Influence of Term of Exposure to High-Fat Diet-Induced Obesity on Myocardial Collagen Type I and III

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Abstract

Background: Obesity is a risk factor for many medical complications; medical research has shown that hemodynamic, morphological and functional abnormalities are correlated with the duration and severity of obesity.

Objective: Present study determined the influence of term of exposure to high-fat diet-induced obesity on myocardial collagen type I and III.

Methods: Thirty-day-old male Wistar rats were randomly distributed into two groups: a control (C) group fed a standard rat chow and an obese (Ob) group alternately fed one of four palatable high-fat diets. Each diet was changed daily, and the rats were maintained on their respective diets for 15 (C_{15} and Ob_{15}) and 30 (C_{30} and Ob_{30}) consecutive weeks. Obesity was determined by adiposity index.

Results: The Ob_{15} group was similar to the C_{15} group regarding the expression of myocardial collagen type I; however, expression in the Ob_{30} group was less than C_{30} group. The time of exposure to obesity was associated with a reduction in collagen type I in Ob_{30} when compared with Ob_{15}. Obesity did not affect collagen type III expression.

Conclusion: This study showed that the time of exposure to obesity for 30 weeks induced by unsaturated high-fat diet caused a reduction in myocardial collagen type I expression in the obese rats. However, no effect was seen on myocardial collagen type III expression. (Arq Bras Cardiol. 2014; 102(2):157-164)

Keywords: Obesity; High-fat diet; Collagen Type I; Collagen Type III; Myocardium.

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 developed as well as developing countries[1,2]. Behaviors associated with a modern industrialized society, including a sedentary lifestyle, inadequate eating habits or a combination of both, have led to an increasing prevalence of obesity[3].

Obesity is also considered a risk factor for many medical complications, among them cardiovascular diseases[4,5]. Hemodynamic changes associated with hormonal ones alter myocardial gene expression, promoting myocardial extracellular matrix remodeling[6]. Studies using either rabbits made obese by a high-fat diet[7] or by genetic engineering, Zucker rats[8], have reported an increase in myocardial collagen types I and III over 12 and 24 weeks, respectively. In contrast, Carroll et al[9], found no change in the fraction of total collagen in obese rats subjected to a high-fat diet for 12 weeks. Previous studies carried out in our laboratory showed that *Wistar* rats made obese by a 15-week high fat diet[10] and *Wistar-Kyoto* rats made obese by 20-week high fat and carbohydrate diet[11] showed increased myocardial total collagen. In these studies, however, the fractions of collagen types I and III were not evaluated.

Medical research has shown that hemodynamic, morphological and functional abnormalities are correlated with the duration and severity of obesity[12-14]. Because of the scarcity of studies that have evaluated the influence of time of exposure to high fat diet-induced obesity on the fractions of myocardial collagen types I and III, this current study was designed to test the hypothesis that the time of exposure to obesity promotes a progressive increase in the amount of type I and type III myocardial collagen.

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Methods

Animals 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) and obese (Ob). 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 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. Each diet was changed daily, and the rats were maintained on their respective diets for 15 (C_{15} and Ob_{15}; n = 22) and 30 (C_{30} and Ob_{30}; n = 25) consecutive weeks. The high-fat diet was calorically rich compared to the standard diet (3.65 kcal/g vs. 2.95 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) and were approved by the Faculdade de Medicina de Botucatu Ethics Committee (UNESP, Botucatu, SP, Brazil).

Nutritional, metabolic and endocrine profiles

Nutritional and metabolic profiles included adiposity index, final body weight (FBW), and glucose tolerance; endocrine profiles included leptin and insulin concentrations. As obesity is defined as an excessive amount of body fat in relation to lean mass[15], a criterion based on the adiposity index was used to determine obesity, according to data from earlier studies[9,16].

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[17]. Body fat was calculated as the sum the weight of the individual fat pads as follows: BF = epididymal fat + retroperitoneal fat + visceral fat.

