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Obesity progression causes liver steatosis and co-morbidities without apparent cardiac metabolic and functional decline

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Abstract. The goal of this study was to test if obesity progression can be a risk factor to alter cardiac metabolism and function along the time. Male Wistar rats were randomly divided to receive either chow diet (12.0% calories from fat) [C group] or high-fat diet (49.7% calories from fat) plus sucrose in the drinking water (100% from carbohydrate) [H group] for 6, 12 and 24 weeks. The Western diet significantly increased adiposity index of rats in all three experimental periods compared to C group. This was associated with increased plasma levels of insulin, resistin, leptin, glucose, triacylglycerol and decreased adiponectin, however, all variables were stable along the time except insulin and leptin. Plasma free fatty acid was only elevated with 24 weeks treatment. The obesity status resulted in hepatic steatosis progression in H group, while oxidative stress, hepatic inflammatory foci as well as TNF-α and IL-6 mRNA levels were not affected. There are no cardiac performance decline as well as metabolism cardiac changes in H group when compared with C. In conclusion, Western diet induced and promoted obesity, co-morbidities and hepatic steatosis progression while was not associated

with apparent alterations of cardiac metabolism and function. These results suggest that obesity progression seems to affect the organs of distinct ways, and cardiac dysfunction is a question of time.

Keywords: obesity, cardiac function, diet-induced obesity, Western diet.

Introduction

Obesity is defined as abnormal or excessive fat accumulation and has been involved with a strong risk for the development of heart failure (KENCHAIAH et al. 2002; LOEHR et al. 2009). Despite obstructive coronary heart disease is a likely contributor to heart failure in obese subjects, analysis of the Framingham Heart Study (KENCHAIAH et al. 2002) and Atherosclerosis Risk in Communities Study (LOEHR et al. 2009) also demonstrated a significant association between obesity and heart failure, even when analyses were adjusted for myocardial infarction and baseline covariates known to increase the risk of coronary heart disease, including diabetes and hypertension. This suggests that structural and functional alterations of heart among obese individuals are independent of coronary heart disease and reflect pathophysiological alterations in obesity that are both extrinsic and intrinsic to the cardiac tissue.

Overnutrition is one of the most costly challenges for public health. So, understanding the relationship between mechanisms promoting effective calorie storage and adverse metabolic consequences of obesity such as cardiac metabolic dysfunction is emergently necessary. Storage of extra calories in adipose tissue as triglycerides is advantageous, since the ability to sequester lipid effectively inside adipocytes prevents toxic lipid accumulation in other non-adipose tissues, such as muscle, liver and heart, a phenomenon called lipotoxicity (MARRA & SVEGLIATI-BARONI 2018; GHABEN & SCHERER 2019; LOPASCHUK et al. 2007; NISHI et al. 2019; SLETTEN et al. 2018). However, under “metabolically unhealthy obesity” (GHABEN & SCHERER 2019), inappropriate storage of calories increases levels of glucose and lipids in circulation and, consequently, alters cardiac substrate metabolism (WENDE & ABEL et al. 2010). This fact offers a risk for cardiac dysfunction (NISHI et al. 2019; CAROBBIO et al. 2017; D'SOUZA et al. 2016). Although the role of obesity on cardiac metabolism and function is established, the relationship between cardiac metabolism/function with the development of long-term of obesity are not entirely understood. The goal of this study was to test if obesity progression can be a risk factor to alter cardiac metabolism and function along the time.

For inducing obesity progression, we fed Wistar rats with a Western type diet, that mimics food habits of humans living in Western countries, for 6, 12 and 24 weeks. Obesity and co-morbidities were characterized. The cardiac morphology and function were evaluated through echocardiography. Metabolism cardiac was evaluated by measuring enzymes of energy metabolism in cardiac tissue.

Materials and Methods

Animals and experimental model

Ten-weeks old male Wistar rats (n=90) (São Paulo State University Animal Center - UNESP – Botucatu/SP) were randomly divided to receive either commercial chow diet [C group; 3.77 kcal/g, being 12.0% energy from fat] or high-fat diet [H group; 5.25 kcal/g, being 49.7% energy from fat] for 6, 12 and 24 weeks. The composition of the control and high-fat diet was described in detail in our previous study (LUVIZOTTO et al. 2013). H rats were also given 30% sucrose in drinking water along with the diet, whereas normal drinking water without any supplementation was given to C rats. The diet model was used for mimicking food habits of humans living in Western countries.

