Obesity Preserves Myocardial Function During Blockade of the Glycolytic Pathway

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Abstract

Background: Obesity is defined by excessive accumulation of body fat relative to lean tissue. Studies during the last few years indicate that cardiac function in obese animals may be preserved, increased or diminished.

Objective: Study the energy balance of the myocardium with the hypothesis that the increase in fatty acid oxidation and reduced glucose leads to cardiac dysfunction in obesity.

Methods: 30-day-old male Wistar rats were fed standard and hypercaloric diet for 30 weeks. Cardiac function and morphology were assessed. In this paper was viewed the general characteristics and comorbities associated to obesity. The structure cardiac was determined by weights of the heart and left ventricle (LV). Myocardial function was evaluated by studying isolated papillary muscles from the LV, under the baseline condition and after inotropic and lusitropic maneuvers: myocardial stiffness; postrest contraction; increase in extracellular Ca2+ concentration; change in heart rate and inhibitor of glycolytic pathway.

Results: Compared with control group, the obese rats had increased body fat and co-morbities associated with obesity. Functional assessment after blocking iodoacetate shows no difference in the linear regression of DT, however, the RT showed a statistically significant difference in behavior between the control and the obese group, most notable being the slope in group C.

Conclusion: The energy imbalance on obesity did not cause cardiac dysfunction. On the contrary, the prioritization of fatty acids utilization provides protection to cardiac muscle during the inhibition of glycolysis, suggesting that this pathway is fewer used by obese cardiac muscle. (Arq Bras Cardiol. 2014; 103(4):330-337)

Keywords: Obesity; Rats; Myocardial; Metabolism; Fatty Acid.

Introduction

Obesity is a chronic metabolic disease defined by an excessive accumulation of body fat compared to lean tissue[1]. According to estimates by the World Health Organization, in 2015, about 2.3 billion adults are overweight and of these, at least 700 million are considered obese (body mass index [BMI]> 30 g/m^2)[1]. The increased availability and consumption of energy and highly palatable diets has led to increased body weight in the population[2-4]. For this reason, experimental studies, diet-induced obesity is the most appropriate model to study the consequences of this disease[2-4].

Obesity is associated with increased risk of mortality and reduced life expectancy[5,6] and cardiovascular disease[7,8]. Clinical studies or experimental show that obesity can lead to depression of cardiac function, the pathophysiological mechanisms responsible for this change are not entirely clear[9]. In normal aerobic conditions, approximately 70% of myocardial energy production derived from fatty acid metabolism, glycolytic contribution being about 30%[10-13]. In obesity, the increased uptake and oxidation of fatty acids is associated with decreased myocardial glucose utilization[2,12,14-16]. These changes in energy substrates can be held responsible for cardiac dysfunction observed in obese[2,9,17-21]. In mice db/db obese and diabetic patients, normalization of energy metabolism, increased utilization of glucose, reversed the contractile dysfunction in these animals[20,21]. This finding concurs with studies

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Manuscript received December 06, 2013; revised March 14, 2014; accepted May 19, 2014.

DOI: 10.5935/abc.20140135

suggesting that ATP generated by the oxidation of glucose is used preferentially by the calcium pump (Ca^{2+}) from the sarcoplasmic reticulum (SERCA2)[12,13,22-24], the protein responsible for reuptake of cytosolic calcium.

The objective of this study was to test the hypothesis that the myocardial energy balance, increased fatty acid oxidation and decreased glucose in obese animals leads to cardiac dysfunction resulting ATP deficit for SERCA2. In order to test this premise, the glycolytic pathway was inhibited by myocardial administration of iodoacetate (IAA), a drug that blocks the activity of the enzyme glyceraldehyde 3-phosphate dehydrogenase[25]. The inhibition of glycolysis enhance cardiac dysfunction in obese mice.