As obesity can be accompanied by metabolic and endocrine disturbances[18], 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 through the 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 blood glucose (as the basal condition) concentration of each animal was immediately determined using a handheld glucometer (Accuchek Advantage; Roche Diagnostics Co., Indianapolis, IN, USA). Subsequently, 2 g/kg of glucose (Sigma-Aldrich®, St Louis, MO, USA) was given intravenously and blood glucose concentrations were measured after 15, 30, 60, 90, and 120 minutes[19]. 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 3000 g for 15 minutes at 4°C. Serum leptin and insulin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Linco Research Inc., St. Louis, MO, USA).

Cardiovascular profile

As obesity can be associated with cardiovascular comorbidities, the cardiovascular profile of the animals was also assessed, using systolic blood pressure, cardiac tissue morphology and left ventricular collagen types I and III protein expression.

Systolic blood pressure

At the end of the experiment, the systolic blood pressure was assessed using the non-invasive tail-cuff method with a Narco BioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA)[20]. The average of two readings was recorded for each measurement.

Morphological studies

The heart was removed and dissected at the time of euthanasia. LV weights, as well as their respective ratios with the tibia were determined as indexes of cardiac remodeling.

Myocardial collagen types I and III protein expression

Left ventricular tissue was analyzed by Western Blot[7] to quantify collagen types I and III protein expression. Briefly, ventricles isolated from control (C_{15} and C_{30}; n = 6 each group) and obese (Ob_{15} and Ob_{30}; 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% 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 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% 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 and III (1:10000) and mouse monoclonal antibodies to β-actin (1:1000) were obtained from Abcam (UK, Cambridge) and Santa Cruz Biotechnology (CA, USA), respectively. Targeted bands were normalized to the expression of cardiac β-actin.

Statistical analysis

All results were reported as mean ± standard deviation and groups were evaluated using two-way analysis of variance (ANOVA) for independent samples. When significant differences were found ($p < 0.05$), Bonferroni *post hoc* test for multiple comparisons was carried out[21]. The level of significance considered was 5% ($\alpha = 0.05$).

Results

Figure 1 shows the general characteristics of animals in groups C_{15}, C_{30}, Ob_{15} and Ob_{30}. The high-fat diet caused an increase in FBW and in the index of adiposity in obese animals compared to respective controls at all times evaluated. The duration of exposure to obesity did not result in a significant difference in FBW and adiposity index between the C_{15} and C_{30} groups and between the Ob_{15} and Ob_{30} groups. There was no difference in systolic blood pressure between the C and Ob groups in the two study periods and that did not change with the time of exposure to obesity. The glucose AUC was higher in Ob groups compared with controls; the time of exposure did not affect glucose levels. Obesity promoted an increase in insulin and leptin levels in Ob groups compared to respective controls. Insulin levels did not change with the time of exposure to obesity; leptin levels were higher in the Ob_{30} group compared with the Ob_{15} groups.

As shown in Figure 2, the presence of obesity increased the weight of the left ventricle and as well as LV/tibia length ratio in the Ob_{15} group as compared to the C_{15} group. However, the LV/tibia length ratio was similar in the Ob_{30} and C_{30} groups, suggesting that exposure to obesity did not have any effect on this variable.

There was no difference in myocardial type I collagen expression between the groups Ob_{15} and C_{15} (Figure 3); however, protein expression was lower in the Ob_{30} group compared with the C_{30} group. The time of exposure to obesity also resulted in a reduction of collagen type I expression in the Ob_{30} group as compared with the Ob_{15} group. Obesity did not alter collagen type III expression, and the time of exposure to obesity did not influence expression of this collagen.

Discussion

The main finding of this study was that the time of exposure to obesity induced by an unsaturated high-fat diet affected the expression of myocardial collagen type I, but had no effect on myocardial collagen type III. Obesity that is experimentally induced by diet most closely resembles obesity found in the human population and it has been frequently used to reproduce possible morphological, molecular, biochemical and functional changes in different organs of the human body[9,16,22].

