Influence of Long-Term Obesity on Myocardial Gene Expression

Ana Paula Lima-Leopoldo[1], André Soares Leopoldo[1], Danielle Cristina Tomaz Silva[2], André Ferreira do Nascimento[2], Dijon Henrique Salomé de Campos[2], Renata de Azevedo Melo Luvizotto[2], Silvio Assis de Oliveira Júnior[3], Carlos Roberto Padovani[2], Célia Regina Nogueira[2], Antonio Carlos Cicogna[2]

Universidade Federal do Espírito Santo[1], Vitória, ES; Universidade Estadual Paulista[2], Botucatu, SP; Universidade Federal do Mato Grosso do Sul[3], Campo Grande, MS, Brazil

Abstract

Background: **Several authors have shown that deterioration of cardiac function is associated with the degree and duration of obesity. It is necessary to establish the gene expression patterns after prolonged periods of obesity.**

Objective: **This study tested the hypothesis that increased duration of exposure to obesity leads to a reduction in the mRNA levels of proteins involved in regulation of myocardial Ca^{2+} homeostasis. In addition, this study verified whether the decrease in mRNA expression was caused by a reduction in thyroid hormone.**

Methods: **Thirty-day-old male Wistar rats were distributed in two groups: control (C) and obese (Ob). The C group was fed a standard diet and the Ob was fed with high-fat diets for 15, 30 and 45 weeks. Obesity was defined by adiposity index. The gene expression was assessed by quantitative real-time PCR.**

Results: **The adiposity index was higher in the Ob compared to the C after all periods. While obesity at 15 and 45 weeks resulted in a reduction in mRNA of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), Na^+/Ca^{2+} exchanger (NCX), and calsequestrin (CSQ), L-type Ca^{2+} channels, ryanodine receptor, SERCA2a, phospholamban (PLB), NCX, and CSQ expression were increased compared to the C after 30 weeks. There was no significant association between T3 levels and mRNA expression.**

Conclusions: **Our data indicate that obesity over the short and long periods of time may promote alteration in gene expression of Ca^{2+} homeostasis regulatory proteins without influence by thyroid hormone (Arq Bras Cardiol. 2013;100(3):229-237).**

Keywords: **Obesity; Gene Expression; Thyroid Hormones; Myocytes, Cardiac; Myocardium.**

Introduction

Obesity is the most prevalent chronic metabolic disorder in developed and underdeveloped countries[1]. This disease plays an important role in cardiovascular morbidity through multiple mechanisms, including intracellular calcium (Ca^{2+}) homeostasis[2-4]. Several proteins and channels, such as sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), ryanodine receptor (RyR), phospholamban (PLB), L-type Ca^{2+} channel, calsequestrin (CSQ) and Na^+/Ca^{2+} exchanger (NCX) regulate Ca^{2+} homeostasis in cardiac muscle[3,4]. Thus, alterations in the expression and/or function of these Ca^{2+} proteins could be responsible for cardiac dysfunction in human and experimental models.

Previous studies with short periods evaluating the gene expression of Ca^{2+} proteins in experimental obese models have shown disparate results[5-7]. Studies using dogs which were fed a high fat diet for 6 weeks, showed similar levels of RyR mRNA between control and high-fat diet groups[5]. Other studies using dogs fed a high-fat diet for 9 weeks, showed decreased mRNA expression of SERCA2a and PLB in the myocardium[6]. Lima-Leopoldo et al[7] showed that obese rats fed a cycle of high-fat diets for 15 weeks, plus a solution of sugar water, had increased mRNA levels of SERCA2a, PLB and RyR. Since several authors have shown that cardiac dysfunction is associated with the degree and duration of obesity, it has become necessary to establish the gene expression patterns after prolonged periods of obesity.

The heart is an important target organ for thyroid hormone (TH) action[8-10]. The ability of TH to regulate cardiac function has been suggested to involve its genomics effects[11,12]. In this regard, the influence and/or role of TH on proteins of Ca^{2+} handling has been an area of intense investigation[12,13] and several studies have reported the involvement of TH as modulators of gene transcription[8,9,14,15]. However, little is known about the relationship between the duration of obesity, gene expression of cardiac Ca^{2+} proteins and TH.

Considering the lack of information, the purpose of this study was to test the hypothesis that long-term exposure to obesity leads to a reduction in the mRNA expression levels of proteins involved in myocardial Ca^{2+} homeostasis. Furthermore, this study verified whether the decrease in gene expression was accompanied by a reduction in TH levels.

Mailing Address: Ana Paula Lima-Leopoldo •
Av. Fernando Ferrari, 514, Goiabeiras. Postal Code 29075-910, Vitória, ES - Brazil
E-mail: anapaula@cefd.ufes.br, anapaulalimaleopoldo@gmail.com
Manuscript received August 10, 2012; manuscript revised September 5, 2012; accepted October 26, 2012.

DOI: 10.5935/abc.20130045

Material and Methods

Animals and procedures

All procedures involving animals were approved by the Ethics Committee of Botucatu Medical School (UNESP, SP, Brazil) under number (573) and performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council in the 1996[16].

Thirty-day-old male *Wistar* rats, weighing approximately 100 g, were obtained from the Central Animal House of Botucatu Medical School (São Paulo, Brazil). Rats were individually caged with free access to water and subjected to different dietary regimens as described below. Animals were kept under standard environmental conditions of controlled light (12 h light/dark schedule; lights on at 6 am), clean-air room temperature (23 ± 3°C), and relative humidity (60 ± 5%).

Experiment design

After 7 days of acclimation, the rats were randomized into two groups: control (C, n = 30) and obese (Ob, n = 30). The C group was fed a standard diet and Ob group were alternately submitted to four palatable high-fat diets. Weight gain and body weight were monitored once a week.

