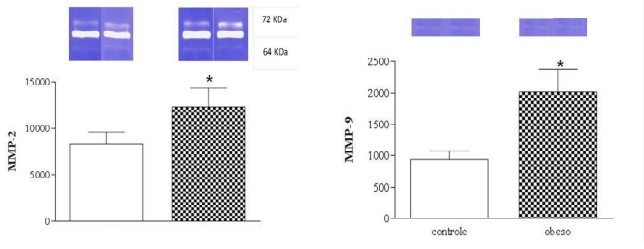

Figure 3. Relation between active MMP-2 (active and intermediate active) and inactive (A) and MMP-9 activity (B) of control (n = 6) and obese rats (n = 6). Inactive MMP-2 = 72 kDa and active MMP-2 = 64 kDa. Data expressed as mean ± standard deviation. Student "t" test for independent samples. * p=0,002; # p=0,001.

Linear Association Between Cardiac Variables: (Table 3) shows that there was a significant correlation between the decrease in type I collagen and increased activity of MMP-2 and -9 and also between the elevation of MMP-2 and -9 activity and leptin. There was also a correlation between the increase in MMP-2 and the decrease in TIMP-1 and -2, increased MMP-9 and the reduction in TIMP-1 and finally decreased TIMP-1 and increased leptin protein levels. There was no significant correlation between increased MMP-9 and decreased TIMP-2 and the decreases in TIMP -2 and increased leptin.

Association	Coefficient of correlation	p value
Collagen I X MMP-2	- 0.723	0.008
Collagen I X MMP-9	- 0.660	0.019
MMP-2 X Leptin	0.766	0.004
MMP-9 X Leptin	0.804	0.002
MMP-2 X TIMP 1	- 0.815	0.001
MMP-2 X TIMP 2	- 0.597	0.040
MMP-9 X TIMP 1	- 0.779	0.003
MMP-9 X TIMP 2	- 0.344	0.274
TIMP 1 X Leptin	- 0.656	0.020
TIMP 2 X Leptin	- 0.273	0.390
MMP: Metalloproteinase; TIMP: Tissue Inhibitor of Metalloproteinases. Pearson's correlation test. Control, n=6 and Obese, n=6.		

Table 3: Linear association between cardiac variables.

Discussion

The high-fat diet in this study, rich in unsaturated fatty acids was effective on promote obesity because the total body and fat weights and adiposity index of obese animals were higher than controls, and these data are in agreement with some authors [26,27].

The main causes of obesity are larger food offerings, the larger intake of energy dense and palatable food and/or reduction in energy expenditure. The high-fat diet used in the current study was rich in mono- and polyunsaturated fatty acids with an energy content of 3.65 kcal/g, while the standard diet fed to the control group consisted of 2.95kcal/g, generating a difference of 24% in caloric content. Research has shown that consumption of a high-fat diet promotes less satiety and thus increased food intake [28]. These data differ from our results because the Ob animal ate smaller amount of food and calories compared to C. However, feed efficiency was higher in Ob rats due to the thermic effect of food.

Lipids require low amounts of energy (2-3%) to be metabolized and therefore lipids not required for energy production are deposited in the form of triglycerides in adipocytes [29] resulting in obesity.

Several studies have reported some comorbidities related to experimental obesity [28-30], such as glucose intolerance, hyperinsulinemia, hyperleptinemia and arterial hypertension. The glucose tolerance test in the present study showed that obese animals exhibited glucose AUC and serum insulin levels higher than controls, indicating that obesity promoted glucose intolerance and hyperinsulinemia. Insulin has a fundamental role in the regulation of glucose and lipid metabolism [31], increasing the synthesis and attenuating the release of triglycerides from fat cells [32]. Glucose intolerance, associated with increased serum insulin, indicated that OB animals in the current study suffered from resistance to the action of insulin. These results are in accordance with previous reports of rats fed a high-fat unsaturated diet [13,26,27,33]. In the present study, in disagreement with previous research [34], there was no indication of dyslipidemia. Leptin is a hormone produced by adipose tissue, participating in energy balance, ultimately by regulating food intake and the oxidation of lipids [35] and the biology of collagens [6,14-16]. Several studies have shown that leptin levels positively correlate with the amount of body fat [35]. The induced obesity as a result of a high-fat diet promotes a state of leptin resistance [35]. Studies have shown that this resistance is due to the inability of leptin to act in the hypothalamus as a result of injury in the transport of the blood-brain barrier.