Food consumption (g) was measured every day. Rats were housed in individual cages in a temperature- (24 ± 2ºC) and humidity- (55 ± 5%) controlled environment on a 12-12 hour light-dark cycle. The study protocol was approved (CEEA 891-2011) by Botucatu School of Medicine Research Ethics Committee – UNESP and followed the Guide for Care and Use of Experimental Animals.

Characterization of obesity and co-morbidities

Adiposity index and plasma analyses

Caudal systolic arterial pressure (mmHg) was evaluated two days before of the sacrifice using a semi-automated tail cuff device Narco Bio-System® PE 300 (International Biomedical, Inc, Houston, TX, USA). At the end of feeding period, all animals underwent a 12- to 15-hour fast. After, they were anesthetized with sodium pentobarbital (40 mg/kg/ip) and sacrificed by decapitation. Adipose tissue (epididymal (g), visceral (g) and retroperitoneal (g) fat pads) was isolated and weighed, which was used to obtain the adiposity index (%) to evaluate obesity status (NASCIMENTO et al. 2011). Blood samples were collected for evaluation of plasma concentrations of insulin (ng/dL), resistin (ng/ml), leptin (ng/dL), glucose (mg/dL), adiponectin (ng/ml), triacylglicerol (mg/dL) and free fatty acid (mmol/L). Enzymatic colorimetric kits were used to measure glucose (Bioclin®, Cat. #K082-3, Belo Horizonte, Minas Gerais, Brazil), triacylglycerol (Bioclin®, Cat. #K117-3, Belo Horizonte, Minas Gerais, Brazil) and free fatty acid (WAKO®, HR Series NEFA-HR(2), Osaka, Japan). Spectrophotometry was performed with the Chemistry Analyser BS 200 automatic spectrophotometer (Mindray Medical International Limited, China). Plasma insulin (Millipore Corporation, Cat. #EZRMI-13K, Billerica, MA, USA), leptin (Millipore Corporation, Cat. #EZRL-83K, Billerica, MA, USA), adiponectin (Millipore

Corporation, Cat. #EZRADP-62K, Billerica, MA, USA) and resistin (BioVendor R&D, Cat. #RD391016200R, Candler, NC, USA) were measured by ELISA method.

Hepatic analyses

Formalin-fixed and paraffin-embedded liver tissues were routinely processed for hematoxylin and eosin (H&E) staining. Liver histology was examined and graded by two blinded independent investigators, as previously described (WANG et al. 2010). Briefly, the degree of steatosis was graded 0–3 based on the average percentage of fat-accumulated hepatocytes per field at 1003 magnification under hematoxylin and eosin staining (0: <6%, 1: 6–33%, 2: 33–66%, and 3: >66%).

Hepatic concentrations of triacylglycerol, caspase-3 activity and oxidative stress markers were determined. Liver samples (~100 mg) were homogenized in 1 ml of a cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 14,000 g for 10 minutes at 4ºC. The supernatant was utilized to determine triacylglycerol (mg/g of tissue) (Bioclin®, Cat. #K117-3, Belo Horizonte, Minas Gerais, Brazil) by enzymatic colorimetric assay. Caspase-3 activity (% of control) was determined by incubating homogenate (10µg of protein) into 100µl of PBS containing 20µg of caspase-3 specific fluorogenic substrate Ac-DEVD-amc (Calbiochem®, Cat. #235425, Billerica, MA, USA). The fluorescence at 440 nm (excitation at 380 nm) was measured in a 1420-multilabel counter (Wallac Victor 2; Perkin-Elmer Life Sciences, Boston, MA). Hepatic total antioxidant performance (% of protection/g of tissue) (TAP) assay was quantified by comparing the area under the curve relative to the oxidation kinetics of phosphatidylcholine liposome control suspension used as the reference biological matrix (RONCHI et al. 2012). Malondialdehyde (pmol/mg) (Cell Biolabs Inc., Cat. #STA-332, San Diego, CA, USA) and nitrotirosine (nM) (Cell Biolabs Inc., Cat. #STA-305, San Diego, CA, USA) were determined by Elisa assay.