Methods

Animal Model and Experimental Protocol

Thirty-day-old male Wistar rats were randomly assigned to one of two groups: control (C, n=12) and obese (Ob, n=12). The control group was fed a standard rat chow containing 11.2% fat, 55.5% carbohydrate, and 33.3% protein; whereas the obese animals received a high-fat diet containing 45.2% kcal fat, 28.6% carbohydrate, and 26.2% protein. Each group was fed the diet for 30 consecutive weeks. High fat diet was designed in our laboratory and contained powdered commercial Agroceres rat chow (Agroceres®, Rio Claro, SP, Brazil), industrialized feed, protein supplement, vitamins and minerals. The high-fat diet was calorically rich (high-fat diet = 3.65 kcal/g versus standard diet = 2.95 kcal/g) due to the higher fat composition, made with saturated (20.2%) and unsaturated fatty acid (79.8%). All rats were housed in individual cages in an environmentally-controlled clean-air room at 23 ± 3°C with a 12 h light/dark cycle and 60 ± 5 % relative humidity. Food consumption was measured daily and water ad libitun. Initial and final body weights (IBW and FBW, respectively) were recorded. Weekly caloric intake was calculated as the average weekly food consumption x caloric value of each diet. Feed efficiency, the ability to translate calories consumed into body weight, was also evaluated.

All experiments and procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals,* published by the U.S. National Institutes of Health[26], and were approved by the Botucatu Medical School Ethics Committee (UNESP, Botucatu, SP, Brazil).

Composition of Experimental Diets

The experimental diets provided sufficient amounts of protein, vitamins, and minerals according to the *Nutrient Requirements of Laboratory Animals* (1995). The standard and the four high-fat diets used in the study were formulated by Agroceres (Rio Claro, SP, Brazil). The ingredients were first ground and then mixed with vitamins and minerals. The mixture was made into pellets, dried in a ventilated drying oven at 55 ± 5°C, and stored at -20°C. The standard diet (RC Focus 1765) contained soybean oil, whole corn, wheat bran, soybean bran, dicalcium phosphate, sodium chloride, fish and meat flour, antioxidant additive, and a vitamins and minerals mixture. Meanwhile, the dietary ingredients used to prepare the high-fat diets were sodium chloride, casein, powdered milk, soybean protein concentrate, whole corn, cracker flour, dicalcium phosphate, Ca2+ carbonate, additives emulsifier, antioxidants and flavoring (cheese, vanilla, chocolate, and bacon), and a vitamins and minerals mixture. The composition of the high-fat diet consisted of saturated and unsaturated fatty acids, which provided 20% and 80% of the fat-derived calories, respectively[9,26].

Determination of Obesity

A criterion based on the adiposity index was used to determine obesity according to several authors[9,26-28]. After animals had been anesthetized (sodium pentobarbital 50 mg/kg intraperitoneal [i.p.]), decapitated, and thoracotomized, the fat pads of adipose tissue 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 [9,26].

Comorbidities Associated with Obesity

As the rat models of diet-induced obesity may develop some of the characteristics of human obesity, such as hypertension, glucose intolerance, insulin resistance, dyslipidemia, hyperinsulinemia, and hyperleptinemia, the following evaluations were performed in all groups. For biochemical analysis, ten animals of each group were used.

Systolic Blood Pressure

At the conclusion of the experiments, the systolic blood pressure was assessed by using the non-invasive tail-cuff method[27] with a Narco BioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA). The average of two pressure readings was recorded for each animal.

Oral Glucose Tolerance Test

At the end of the 30-week feeding period, an oral glucose tolerance test was performed. Rats fasted overnight (12-15 h) and blood samples were drawn from the tip of the tail. Blood glucose was collected under basal conditions and after intraperitoneal [i.p.] administration of 2 g/kg glucose load[9]. Blood samples were collected at 0, 15, 30, 60, 90 and 120 minutes, and analyzed using a glucometer (Accu-Check Go Kit; Roche Diagnostic Brazil Ltda, SP, Brazil).