The high-calorie diet used in this experiment contained enough calories and was of sufficient duration to promote obesity in rats; it was likely due to the high unsaturated fat content of the diet, which has a higher energy density and greater storage capacity in the body. This study showed that the final body weight and the index of adiposity of obese animals were increased as compared to respective controls at all times evaluated, and these data are consistent with the literature[22]. However, the time of exposure to the diet did not alter these two variables, which were similar in the two time periods studied - 15 and 30 weeks.

The glucose load in the obese groups resulted in glucose intolerance during the two treatment periods, 15 and 30 weeks. Another important aspect is that time of exposure to obesity did not influence the glycemic profile between obese animals, as glucose intolerance seen in the first 15 weeks remained stable until the 30th week of the experimental protocol. Impaired glucose tolerance, an intermediate stage between normal glucose homeostasis and type 2 diabetes mellitus[23], may be related to the development of insulin resistance in obese animals. In support of this assumption, our data show that obesity led to hyperinsulinemia at both evaluations. The results of this study are in agreement with authors who observed that obesity induced by a diet high in unsaturated fat promotes glucose intolerance, hyperinsulinemia and insulin resistance in a short period of time[24-27] and these effects are prolonged[28].

An increase in leptin serum concentrations after 15 and 30 weeks was seen in obese animals compared with their controls. The time of exposure to obesity influenced the level of this hormone, with increases seen from 15 weeks onward. Leptin concentrations are correlated with body fat, thereby influencing both lipogenesis and lipolysis[29]. In this study, although the index of adiposity remained the same in the Ob_{15} and Ob_{30} groups, leptin concentrations were higher in the Ob_{30} compared with the Ob_{15}. This fact was likely due to the emergence of resistance to the action of this hormone after the 30th week. Other studies have shown that long periods of obesity promote leptin resistance, which consequently leads to an increase in this hormone[14,24,30].

Although the increase in adipose tissue led to metabolic and hormonal alterations, obesity did not result in an increase in blood pressure after 15 and 30 weeks, which remained stable over the two periods studied. The mechanisms responsible for altering blood pressure include hyperactivity of the sympathetic nervous system[31], increased activity of the renin-angiotensin-aldosterone system (RAAS)[16,32,33] and oxidative stress[31], which can result in peripheral vasoconstriction and increased renal sodium reabsorption. The lack of effect on blood pressure suggests that obesity does not alter those factors involved in blood pressure control. These results are in agreement with some authors who observed no change in blood pressure in obese