Cardiac morphology, performance and energy metabolism

Analyze of morphology and function

Echocardiographic evaluation was performed using a commercially available echocardiograph (General Electric Medical Systems, Vivid S6, Tirat Carmel, Israel) equipped with a 5 - 11.5 MHz multifrequency probe, as described before (NASCIMENTO et al. 2011). The analysed variables were: the left ventricular (LV) end-diastolic dimension (LVDD), posterior wall thickness (PWTd), and anterior wall thickness (AWTd) in diastole were measured at themaximum diastolic dimension. The LV end-systolic dimension (LVSD), posterior wall thickness (PWTs), and anteriorwall thickness (AWTs) in systole were taken at the maximum anterior motion of the posterior wall. The left atrial dimension (LA), aortic dimension (AO) and heart rate (HR) were also measured. Relative wall thickness (RWT) was determined by PWT/LVDD. Left ventricular mass (LVM) was calculated using the following formula: [(LVDD+AWTd+PWTd)3−LVDD3]×1.04, where the value 1.04 indicates the specific density of the myocardium. Indices of left ventricular systolic function were assessed by calculating endocardial fractional shortening (EFS)=(LVDD−LVSD)/LVDD×100 and midwall fractional shortening (MFS)=(LVDD+½ PWTd+½ AWTd)−(LVSD+PWTs+½xAWTs)/(LVDD+½PWTd+ AWTd). Indices of left ventricular diastolic function were assessed by early peak transmitral flow velocity (E), late peak transmitral flow velocity (A), ratio E/A, E-wave deceleration time (EDT) and isovolumetric relaxation time (IVRT).

Analyze of energy metabolism

Left ventricle samples (200 mg) were homogenized in 5 ml of a cold 0.1 mol/l phosphate buffer, pH 7.4. Tissue homogenates was centrifuged at 10,000g for 15 min. The energy metabolism was assessed by NADH-pyruvate dehydrogenase activity (PDH E.C.1.2.4.1.), β-hydroxyacyl coenzyme-A dehydrogenase (OHADH; E.C.1.1.1.35.) and citrate synthase (CS; E.C.4.1.3.7.) determinations (NOVELLI et al. 2010). β-hydroxyacyl coenzyme-A dehydrogenase was assayed in a medium containing 50 mmol/l Tris-HCl pH 7.0, 5 mmol/l EDTA, 0.45 mmol/l NADH, and 0.1 mmol/l acetoacetyl-coenzyme-A. For CS activity, the assay medium consisted of 50 mmol/l Tris-HCl pH 8.1, 0.3 mmol/l acetyl-CoA, 0.1 mmol/l DTNB (5,5′ dithiobis-2-nitrobenzoic acid), and 0.5 mmol/l oxaloacetate. The rate of glucose oxidation was determined by the NADH-pyruvate dehydrogenase activity (PDH, E.C.1.2.4.1.) in an assay medium using 2.5 mmol/l NAD, 0.2 mmol/l thiamin pyrophosphate, 0.1 mmol/l coenzyme-A, 0.3 mmol/l dithiothreitol, 5 mmol/l pyruvate, 1 mmol/l magnesium chloride, and 1 mg/ml of bovine serum albumin in 0.05 mol/l potassium phosphate buffer, pH 7.8. Enzyme determinations were performed at 25 °C using a microplate reader (µQuant-MQX 200 with KCjunior software to computer system control; BioTek Instruments, Winooski, VT). The spectrophotometric determinations were performed in a Pharmacia Biotech spectrophotometer with temperature-controlled cuvette chamber (UV/visible Ultrospec 5,000 with Swift II Applications software to computer system control, 974213; Pharmacia Biotech, Cambridge, England, UK). All reagents were from Sigma (St. Louis, MO).

Statistical analysis

Data are reported as means ± standard deviation. Comparisons between groups were performed using two-way analysis of variance (ANOVA) for independent groups and completed using the post hoc Tukey test. A 5% significance level was adopted. Score of steatosis was presented

as median ± semi-range and descriptive statistic was used.

Results and discussion

Final body weight was higher in the H rats than in the C after 12 and 24 weeks (Figure 1). Western diet also up-regulated adiposity index and plasma glucose, triacylglycerol (Figure 1), insulin, leptin and resistin in H group, while down-regulated plasma adiponectin (Table 1), at all experimental time points. Plasma free fatty acid was elevated only at 24 weeks in H group when compared to C (Figure 1). There was no difference between H and C rats for systolic blood pressure (data not shown).