At week 3 of this study, the beginning of obesity based on body weight gain was established, previously determined by our group (*unpublished data*). At this time point, weeks 3, control and obese rats were randomized into six new groups (C_{15}, Ob_{15}, C_{30}, Ob_{30}, C_{45} and Ob_{45}) and maintained on their respective diets for 15, 30 and 45 consecutive weeks.

Diets

Experimental diets were prepared in pellet form by Agroceres (Rio Claro, SP, Brazil). The standard diet (RC Focus 1765) consisted of 12.3% kcal from fat, 57.9% kcal from carbohydrates, and 29.8% kcal from protein. Four palatable high-fat diets (RC Focus 2413, 2414, 2415, and 2416) contained 49.2% kcal from fat, 28.9% kcal from carbohydrates, and 21.9% kcal from protein. High-fat diets were calorically rich (high-fat diet = 3.65 kcal/g *versus* low-fat diet = 2.95 kcal/g) due to higher fat energy. The composition of the high-fat diets consisted of saturated and unsaturated fatty acids, which provided 20% and 80% of the fat-derived calories, respectively. Standard and high-fat diets components have been previously described[17].

Characterization of obesity

A criterion based on adiposity index was utilized to confirm animals obesity. After animals had been anesthetized (sodium pentobarbital 50 mg/kg intraperitoneal [i.p.]), decapitated, and thoracotomized, the adipose tissue fat pads were dissected and weighed. The adiposity index was calculated by the following formula: adiposity index = (total body fat (BF)/final body weight) × 100. BF was measured from the sum of the individual fat pad weights as follows: BF = epididymal fat + retroperitoneal fat + visceral fat.

Comorbidities associated with obesity

As rat models of diet-induced obesity may develop some characteristics of human obesity, such as hypertension, glucose (GL) intolerance, insulin resistance, dyslipidemia, hyperinsulinemia, and hyperleptinemia, the following evaluations were performed in all groups:

Systolic blood pressure (SBP)

After observation periods, the tail systolic blood pressure (SBP) was assessed in each animal by semi automated tail cuff device Narco BioSystems® PE 300 (International Biomedical, Austin, TX, USA). The average of two pressure readings was recorded.

GL tolerance test (GTT) and homeostatic model assessment index (HOMA)

After 15, 30 and 45 weeks of treatment, GL tolerance and insulin resistance were evaluated by GTT and HOMA-IR, respectively. All rats were fasted for 4-6 h prior to GL tolerance test[18]. After fasting, a blood sample was collected from the tip of the tail in a heparinized tube. The basal blood GL level of each animal was immediately determined using a handheld glucometer (Accuchek Advantage; Roche Diagnostics Co., Indianapolis, IN). Subsequently, an injection of 2 g/kg GL (Sigma-Aldrich®, St Louis, MO, USA) was given intravenously and blood GL levels were measured after 15, 30, 60, 90, and 120 min[18]. GL intolerance was evaluated by the area under the curve (AUC) for GL. The HOMA-IR was expressed as an index of insulin resistance and calculated by the following formula: HOMA-IR = [fasting GL (mmol/l) × fasting insulin (μU / ml)]/22.5. All rats ate normally and regained their body weights within 1 d after this regimen.

Triglyceride determination and Hormonal analysis

At the end of each experimental period (15, 30 and 45 weeks), animals were fasted for 12–15 h, anesthetized with sodium pentobarbital (50 mg/kg i.p.), and euthanized by decapitation. Blood samples were collected in heparinized tubes, and the serum was separated by centrifugation at 3000 × *g* for 15 minutes at 4°C and stored at −80°C until further analysis. Serum was analyzed for triglyceride levels (TG) and insulin and leptin hormones. Serum concentration of TG was assayed using a commercial kit (CELM,® São Paulo, Brazil) and measured with an automatic enzymatic analyzer system (Technicon, RA-XT™ System, Global Medical Instrumentation, Minnesota, USA). Leptin and insulin levels were determined by ELISA using commercial kits (Linco Research Inc., St. Louis, MO, USA).

The serum concentrations of free triiodothyronine (T_3) and thyroid stimulating hormone (TSH) were analyzed using Diagnostics Products Genese (São Paulo, SP, Brazil) with Luminex Corporation's xMAP Technology™, by reaction multiplex immunoassay, Lincoplex kits (Linco Research Inc, St. Louis, MO, USA).

Gene expression of key intracellular Ca^{2+}-cycling proteins

Whole RNA was extracted from cardiac tissue (left ventricle) using Trizol (Invitrogen), according to the manufacturer's instructions. High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) was utilized for the synthesis of complementary DNA (cDNA) from 1000 ng of whole RNA. Real-time PCR was used to quantitatively measure the messenger RNA (mRNA) levels of SERCA2a (Rn00568762_m1), RyR (Rn01470303_m1), PLB (Rn01434045_m1), L-type Ca^{2+} channel (Rn00709287_m1), CSQ (Rn00567508_m1) and NCX (Rn00570527_m1). Quantitative mRNA was measured using TaqMan Universal PCR Master Mix (Applied Biosystems, CA, USA), according to the manufacturer's instructions, and the Applied Biosystems StepOne Plus detection system. All samples were assayed in triplicate. Cycling conditions were as follows: enzyme activation at 50°C for 2 min, denaturation at 95°C for 10 min, the cDNA products were amplified for 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Gene expression was quantified in relation to the values of the control groups (C_{15}, C_{30} and C_{45}, respectively) after normalization by an internal control (β-actin, Rn00667869_m1) and determined by the method $2^{-\Delta\Delta Ct}$, as previously described[19].