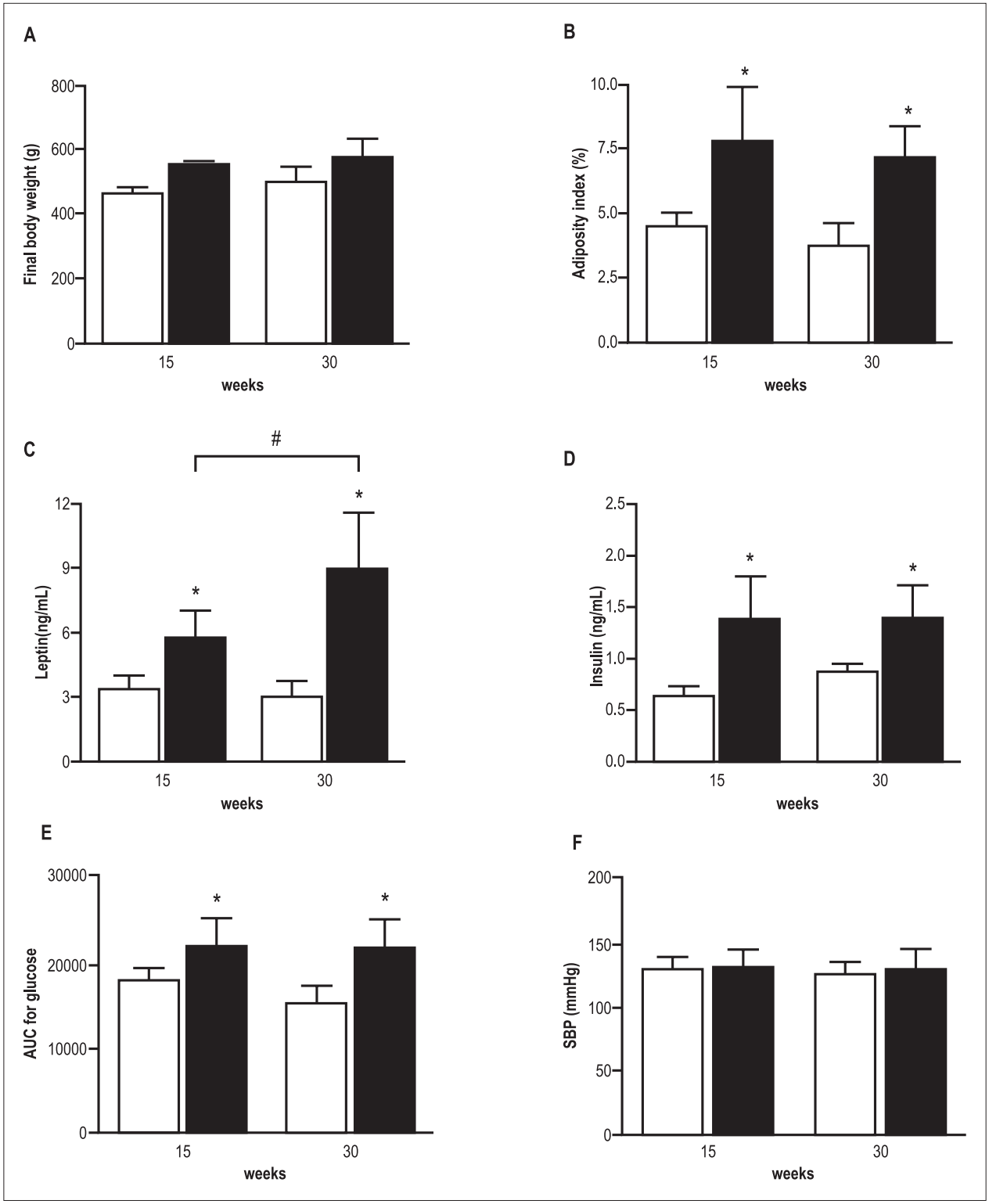

Figure 1 - *Final body weight (A), adiposity index (B), leptin (C), insulin (D), area under curve (AUC) of intraperitoneal glucose tolerance test (E), systolic blood pressure (SBP) (F) in control (white bars) and obese rats (black bars) after 15 and 30 weeks of treatment. Date are mean ± SD; two-way ANOVA and Bonferroni post hoc test. *p < 0.05 vs control group; # p < 0,05 Ob_{15} vs Ob_{30}*