Plasma Analysis of Hormones

At the end of treatment, animals were subjected to 12-15 h fast, anaesthetized with sodium pentobarbital (50 mg/kg intraperitoneal) and euthanized by decapitation. Blood was collected in heparinized tubes, centrifuged at 3000 × g for 15 minutes at 4°C, and then stored at -80°C. Plasma leptin and insulin concentrations were determined by ELISA[9] using specific commercial kits (Linco Research Inc., St. Louis, MO, USA).

Cholesterol, Triacylglycerol, non-esterified Tatty Acid, Insulin, and Leptin

At the end of the experimental period, animals were submitted to 12–15 h of fasting, 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 levels of GL, triglycerides (TG), total cholesterol (T-Chol), non-esterified fatty acid (NEFA), and hormones (insulin and leptin). Serum concentrations of GL, TG, and T-Chol were measured with an automatic enzymatic analyzer system (Technicon, RA-XTTM System, Global Medical Instrumentation, Minnesota, USA). NEFA levels were determined by method of Johnson and Peters (1993) by using colorimetric kits (WAKO NEFA-C, Wako Pure Chemical Industries, Osaka, Japan). Leptin and insulin levels were determined by ELISA using specific commercial kits (Linco Research Inc., St. Louis, MO, USA).

Body Fat Analysis

After animals had been anesthetized (sodium pentobarbital 50 mg/kg i.p.), decapitated and thoracotomized, the fat pads of adipose tissue were dissected and weighed. The total body fat was measured from the sum of the individual fat pad weights: epididymal fat + retroperitoneal fat + visceral fat. The adiposity index was calculated from: (body total fat/final body weight)*100 [9,26].

Cardiac Structure and Function

The heart and left ventricle (LV) weights were determined as indices of cardiac structure in absolute values and after normalization with the length of the shin-bone (SB).

Myocardial function was evaluated by studying isolated papillary muscles from the LV. This procedure has been utilized by various authors[9,17,29]. This preparation allows us to measure the capacity of cardiac muscle to shorten and develop force independently of influences that can modify *in vivo* mechanical performance of the myocardium, such as heart rate, preload, and afterload. Briefly, at the time of investigation, rats were anesthetized with sodium pentobarbital (50 mg/kg IP) and sacrificed by decapitation. The hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. LV papillary muscles from C (n = 12) and Ob rats (n = 12) were dissected, mounted between two spring clips, and placed vertically in a chamber containing Krebs-Henseleit solution (118.5 mM NaCl; 4.69 mM KCl; 2.5 mM CaCl2; 1.16 mM MgSO4; 1.18 mM KH2PO4; 5.50 mM GL, and 24.88 mM NaCO3) maintained at 28°C with a thermostatic water circulator. The bathing solution was bubbled with 95% oxygen and 5% carbon dioxide, with a pH of 7.4. The lower spring clip was attached to a 120T-20B force transducer (Kyowa, Tokyo, Japan) by a thin steel wire (1/15,000 inch), which passed through the mercury seal at the bottom of the chamber. The upper spring clip was connected by a thin steel wire to a rigid lever arm, above which a micrometer stop was mounted for adjusting the muscle length. The muscle preparation was placed between two parallel platinum electrodes (Grass E8, GRASS Technologies, An Astro-Med, Inc. Product Group, West Warwick, RI, USA) and stimulated at a frequency of 0.2 Hz (12 pulses/min) by using square-wave pulses of 5 ms in duration. Voltage was set to a value 10% greater than the minimum required to produce a maximal mechanical response.

The muscles were contracted isotonically with light loads for 60 min and then loaded (50 g) to contract isometrically and stretched to the maximum of their length-tension curves. After a 5-min period during which preparations underwent isotonic contractions, muscles were again placed under isometric conditions, and the peak of the length-tension curve (*Lmax*) was carefully determined. A 15-min period of stable isometric contraction was imposed prior to the experimental period, during 10 which one isometric contraction was then recorded.

