BASIC RESEARCH

Simvastatin-induced cardiac autonomic control improvement in fructose-fed female rats

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OBJECTIVE: Because autonomic dysfunction has been found to lead to cardiometabolic disorders and because studies have reported that simvastatin treatment has neuroprotective effects, the objective of the present study was to investigate the effects of simvastatin treatment on cardiovascular and autonomic changes in fructose-fed female rats.

METHODS: Female Wistar rats were divided into three groups: controls (n = 8), fructose (n = 8), and fructose+simvastatin (n = 8). Fructose overload was induced by supplementing the drinking water with fructose (100 mg/L, 18 wks). Simvastatin treatment (5 mg/kg/day for 2 wks) was performed by gavage. The arterial pressure was recorded using a data acquisition system. Autonomic control was evaluated by pharmacological blockade.

RESULTS: Fructose overload induced an increase in the fasting blood glucose and triglyceride levels and insulin resistance. The constant rate of glucose disappearance during the insulin intolerance test was reduced in the fructose group (3.4 ± 0.32%/min) relative to that in the control group (4.4 ± 0.29%/min). Fructose+simvastatin rats exhibited increased insulin sensitivity (5.4 ± 0.66%/min). The fructose and fructose+simvastatin groups demonstrated an increase in the mean arterial pressure compared with controls rats (fructose: 124 ± 2 mmHg and fructose+simvastatin: 126 ± 3 mmHg vs. controls: 112 ± 2 mmHg). The sympathetic effect was enhanced in the fructose group (73 ± 7 bpm) compared with that in the control (48 ± 7 bpm) and fructose+simvastatin groups (31 ± 8 bpm). The vagal effect was increased in fructose+simvastatin animals (84 ± 7 bpm) compared with that in control (49 ± 9 bpm) and fructose animals (46 ± 5 bpm).

CONCLUSION: Simvastatin treatment improved insulin sensitivity and cardiac autonomic control in an experimental model of metabolic syndrome in female rats. These effects were independent of the improvements in the classical plasma lipid profile and of reductions in arterial pressure. These results support the hypothesis that statins reduce the cardiometabolic risk in females with metabolic syndrome.

KEYWORDS: Female; Fructose; Arterial pressure; Statin; Autonomic function.

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INTRODUCTION

The consumption of high levels of fructose in humans and animals causes insulin resistance, lipid abnormalities, obesity, hypertension, and renal changes.[1-5] The combination of these metabolic and cardiovascular alterations observed in fructose-fed subjects is collectively known as metabolic syndrome (MS). To model the development of MS experimentally, long-term fructose overload in rats has been used.[1,2]

Statins (or HMG-CoA reductase inhibitors) have been shown to lower arterial pressure (AP) in borderline hypertensive dyslipidemic humans. This favorable effect of statins may be a result of both lipid-based mechanisms and non-lipid-based mechanisms affecting endothelial vasoregulation and the sympathovagal balance in the disease state.[6] Experimental studies have shown that simvastatin improves baroreflex sensitivity (BRS).[7] Findings from several studies have strongly suggested that simvastatin normalizes the autonomic function in individuals with heart failure, inhibiting the central mechanisms of angiotensin II and, consequently, the superoxide production pathway.[8] Moreover, simvastatin may improve left ventricular function[8] and reduce vascular dysfunction in mice with dyslipidemia.[9]

Despite these positive results, the effects of statin therapy on autonomic function have not been established to date,

No potential conflict of interest was reported.

particularly in females with MS. It is important to emphasize that significant advances in the management of cardiovascular disease and MS have been made in recent years.[1,2,10,11] However, cardiovascular diseases remain the leading cause of death among women in the most developed areas of the world,[12] exceeding the number of deaths in men and the combined number of deaths due to the next seven causes in women.[10] Because autonomic dysfunction leads to cardiometabolic disorders[11] and because statins have demonstrated neuroprotective effects,[12-15] we hypothesized that chronic simvastatin administration in female rats submitted to long-term fructose overload (18 weeks) would improve cardiac autonomic control and reduce the cardiometabolic risk. Therefore, the aim of the present study was to investigate the effects of simvastatin on the metabolic, cardiovascular and autonomic changes induced by fructose overload in female rats.