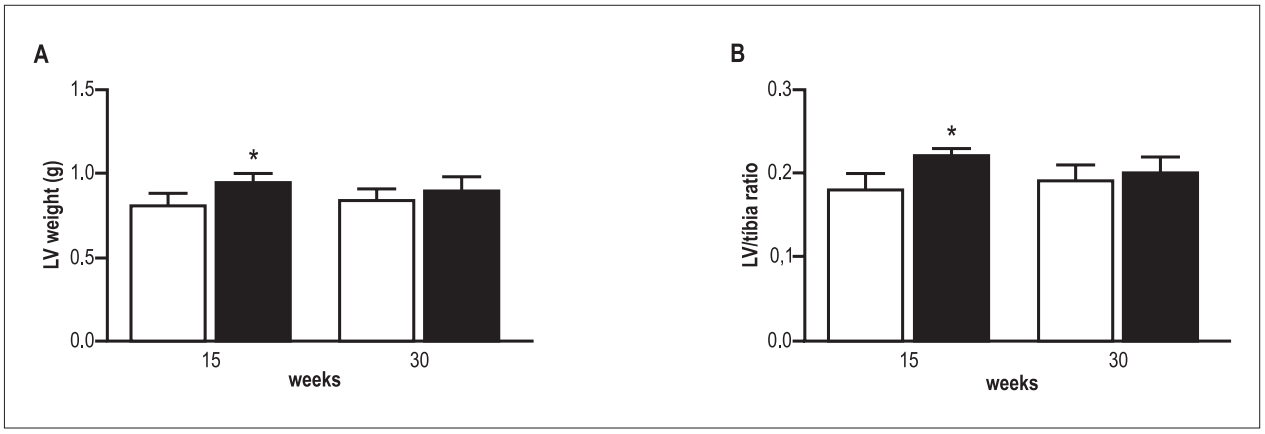

Figure 2 - *Left ventricle dimensions. Left ventricle (LV) weight (A), and left ventricle weight/tibia ratio (B) in control (white bars) and obese rats (black bars) after 15 and 30 weeks of treatment. Data are mean ± SD; two-way ANOVA and Bonferroni post hoc test. *p < 0.05 vs control group.*

