ORIGINAL ARTICLE

Cardiometabolic Alterations in Wistar Rats on a Six-Week Hyperlipidic, Hypercholesterolemic Diet

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Abstract

Background: The diet of modern society is composed by large intakes of lipids and cholesterol, involved in the development of cardiometabolic diseases. However, there are gaps in the literature regarding the existence of dyslipidemia models in Wistar rats.

Objectives: To analyze the cardiometabolic profile of Wistar rats on a six-week hyperlipidic, hypercholesterolemic diet.

Methods: Young Wistar rats were kept on a hyperlipidic, hypercholesterolemic diet for six weeks to induce hyperlipidemia. The rats underwent catheterization of the carotid artery to determine blood pressure. After fasting, blood samples were drawn through the catheter, and concentrations of total cholesterol, HDL cholesterol, triglycerides and glucose were determined. Cardiac tissue samples were taken for a histological analysis to check for ventricular hypertrophy.

Results: The six-week diet was effective in inducing cardiometabolic alterations. The dyslipidemic profile presented by the Wistar rats was accompanied by hyperinsulinemia, moderate hypertension and cardiac ventricular hypertrophy. There were no alterations in glycemia.

Conclusion: The six-week hyperlipidic, hypercholesterolemic diet in young Wistar rats induced cardiometabolic alterations, becoming an effective model for disorders of this nature. (Int J Cardiovasc Sci. 2016;29(5):362-369)

Keywords: Rats; Diet-High-Fat; Caloric Restriction; Hypercholesterolemia; Hypertension, Hypertrophy, Left Ventricular.

Introduction

Obesity, atherosclerosis and hypertension, when associated in one patient, constitute a series of metabolic risk factors known as metabolic syndrome. This syndrome is related to disorders in insulin and lipid metabolism such as hyperinsulinemia, diabetes mellitus type II and dyslipidemias characterized by elevated plasma concentrations of triglycerides and LDL, associated to reductions in HDL concentrations.[1-3]

Conditions found especially in wealthy western countries such as social life-style, high-lipid diets, sedentariness and excessive work contribute to an increase in the incidence of the disease in people of all ages, especially active workers.[4-6]

The beginning of this complex syndrome is not yet completely clear, and an important question to be answered is: What is the triggering factor for this disease? Studies show that there is a close relation between this syndrome and insulin resistance[7,8] and high-fat diets.[1,4]

A unique characteristic of this study is that, most studies found in literature that involve the administration of hyperlipidic diets and cardiovascular alterations are done in mice. Published studies that were done in Wistar

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rats present long treatment protocols such as 20[9,10] or 30[11] weeks, generating a high cost to maintain the rats. Thus, in this study, we attempted to evaluate the efficacy of the short-period dietary treatment to trigger cardiovascular and metabolic disorders in Wistar rats.

Methods

Animals

We used male Wistar rats that weighed 150 g (4 weeks old) at the beginning of the study. The animals were kept in a room with temperature control (22±2ºC), and 12-hour dark light cycle (lights on at 6:30 am). The experiments were performed in accordance with the principles adopted by COBEA (Brazilian College of Animal Experimentation) for the use of animals in research and education, under number 226-1,2561-1, according to the Declaration of Helsinki of 1975, revised in 2008.

Treatments

Young Wistar rats (n=9), at four weeks of age, were randomly distributed into groups: normolipidemic (n=4) and hyperlipidemic (n=5). The normolipidemics were fed *ad libitum*, for six weeks, with standard laboratory feed (4% fat, 0% cholesterol and 23% protein; Labina-Purina, Brazil). The animals from the hyperlipidemic group received a hyperlipidic hypercholesterolemic diet, with 15% cocoa butter fat, 1.25% cholesterol, 0.5% cholic acid, 27.9% protein.[12,13]

Catheterization of blood vessels

The rats were anaesthetized with xylazine (50 mg/Kg, i.m.) and ketamine hydrochloride (0.01 mg/Kg, i.m.), and the left carotid artery was catheterized with a siliconized polyethylene cannula (PE 20) filled with 5 mM sodium citrate. The catheter was connected to a PE 50 tube (with 25 cm of length, filled with 0.9% NaCL solution), exteriorized in the interscapular region, fixed on the animal's skin.[14] This method allowed us to collect blood samples from non-anaesthetized rats with no movement restriction. Before the experiment, all rats had a 48-hour surgery recovery time in individual cages.