Furthermore, altering the leptin molecular signaling pathways, resulting in hyperleptinemia can also induce resistance to this hormone [36]. In terms of the effect of obesity on the cardiovascular system, obesity did not promote cardiac remodeling. There was however, an increase in SBP in the obese animals. Since the SBP control involves the neurohumoral system, such as the sympathetic nervous system and the renin-angiotensin-aldosterone which are increased in obesity [37], it may be inferred that the neurohumoral system is activated in obese animals. These findings are consistent with previous researchers who investigated obese animals fed a high-fat diet with a balance of saturated and unsaturated fatty acids [38], however in disagreement with authors who reported no significant change in SBP in obese animals fed an unsaturated high-fat diet [27].

The main objective of this study was to investigate if the increased MMP-2 and MMP-9 activity, by leptin, is responsible for the reduction in myocardial type I collagen in obese rats. The results of this investigation indicated that there was reduction of protein levels of type I collagen and that, was accompanied by increase in leptin protein levels and in MMP-2 and -9 activities, and also decrease in TIMP-1 and -2 protein levels in the heart. As indicated above, few studies evaluated the behavior of type I collagen in obese animal's myocardium by high-fat diet; while Carroll [12] and Martínez-Martínez [32] found increased, Silva13 found decreased in myocardial type I collagen.

The changes in myocardial collagen may result from elevation of the synthesis and/or decreased degradation of myocardial collagen. The data from this study showed that should have prevailed the degradation of type I collagen in the obese rats, whereas there was a significant association between the reduction of type I collagen and the increase in MMP-2 and -9 activities. Although there are studies which show that the increase in MMP-2 activity enhance collagen synthesis [39], most information in the literature show the opposite behavior, the increase in MMP-2 activity promotes the degradations of type I collagen [6,32,40]. This elevation of MMP-2/-9 may have been consequent to cardiac leptin increase because there was a close association between these two variables, although these findings do not necessarily reflect a cause and effect ratio. Nevertheless, several studies have reported a direct relationship between leptin, MMP-2 activity [6,15,16] in cardiomyocytes and MMP-9 gene expression in cardiomyocytes [38], bone marrow, vessel wall and different human cells [16,17].

Although increased MMP activity is associated with elevated cardiac leptin hormone, another modifying factor of this enzyme is the behavior of TIMPs. The results of the current study showed a decrease of TIMP-1/-2 protein levels in obese animals, which may have influenced the increase of MMP-2/-9 as there was a significant association between MMP-2 and TIMP-1/-2, and MMP-9 and TIMP-1. The decrease of TIMP-1 may be related the increase in leptin as there was a significant association between these variables. This finding is consistent with Schram et al. who found a significant reduction in TIMP-1 mRNA expression after the elevation of leptin concentrations in cultured cardiac cells [15]. We did not find studies that evaluated the association between type I collagen, leptin, MMP-2/-9 and TIMP-1/-2 in myocardium of obese animals fed an unsaturated high-fat diet.

One limitation of this study is to have an associative character, which does not allow claim a cause and effect ratio. Therefore, future work is required *in vitro* to confirm these findings, using blockade and/or stimulation of leptin and MMPs.

Conclusion

In conclusion, the hypothesis of this study was confirmed, as the reduction in type I collagen is associated with increased MMP-2/-9 activity which in turn is linked to elevation of leptin in obese rat's myocardium. This study allows evaluate the mediators involved in cardiac remodeling, which can trigger a damage of heart function in obese patients. The identification of these deleterious factors may enable a possible therapeutic action.

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Conflict of Interest

None.

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