Myocardial Dysfunction and Abnormalities in Intracellular Calcium Handling in Obese Rats


Departamento de Desportos, Centro de Educação Física e Desportos, UFES - Universidade Federal do Espírito Santo, Vitória, ES; Departamento de Clínica Médica, Faculdade de Medicina, UNESP - Universidade Estadual Paulista “Júlio Mesquita Filho”, Botucatu, SP; Instituto de Ciências da Saúde, UFMT - Universidade Federal de Mato Grosso, Sinop, MT; Departamento de Fisioterapia, UFMS - Universidade Federal do Mato Grosso do Sul, Campo Grande, MS; Departamento de Bioestatística, Instituto de Biociências, UNESP - Universidade Estadual Paulista “Júlio Mesquita Filho”, Botucatu, SP - Brazil

Abstract

Background: Several mechanisms have been proposed to contribute to cardiac dysfunction in obesity models, such as alterations in calcium (Ca\textsuperscript{2+}) handling proteins and β-adrenergic receptors. Nevertheless, the role of these factors in the development of myocardial dysfunction induced by obesity is still not clear.

Objective: The purpose of this study was to investigate whether obesity induced by hypercaloric diets results in cardiac dysfunction. Furthermore, it was evaluated whether this functional abnormality in obese rats is related to abnormal Ca\textsuperscript{2+} handling and the β-adrenoceptor system.

Methods: Male 30-day-old Wistar rats were fed with standard food (C) and a cycle of five hypercaloric diets (Ob) for 15 weeks. Obesity was defined as increases in body fat percentage in rats. Cardiac function was evaluated by isolated analysis of the left ventricle papillary muscle under basal conditions and after inotropic and lusitropic maneuvers.

Results: Compared with the control group, the obese rats had increased body fat and glucose intolerance. The muscles of obese rats developed similar baseline data, but the myocardial responsiveness to post-rest contraction stimulus and increased extracellular Ca\textsuperscript{2+} were compromised. There were no changes in cardiac function between groups after β-adrenergic stimulation.

Conclusion: Obesity promotes cardiac dysfunction related to changes in intracellular Ca\textsuperscript{2+} handling. This functional damage is probably caused by reduced cardiac sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA2) activation via Ca\textsuperscript{2+} calmodulin kinase. (Arq Bras Cardiol 2011; 97(3) : 232-240)

Keywords: Myocardial dysfunction; rats; obesity; calcium elevation; receptors, adrenergic, beta.

Introduction

Obesity can be defined as a chronic metabolic disease characterized by excess fat that compromises healthy individuals\textsuperscript{1}. It is associated with various co-morbidities and has been recognized as an independent risk factor for several cardiovascular diseases\textsuperscript{2-4}.

Previous research attempting to relate cardiac function and diet-induced obesity present controversial results. Rats subjected to hypercaloric diet for 8 to 14 weeks revealed, on echocardiogram\textsuperscript{1,6} and myocyte\textsuperscript{7}, that obesity did not alter the cardiac function. However, Ouwens et al\textsuperscript{8} showed in papillary muscles from rats submitted to hypercaloric diet for 7 weeks higher basal contractile force and an impaired recovery after increase of workload. Moreover, other investigators have found impaired cardiac contraction in isolated hearts\textsuperscript{9}, papillary muscles\textsuperscript{10}, and cardiomyocytes\textsuperscript{11} in obese rabbits and rats, respectively.

Few studies have evaluated the relationship between Ca\textsuperscript{2+} handling, the β-adrenoceptor system, and myocardial function in obese animals\textsuperscript{11,12}. Ren et al\textsuperscript{12} reported depression in the myocardial functional state of genetically obese rats after inotropic maneuvers. Therefore, it is unclear whether similar mechanisms operate in humans and animal models of diet-induced obesity.