Statistical analysis

All results were reported as mean ± standard deviation. General characteristics, comorbidities, hormonal measurements and gene expression of intracellular Ca^{2+}-cycling proteins were evaluated using two-way analysis of variance (ANOVA) for independent samples. When significant differences were found ($p < 0.05$), Bonferroni's *post hoc* test for multiple comparisons was carried out. The association analysis between the levels of thyroid hormone and intracellular Ca^{2+}-cycling protein gene expression was determined by Pearson's linear correlation test. The level of significance was 5%.

Results

General characteristics and comorbidities

The general characteristics of rats are shown in Figure 1. Obese animals had significantly greater final body weight, weight gain, body fat, and adiposity index compared to control rats in all experimental periods (Figure 1, Panel A, B, C and D). Using the adiposity index as the indicator of obesity, after 15, 30 and 45 weeks Ob rats had 79.5%, 82% and 69.5% more fat, respectively, compared with their respective C rats (Figure 1D). Long-term to exposure to high-fat diet did not influence the degree of obesity, since the body fat and adiposity index were similar between Ob rats in different Ob groups (Figure 1C and D).

The comorbidities associated with obesity are summarized in Figure 2. The area under the curve for glucose (AUC) and HOMA index were significantly affected by exposure to obesity. The Ob rats had significantly greater glucose

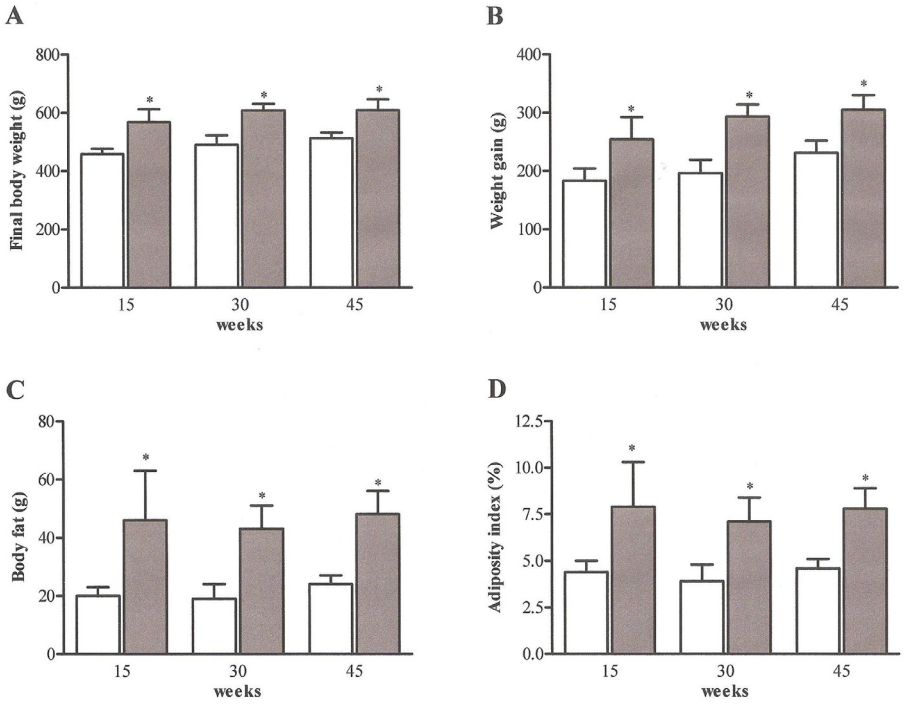

Figure 1 - *Final body weight (A), weight gain (B), body fat (C), and adiposity index (D) in control (white bars) and obese rats (gray bars) after 15, 30 and 45 weeks of experiment; Data are mean ± SD; two-way ANOVA and Bonferroni post hoc test; * p<0.05 vs control group.*

AUC than their respective C group (Ob_{15}>C_{15}, Ob_{30}>C_{30} and Ob_{45}>C_{45}; $p < 0.05$), Figure 2A. HOMA index were also significantly greater in the Ob rats compared to control group (Ob_{15}>C_{15} and Ob_{45}>C_{45}; $p < 0.05$), Figure 2B. Although, the levels of HOMA index were not significantly different between groups at 30 week, there was a trend this measurement to be greater ($p = 0.054$) in Ob rats than C rats (Figure 2B).

Glucose tolerance test revealed compromised glucose tolerance in the Ob group in all experimental periods, consistent with the notion that obesity is often accompanied by insulin resistance, which was observed in this study determined by HOMA index. Furthermore, Figure 2D shows that high-fat diet-induced obesity significantly altered serum triglycerides levels in relation to the 3 C groups (Ob_{15}>C_{15} and Ob_{30}>C_{30}; $p < 0.05$). At weeks 45, there was no significant difference in TG levels between groups ($p = 0.086$). The duration of obesity had no effect on systolic blood pressure in the Ob groups (Figure 2C).

Hormonal analysis

Table 1 presents the values for leptin, insulin, T_3 and TSH. The high-fat diet caused a significant elevation in leptin and insulin levels in all experiments periods. The duration of obesity affected leptin levels significantly between 15 and 45 week (Ob_{45}>Ob_{15}; $p < 0.05$), however, leptin levels were not significantly different between obese groups at 15 *vs* 30 weeks or 30 *vs* 45 weeks (Ob_{15} *versus* Ob_{30}, $p = 0.055$; and Ob_{30} *versus* Ob_{45}, $p = 0.09$). There was a trend of increased leptin levels over time which was influenced by duration obesity exposure (Table 1). Furthermore, long-term obesity exposure (Ob_{45} > Ob_{15} and Ob_{30}, $p < 0.05$) promoted a greater rise in insulin levels compared to short- and mid-term obesity exposure, indicating that the duration of obesity resulted in higher insulin resistance (Table 1). According to these leptin and insulin results obtained, obesity in all time periods was accompanied by hyperinsulinemia and hyperleptinemia.