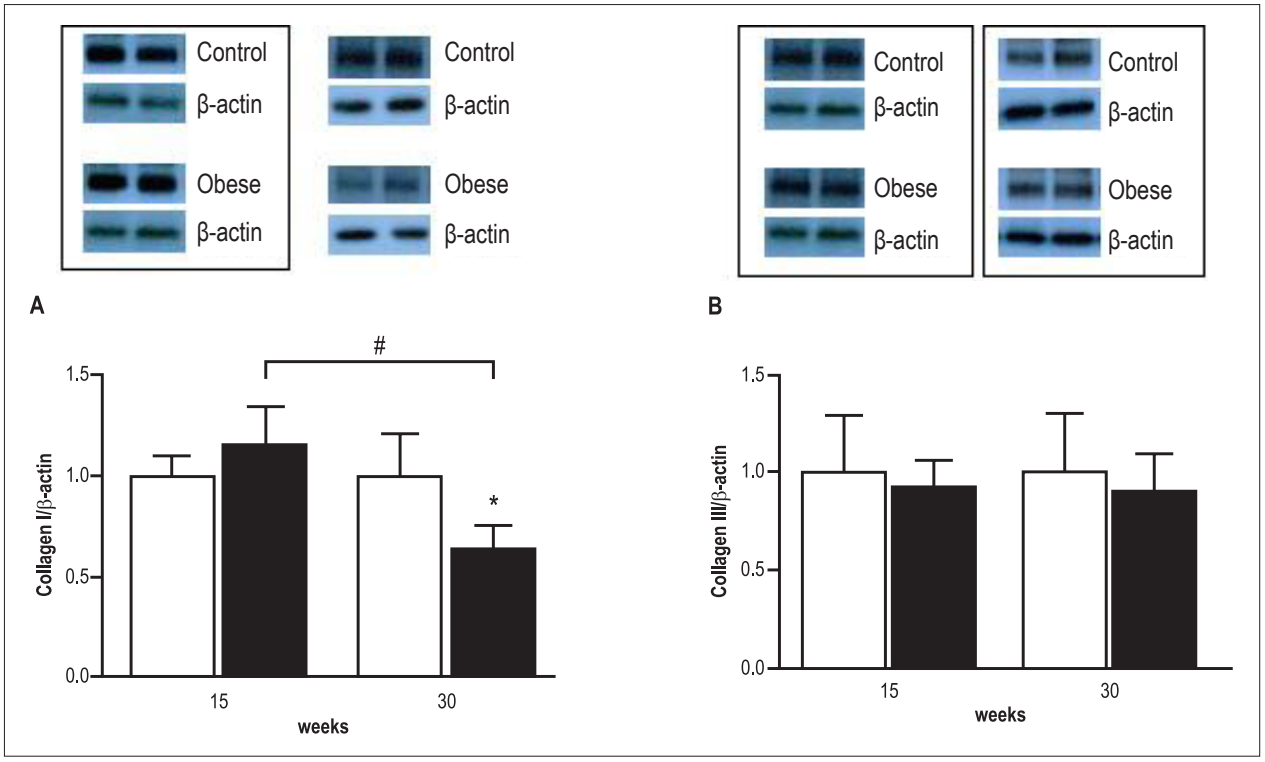

Figure 3 - *Westem bolt analysis of collagen I and III in the heart of control (white bars) and obese rats (black bars) after 15 and 30 weeks of treatment. Blots were scanned. Histographic presentation of collagen I/β-actin (A) and collagen III/β-actin ratios. (B). Data are mean ± SD from different animals per group; two-way ANOVA and Bonferroni post hoc test. *p < 0.05 vc control group; # p < 0,05 Ob_{15} vs Ob_{30}.*

animals[9]; but differ from studies that showed elevated levels of this parameter[32-34]. However, although obesity did not alter blood pressure, there was a slight remodeling of the left ventricle, probably due to an increase in the neurohormonal factors mentioned above.

The most important finding of this study was that the time of exposure to obesity caused a reduction in myocardial collagen type I expression in the Ob_{30} group compared with the Ob_{15} group and, in contrast, did not modify the expression of myocardial collagen type III. We found no studies that investigated the association between the duration of obesity and expression of myocardial collagen types I and III. The mechanisms responsible for the decrease in collagen type I expression remain unclear. A possible explanation for this reduction may be due to the decrease in synthesis and/or an increase in degradation of collagen. Adipose tissue secretes several substances that are involved in the regulation

of myocardial collagen, including the hormone leptin, which is produced mainly by adipocytes and is also synthesized by various tissues including the heart[35]. The effects of leptin on the heart have not been fully elucidated, but it has been thought to influence cardiomyocyte hypertrophy and regulate production of various components of the extracellular matrix of the myocardium that act on cardiac fibroblasts[35]. Although there is controversy about the association between leptin and myocardial collagen type I, such as increased expression of procollagen[35-37] and decreased synthesis[38], there is an agreement among authors that leptin increases the activity of metalloproteinases (MMP-2) 2[35-38] and mRNA expression of MMP-9[39,40], participants in myocardial collagen type I degradation. Therefore, it is possible that increased activity of both MMP-2 and MMP-9 is responsible for the reduction in myocardial collagen type I. As stated above, myocardial collagen type III did not change in obesity. No information was found in the literature as a possible explanation for this finding.

Importantly, our finding may have clinical relevance, as it shows that long-term obesity, common in patients, not accompanied by arterial hypertension, can cause a decrease in ventricular compliance due to the reduction in collagen type I. This phenomenon may result in a better adaptation of the heart to obesity because this pathology is associated with increased blood volume.

In conclusion, this study shows that the time of exposure to obesity induced by an unsaturated high-fat diet causes a reduction in myocardial collagen type I expression after 30 weeks, but no changes were seen in myocardial collagen type III. Future studies are needed to determine the mechanism responsible for the decrease in myocardial collagen type I and the lack of effect on collagen type III.

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Author contributions

Conception and design of the research: da Silva DCT, Padovani CR, Cicogna AC; Acquisition of data: da Silva DCT, Lima-Leopoldo AP, Leopoldo AS, de Campos DHS; Analysis and interpretation of the data: da Silva DCT, Lima-Leopoldo AP, Leopoldo AS, de Campos DHS, Nascimento AF, Oliveira Junior SA, Padovani CR, Cicogna AC; Statistical analysis: da Silva DCT, Padovani CR; Obtaining funding: Cicogna AC; Writing of the manuscript: da Silva DCT; Critical revision of the manuscript for intellectual content: da Silva DCT, Lima-Leopoldo AP, Nascimento AF, Oliveira Junior SA, Cicogna AC.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