Absent of steatosis was observed in the livers of rats fed with the control diet in all periods (Score= 0±0), whereas a significant accumulation of fat droplets in the liver was observed in H groups at 6, 12 and 24 weeks (Score: 1±1, 1±1 and 3±1, respectively). Hepatic inflammatory foci were not found in all experimental groups (data not shown); also, TNF-□ and IL-6 mRNA levels, in liver, were not altered in H groups when compared to C (data not shown). The hepatic triacylglycerol accumulation was significantly elevated in H group when compared to C at 6, 12 and 24 weeks (Figure 2). There were no alteration of TAP, MDA, nitrotirosine and caspase-3 in H group when compared to the C group at all experimental periods (Figure 2). Together, all data showed there is no hepatic inflammation and injury, suggesting isolated liver steatosis, however, absent of nonalcoholic steatohepatitis and/or advanced liver injury.

H group showed higher left ventricular diastolic dimension than C, while presented lower ratio between left ventricular posterior wall thickness and diastolic dimension, after 12 and 24 weeks of Western diet, suggesting a subtle change in the left ventricular geometry (Table 2). In relation to function, there were no differences in parameters involved with both left ventricular diastolic and systolic function, except cardiac output that increased in H group at 12 and 24 weeks (Table 2). In relation to cardiac metabolic enzymes, Western diet up-regulated both pyruvate dehydrogenase and β-hydroxyacyl coenzyme-A dehydrogenase enzymes in H group, however, just in six week's period. There was no difference to citrate synthase between H and C groups at all experimental moments (Table 3).

Western diet used in this study caused obesity progression in animals, which was confirmed by the elevated adiposity index in association with higher body weight in the H group along the time (Figure 1). Obesity was not related with apparent cardiac metabolic and functional decline, even in the presence of systemic metabolic abnormalities such as hyperglycemia and dyslipidemia (Figure 1) as well as increased leptin, insulin and resistin plasma levels and decreased adiponectin plasma levels (Table 1), and liver steatosis progression. These results provide new insights relating the evolution of obesity and metabolism and cardiac function.

Table 1. Hormone determination of control (C) and obese (H) groups at end of experimental periods 6, 12 and 24 weeks

Variables		Weeks		
		6	12	24
Insulin (ng/dL)	C	1.9±0.5Aa	2.1±0.7Aa	2.1±0.5Aa
	H	3.9±1.6Ab	4.2±1.4ABb	5.4±1.8Bb
Adiponectin (ng/ml)	C	19.4±3.9Aa	20.8±4.4Aa	19.0±4.3Aa
	H	11.4±1.2Ab	11.7±2.6Ab	13.1±1.3Ab
Resistin (ng/ml)	C	11.1±3.2Aa	4.9±2.4Ba	6.1±1.7Ba
	H	14.7±3.2Ab	11.6±3.1Ab	12.5±3.1Ab
Leptin (ng/dL)	C	2.3±0.5Aa	2.9±0.7Aa	3.7±2.3Aa
	H	6.2±1.0Ab	11.4±5.9Bb	13.9±3.5Bb

C: control group; H: obese group; IL-6: interleukin 6; TNF-α: tumor necrosis factor α. Data are expressed as means ± SD. Capital letters compare 6 vs 12 vs 24 weeks; lower letters compare C vs H in each moment. Different letters indicate significant difference (p<0,05, Two Way ANOVA, post hoc Tukey Test).

Table 2. Cardiac structure and function of control (C) and obese (H) groups at end of experimental periods 6, 12 and 24 weeks