MATERIAL AND METHODS

Experiments were performed using 24 female Wistar rats (70 days old, approximately 50 g) that were obtained from the Animal Shelter of Sao Judas Tadeu University in Sao Paulo, Brazil. The rats received standard laboratory chow (Nuvital, Colombo, Brazil) and water ad libitum. The animals were housed in individual cages in a temperature-controlled room (22℃) with a 12-h dark-light cycle. All rats were treated similarly in terms of daily manipulation. All surgical procedures and protocols were in accordance with the Ethical Care Guidelines for Experimental Animals and the International Animal Care and Use Committee and were approved by the Sao Judas Tadeu University Ethical Committee (protocol number 058/2007). Three experimental groups were used in this study: control (C; n = 8), fructose (F; n = 8), and fructose+simvastatin (FS, n = 8). Fructose overload was induced via dilution of D-fructose in the drinking water (100 g/L) for 18 weeks. At 16 weeks, the presence of fructose-induced metabolic and cardiovascular dysfunctions[16] was analyzed. Simvastatin (5 mg/kg/day) treatment was performed by gavage for the last two weeks of fructose overload.

After 18 weeks of fructose overload, the blood glucose and triglyceride concentrations were measured using a Roche device (Accutrend GCT, Roche, Sao Paulo, Brazil) after four hours of fasting at the end of the protocol. For the insulin tolerance test (ITT), the rats were fasted for two hours and then anesthetized with thiopental (40 mg/kg body weight, ip). A drop of blood was collected from the tail to measure the blood glucose concentration using the Accucheck system (Roche, Sao Paulo, Brazil) before and 4, 8, 12, and 16 minutes after insulin injection (0.75 U/kg). The constant rate of decrease of the blood glucose concentration (Kitt) was calculated using the $0.693/t^1/_2$ formula. The $t^1/_2$ for blood glucose was calculated from the slope of the least squares analysis of the blood glucose concentrations during the linear phase of decline.[16,17]

After metabolic measurements, two catheters filled with 0.06 ml of saline were implanted in anesthetized rats (ketamine 80 mg/kg+xylazine 12 mg/kg) into the carotid artery and jugular vein (PE-10) for direct measurements of the AP and for drug administration, respectively. One day after the catheter placement, the rats were conscious and allowed to move freely during the experiments. The arterial cannula was connected to a strain-gauge transducer (Blood Pressure XDCR, Kent© Scientific, Litchfield, CT, USA), and AP signals were recorded over a 30-min period by a microcomputer equipped with an analog-to-digital converter board (CODAS, 2-kHz sampling frequency, Dataq Instruments, Inc., Akron, OH, USA). The recorded data were analyzed on a beat-to-beat basis to quantify the changes in the mean AP (MAP) and the heart rate (HR).[16,18]

The vagal and sympathetic effects were studied by injecting methylatropine (3 mg/kg IV, Sigma-Aldrich, St. Louis, MO, USA) and propranolol (4 mg/kg IV, Sigma-Aldrich) in a volume of 0.1 ml/100 g of body weight. The resting HR was recorded while the rats were in their cages in an unrestrained state. Methylatropine was injected immediately after the recording. Because the HR response to these drugs reaches its peak within 10 to 15 minutes, this time interval was allowed to elapse before the HR measurement was taken. On the next day, the sequence of the injections was inverted, and propranolol was injected before methylatropine.[16] The sympathetic effect was determined by calculating the difference between the basal HR and the lowest HR after the administration of propranolol. The vagal effect was obtained based on the difference between the maximum HR after methylatropine injection and the basal HR.

The data were expressed as the means ± SEM and were compared using one-way analysis of variance (ANOVA) or repeated one-way ANOVA followed by the Student Newman-Keuls test. The significance level was set at $p<0.05$.