References

1. Eckel RH, Barouch WW, Ershow AG. Report of the National Heart, Lung, and Blood Institute-National of Diabetes and Digestive and Kidney Diseases Working Group on the pathophysiology of obesity-associated cardiovascular disease. Circulation. 2002;105(24):2923-8.
2. Wong CY, O' Moore-Sullivan T, Leano R, Byrne N, Beller E, Marwick TH. Alterations of left ventricular myocardial characteristics associated with obesity. Circulation. 2004;110(19):3081-7.
3. Stein CJ, Colditz GA. The epidemic of obesity. J Clin Endocrinol Metab. 2004;89(6):2522-5.
4. Malnick SD, Knobler H. The medical complications of obesity. QJM. 2006;99(9):565-79.
5. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer X, et al; American Heart Association; Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2006;113(6):898-918.
6. Galinier M, Pathak A, Roncalli J, Massabuau P. [Obesity and cardiac failure]. Arch Mal Coeur Vaiss. 2005;98(1):39-45.
7. Carroll JF, Tyagi SC. Extracellular matrix remodelling in the heart of the homocysteinemic obese rabbit. Am J Hypertens. 2005;18(5 Pt 1):692-8.
8. Toblli JE, Cao G, DeRosa G, Forcada P. Reduced cardiac expression of plasminogen activator inhibitor 1 and transforming growth factor b1 in obese Zucker rats by perindopril. Heart. 2005;91(1):80-6.
9. Carroll JF, Zenebe WJ, Strange TB. Cardiovascular function in a rat model of diet-induced obesity. Hypertension. 2006;48(1):65-72.
10. Leopoldo AS, Sugizaki MM, Lima-Leopoldo AP, do Nascimento AF, Luvizotto Rde A, de Campos DH, et al. Cardiac remodeling in a rat model of diet-induced obesity. Can J Cardiol. 2010;26(8):423-9.
11. Oliveira SA Jr, Okoshi K, Lima-Leopoldo AP, Leopoldo AS, Campos DH, Martinez PF, et al. Nutritional and cardiovascular profiles of normotensive and hypertensive rats kept on a high fat diet. Arq Bras Cardiol. 2009;93(5):526-33.
12. Alpert MA. Obesity cardiomyopathy: pathophysiology and evolution of the clinical syndrome. Am J Med Sci. 2001;321(4):225-36.

13. Scaglione R, Dichiara A, Indovina A, Lipari R, Ganguzza A, Parrinello G, et al. Left ventricular diastolic and systolic function in normotensive obese subjects: influence of degree and duration of obesity. Eur Heart J. 1992;13(6):738-42.

14. Nascimento AF, Luvizotto RA, Leopoldo AS, Lima-Leopoldo AP, Seiva FR, Justulin LA Jr, et al. Long-term high-fat diet-induced obesity decreases the cardiac leptin receptor without apparent lipotoxicity. Life Sci. 2011;88(23-24):1031-8.

15. Nascimento TB, Baptista Rde F, Pereira PC, Campos DH, Leopoldo AS, Leopoldo AP, et al. Vascular alterations in high-fat diet-obese rats: role of endothelial L-arginine/NO pathway. Arq Bras Cardiol. 2011;97(1):40-5.

16. Boustany-Kari CM, Gong M, Akers WS, Guo Z, Cassis LA. Enhanced vascular contractility and diminished coronary artery flow in rats made hypertensive from diet-induced obesity. Int J Obes (Lond). 2007;31(11):1652-9.

17. Taylor BA, Phillips SJ. Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. Genomics. 1996;34(3):389-98.

18. Kopelman PG. Obesity as a medical problem. Nature. 2000;404(6778):635-43.

19. Ouwens DM, Boer C, Fodor M, de Galan P, Heine RJ, Maassen JA, et al. Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. Diabetologia. 2005;48(6):1229-37.

20. Pfeffer JM, Pfeffer MA, Frohlich ED. Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. J Lab Clin Med. 1971;78(6):957-62.

21. Bayley BJ. Tables of the Bonferroni "t" statistic. J Am Stat Assoc. 1977;72:469-78.

22. Ghibaudi L, Cook J, Farley C, van Heek M, Hwa JJ. Fat intake affects adiposity, comorbidity factors, and energy metabolism of sprague-dawley rats. Obes Res. 2002;10(9):956-63.