Blood pressure measurement

After recovering from surgery, the catheterized rats were kept in the cages, conscious, and with no movement restriction in a quiet environment. Blood pressure was registered at every beat, with a pressure transducer (Gould-Strain Gauge) connected to a pre-amplifier linked to an intra-arterial catheter. The measurements were recorded in a computer to be later analysed by WINDAQ-PRO Data Acquisition software (DI220 AT CODAS data acquisition system, Data Instruments Co). Systolic, diastolic and mean blood pressures and heart rate in basal conditions were calculated with the software Advanced CODAS.

Blood samples

After 16 hours of fasting, blood samples were collected with a catheter. Total cholesterol, HDL cholesterol, triglycerides (TAG) and glucose concentrations were determined in the serum by a colorimetric method with diagnostic kits (laborlab, Barueru, SP, Brazil). An indirect method was used to determine the concentrations of LDL and VLDL cholesterol.[15] Free fatty acids (FFA) were also determined by the colorimetric method with a specific diagnostic kit (Wako Chemicals GmbH; Neuss, Germany). Insulin concentrations were determined by radioimmunoassay (RIA), as described in the literature.[16] The atherogenic index (AI), a measurement that indicates the organism's tendency to develop atherosclerosis, was determined by the calculation: AI = [(Total cholesterol – HDL)/HDL].

Histologic analysis

The rats were euthanized by anaesthetic overdose, and cardiac tissue samples were taken for the histologic analysis and verification of ventricular hypertrophy. The ventricle was washed in a physiological solution, weighed and immediately placed in a container with formaldehyde. Ventricle slices were dehydrated with alcohol in gradual concentrations (70% to absolute), followed by diaphonization in xylene, immersion in Bouin solution and paraffin embedding (58°C – 60°C). Blocks were sectioned 5 μm thick and stained with Trichrome Masson. Chamber diameter, and ventricular area and wall thickness were determined with the software Scion Image (NIH Image Software).

Statistical analysis

Calculation of the sample number of experiments for each of the different conditions was done according to the Lenth,[17] with the software Statistica 7.0 (Stat Soft, Inc. 2004: version 7. www.statsoft.com). This calculation was done to ensure the following parameters: minimum

power of the test of 0.80 and alpha preset at 0.05. The animals were removed from the vivarium *Biotério Central da Universidade Estadual de Campinas* and, upon arriving at the department of functional and structural biology, four animals were randomly separated to form the normolipidemic group. The five remaining animals constituted the dyslipidemic group. After indication of normal distribution of data through Kolmogorov Smirnov analysis, results were analysed by unpaired Student's t-test as stated in the legends. Statistical significance level was set at 5%. Results were expressed as mean ± standard error of the mean (SEM). Statistical study was done with the software Prisma.

Results

Serum concentrations of total, LDL, VLDL and TAG cholesterol

The rats that were on the hyperlipidic and hypercholesterolemic diet during six weeks (from their 4th to their 10th week of life) presented significant increases in serum concentrations of total, LDL, VLDL cholesterol and triglycerides (Table 1). No significant differences were observed for HDL cholesterol concentrations. Moreover, rats in the hyperlipidic, hypercholesterolemic diet presented higher AI than those in the control group (Table 1).

Plasma concentrations of glucose

Plasma concentrations of glucose were not altered in rats on the hyperlipidic, hypercholesterolemic diet. On the other hand, insulin concentrations were significantly higher, in comparison to rats that were on a standard feed diet (Table 2).

Blood pressure

Mean, systolic and diastolic blood pressures and heart rate were significantly higher in rats on the six-week hyperlipidic, hypercholesterolemic diet (Table 3).

Table 1
Serum levels of total, HDL-, LDL-, VLDL-cholesterol, triglycerides, FFA and AI in rats on standard and hyperlipidic, hypercholesterolemic diets for six weeks

Diet	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	TAG (mg/dL)	FFA (mmol/L)	AI
Standard (4)	36.2 ± 4.2	5.2 ± 1.2	24.2 ± 5.5	6.7 ± 0.8	20.9 ± 1.2	0.65 ± 0.06	7.1 ± 1.8
Hyperlipidic and Hypercholesterolemic (5)	95.7 ± 9.5*	6.6 ± 0.4	83.2 ± 9.4*	10.5 ± 0.5*	51.3 ± 1.7*	1.02 ± 0.14	15.3 ± 1.3*