The following parameters were measured from the isometric contraction at *Lmax*: peak developed tension (DT [g/mm^2]), resting tension (RT [g/mm^2]), maximum rate of tension development (+dT/dt [g/mm^2/s]) and maximum rate of tension decline (-dT/dt [g/mm^2/s]) normalized per cross-sectional area (CSA). The myocardial stiffness was determined by the ratio between the muscle length variation and resting tension. Resting tension was analyzed in muscle length corresponding to 90, 92, 94, 96, 98, and 100 % of the L_{max}. Resting tension-length curves were plotted using exponential regression analysis: log (RT) = -51.1118 + 25.5425 log(L_{max}) for the C group and log (RT) = -58.1992 + 29.1455 log(L_{max}) for the Ob group.

The parameters used to characterize papillary muscle were length (mm), weight (mg), and CSA (mm^2). After the end of each experiment, the muscle length at L_{max} was measured with a cathetometer (Gartner Scientific Corporation, Chicago, IL, USA) and the muscle between the two clips was blotted dry and weighed. The cross-sectional area was calculated from the muscle weight and length by assuming uniformity and a specific gravity of 1.0. All force data were normalized for the muscle cross-sectional area.

To determine the mechanism by which obesity induces negative inotropic effects on contractile function, the papillary muscles were evaluated under the baseline condition of 2.5 mmol/L Ca2+ and after inotropic and lusitropic maneuvers: postrest contraction of 10, 30 and 60 seconds; increase in extracellular Ca2+ concentration from 0,5 to 2.5 mmol/L; change in heart rate from 0.1 to 2.0 Hz; addition of the acid iodo acetic; the evaluation of glycolytic pathway was performed using a specific inhibitor, acid iodo acetic, 10^{-3} mM/L. The myocardial function was recorded 5, 10, 15, 20, 25, 30, 35, 40 and 45 min after the addition of acid iodo acetic at the solution nutrient. The behavior of the TD and TR after blocker administration of the glycolytic pathway, was carried through the construction of the linear regression model.

Statistical Analysis

General, nutritional, hormonal and morphologic characteristics, and cardiac function evaluation were reported as means ± standard deviation and the comparison between groups was analyzed using Student's t-test for independent

samples. The glucose profile of the groups was compared by ANOVA for repeated measures. When significant differences were found ($p < 0.05$), the Bonferroni test post hoc for multiple comparisons was carried out. The regression curves of the blockade of the glycolytic pathway and myocardial stiffness were compared by the angular coefficient test and linear regression. The program used for statistical analysis was the Sigma Plot 3.5 for Windows (Systat Software Inc., San Jose, CA, USA). The normality of variables was analyzed by the Kolmogorov-Smirnov test. The level of significance considered was 5 %.

Results

General characteristics and comorbities associated to obesity

The general characteristics and comorbities associated to obesity are shown in Table 1. The final body weight, total body fat, adiposity index and glucose, leptin and insulin levels were higher in Ob than in C. There was no significant difference between groups in protein and lipids levels. The test results of glucose tolerance are shown in Figure 1. Glucose levels were similar at baseline between groups. After intraperitoneal

Table 1 – General characteristics and comorbities associated to obesity

Variables	Groups		p-value
	C (n = 12)	Ob (n = 12)	
FBW (g)	498 ± 25	562 ± 36*	0.03467
Adiposity index (%)	4.28 ± 1.65	5.96 ± 1.54*	0.02872
Glucose (mg/dL)	107 ± 22	126 ± 19*	0.00548
Triglicérides (mg/dL)	60.1 ± 15.2	66.1 ± 24.4	0.30161
Colesterol (mg/dL)	58.3 ± 10.5	59.0 ± 8.1	0.78859
HDL (mg/dL)	24.8 ± 4.3	27.1 ± 4.9	0.10570
LDL (mg/dL)	14.0 ± 3.3	12.2 ± 2.8	0.13769
NEFA (mmol/L)	0.27 ± 0.05	0.28 ± 0.06	0.66658
Leptin (ng/dL)	2.40 ± 0.36	7.60 ± 0.81*	0.00005
Insulin (ng/dL)	0.23 ± 0.08	0.54 ± 0.07*	0.00067
SBP (mmHg)	127 ± 12	129 ± 13	0.87912