Serum T_3 and TSH were determined to evaluate the role of thyroid hormones on gene transcription induced by high-fat diet induced obesity (Table 1). The duration of obesity promoted different responses on serum TSH levels at 15 and 45 weeks between the C and Ob groups (C_{15} > Ob_{15}; C_{45} < Ob_{45}). TSH values were not significantly different between the C and Ob groups groups after 30 weeks of obesity. Furthermore, long periods of obesity did influence TSH levels within the obese groups at 15 *vs* 45 weeks (Ob_{15} < Ob_{45}). However, long-term exposure to high-fat diet induced obesity did not cause statistically significant changes in T_3 levels between C and Ob groups at all experimental time periods (Table 1). T_3 values were reduced at 15 weeks (Ob_{15} < C_{15}, $p = 0.068$) and elevated at 45 weeks (Ob_{45} > C_{45}, $p = 0.007$) in the Ob groups compared to their respective C groups. These results showed that T_3 and TSH respond similarly in regards to duration of exposure to obesity (C_{15} > Ob_{15}; C_{45} < Ob_{45}).

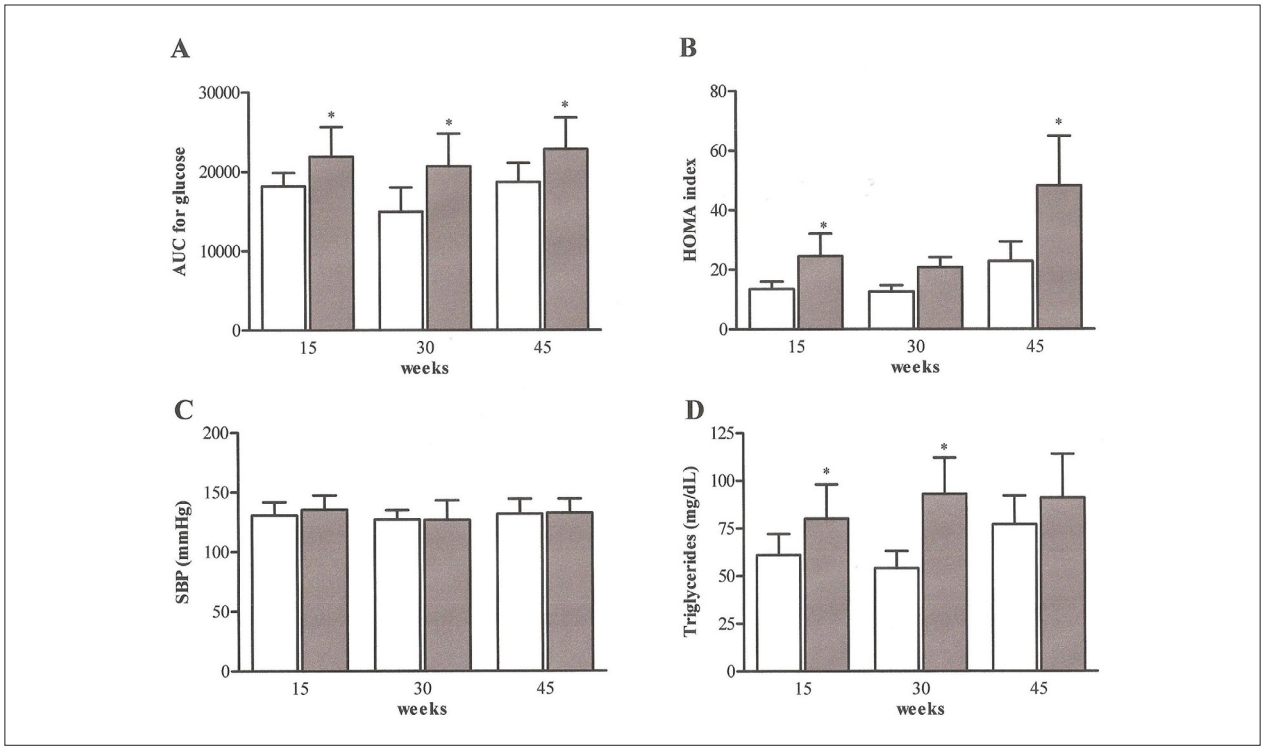

Figure 2 - *AUC - Area under curve of intraperitoneal glucose tolerance test (A); HOMA index (B), SBP - Systolic blood pressure (C), and lipid profile (D) in control (white bars) and obese rats (gray bars) after 15, 30 and 45 weeks of experiment; Data are mean ± SD; two-way ANOVA and Bonferroni post hoc test; * p<0.05 vs control group.*

Table 1 - Hormonal analysis of experimental groups

Hormone	Groups	Treatment		
		15 weeks	30 weeks	45 weeks
Leptin (ng/ml)	C	3.1 ± 0.9	3.0 ± 0.8	4.5 ± 0.6
	Ob	6.2 ± 1.5*#	8.2 ± 2.7*	9.9 ± 1.9*
Insulin (ng/ml)	C	0.86 ± 0.19	0.81 ± 0.10	1.35 ± 0.27
	Ob	1.39 ± 0.37*#	1.18 ± 0.18*#	2.86 ± 0.54*
T3 (pg/ml)	C	20223 ± 5415	14294 ± 3373	19353 ± 5213
	Ob	15874 ± 1390#	18087 ± 4413	23783 ± 3042
TSH (pg/ml)	C	1837 ± 574	1387 ± 93	1552 ± 456
	Ob	1143 ± 381*#	1694 ± 390	2135 ± 492*