*TC: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein; TAG: Triglycerides; FFA: Free fatty acids; AI: Atherogenic index. Blood samples were collected after 16 hours of fasting, from conscious rats, with no movement restriction; AI = [(total cholesterol – HDL)/HDL]. Results are expressed as mean ± SEM. The number of animals is stated between parenthesis; * significantly different mean of the control group; ($p < 0.05$; unpaired Student's t-test).*

Table 2
Serum concentrations of glucose and plasma concentrations of insulin in rats on the standard or hyperlipidic, hypercholesterolemic diets for six weeks

Diet	Glucose (mg/dL)	Insulin (ng/mL)
Standard (4)	112.9 ± 11.9	0.75 ± 0.02
Hyperlipidic and Hypercholesterolemic (5)	110.5 ± 4.9	1.30 ± 0.12*

*Blood samples were taken after 16 hours of fasting, from conscious rats, with no movement restriction. Results are expressed as mean ± SEM. The number of animals is stated between parenthesis; * significantly different mean from the control group; ($p < 0.05$; unpaired Student's t-test).*

Table 3
Mean, systolic and diastolic blood pressures and heart rate of conscious rats that were on the standard or hyperlipidic, hypercholesterolemic diets for six weeks

Diet	MBP (mmHg)	SP (mmHg)	DP (mmHg)	HR (bpm)
Standard (3)	105 ± 2	120 ± 2	91 ± 1	346 ± 7
Hyperlipidic and Hypercholesterolemic (4)	112 ± 1*	130 ± 1*	98 ± 2*	368 ± 4*

*MBP: Mean blood pressure; SP: Systolic pressure; DP: Diastolic pressure; HR: Heart rate. Results are expressed as mean ± SEM. The number of animals is stated between parenthesis; * significantly different mean from the control group; (p < 0.05; unpaired Student's t-test).*

Histologic analysis of the heart

Left ventricular mass was significantly higher in rats on the hyperlipidic, hypercholesterolemic diet. Similar results were found for the ventricular mass index and for the left ventricular wall thickness ($p < 0.0001$), internal diameter ($p < 0.0001$) and ratio of the wall thickness to the internal diameter ($p < 0.0001$). The area of the left ventricular chamber, on the other hand, showed no alterations (Table 4). Morphological changes are depicted in Figure 1: Column A: images of the cross-section of half the organ, obtained by analytical microscope (stained with Trichrome Masson); Column B: surface area of cardiomyocytes; Column C: longitudinal view of cardiomyocytes; Column D: myocardial fibrosis; and Column E: perivascular fibrosis

Table 4
Body weight, left ventricular mass, ventricular mass index, left ventricular wall thickness, internal diameter, chamber area and ratio of the left ventricular thickness and the internal diameter of the left ventricle of rats on standard or hyperlipidic and hypercholesterolemic diets for six weeks

Diet	BW (g)	VM (mg)	VMI (mg/g)	VT (mm)	ID (mm)	VT / ID	CA (mm)
Standard (4)	292.4 ± 13.2	546.6 ± 23.9	1.89 ± 0.15	11.5 ± 0.12	28.8 ± 0.2	0.40 ± 0.004	218.1 ± 4.4
Hyperlipidic and Hypercholesterolemic (4)	307.8 ± 7.5	653.8 ± 28.2*	2.22 ± 0.10	15.1 ± 0.51*	20.6 ± 0.3*	0.64 ± 0.010*	220.1 ± 4.7

*BW: Body weight; VM: Left ventricular mass; VMI: Ventricular mass index; VT: Left ventricle was thickness; ID: Internal diameter; CA: Chamber area; VT/ID: Ratio of the ventricular thickness and internal diameter of the left ventricle. Results are expressed as mean ± SEM. Ventricular mass index = ventricle weight / body weight. The number of animals is stated between parenthesis; * significantly different mean from the control group; (p < 0.05; unpaired Student's t-test).*

Discussion

Experimental and epidemiological studies have confirmed that primary dyslipidemia (genetic) and secondary dyslipidemia (hormonal and/or metabolic alterations caused by environmental factors) are atherogenic, and the main risk factor for atherosclerosis and coronary diseases.[18-20] After six weeks of a hyperlipidic, hypercholesterolemic diet, the rats presented dyslipidemia associated to hyperinsulinemia and moderate hypertension. Elevations in serum concentration of triglycerides, total, LDL and VLDL cholesterol characterize atherogenic dyslipidemia. AI was higher in rats on the hyperlipidic, hypercholesterolemic diet. Serum concentrations of HDL and FFA were not affected by the diet.