Values expressed as mean ± standart deviation. C: control; Ob: obese; FBW: final body weight; HDL: lipoprotein of high density; LDL: lipoprotein of low density; NEFA: non-esterified fatty acids; SBP: systolic blood pressure. Student's t-test for independent samples.

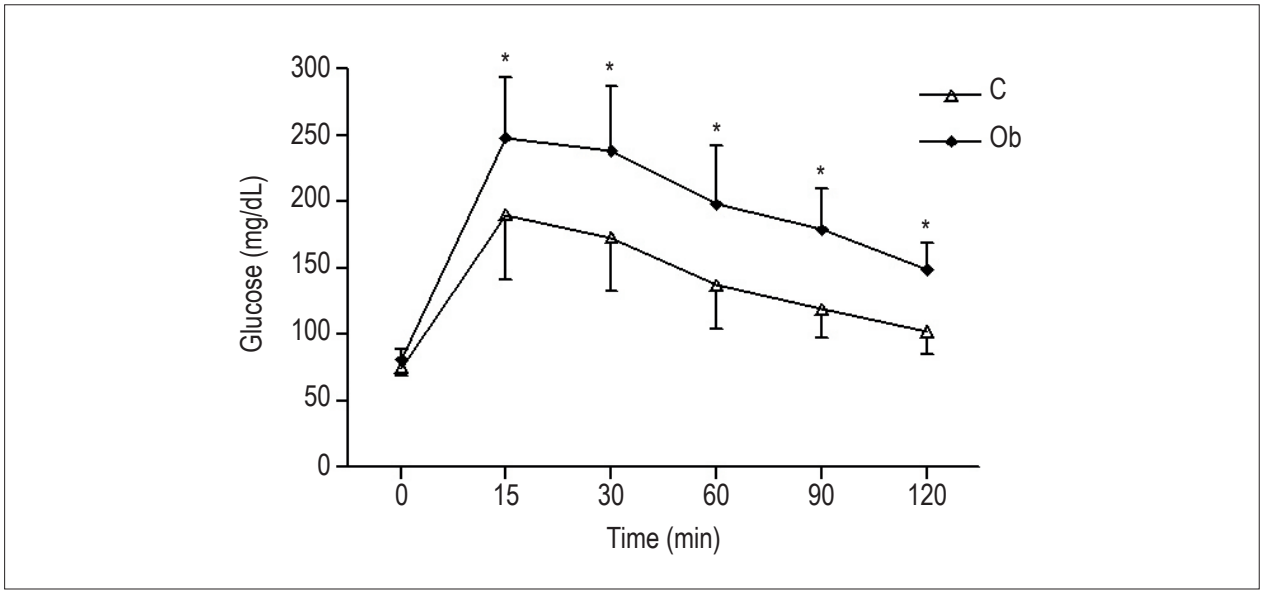

Figure 1 – *Oral glucose tolerance test in control animals (C, n = 12) and obese (Ob, n = 12). Values expressed as mean ± standart deviation. ANOVA and Bonferroni test. * p < 0.05 vs C.*

administration of glucose, blood glucose was higher in Ob group at all times evaluated.

Cardiac structure and function

The macroscopic cardiac structure is presented in Table 2; there was no significant difference between the two groups. Basal papillary myocardial function is presented in the Table 3; there were no significant differences in mechanical data. Moreover both groups, control and obese, presented same behavior with inotropic stimulations. The influence of variation of muscle length on the resting tension of the papillary muscle is shown in Figure 2; there were no significant differences between the two groups. This result shows that obesity did not increase the stiffness of the myocardium. Functional evaluation after blockade by iodoacetate is shown in Figure 3A and 3B; while there was no difference between linear regression of DT (Figure 3A), the RT showed a statistically significant difference in behavior between control and obese group, being the slope of the more remarkable in group C (Figure 3B).