*Serum hormones levels in C - control and Ob - obese groups at 15, 30 and 45-weeks; leptin and insulin - 8 animals each groups; TSH - thyroid stimulating hormone and T_3 - triiodothyronine: 6 rats per group. Data are expressed as means ± SD; Two-way ANOVA and Bonferroni post hoc-test; * p<0.05 vs. C. # p< 0.05 vs. Ob_{45} group.*

Gene expression of key intracellular Ca^{2+}-cycling proteins

Analysis of the mRNA levels for intracellular Ca^{2+}-cycling proteins was performed to understand the mechanism by which long-term exposure to obesity modulates cardiac function. The mRNA measurements of SERCA2a, RyR, PLB, L-type Ca^{2+} channel, CSQ and NCX are summarized in Figures 3A, B, C, D, E and F. Figures 3A, E and F show that different periods of high-fat diet induced obesity resulted in significant decrease in gene expression of SERCA2a, CSQ and NCX at 15 and 45 weeks ($C_{15} > Ob_{15}$; $C_{45} > Ob_{45}$; $p < 0.05$). Furthermore, the levels of PLB and L-type Ca^{2+} channel mRNA were decreased only with exposure to obesity in the short-term (PLB: C_{15}: 1.00 ± 0.18 *versus* Ob_{15}: 0.53 ± 0.13 and L-type Ca^{2+} channel: C_{15}: 1.00 ± 0.18 *versus* Ob_{15}: 0.54 ± 0.10; $p < 0.05$). The RyR mRNA levels were similar between the C and Ob groups after 15 and 45 weeks of obesity. At week 45, the gene expression of PLB and L-type Ca^{2+} channel was not significantly different between the C and Ob groups (Figures 3C and D). Importantly after 30 weeks of high-fat diet induced obesity, the mRNA levels of all intracellular Ca^{2+}-cycling proteins (SERCA2A, RyR, PLB, L-type Ca^{2+} channel, CSQ and NCX) were elevated compared to the C group (Figure 3A, B, C, D, E and F). Long-term exposure to high-fat diet induced obesity also resulted in statistically significant differences in mRNA levels of SERCA2A, RyR, L-type Ca^{2+} channel, CSQ and NCX within the obese groups as shown in Figure 3 ($Ob_{30} > Ob_{15} = Ob_{45}$).

The potential role of the thyroid hormones altering gene expression of intracellular Ca^{2+}-cycling proteins was also evaluated. Table 2 shows the results of Pearson's linear correlation test. There were no significant correlations between free T_3 concentrations and mRNA levels of SERCA2a, RyR, PLB, L-type Ca^{2+} channel and NCX. These results demonstrate that free T_3 did not influence and/or regulate transcription of intracellular Ca^{2+} handling proteins after long-term exposure to obesity (Table 2).

Discussion

Obesity is a condition that has reached epidemic levels in recent years, and its adverse effects have been extensively studied in experimental animals. Interestingly, little information is available on the relationship between the duration of obesity and Ca^{2+} regulation. The major contribution of this current study is that we demonstrated that exposure to obesity alters the gene expression of Ca^{2+} handling proteins differently. While exposure to obesity for 30 weeks promoted elevations in mRNA levels, in contrast, exposure to obesity for 15 and 45 weeks, resulted in decreased mRNA levels, which suggests a different cardiac remodeling process may occur in short-term *vs* long-term exposure to obesity. Furthermore, there was not an influence of thyroid hormones on the gene expression of Ca^{2+} handling proteins after long periods of exposure to obesity. We believe that this is the first study to report the cardiac mRNA expression of Ca^{2+} handling proteins in obese animals as well as the role of thyroid hormones regulating the gene expression of Ca^{2+} handling proteins over long periods of time.

Fat-enriched diets have been used for decades to model obesity in rodents. The high-fat diet used was of sufficient intensity and duration to promote obesity in rats at all experimental time periods, 15, 30 and 45 weeks. The development of obesity over time was characterized by significant differences in body weight, body fat, fat pads and adiposity index in comparison to control rats. According to the literature, increased fat consumption may not be accompanied by an elevation of oxidation, which favors greater deposition of fat in animals fed a high-fat diet[20]. Nevertheless, in this study, the time of exposure to a high-fat diet did not exacerbate this condition, since obesity was not accompanied by an elevation of the intensity or degree of adiposity. Consistent with previous investigations[21-26], the high-fat diet used in this study was effective at promoting numerous comorbidities associated with short and long-term obesity, including glucose intolerance, hyperinsulinemia, insulin resistance, hyperleptinemia, and dyslipidemia observed by elevation in serum TG. Although there were no significant changes in TG levels after 45 weeks ($p = 0.086$), TG levels showed an increasing trend over time and exposure to obesity. These results demonstrate that obesity over the long periods of time promotes alterations in metabolic and hormonal parameters without effecting on systolic blood pressure.