Variables		Weeks		
		6	12	24
Structure				
LVDD (mm)	C	8,02±0,37Aa	7,86±0,44Aa	8,04±0,40Aa
	H	8,19±0,31Aa	8,25±0,42Ab	8,36±0,33Ab
LVSD (mm)	C	3,88±0,44Aa	3,75±0,51ABa	3,44±0,58Ba
	H	4,00±0,28Aa	3,95±0,44Aa	3,71±0,35Aa
PWTd (mm)	C	1,43±0,06Aba	1,41±0,07Aa	1,47±0,06Ba
	H	1,41±0,05Aa	1,40±0,04Aa	1,44±0,07Aa
PWTd / LVDD	C	0,36±0,02Aa	0,36±0,02Aa	0,37±0,03Aa
	H	0,34±0,02Aa	0,34±0,02Ab	0,34±0,02Ab
LA/AO	C	1,42±0,19Aa	1,33±0,13Aa	1,39±0,15Aa
	H	1,57±0,18Ab	1,29±0,14Ba	1,32±0,15Ba
LVDD/BW	C	17,8±0,91Aa	16,1±1,50 Ba	14,4±1,59Ca
	H	17,8±1,96Aa	15,3±1,47Ba	12,75±1,53Cb
LVM (g)	C	0,81±0,08Aa	0,77±0,09Aa	0,84±0,06Aa
	H	0,82±0,06Aa	0,83±0,07Aa	0,88±0,08Aa
LVM index	C	1,78±0,11Aa	1,58±0,14Ba	1,50±0,16Ba
	H	1,78±0,20Aa	1,53±0,16Ba	1,33±0,16Cb
Function				
Heart rate (beats/min)	C	286±27Aa	253±23Ba	263±27Ba
	H	299±21Aa	279±15Bb	273±20Ba
Cardiac output (ml/min)	C	131±21Aa	109±17Ba	126±23Aba
	H	145±16Aa	140±24Ab	145±18Ab
Cardiac index (%)	C	289±32Aa	223±28Ba	226±51Ba
	H	316±47Aa	259±45Bb	221±30Ca
EFS (%)	C	51,7±4,9Aa	52,3±5,0Aa	57,2±6,9Ba
	H	51,1±2,6Aa	52,2±4,4ABa	55,5±4,3Ba
MFS (%)	C	59,0±4,2Aa	59,7±4,4Aa	63,9±5,9Ba
	H	58,3±2,4Aa	59,2±3,9Aa	62,1±3,7Ba
PWSV (mm/s)	C	40,3±4,0Aa	36,5±3,9Ba	43,6±4,8Aa
	H	41,2±3,4ABa	38,6±4,0Aa	44,7±4,7Ba
E/A	C	1,64±0,25Aa	1,72±0,27Aa	1,74±0,21Aa
	H	1,65±0,29Ba	1,90±0,36Aa	1,58±0,27Ba
EDT (ms)	C	44,4±8,5Aa	47,5±6,5Aa	46,7±7,9Aa
	H	43,3±6,1Aa	43,7±3,7Aa	45,1±9,2Aa
IVRT/R-R	C	53,0±7,6Aa	54,6±5,5Aa	53,1±4,8Aa
	H	52,5±7,1Aa	53,1±7,5Aa	51,2±5,0Aa
Tei index	C	0,48±0,06Aa	0,50±0,06Aa	0,48±0,04Aa
	H	0,53±0,08Aa	0,53±0,07Aa	0,47±0,06Aa

C: control group; H: obese group; BW, body weight; LVDD, the left ventricular end-diastolic dimension; PWTd, posterior wall thickness; LVSD, the left ventricular end-systolic dimension; LA, the left atrial dimension; AO, aortic dimension; HR, heart rate; PWSV, posterior wall shortening velocity; LVM, left ventricular mass; R-R, heart rate interval; EFS, endocardial fractional shortening; MFS, midwall fractional shortening; E, early peak transmitral flow velocity; A, late peak transmitral flow velocity; E/A, ratio between E and A; EDT, E-wave deceleration time; IVRT, isovolumetric relaxation time. Data are expressed as means ± SD. Capital letters compare 6 vs 12 vs 24 weeks; lower letters compare C vs H in each moment. Different letters indicate significant difference ($p<0,05$, Two Way ANOVA, post hoc Tukey Test).

Table 3. Cardiac energy metabolism enzymes of control (C) and obese (H) groups at end of experimental periods 6, 12 and 24 weeks

Variables		Weeks		
		6	12	24
PDH (nmol/100g of protein)	C	0,85±0,16Aa	0,83±0,13Aa	0,94±0,17Aa
	H	1,06±0,19Ab	0,73±0,13Ba	0,85±0,25ABa
CS (nmol/g of protein)	C	25±10Aa	32±14ABa	52±24Ba
	H	30±10Aa	34±10Aa	47±26Aa
OHADH (nmol/g of protein)	C	1,31±0,27Aa	3,27±2,69Aa	2,84±1,15Aa
	H	4,59±1,43Ab	2,38±1,64Aa	4,65±4,55Aa
PDH (nmol/100g of protein)	C	0,85±0,16Aa	0,83±0,13Aa	0,94±0,17Aa
	H	1,06±0,19Ab	0,73±0,13Ba	0,85±0,25ABa

C: control group; H: obese group; PDH: pyruvate dehydrogenase; CS: citrate synthase; OHADH: β-hydroxyacyl coenzyme-A dehydrogenase. Data are expressed as means ± SD. Capital letters compare 6 vs 12 vs 24 weeks; lower letters compare C vs H in each moment. Different letters indicate significant difference (p<0,05, Two Way ANOVA, post hoc Tukey Test).