It is apparent that a variety of alterations in cardiac structure and function occur with elevations in adipose tissue. However, the mechanisms responsible for these alterations are not well established. Several factors have been proposed to contribute to cardiac dysfunction in obesity models, such as reduced myofilament Ca\textsuperscript{2+} sensitivity, as well as alterations in Ca\textsuperscript{2+} handling proteins and β-adrenergic receptors\textsuperscript{11-14}. Nevertheless, it is unclear whether these factors play a critical role in the development of myocardial dysfunction induced by obesity.
Considering divergent data, the purpose of this study was to obtain further information about the influence of obesity induced by hypercaloric diets on myocardial function. In addition, the objective of this work was to verify the participation of Ca$^{2+}$ handling and the β-adrenoceptor system on myocardial function in obese rats. Accordingly, the hypothesis of this study was that obesity induces myocardial dysfunction through Ca$^{2+}$ handling and β-adrenoceptor system alterations.

**Methods**

**Animal models and experimental protocol**

Thirty-day-old male Wistar rats were randomly assigned to one of two groups: control (C, n = 8) and obese (Ob, n = 8). The control group was fed a standard diet (Purina, Paulínia, SP, Brazil) and the Ob group was fed with cycles of five hypercaloric diets on a weekly basis as previously described. The hypercaloric diets provided to the Ob group were calorically rich (hypercaloric diets mean = 4.6 kcal/g versus standard diet = 3.5 kcal/g) due to the higher fat energy. In the hypercaloric diet 3, the increased caloric value was through suroce addition in the water (1.2 kcal/ml). Each group was fed with their relevant diets for 15 consecutive weeks. All rats were housed in individual cages in an environmentally-controlled clean-air room at 23 ± 3°C, 12 h light/dark cycle and 60 ± 5% relative humidity. Food intake was measured daily and body weight was recorded weekly. Weekly caloric intake was calculated as the average weekly food intake x calorie value of each diet. Feed efficiency, the ability to translate calories consumed into body weight, was measured by the following formula: mean body weight gain (g) / total caloric intake. Initial and final body weight (IBW and FBW, respectively), body length (L), left ventricle weight (LVW), right ventricle weight (RVW), and papillary muscle cross-sectional area (CSA) were recorded.

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, and were approved by the Botucatu Medical School Ethics Committee in Animal Experiments (Unesp, Botucatu, SP, Brazil).

**Systolic blood pressure (SBP)**

After 15 weeks of the experiments (just prior to sacrifice), the systolic blood pressure was assessed by tail-cuff plethysmography with Narco BioSystems™ Electro-Sphygmomanometer, model 709-0610 (International Biomedical, Austin, TX, USA).

**Oral glucose tolerance test (OGTT)**

At the end of the 15-week feeding period, an oral glucose tolerance test (OGTT) was performed. Rats were fasted overnight (12-15 h) and blood samples were drawn from the tip of the tail. Blood was collected under basal conditions and after gavage administration of 3 g/kg glucose load. Blood samples were collected at 0, 60, 120, 180 and 240 minutes, and analyzed using a glucometer (Accu-Check Go Kit; Roche Diagnostic Brazil Ltda, SP, Brazil).

**Body fat analysis and Lee’s index**

After animals had been anesthetized with sodium pentobarbital (50 mg/kg IP), decapitated, and thoracotomized, the viscera were disposed of, leaving only the carcass. Carcasses were dried at 100 ± 5°C for 72 h in a ventilated Fanem dryer (Fanem, Guarulhos, SP, Brazil). After drying, the carcass was wrapped in filter paper and the fat was extracted in a Soxhlet Extractor (Corning Incorporated Life Sciences, Lowell, MA, USA). The percentage of body fat in each carcass was calculated by the following formula: ([PP–PSSG]/PP) x 100, where PP = post-drying weight, PSSG = dry weight after fat extraction, and PP = pre-drying weight. The Lee’s index was calculated from: $\sqrt[3]{\text{body weight (g)/nasoanal length (cm)}}$.