Discussion

In the study period the diet was able to develop obesity because the adiposity index was 39.2% higher than the control. Data from this study are consistent with work done in our laboratory using the same methodology[9,17,26] . In this paper was viewed some comorbidities associated with obesity experimental as glucose intolerance, hyperinsulinemia and hyperleptinemia. These results reveal that our model promotes changes in metabolic and hormonal parameters and are in agreement with the findings of several researches[9,30,31].

Obesity did not cause structural remodeling in heart rate and systolic blood pressure. The data differ from studies that showed that obesity induced by high-fat diet promoted cardiac hypertrophy[9,32,3326] and increased blood pressure[34]. The change in these variables do not suggest that obesity did not cause significant change neuro hormone capable of producing cell proliferation, vasoconstriction, and hydro-saline retention. In this study, myocardial function was assessed in vitro using isolated LV papillary muscles. Papillary muscle preparations permit the measurement of cardiac muscle force ability to develop and to shorten, independent of changes in cardiac load and heart rate that might modify mechanical performance of the myocardium in vivo. Inotropic stimulation allows the identification of alterations in contraction and relaxation phases observed that can not be read help under basal conditions and in the under-standing of the mechanisms involved alterations in myocardial function. The animals used in both groups had similar cross-sectional area and this allows to avoid the homogenizing influence of cross-sectional area on the results[35]. The results show that obesity did not cause deterioration of cardiac function and myocardial stiffness in basal and with inotropic stimulation. This behavior is similar to that obtained by other authors, who used isolated hearts of obese rabbits by 12 weeks[36] and isolated myocytes of obese mice for 14 weeks[37]. However, researchers differ from that observed depression of mechanical function in isolated myocytes of obese mice for 12 weeks[38] and the authors found that elevation of basal contractile performance of papillary

Table 2 – Macroscopic cardiac structure

Variables	Groups		p-value
	C (n = 12)	Ob (n = 12)	
LV (g)	0.18 ± 0.02	0.20 ± 0.02	0.82766
Heart (g)	0.055 ± 0.01	0.060 ± 0.01	0.06416
LV/SB (g/mm)	0.021 ± 0.003	0.023 ± 0.002	0.12565
Heart/SB (g/mm)	0.29 ± 0.02	0.32 ± 0.02	0.32872

Values expressed as mean ± standart deviation. C: control; Ob: obese; LV: left ventricle; SB: shin-bone. Student's t-test for independent samples.

Table 3 – Basal isometric contraction

Variables	Groups		p-value
	C (n = 12)	Ob (n = 12)	
DT (g/mm^2)	7.14 ± 1.77	6.89 ± 1.54	0.22756
RT (g/mm^2)	0.68 ± 0.24	0.67 ± 0.20	0.61639
+dT/dt (g/mm^2/s)	69.4 ± 18.1	62.9 ± 14.6	0.19907
-dT/dt (g/mm^2/s)	22.1 ± 5.9	21.6 ± 4.9	0.52313
CSA (mm^2)	0.97 ± 0.24	0.98 ± 0.27	0.38607

Values expressed as mean ± standart deviation. C: control; Ob: obese; DT: peak developed tension; RT: resting tension; +dT/dt: maximum rate of tension development; -dT/dt: maximum rate of tension decline; CSA: cross sectional area. Student's t-test for independent samples.