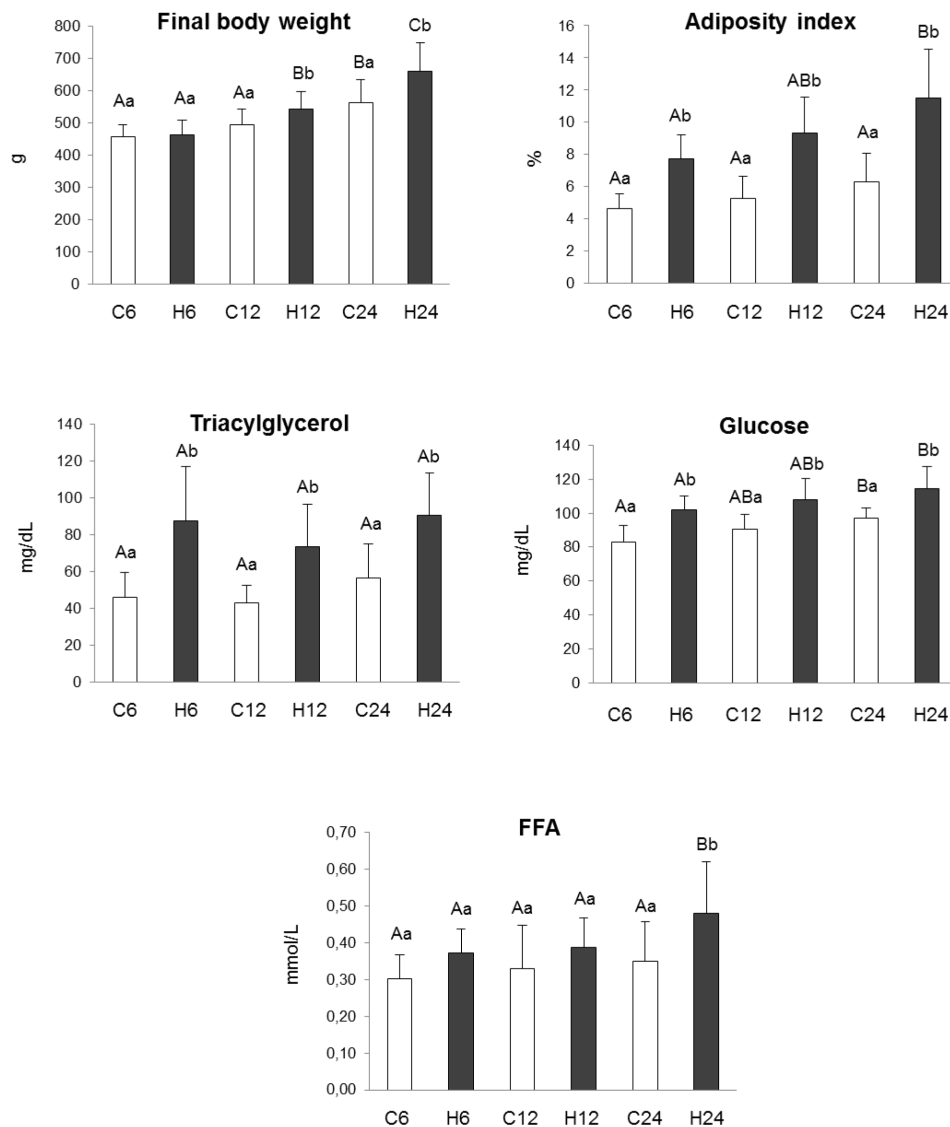

Figure 1. General characteristics of control (C) and diet-induced obese (H) groups at end of experimental periods 6, 12 and 24 weeks. Data are expressed as means ± SD. Capital letters compare 6 vs 12 vs 24 weeks; lower letters compare C vs H in each moment. Different letters indicate significant difference (p<0,05, Two Way ANOVA, post hoc Tukey Test).