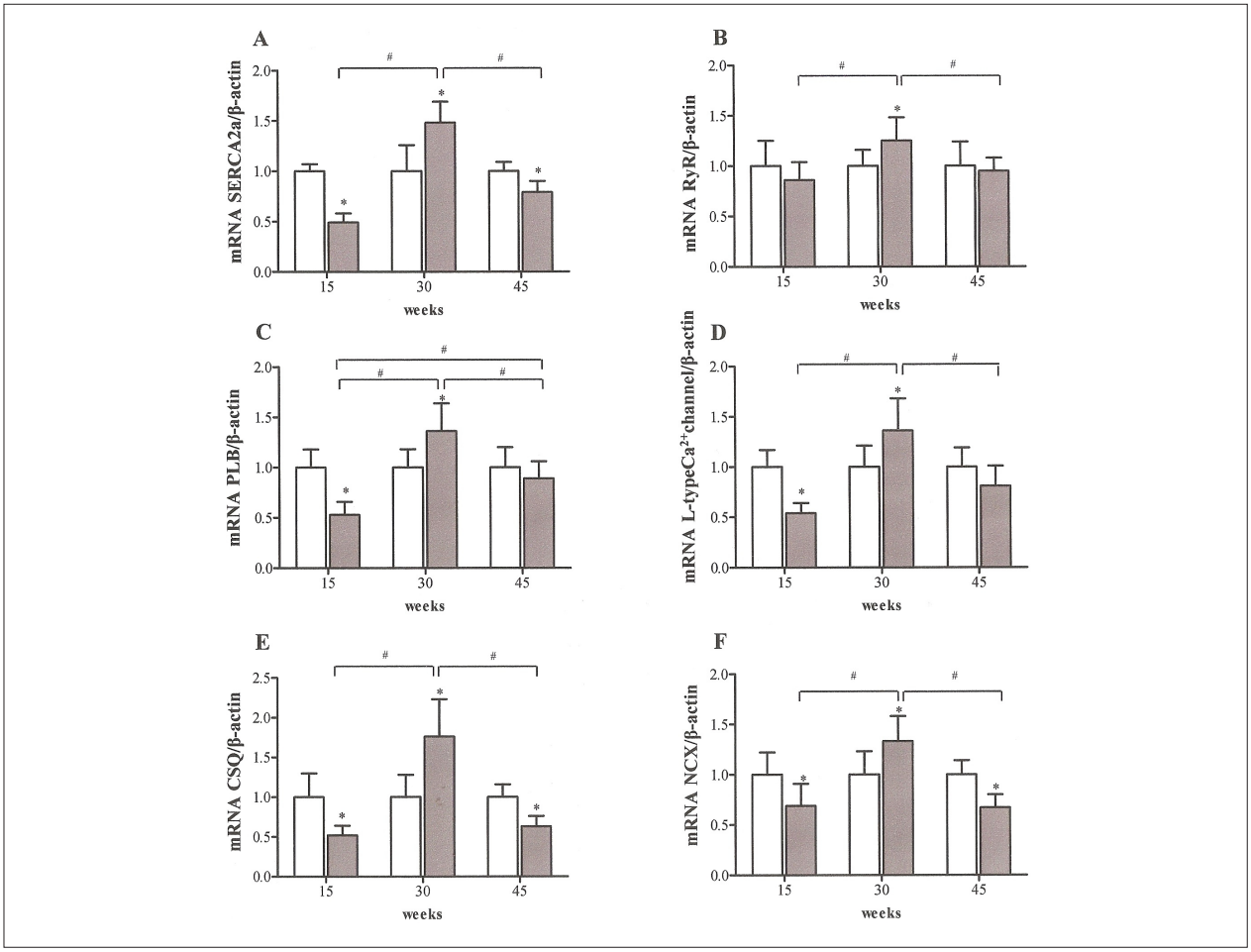

Figure 3 - *mRNA levels of key intracellular Ca^{2+}-cycling proteins determined by real-time PCR from control (white bars) and obese rats (gray bars) after 15, 30 and 45 weeks of experiment; A: SERCA2a - sarcoplasmic reticulum Ca^{2+}-ATPase; B: RyR - ryanodine receptor; C: PLB - phospholamban; D: L-type Ca^{2+} channel; E: CSQ - calsequestrin; and F: NCX - Na^{+}/Ca^{2+} exchanger; Data are mean ± SD; two-way ANOVA and Bonferroni post hoc test; * p<0.05 vs control group; # p<0.05 vs respective obese group.*

Table 2 - Correlations between free triiodothyronine (T_3) and mRNA levels of Ca^{2+} handling proteins

mRNA	Free triiodothyronine (T_3)	
	correlation coefficient	p *value*
SERCA2a	-0.021	0.89
RyR	-0.040	0.81
PLB	0.056	0.74
L-type Ca^{2+} channel	-0.168	0.33
NCX	-0.254	0.14

SERCA2a - sarcoplasmic reticulum Ca^{2+}-ATPase; PLB - Phospholamban; RyR - ryanodine receptor; CSQ - calsequestrin; NCX - Na^{+}/Ca^{2+} exchanger; Pearson's linear correlation test.

It is well known that obesity is accompanied by many hormonal changes including thyroid hormone levels. In this study, obesity promoted an increase in serum TSH after 45 weeks ($Ob_{45} > C_{45}$), probably caused by high levels of leptin in the obese group. Studies in experimental animals, as well as in human subjects have shown that leptin stimulates TSH release[27,28]. In addition, previous studies have shown a positive relationship between serum TSH and leptin levels indicating that thyroid hormones undergo a process of adaptation with increasing adiposity[29,30]. The absence of alterations in leptin and TSH levels in the control group over time corroborates this assertion. Another important finding from our current study is that T_3 and TSH response showed similar trends in the obese groups over time. As reported in the literature[31], changes in T_3

and TSH levels are often associated with alterations in body fat, our results suggest that this effect may be influenced by elevated of leptin levels over time.