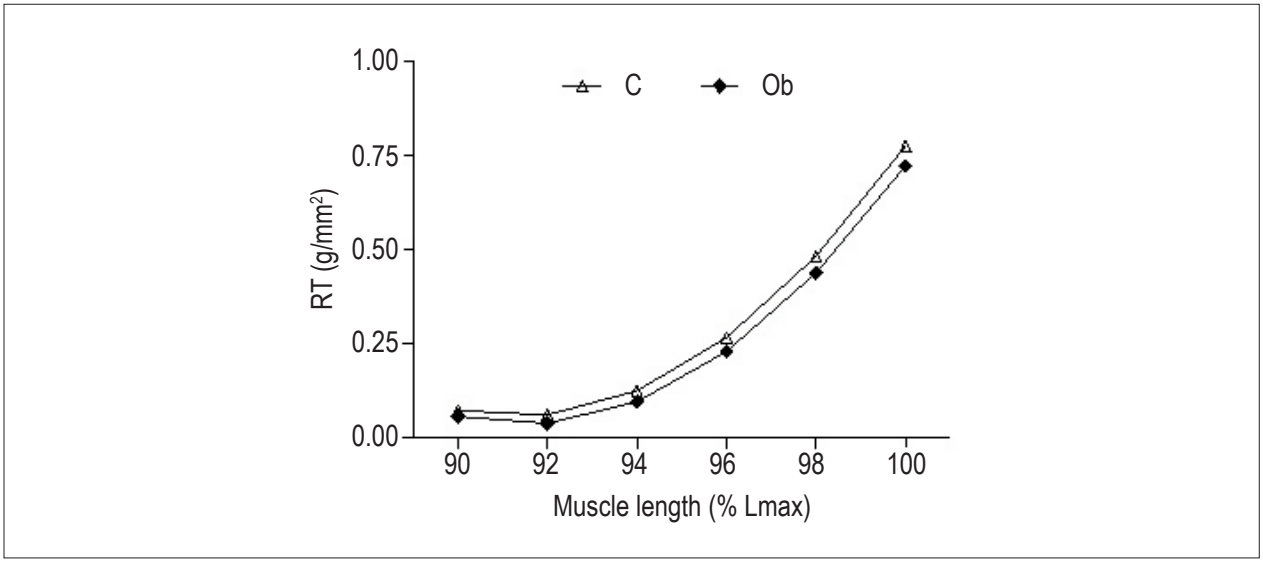

Figure 2 – *The influence of variation of muscle length on the resting tension in control animals (C, n = 12) and obese (Ob, n = 12). Linear regression.*