High concentrations of lipids may be a triggering factor to other metabolic alterations. It has been suggested that the intracellular accumulation of triglycerides,[21] which increases glucose production, precedes the effects of lipids in the peripheral insulin action.[22-24]

Excessive nutrient intake, especially glucose and lipids, may cause insulin resistance in the adipose tissue and muscles, just as it compromises endogenous glucose production. Experimental models with animals on hyperlipidic diets have shown a reduction in glucose tolerance associated to a lower basal glucose uptake,

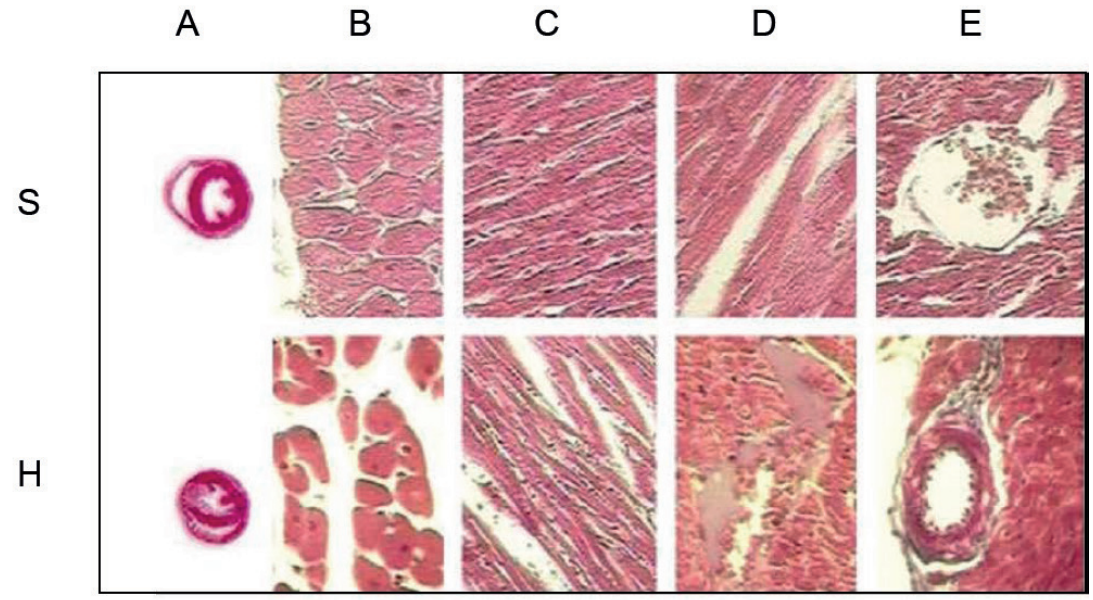

Figure 1
Representative figures of the histologic analysis of the rats treated during six weeks with standard (S) or hyperlipidic, hypercholesterolemic (H) diets. Tissues were stained with Trichrome Masson. Column A: images of the cross-section of half the heart. Left ventricular wall thickness was significantly increased in rats on the hyperlipidic, hypercholesterolemic diet; Column B: surface area of cardiomyocytes (scale = 7μmm); Column C: longitudinal view of cardiomyocytes; Column D: myocardial fibrosis; and Column E: perivascular fibrosis (Column B-E – 400x increase).

stimulated by insulin.[25] The results seen here showed that rats on the hyperlipicid, hypercholesterolemic diet were hyperinsulinemic and euglycemic, which likely suggests a reduction of glucose tolerance. 2-deoxyglucose uptake by adipocytes, as the euglycemic-hyperinsulinemic clamp, is being evaluated in our laboratories to confirm this condition in the experimental model.