**Isolated muscle performance**

Myocardial performance was evaluated by studying the isolated papillary muscle from the left ventricle (LV) as described previously. Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal) and euthanized. The hearts were promptly removed and placed in an oxygenated Krebs-Henseleit solution at 28°C. Papillary muscles were dissected from the LV, mounted between two stainless steel spring clips, placed vertically in a glass chamber containing Krebs-Henseleit solution at 28°C, and gassed with 95% O$_2$ and 5% CO$_2$. The composition of the Krebs-Henseleit solution was the following: 118.5 mM NaCl; 4.69 mM KCl; 1.25 mM CaCl$_2$; 1.16 mM MgSO$_4$; 1.18 mM KH$_2$PO$_4$; 5.50 mM glucose; and 24.88 mM NaCO$_3$. The lower spring clip was attached to a 120T-20B force transducer (Kyowa, Tokyo, Japan) by a steel wire (1/15,000 inch), which passed through a mercury seal at the bottom of the glass chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm, above which a micrometer was mounted to adjust the muscle length. The papillary muscle was placed between two parallel platinum electrodes and stimulated at a frequency of 0.2 Hz (12 times per minute), using square-wave pulses of 5 ms duration. Voltage was set to a value 10% greater than the minimum required to produce a maximum mechanical response.

The muscles were isotonically contracted with light loads continuously for 60 minutes, then loaded to contract isometrically and stretched to the maximum of their length-tension curves. After a 5-minute period during which preparations underwent isometric contractions, muscles were again placed under isometric conditions, and the peak of the length-tension curve (tension) was carefully determined. A 15-minute period of stable isometric contraction was imposed prior to the experimental period and one isometric contraction was then recorded.

The mechanical behavior of the papillary muscles was evaluated under basal conditions with 1.25 mM calcium ([Ca$^{2+}$]) and after inotropic maneuvers: post-rest contraction (PRC), increase in extracellular [Ca$^{2+}$] concentration from 0.625 to 8.0 mM, and during β-adrenergic stimulation with 10$^{-6}$ to 10$^{-4}$ M isoproterenol. Post-rest contraction (PRC) was studied at an extracellular calcium concentration ([Ca$^{2+}$]) of 1.25 mM, where the stimulus was paused for 30s before restarting the stimulation. During rest in the myocardium in...
rats, the sarcoplasmic reticulum (SR) accumulates calcium above and beyond that accumulated during regular stimulation, and the first beat after the rest interval (B1) is stronger than the beat before the rest interval (B0)\(^2\). The study of PRC may provide some insight into SR function in a biochemically intact preparation\(^4\). After the PRC, the response of the muscle after increasing extracellular calcium concentration \([Ca^{2+}]_o\) was assessed to test its effect on myofilament machinery\(^2\). Isometric contractile parameters were recorded 10 min after the addition of each dose of extracellular calcium (0.625 to 8.0 mM) to the bathing solution. Moreover, stimulation of the \(\beta\)-adrenoceptor system was also studied to test the integrity of the beta-adrenergic complex system, the sensitivity to troponin-C, and the calcium uptake by sarcoplasmic reticulum\(^5\). The \(\beta\)-adrenoceptor stimulation was induced by using cumulative concentrations of isoproterenol (10\(^{-8}\) to 10\(^{-4}\)M) in the presence of 1.0 mM \([Ca^{2+}]_o\).

#### Mechanical parameters

Conventional mechanical parameters at \(L_{\text{max}}\) were calculated from isometric contraction: maximum developed tension normalized per cross-sectional area of the papillary muscle (DT [g/mm\(^2\)]), resting tension normalized per cross-sectional area of the papillary muscle (RT [g/mm\(^2\)]), time-to-peak tension (TPT [ms]), peak of the positive (+dT/dt [g/mm\(^2\)/s]) and negative (-dT/dt [g/mm\(^2\)/s]) tension derivatives normalized per cross-sectional area of the papillary muscle, and time to peak tension to 50% relaxation (RT\(_{50}\) [ms]).

The parameters used to characterize the size of the papillary muscles were length (mm), weight (mg), and cross-sectional area (CSA [mm\(^2\)]). After each experiment, the muscle length at \(L_{\text{max}}\) was measured with a Gartner cathetometer (Gartner Scientific Corporation, Chicago, IL, USA) and the muscle between the two steel clips was blotted dry and weighed. The CSA was calculated by dividing muscle weight by its length, assuming uniformity and specific gravity of 1.0.