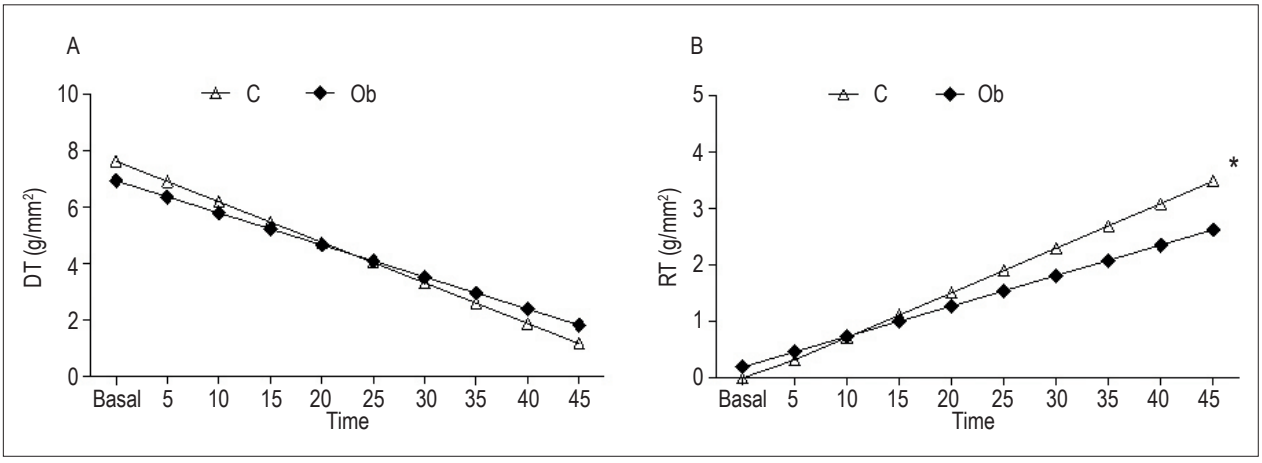

Figure 3 – *Effects of blockade of glyceraldehyde 3-phosphate dehydrogenase with iodoacetate in the myocardium of rats by linear regression model in control animals (C, n = 12) and obese (Ob, n = 12). A: DT: peak developed tension. B: RT: resting tension. Linear regression. * p < 0,05 vs Ob.*

muscle in obese mice by 7 weeks. Leopoldo et al[9] did not observe change in basal myocardial function in obeses rats by 15 weeks; however, these authors found differences between obese and control groups after L-type Ca2+ channel and SERCA2 blockers.

The blockade of the glycolytic pathway with iodoacetate on myocardial function showed that the slope of developed tension in obese animals was similar to control group; however, this slope of the RT in obese rats was significantly lower than controls. This finding suggests, contrary to expectations, that the control rats showed myocardial stiffness higher than the obese. This behavior could be related to the highest elevation of cytosolic calcium, possibly resulting from a lower recapture by SERCA2 and/or greater affinity between calcium and troponin C. The smallest contracture observed in obese rats suggests that prioritization of fatty acids in the myocardium at the expense of glucose increased energy availability for SERCA2 provided by fatty acids led to myocardial protection during the blockade of the glycolytic pathway. This suggests that in situations that occur in increased concentrations of fatty acids, the SERCA2 also uses energy from the beta-oxidation. Therefore, in this experiment, obesity promoted metabolic changes that resulted in myocardial protection.

The difference in behavior between the TR and TD, the TD being equal between the groups could be related to the ratio between the amount of calcium that diffuses and is removed from the cytosol during a cardiac cycle. The reason, according to Katz[39], approximately 150 shows that the amount of calcium that is removed is less than the spread to the interior of the cell[39]. Since SERCA2, largely responsible for the reuptake of

calcium ions in rodents[40], uses ATP to its basic function, an energy deficit for this organelle would have severe deleterious consequences for diastole than in systole[40].

Conclusion

In conclusion, the energy imbalance in obesity does not cause cardiac dysfunction. Rather, the prioritization of the use of fatty acids provides protection to the heart muscle during the blockade of glycolysis, suggesting that this pathway is lesser utilized by myocyte in obese animals.

The results of this study suggest that the use of fatty acids by the myocardium, in certain situations, may have beneficial effects; thus, could do his job in models of cardiac remodeling as a therapy of low cost and easy implementation.

Acknowledgments

We are grateful to José C. Georgette, Mário B. Bruno, Sandra A. Fabio, Camila R. C. Camacho and Corina J. Correa for their technical assistance. This manuscript has been proofread and edited by native English speakers with a related biomedical background in BioMed Proofreading (Cleveland, Ohio, USA).

Author contributions

Conception and design of the research: Campos DHS; Nascimento AF; Cicogna AC. Acquisition of data: Campos DHS; Leopoldo AS; Lima-Leopoldo AP; Nascimento AF; Oliveira-Júnior AS; SILVA DCT. Analysis and interpretation of the data: Campos DHS; Leopoldo AS; Lima-Leopoldo AP; Oliveira-Júnior AS; SUGIZAKI MM; Cicogna AC. Statistical analysis: Campos DHS; Leopoldo AS; Padovani CR. Obtaining fundung: Campos DHS; Cicogna AC. Writing of the manuscript: Campos DHS; Cicogna AC. Critical revision of the maniscript for intelectual contente: Campos DHS; Sugizaki MM; 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 - process n° 07/59747-7 e 07/46595-0.

Study Association

This article is part of the thesis of master submitted by Dijon Henrique Salomé de Campos, from Faculdade de Medicina de Botucatu – UNESP.

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