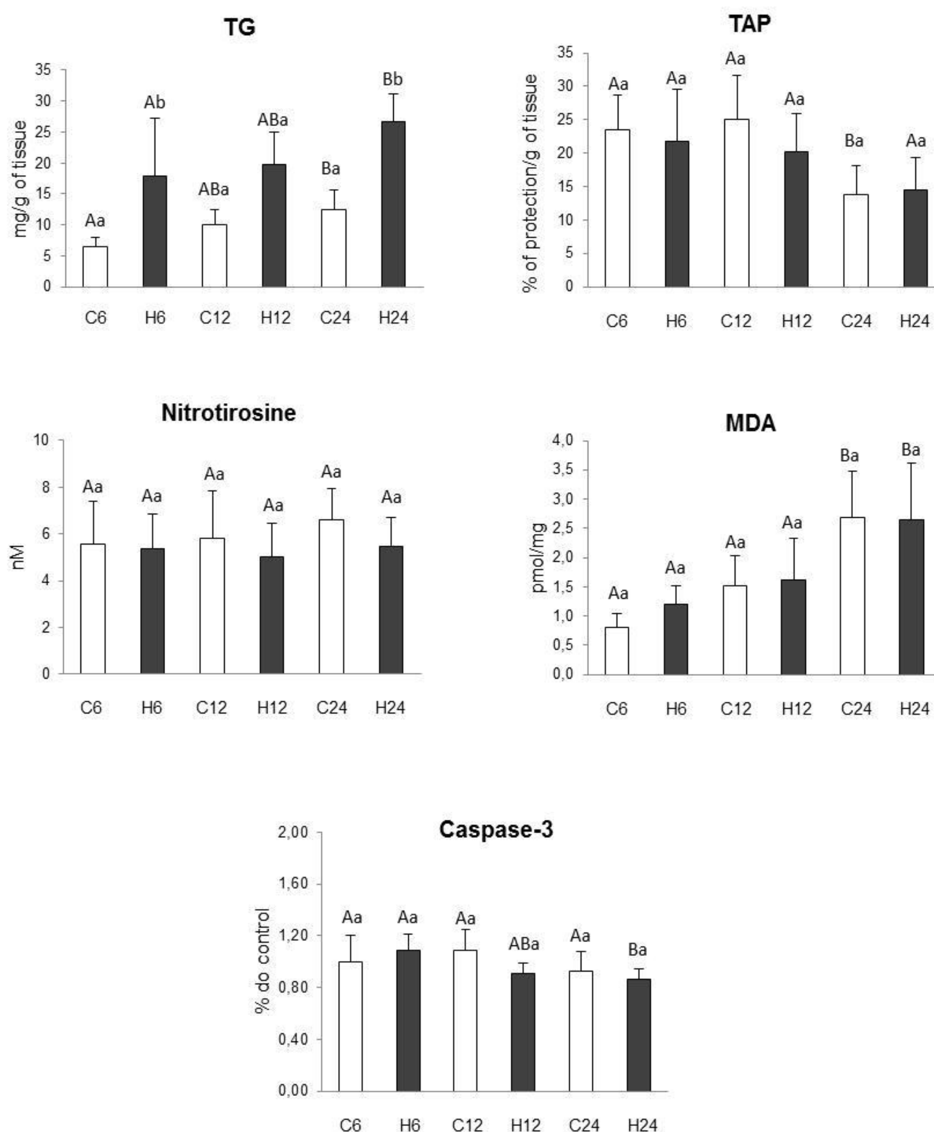

Figure 2. Hepatic analyses in control (C) and diet-induced obese (H) groups at end of experimental periods 6, 12 and 24 weeks. Data are expressed as means ± SD. Capital letters (6 vs 12 vs 24 weeks); lower letters (C vs H). Different letters indicate significant difference (p<0,05, Two Way ANOVA, post hoc Tukey Test). TG, triacylglycerol; TAP, total antioxindat performance; MDA, malondialdehyde.

Obesity has been associated with cardiac morphology and function abnormalities in a variety of ways (ALPERT et al. 2016; ALPERT et al. 2018). This fact reflects several hemodynamic, neurohormonal and metabolic abnormalities, as well as degree and duration, associated with obesity. In our study, 12 and 24 weeks of obesity were related to higher left ventricular diastolic dimension and increased cardiac output, suggesting a supra-normal left ventricular ejection and subtle change in the left ventricular geometry. Heart rate was mildly elevated in 12 and 24 weeks. Thus, the rise in cardiac output in obese group, in part, should be due the increased heart rate. In other hand, higher left ventricular diastolic dimension could be involved with increased cardiac preload, increasing cardiac output by Frank-Starling mechanism. Another mechanism present in this change is that the excess adipose accumulation in obese individuals leads to an increase in total and central blood volume (ALEXANDER & ALPERT 1998), which in turn predisposes to higher preload and, consequently, elevated cardiac output; also, it explain partially the higher left ventricular diastolic dimension in this study as an eccentric left ventricular hypertrophy that occurs with left ventricular volume overload states. Additionally, the influence of adipose tissue on other aspects of systemic metabolic homeostasis has been appreciated (GHABEN & SCHERER 2019). Adipose tissue expansion by hyperplasia is generally considered healthy and adaptive, preventing toxic lipid accumulation in other tissues, such as liver and heart, and consequently function of the organ. Although liver steatosis has increased along the time