#### Statistical analysis

Data were reported as means ± standard deviation. Comparisons between groups were performed using Student’s t-test for independent samples. The glucose profile between groups was compared by analysis of variance (ANOVA) for repeated measures. When significant differences were found (p < 0.05), Bonferroni’s posthoc test for multiple comparisons was carried out\(^6\). All mechanical values of maneuvers were expressed as mean percent of baseline data and were calculated as follows: D = (\(M2-M1\))/\(M1\)×100, where \(M1\) was the value in baseline condition and \(M2\) was the value after the inotropic and lusitropic maneuvers. Effects of increased extracellular calcium and isoproterenol concentration were analyzed with repeated-measures two-way analysis of variance (ANOVA) and complemented by Tukey’s posthoc test for specific differences. The comparison of post-rest contraction was performed using the Student’s t-test. The level of significance considered was 5% (\(\alpha = 0.05\)).

### Results

#### General characteristics of rats

The general characteristics of rats are displayed in Table 1. After 15 weeks, there were no significant differences in final body weight (FBW), final body weight-to-length (FBW/L), Lee’s index, systolic blood pressure (SBP), calorie intake, left ventricular weight (LVW), or right ventricular weight (RVW) between the rats. The Ob rats ingested less food than the control rats and feed efficiency was higher in Ob rats compared with control rats, although these differences were not statistically significant (p = 0.06). Furthermore, the percentage of body fat found in the carcasses was significantly increased (96.1%) in Ob rats. Glucose intolerance and comorbidity associated with obesity were observed in this study (Figure 1).

#### Isolated muscle performance

##### Basal conditions

Baseline values of various parameters were obtained for isolated papillary muscle preparations (Table 2). The contraction performance of papillary muscles at basal conditions was similar for all parameters (DT, RT, TPT, +dT/dt, -dT/dt, and RT\(_{50}\)) between the two groups.

##### Extracellular calcium stimulation

The effects of increasing concentrations of calcium on papillary muscle function are summarized and illustrated in Figure 2. Increasing \([Ca^{2+}]_o\) from 0.625 to 8.0 mM promoted a greater response in DT and -dT/dt in control than obese rats. At calcium concentrations of 2.5 mM, 5.0 mM, and 8.0 mM, the mean percent of DT was 59 ± 20%, 51 ± 14%, and 43 ± 14%, respectively, of baseline in the obese group vs 105 ± 43%, 101 ± 47%, and 92 ± 50%, respectively, of baseline in the control group. At a \([Ca^{2+}]_o\) of 1.25 mM, the myocardium

<table>
<thead>
<tr>
<th>Table 1 - General characteristics of experimental groups</th>
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<tr>
<td>(C (n = 8))</td>
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<tr>
<td>Initial body weight (g)</td>
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<td>Final body weight (g)</td>
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<td>Final body weight / body length</td>
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<td>Lee’s index</td>
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<td>Body fat (%)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<td>Food intake (g/day)</td>
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<td>Energy consumption (kcal/day)</td>
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<tr>
<td>Feed efficiency (%)</td>
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<tr>
<td>LVW (g)</td>
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<td>RVW (g)</td>
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Values are means ± SD. control (C); obese (Ob); n – number of animals per group; LVW – left ventricular weight; RVW – right ventricular weight. * p < 0.05 vs C; Student’s t-test for independent samples.
Table 2 - Baseline data from isolated muscle preparation

<table>
<thead>
<tr>
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<th>C (n = 8)</th>
<th>Ob (n = 7)</th>
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<tbody>
<tr>
<td>DT (g/mm²)</td>
<td>4.87 ± 1.22</td>
<td>5.09 ± 1.00</td>
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<tr>
<td>RT (g/mm²)</td>
<td>1.04 ± 0.25</td>
<td>1.18 ± 0.29</td>
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<tr>
<td>TPT (ms)</td>
<td>157 ± 10</td>
<td>153 ± 8</td>
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<tr>
<td>+dT/dt (g/mm²/s)</td>
<td>53 ± 14</td>
<td>58 ± 11</td>
</tr>
<tr>
<td>-dT/dt (g/mm²/s)</td>
<td>18 ± 4</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>RT₅₀ (ms)</td>
<td>172 ± 19</td>
<td>175 ± 22</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>1.05 ± 0.22</td>
<td>0.88 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± SD; control (C) and obese (Ob) groups; n - number of animal per group; peak developed tension normalized per cross-sectional area (DT, g/mm²), resting tension normalized per cross-sectional area (RT, g/mm²), time-to-peak tension (TPT, ms), peak of the positive (+dT/dt, g/mm²/s) and negative (-dT/dt, g/mm²/s) tension derivatives normalized per cross-sectional area, and time from peak tension to 50% relaxation (RT₅₀, ms). CSA- cross-sectional area. There were no statistical differences between groups (p>0.05) Student’s t-test for independents sample.