A compromise in insulin sensitivity and/or in glucose transport were related to changes in the composition of fatty acids of the plasma membrane induced by a lipid based diet. Saturated fats, present in hyperlipidic and hypercholesterolemic diets, seem to be more deleterious than monounsaturated or polyunsaturated fats[26-28] because lipids induce a reduction of insulin sensitivity. Studies with rabbits have demonstrated that a diet-induced hypercholesterolemia resulted in depressed systolic and diastolic functions as a consequence of the increased amount of cholesterol in the cardiac sarcolemma, alterations in calcium permeability, and/or SERCA-2 activity.[18] Therefore, animal fat intake, associated to a sedentary life-style, predisposes atherogenic lipoprotein elevation and metabolic syndrome.[1,4]

Rats on the hyperlipidic, hypercholesterolemic diet, in comparison to the control group, also showed a significant increase in HR and MBP, with a moderate, but significant, increase in SP and DP. HR increase is an indicator of sympathetic nervous system (SNS) hyperactivity,[29,30] and this SNS activity increases with calorie intake.[22,31] Elevated concentrations of insulin appear to be the link between nutrient absorption and sympathetic activity. HR increase, associated to dyslipidemia, is a factor involved in the genesis of hypertension and atherosclerosis.

Insulin resistance and hyperinsulinemia have also been described as important mechanisms in the pathophysiology of dyslipidemia and atherosclerosis.[32,33] It has been suggested that insulin regulates the peripheral vascular resistance, leading to vasoconstriction through the activation of the SNS. In a situation of insulin resistance, nerve endings of the sympathetic system are not significantly affected, thus, the sympathetic stimulus is continuous.[33] On the other hand, insulin increases nitric oxide (NO) synthesis,[34,35] reducing catecholamine-mediated vasoconstriction and potentiating acetylcholine-mediated vasodilatation in

the blood vessels. These actions seem protective against the development of arterial hypertension. However, in the condition of insulin resistance, such effects are reduced,[32,36] leading to hypertension. Moreover, the stimulation of growth and migration of smooth muscle cells in the vessel wall is directly associated to the beginning and progression of this process,[37] in which the NO synthesis is reduced. Evidence that hyperinsulinemia precedes the development of vascular disorders has been previously described.[38]

It has been demonstrated that nitric oxide is an effective anti-hypertrophic and cardiac remodeling inhibitor.[39] However, the elevated plasma concentrations of LDL observed in rats fed with the hyperlipidic diet can promote an increase of this oxidative stress through lipid peroxidation, reducing NO availability,[40] and increasing concentrations of nitrite and nitrate.[41] Moreover, molecules such as tumor necrosis factor (TNF-α), which is secreted by the adipose tissue and is a factor that contributes to insulin resistance and dyslipidemia,[42] are also additional inducers of apoptosis of myocytes. Even though we did not verify apoptosis in the evaluated hearts, this condition is associated to the progressive reduction of ventricular function or myocardial lesion,[43] and concentric hypertrophy due to the pressure overload, as shown in our results.

Rosen et al.,[44] in a study with chronic heart failure and hypertrophic cardiomyopathy patients, confirmed the normal activation of the SNS with density reduction of beta-adrenoceptor of the myocardium. In preliminary studies, we identified that rats on the hyperlipidic diet, for six weeks, presented subsensitivity to b-adrenergic agonists in the right atrium.[45]

The main limitation of this study is the lack of investigation of mechanisms involved in the presented disorders. However, considering this is a model of dyslipidemia induction by a diet in only six weeks, further studies are necessary to unravel the mechanisms related to this syndrome.

Conclusion

Results show that a six-week hyperlipidic, hypercholesterolemic diet induced dyslipidemia associated to hyperinsulinemia, hypertension and left ventricular hypertrophy. This condition is associated to cardiometabolic diseases. Therefore, this could be a short-term experimental model to induce cardiometabolic disorders through a diet in Wistar rats, which can contribute to elucidate the mechanisms of this complex pathology.

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Author contributions

Conception and design of the research: Crege DRXO, Miotto AM, Kassisse DMG. Acquisition of data: Crege DRXO, Miotto AM, Wolf-Nunes V, Kassisse DMG. Analysis and interpretation of the data: Crege DRXO, Miotto AM, Borghi F, Wolf-Nunes V, Kassisse DMG. Statistical analysis: Crege DRXO, Miotto AM, Kassisse DMG. Obtaining financing: Kassisse DMG. Writing of the manuscript: Crege DRXO, Borghi F, Kassisse DMG. Critical revision of the manuscript for intellectual content: Crege DRXO, Miotto AM, Borghi F, Wolf-Nunes V, Kassisse DMG. Guidance of the two doctoral theses (Drs Danilo Roberto Xavier de Oliveira Crege and Alexandre Marcucci Miotto): Kassisse DMG.

Potential Conflict of Interest

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

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Study Association

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