Among research using obese models, we believe that this is the first study to verify the influence of long-term exposure to obesity on gene expression of Ca^{2+} handling proteins. In cardiomyocytes, the proteins associated with myocardial Ca^{2+} handling participate in the release, recapture and extrusion of Ca^{2+}, executing a key role in the regulation of cardiac function[3,4]. The current study showed that the duration of obesity resulted in different responses in gene expression of Ca^{2+} handling proteins after 15, 30 and 45 weeks. Our results showed that obesity, induced by a high-fat caused, initially (15 weeks), decreased gene expression. Consequently, in the 30ª week of induced obesity, the cellular mechanisms involved in functioning of the heart had to increase in an attempt to reestablish molecular cardiac mRNA levels. After normalization of this process, the mRNA transcription returned to normal and/or reduced levels (45 weeks). These findings suggest that, in the current study, there was a remodeling of cardiac processes after long periods of exposure to obesity. Rider et al[32] state that cardiac remodeling is an adaptive characteristic of obesity. According to Cohn et al[33], cardiac remodeling is defined as genome expression resulting in molecular, cellular and interstitial changes, manifested clinically as changes in size, shape and function of the heart resulting from cardiac load or injury. Furthermore, they point out that cardiac remodeling is influenced by hemodynamic overload, neurohormonal activation and other factors still under investigation. Therefore, a possible explanation for our results is that this process may be related to fat overload imposed to heart, where initially, it suffer one aggression after 15 weeks (obesity), then the heart has to remodel to normalize the cardiac molecular and cellular changes in the week 30. Finally, the heart adapts, returning to normal and/or reduced mRNA levels (45 weeks), since, it was no longer necessary to sustain increased RNA levels. These results may be corroborated with our previous and other studies which demonstrate that obesity induced and promoted myocardial dysfunction[17,34,35], where the myocardial dysfunction could be related to changes in mRNA levels after 15 weeks of obesity. Furthermore, researches conducted in our laboratory, shows that cardiac function returns to normal after 30 and 45 weeks of obesity (*unpublished data).*

An alternative explanation for the cardiac remodeling process to normalize after 45 weeks may be related to diet composition. The high-fat diet used in this study was composed of large amounts of unsaturated fatty acids and this could be a mechanism that protects the heart, even in the presence of obesity. The mechanisms underlying the effects of long-term to exposure to obesity on mRNA levels of Ca^{2+} handling proteins remain unknown. Several agents and pathways which may mediate the molecular and cellular responses (alterations in gene expression) involved in cardiac remodeling, include various cytokines, β-adrenergic receptors, neurotransmitters, intracellular messengers and hormones such as insulin[3,36-38].

One important mediator involved in the activation of several transcriptional factors, and, therefore, fundamental in to gene expression responses is thyroid hormone. The literature reports that thyroid hormones may play a positive role in gene expression of SERCA, RyR and L-type Ca^{2+} channels, and a negative role in PLB and NCX expression[9]. However, in disagreement with our hypothesis, thyroid hormone did not affect the gene expression of myocardial Ca^{2+} handling proteins, based on correlation analysis which showed no significant association between T_3 and mRNA levels of Ca^{2+} handling proteins. As shown in the cardiac remodeling process, different external stimuli can activate several transcriptional factors[36]. Therefore, we can infer that the same mediator/stimuli may have activated different transcription factors, leading to similar responses in gene expression. Since obesity is associated with increase in endothelin, angiotensin II, cytokines concentrations, among others[39,40], and they work via different transcriptional factors, it is likely that some of these factors may be involved in changes in mRNA expression of Ca^{2+} handling proteins.

Conclusion

Our data indicate that obesity over the short and long periods of time may promote alteration in gene expression of Ca^{2+} homeostasis regulatory proteins without influence by thyroid hormone. One aspect which is important is that the knowledge of mRNA alterations at different periods during the development and establishment of obesity could contribute to understanding the mechanisms involved in cardiac dysfunction promoted by obesity. Nevertheless, further studies are necessary to determine which mediators influence mRNA behavior at each time period of the remodeling process induced by obesity.

Author contributions

Conception and design of the research: Lima-Leopoldo AP, Leopoldo AS, Nascimento AF, Luvizotto RAM, Padovani CR, Nogueira CR, Cicogna AC; Acquisition of data: Lima-Leopoldo AP, Leopoldo AS, Silva DAT, Nascimento AF, Campos DHS, Luvizotto RAM, Nogueira CR, Oliveira Júnior SA, Cicogna AC; Analysis and interpretation of the data: Lima-Leopoldo AP, Leopoldo AS, Silva DAT, Campos DHS, Luvizotto RAM, Padovani CR, Nogueira CR, Oliveira Júnior SA, Cicogna AC; Statistical analysis: Lima-Leopoldo AP, Leopoldo AS, Padovani CR, Cicogna AC; Obtaining financing: Lima-Leopoldo AP, Leopoldo AS, Nascimento AF, Campos DHS, Cicogna AC; Writing of the manuscript: Lima-Leopoldo AP, Leopoldo AS, Nascimento AF, Nogueira CR, Cicogna AC; Critical revision of the manuscript for intellectual content: Lima-Leopoldo AP, Leopoldo AS, Silva DAT, Luvizotto RAM, Padovani CR, Nogueira CR, Oliveira Júnior SA, Cicogna AC.

Potential Conflict of Interest

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

Sources of Funding

This study was funded by FAPESP.

Study Association

This article is part of the thesis of doctoral submitted by Ana Paula Lima Leopoldo, from Universidade Estadual Paulista.