RESULTS

The body weight was not different between the studied groups at the beginning (C: 48 ± 6 g, F: 43 ± 5 g, and FS: 45 ± 1 g, $p>0.05$) and at the end of the protocol (C: 285 ± 10 g, F: 294 ± 9 g, and FS: 288 ± 8 g, $p>0.05$).

The fasting glucose levels were increased in F (92 ± 2 mg/dL) and FS (93 ± 2 mg/dL) rats compared with that in C rats (82 ± 2 mg/dL, $p<0.05$). The blood triglyceride concentration was also higher in the F and FS groups (142 ± 12 and 187 ± 22 mg/dL, respectively) compared with that in the C group (101 ± 5 mg/dL, $p<0.05$). The constant rate of the plasma glucose disappearance (Kitt) was reduced in the F group (3.4 ± 0.32%/min) compared with that in the C group (4.4 ± 0.29%/min, $p<0.05$) during ITT, which is indicative of the insulin-resistant state in the fructose-fed rats. The simvastatin treatment increased the Kitt (FS: 5.4 ± 0.66%/min, $p<0.05$) in F rats.

The F group exhibited increases in the systolic, diastolic, and mean arterial pressures ($p<0.05$). Simvastatin treatment did not change the AP in the F rats ($p>0.05$) (Table 1). The resting HR showed similar values among groups ($p>0.05$) (Table 1).

The cardiac vagal effect was similar between the C (49 ± 9 bpm) and F groups (46 ± 5 bpm, $p>0.05$). Simvastatin treatment increased the vagal effect in fructose-overloaded animals (FS: 84 ± 7 bpm) compared with that in C and F animals ($p<0.05$) (Figure 1A). The sympathetic effect was enhanced in the F group (73 ± 7 bpm) compared with that in the C group (48 ± 7 bpm, $p<0.05$). This effect was normalized by simvastatin treatment (FS: 31 ± 8 bpm, $p<0.05$) (Figure 1B).

Table 1 - Evaluation of cardiovascular function of the control (C), fructose (F), and fructose+simvastatin (FS) groups.

PARAMETERS	C	F	FS
SAP, mmHg	127±3	140±3*	143±4*
DAP, mmHg	96±2	106±2*	111±4*
MAP, mmHg	112±2	124±2*	126±3*
HR, bpm	354±9	380±20	328±9

Values are the mean ± SEM. SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; and HR: heart rate.
*$p<0.05$ vs. the control group.

DISCUSSION

The aim of this study was to determine the effects of simvastatin treatment on the metabolic, cardiovascular, and autonomic modulation in an experimental model of MS that was induced by long-term fructose overload (18 weeks) in female rats. Although simvastatin treatment did not change the blood metabolic parameters or the AP, this pharmacologic approach improved insulin resistance, reduced the exacerbated cardiac sympathetic effect and increased the vagal effect to the heart. Additionally, our findings in female rats corroborated previous data that have been obtained in male animals submitted to fructose overload; these male rats exhibited enhanced blood glucose and triglyceride levels, insulin resistance, increased AP and sympathetic activation.

We should emphasize that the protocol used in our research differed from those of previous studies. Because fructose overload was performed in animals starting from their 70th day of life through their adult phase, we could simulate the fructose consumption of Western diets over the entire lifespan. Previous investigations have covered a shorter time-span and gathered data from the acute (2-24 h) or mid-term (1-9 weeks) phases of fructose administration.[2-5,16,19-22] The rationale behind our design choice lies in the fact that metabolic and cardiovascular disorders take years to manifest as clinical alterations due to the stepwise compensatory behaviors, physiological adaptations, and new equilibrium levels.