23. Pereira LO, de Francischi RP, Lancha Jr AH. Obesidade: hábitos nutricionais, sedentarismo e resistência à insulina. Arq Bras Endocrinol Metab. 2003;47(2):111-27.

24. Woods SC, Seeley RJ, Rushing PA, D'Alessio DA, Tso P. A controlled high-fat diet induces an obese syndrome in rats. J Nutr. 2003;133(4):1081-7.

25. Huang BW, Chiang MT, Yao HT, Chiang W. The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. Diabetes Obes Metab. 2004;6(2):120-6.

26. Li L, Yang G, Li Q, Tang Y, Li K. High-fat- and Lipid-induced insulin resistance in rats: the comparison of glucose metabolism, plasm resistin and adiponectin levels. Ann Nutr Metab. 2006;50(6):499-505.

27. Winzell MS, Ahrén B. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes. 2004;53 Suppl 3:S215-9.

28. Nivoit P, Morens C, Van Assche FA, Jansen E, Poston L, Remacle C, et al. Established diet-induced obesity in female rats leads to offspring hyperphagia, adiposity and insulin resistance. Diabetologia. 2009;52(6):1133-42.

29. Ainslie DA, Proietto J, Fam BC, Thorburn AW. Short-term, high-fat diets lower circulating leptin concentrations in rats. Am J Clin Nutr. 2000;71(2):438-42.

30. Ren J, Zhu BH, Relling DP, Esberg LB, Ceylan-Isik AF. High-fat diet-induced obesity leads to resistance to leptin-induced cardiomyocyte contractile response. Obesity (Silver Spring). 2008;16(11):2417-23.

31. Pausova Z. From big fat cells to high blood pressure: a pathway to obesity-associated hypertension. Curr Opin Nephrol Hypertens. 2006;15(2):173-8.

32. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. Am J Physiol Regul Integr Comp Physiol. 2004;287(4):R943-9.

33. Smith AD, Brands MW, Wang MH, Dorrance AM. Obesity-induced hypertension develops in young rats independently of the renin-angiotensin-aldosterone system. Exp Biol Med. 2006;231(3):282-7.

34. Dobrian AD, Davies MJ, Prewitt RL, Lauterio TJ. Development of hypertension in a rat model of diet-induced obesity. Hypertension. 2000;35(4):1009-15.

35. Schram K, De Girolamo S, Madani S, Munoz D, Thong F, Sweeney G. Leptin regulates MMP-2, TIMP-1 and collagen synthesis via p38 MAPK in HL-1 murine cardiomyocytes. Cell Mol Biol Lett. 2010;15(4):551-63.

36. Schram K, Wong MM, Palanivel R, No EK, Dixon IM, Sweeney G. Increased expression and cell surface localization of MT1-MMP plays a role in stimulation of MMP-2 activity by leptin in neonatal rat cardiac myofibroblasts. J Mol Cell Cardiol. 2008;44(5):874-81.

37. Madani S, De Girolamo S, Muñoz DM, Li RK, Sweeney G. Direct effects of leptin on size and extracellular matrix components of human pediatric ventricular myocytes. Cardiovasc Res. 2006;69(3):716-25.

38. Schram K, Ganguly R, No EK, Fang X, Thong FS, Sweeney G. Regulation of MT1-MMP and MMP-2 by leptin in cardiac fibroblasts involves Rho/ROCK-dependent actin cytoskeletal reorganization and leads to enhanced cell migration. Endocrinology. 2011;152(5):2037-47.

39. Tao M, Yu P, Nguyen BT, Mizrahi B, Savion N, Kolodgie FD, et al. Locally applied leptin induces regional aortic wall degeneration preceding aneurysm formation in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2013;33(2):311-20.

40. Schroeter MR, Stein S, Heida NM, Leifheit-Nestler M, Cheng IF, Gogiraju R, et al. Leptin promotes the mobilization of vascular progenitor cells and neovascularization by NOX2-mediated activation of MMP9. Cardiovasc Res. 2012;93(1):170-80.