Effects on post-rest contraction

The results shown in Figure 3 indicate that the PRC induced a greater response in DT and -dT/dt in control than obese rats. The DT and -dT/dt were significantly diminished in obese myocardium after 30 s of cessation of the stimulus. The mean percent of DT was 24 ± 7% of baseline in the obese group vs 39 ± 15% of baseline in the control group. In this sense (after PRC), percentage of -dT/dt compared to baseline was 6 ± 3% in the obese group versus 24 ± 15% in the control group. No differences were observed between the two groups for the other parameters (RT, +dT/dt, TPT, RT₅₀).

Stimulation with isoproterenol

For all investigated parameters, the myocardium from the obese group did not exhibit differences in response to β-adrenoceptor stimulation compared to the control group (Figure 4). A minor focal response was observed in +dT/dt in obese rats compared with control rats.

Discussion

This study investigated the effects of diet-induced obesity on myocardial performance, intracellular Ca²⁺ handling and the β-adrenoceptor system. The main finding in this study is that obesity promotes cardiac dysfunction by changes in intracellular Ca²⁺ handling, probably by reduced cardiac sarcoplasmic reticulum Ca²⁺ ATPase (SERCA2) activation via Ca²⁺ -calmodulin kinase. Although there was no difference in the final body weight between groups, the hypercaloric diet used was of sufficient intensity and duration to promote obesity in rats, measured by percentage of body fat (Table 1). The gain of body weight, 8.4%, was accompanied by a major increase in the weight of total body fat by 96.1% more than control rats, describing a model of diet-induced obesity. The
body weight per se can be misleading and, in this case, it may greatly underestimate the actual degree of obesity developed in obese rats\(^\text{19,27}\). These results are in line with several studies that induced obesity through hypercaloric diets in rats\(^\text{5,11,19,28,29}\) and did not observe any difference in final body weight\(^\text{6,8,30}\). Furthermore, Ob animals had the presence of co-morbidity associated with obesity, and impaired glucose tolerance.

The analysis of cardiac morphology showed that obesity did not alter the LV and RV weight. These data are consistent with previous studies\(^\text{5,7}\) that did not show myocyte hypertrophy in obese rats, but inconsistent with other publications that demonstrated higher LV and RV weights in obese rats\(^\text{11,29}\).

One of the primary objectives of this investigation was to study the changes in myocardial performance using an isolated papillary muscle preparation. The isolated preparation measured the capacity of the cardiac muscle to develop force and shorten, regardless of changes in loads, heart rate, and wall geometry that could modify the mechanical performance of the heart in vivo\(^\text{31}\). Additionally, inotropic and lusitropic maneuvers were also used to study the mechanical function of the heart.
of cardiac muscle, in order to identify abnormalities in contraction and relaxation that could not be observed under basal conditions. Moreover, they help understanding the mechanisms involved in myocardial functional alterations. The more commonly used maneuvers are: post-rest contraction, elevation of extracellular Ca$^{2+}$, and β-adrenergic stimulation. In this study, the evaluation of papillary muscle function showed that obesity did not promote changes as compared to the baseline data. This result is not consistent with Relling et al.\textsuperscript{11} which verified depressed peak shortening amplitude and maximal velocity of shortening/relengthening, as well as prolonged duration of shortening and relengthening in cardiomyocytes from obese rats. Other researchers have found reduced baseline tension development in papillary muscles from obese rabbits\textsuperscript{10}.

In this study, the post-rest contraction and elevation of extracellular Ca$^{2+}$ concentration caused a lower response in systolic (DT) and diastolic (-dT/dt) indices of myocardial function in the obese group. PRC findings and extracellular calcium concentration changes suggest that obesity promotes regulatory Ca$^{2+}$ channel dysfunction. The compromised response can be related to changes in the sarcolemmal Na$^+$/Ca$^{2+}$ exchanger.