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. Relling DP, Esberg LB, Fang CX, Johnson WT, Murphy EJ, Carlson EC, et al. High-fat diet-induced juvenile obesity leads to cardiomyocyte dysfunction and upregulation of Foxo3a transcription factor independent of lipotoxicity and apoptosis. J Hypertens. 2006;24(3):549-61.
3. Bers DM. Cardiac excitation-contraction coupling. Nature. 2002;415(6868):198-205.
4. Opie LH. Myocardial contraction and relaxation. In: Opie LH. The heart: physiology from cell to circulation. Philadelphia: Lippincott-Raven; 1998. p. 221-45.
5. Dincer UD, Araiza A, Knudson JD, Shao CH, Bidasee KR, Tune JD. Dysfunction of cardiac ryanodine receptors in the metabolic syndrome. J Moll Cell Cardiol. 2006;41(1):108-14.
6. Philip-Couderc P, Smih F, Hall JE, Pathak A, Roncalli J, Harmancey R, et al. Kinetic analysis of cardiac transcriptome regulation during chronic high-fat diet in dogs. Physiol Genomics. 2004;19(1):32-40.
7. Lima-Leopoldo AP, Sugizaki MM, Leopoldo AS, Carvalho RF, Nogueira CR, Nascimento AF, et al. Obesity induces upregulation of genes involved in myocardial Ca^{2+} handling. Braz J Med Biol Res. 2008;41(7):615-20.
8. Carr AN, Kranias EG. Thyroid hormone regulation of calcium cycling proteins. Thyroid. 2002;12(6):453-7.
9. Dillmann WH. Cellular action of thyroid hormone on the heart. Thyroid. 2002;12(6):447-52.
10. Kahaly GJ, Dillmann WH. Thyroid hormone action in the heart. Endocr Rev. 2005;26(5):704-28.
11. Dillmann WH. Biochemical basis of thyroid hormone action in the heart. Am J Med. 1990;88(6):626-30.
12. Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81(3):1097-142.
13. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. N Engl J Med. 2001;344(7):501-9.
14. Danzi S, Klein I. Thyroid hormone-regulated cardiac gene expression and cardiovascular disease. Thyroid. 2002;12(6):467-72.
15. Klein I, Danzi S. Thyroid disease and the heart. Circulation. 2007;116(15):1725-35.
16. National Research Council. Guide for the care and use of laboratory animals: Washington, (DC): National Academy Press; 1996.
17. Leopoldo AS, Lima-Leopoldo AP, Sugizaki MM, do Nascimento AF, de Campos DH, Luvizotto RA, et al. Involvement of L-type calcium channel and SERCA2A in myocardial dysfunction induced by obesity. J Cell Physiol. 2011;226(11):2934-42.
18. 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 signaling in rats. Diabetologia. 2005;48(6):1229-37.
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-[Delta][Delta] CT method. Methods. 2001;25(4):402-8.
20. Tentolouris N, Pavlatos S, Kokkinos A, Perrea D, Pagoni S, Katsilambros N. Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. Metabolism. 2008;57(3):313-20.
21. Akiyama T, Tachibana I, Shirohara H, Watanabe N, Otsuki M. High-fat hypercaloric diet induces obesity, glucose intolerance and hyperlipidemia in normal adult male *Wistar* rat. Diabetes Res Clin Pract. 1996;31(1-3):27-35.
22. da Silva AA, Kuo JJ, Tallam LS, Hall JE. Role of endothelin-1 in blood pressure regulation in a rat model of visceral obesity and hypertension. Hypertension. 2004;43(2):383-7.
23. Dourmashkin JT, Chang GQ, Gayles EC, Hill JO, Fried SK, Julien C, et al. Different forms of obesity as a function of diet composition. Int J Obes. 2005;29(11):1368-78.
24. 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.
25. 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.
26. 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.
27. Michalaki MA, Vagenakis AG, Leonardou AS, Argentou MN, Habeos IG, Makri MG, et al. Thyroid function in humans with morbid obesity. Thyroid. 2006;16(1):73-8.
28. Ortiga-Carvalho TM, Oliveira KJ, Soares BA, Pazos-Moura CC. The role of leptin in the regulation of TSH secretion in the fed state: in vivo and in vitro studies. J Endocrinol. 2002;174(1):121-5.
29. Matzen LE, Kvetny J, Pedersen KK. TSH, thyroid hormones and nuclear-binding of T3 in mononuclear blood cells from obese and non-obese women. Scand J Clin Lab Invest. 1989;49(3):249-53.
30. Tagliaferri M, Berselli ME, Calò G, Minocci A, Savia G, Petroni ML, et al. Subclinical hypothyroidism in obese patients: relation to resting energy expenditure, serum leptin, body composition, and lipid profile. Obes Res. 2001;9(3):196-201.
31. Reinehr T. Obesity and thyroid function. Mol Cell Endocrinol. 2010;316(2):165-71.
32. Rider OJ, Francis JM, Ali MK, Byrne J, Clarke K, Neubauer S, et al. Determinants of left ventricular mass in obesity; a cardiovascular magnetic resonance study. J Cardiovasc Magn Reson. 2009;11:9.
33. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol. 2000;35(3):569-82.
34. Leopoldo AS, Sugizaki MM, Lima-Leopoldo AP, do Nascimento AF, Luvizotto RA, de Campos DH, et al. Cardiac remodeling in a rat model of diet-induced obesity. Can J Cardiol. 2010;26(8):423-9.
35. 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.
36. Katz AM. Heart failure. In: Katz AM. Physiology of the heart. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 510-48.
37. Proud CG. Ras, PI3-kinase and mTOR signaling in cardiac hypertrophy. Cardiovasc Res. 2004;63(3):403-13.
38. Young LH, Dahl DM, Rauner D, Barrett EJ. Physiological hyperinsulinemia inhibits myocardial protein degradation in vivo in the canine heart. Circ Res. 1992;71(2):393-400.
39. 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.
40. Rondinone CM. Adipocyte-derived hormones, cytokines, and mediators. Endocrine. 2006;29(1):81-90.