in obese group, our results showed no lipid accumulation in myocardium of H animals during all treatment periods (data no shown). In this study, to check the oxidation of fatty acids and glucose by the myocardium metabolic enzymes were also evaluated in cardiac tissue, and was observed that only in short-term of obesity (6 weeks) up-regulated both pyruvate dehydrogenase and β-hydroxyacyl coenzyme-A dehydrogenase; this may be due to the fact that the body is faced with a large supply of fat (hypertriglyceridemia) and glucose (hyperglycemia) to the heart, but there is no relationship with cardiac morphology and function, which was both normal at 6 weeks. To our understanding, chronic obesity might be created a cardiac metabolic homeostasis at 12 and 24 weeks without metabolic dysfunction for promoting cardiac dysfunction. Also, we believe that adipose tissue expansion in our study may have prevented additional systemic metabolic imbalance, buffering additional systemic metabolic dysfunction and, consequently, prevent cardiac disease.

Nonalcoholic fatty liver disease (NAFLD) is a metabolic liver disease characterized by an extensive continuum of liver injury, varying from pure steatosis to nonalcoholic steatohepatitis, fibrosis and cirrhosis, being commonly seen among patients with other metabolic disorders, such as obesity (CLARK et al. 2002; ANGULO 2002). In the last years, two meta-analysis (BORGES-CUNHA et al. 2019; BONCI et al. 2015) demonstrated that NAFLD, especially NASH, associates with adverse structural alterations and cardiac dysfunction, suggesting that liver injury may represent an additional contributor to cardiac alterations. In this study, hepatic steatosis increased along the time, which was not accompanied by inflammation. It was also demonstrated that obesity and steatosis progression was not associated with liver oxidative stress and injury (Figure 2). Additionally, free fatty acid was mildly elevated in 24 weeks (Figure 1). Taken together, these results suggest that hepatic steatosis may be an adaptive response to the higher plasmatic free fatty acid levels from adipose tissue without promoting additional insults to the heart.

Multiple hemodynamic, neurohormonal and metabolic variables, as well as interact between them, play a key role in the development of cardiac alterations, such as -angiotensin-aldosterone and sympathetic nervous system activation, insulin resistance, hyperleptinemia, and others (ALPERT et al. 2018; SLETTEN et al. 2018). Here, we demonstrated that obesity was associated with co-morbidities such as hyperglycemia and hypertriglyceridemia (Figure 1) as well as increased leptin, insulin and resistin plasma levels and decreased adiponectin plasma levels (Table 1). However, these co-morbidities remained stable along the time, except insulin and leptin; both increased in a time-dependent manner in obese (Table 1). As mentioned above, free fatty acid was also elevated in 24 weeks (Figure 1). Recently, in the same experimental model, our group has demonstrated that both IL-6 and TNF-α were increased in epididymal adipose tissue of 24 weeks obese animals when compared to control as well as short-term of obesity (6 weeks). Also, adipocyte hypertrophy and inflammatory cells as well as Toll-like receptor-4 and NF-κB were only observed at 24 weeks (FRANCISQUETTI et al. 2017). These results allowed us to demonstrate that adipose tissue is influenced by time of obesity, and inflammation of the adipose tissue is associated with adipocyte hypertrophy and TLR-4 activation. Thus, Western diet-associated obesity progression seems to affect the organs of distinct ways. While cardiac alterations are subtle with long-term of obesity demonstrated in this study, adipose tissue has been associated with inflammation as well as insulin resistance and lipolysis, as demonstrated before by our group (FRANCISQUETTI et al. 2017), increasing plasmatic free fatty acid and liver steatosis at 24 weeks. Thus, we believe that cardiac dysfunction is time-manner question.

Conclusion

In conclusion, this present study provided further insights into the western diet- long term-induced obesity progression and its related disease. Chronic exposure to western diet induces obesity and hepatic steatosis progression as well as co-morbidities while was not associated with altered myocardial substrate metabolism and apparent cardiac dysfunction along the time. However, adipose tissue inflammation and liver steatosis are conditions already evident. These results suggest that obesity progression seems to affect the organs of distinct ways, and cardiac dysfunction is a question of time.

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