**Figure 3** - Effects of post-rest contraction in the myocardium of control rats (n = 8) and obese rats (n = 7) after the stimulus was paused for 30 seconds. A: DT - peak developed tension; B: RT - resting tension; C: +dT/dt - positive tension derivative; D: -dT/dt - negative tension derivative; E: TPT - time to peak tension; F: RT $^{50}$ - time from peak tension to 50% relaxation. Data are mean percent of baseline value ± SD; * p < 0.05 vs control group; Student’s t-test for independent samples.
Obesity and myocardial dysfunction

(NCX), sarcolemmal L-type channel, sarcoplasmic reticulum (SR), and myofilament Ca$^{2+}$ sensitivity. Post-rest contraction allows us to study aspects of RS participation in the contraction-relaxation cycle of the cardiac muscle. In the rat ventricular muscle, PRC is recognized as a composite function of time- and beat-dependent recycling of Ca$^{2+}$ within the SR, combined with a small net cellular Ca$^{2+}$ gain through the Na$^+$/Ca$^{2+}$ exchange process. The first contraction is larger than that steady-state contraction when stimulated after rest interval. Therefore, this potentiation of PRC is highly dependent on increased Ca$^{2+}$ release from the sarcoplasmic reticulum.

In this study, the PRC induced a significantly diminished response in -dT/dt and DT in obese myocardium (Fig. 3). These data are consistent with a previous study that showed lower contractile response in obese Zuckers after 60s of cessation.
of stimulus. As -dT/dt is influenced by the rate of uptake of calcium ions into the SR, the minor Ca\(^{2+}\) resequestration demonstrated by -dT/dt in obese rats suggests depressed SERCA2 activity. The decrease in the -dT/dt with high amounts of cytosolic Ca\(^{2+}\) suggests that SERCA2 activation via Ca\(^{2+}\)-calmodulin kinase may be depressed by obesity. The significant reduction of developed tension observed in obese rats could be the result of depleted SR Ca\(^{2+}\) stock and diminished Ca\(^{2+}\) release by ryanodine receptors. Furthermore, we cannot reject the hypothesis that obesity reduces the rate of dissociation of Ca\(^{2+}\) from troponin-C. Other studies are necessary to clarify this relationship between troponin-C and calcium affinity.

The elevation of extracellular Ca\(^{2+}\) alters the contraction and relaxation phases due to increases in available cytoplasmic Ca\(^{2+}\) concentration, by interfering with the operation of the L-type channel, NCX, and SR function. The lower response to extracellular Ca\(^{2+}\) elevation in obese rats (Fig. 2) can be related to a reduction of Ca\(^{2+}\) influx across L-type channels and/or changes in the SR function, as verified with post-rest contraction. These results are in line with previous studies that verified cardiac dysfunction and depressed responsiveness to extracellular Ca\(^{2+}\) elevation in myocytes and papillary muscles of obese rats. However, another study found a high response to extracellular Ca\(^{2+}\) increase in rats provided with a high-fat diet.

The β-adrenergic system has an important role in cardiac performance, which is to promote alterations in intracellular Ca\(^{2+}\) cycling. This alteration results from cyclic AMP-stimulated phosphorylations, increasing Ca\(^{2+}\) influx across sarcolemmal L-type channel, Ca\(^{2+}\) uptake by SERCA2, facilitating Ca\(^{2+}\) dissociation from troponin-C. Alterations in one or more components of the β-adrenergic pathway have been implicated in the reduced contractile response to β-adrenergic agonists. Data from isoproterenol stimulation reveals that the β-adrenergic system and cAMP phosphorylation of proteins related to Ca\(^{2+}\) handling, including phospholamban (PLB) and SERCA2, were preserved in obese rats. This result reinforces the idea that the reduced -dT/dt observed in obese rats has probably occurred by decreased phosphorylation of PLB via Ca\(^{2+}\) calmodulin-kinase. Similar cardiac contractile responses to β-adrenergic stimulation have been previously observed in obese rabbits.

**Conclusion**

This investigation shows that obesity causes myocardial dysfunction, and this change may be possibly attributed to reduced SERCA2 Ca\(^{2+}\) uptake due to the lower activation via Ca\(^{2+}\) - calmodulin kinase. Further studies are necessary to confirm this data.

**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 post-graduation program.

**References**