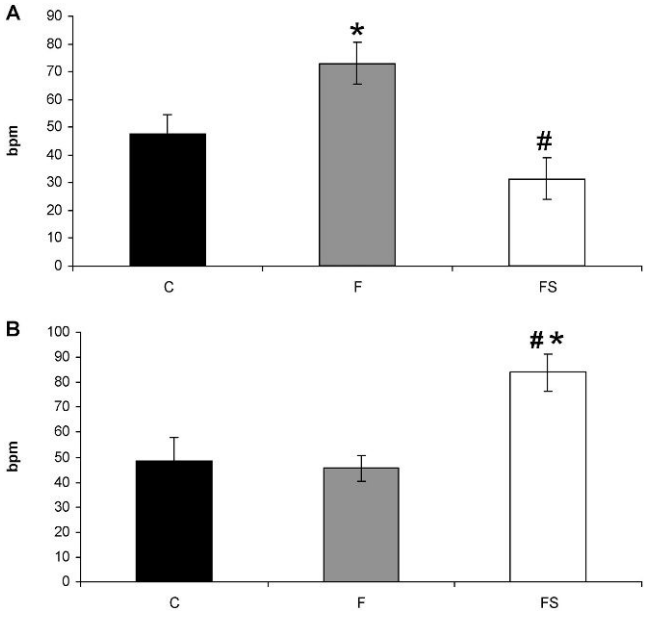

Figure 1 - A) The sympathetic effect and **B**) the vagal effect in the control (C), fructose (F), and fructose+simvastatin (FS) groups. *$p<0.05$ vs. the C group; #$p<0.05$ vs. the F group.

The fructose drinking model in rats resembles a state of early insulin resistance in humans, which is associated with mild hypertension.[16,23] Previous studies have demonstrated that increased body weight is not common in male rat or in fructose-rich chow-fed mouse models. However, fructose-rich chow-fed mouse models can develop hypertriglyceridemia, increased blood glucose concentrations, glucose intolerance or insulin resistance and hyperinsulinemia.[3,5,16,19-21] In our study, the fructose-overloaded female rats displayed increased blood glucose and triglyceride concentrations and insulin resistance. However, the body weight remained unaltered. Although simvastatin treatment did not alter the fasting blood glucose and triglyceride levels in our study, it increased Kitt in fructose-fed rats. These results indicate that simvastatin treatment reduces insulin resistance in fructose-overloaded rats.

Most studies investigating the effects of high fructose consumption on the basal AP in male or female rats have used tail-cuff plethysmography, which is an indirect measurement of AP that can only measure systolic AP.[19-22] Increased fructose consumption leads to increases in the AP in male[20,21] and female rats[16] and in male mice during the dark period.[5] Farah et al.[5] have demonstrated an increase in the low-frequency component of systolic AP in fructose-overloaded (eight weeks) male mice. These results show that increased AP is associated with sympathetic modulation in the circulation that is limited to the dark (active) period. In the present study, we observed an increase in the AP and in the sympathetic effect in the heart in fructose-fed female rats after 18 weeks of fructose consumption. The AP change in fructose-fed animals is mediated by activation of the sympathetic nervous system,[5,24] impairment of the cardiac parasympathetic tonus[16] and of endothelium-dependent relaxation[25] and dysfunction in the angiotensin-renin system.[5]

Our results show that the simvastatin treatment normalizes the cardiac sympathetic effect and insulin resistance. In addition, simvastatin treatment increased the cardiac vagal effect in fructose-fed female rats. However, simvastatin treatment did not change the basal AP or the blood triglyceride level. These results suggest that these statin-induced improvements might result from a pleiotropic effect that is independent of the drug's classical effect on lipids. Pliquett et al.[26] have also demonstrated improvements in the BRS in rabbits with heart failure after statin treatment without changes in the total plasma or high-density cholesterol levels. Additionally, if decreased AP was observed in the present study in simvastatin-treated fructose-fed rats, the autonomic improvement observed in these animals may be attributed, at least in part, to this change. Previous studies have reported lower APs in borderline hypertensive dyslipidemic humans who were treated with statins and have attributed this favorable alteration to both lipid-based mechanisms and non-lipid-based mechanisms affecting endothelial vasoregulation and the sympathovagal balance in the disease state.[6]

Based on these findings, we hypothesize that the role of simvastatin in the autonomic nervous system is vast and includes enhancing NO synthesis in the endothelium[27,28] and reducing angiotensin II–induced injury, AT1 receptor expression,[29,30] and ETA receptor expression.[31] These functions indicate a potential role for statins in regulating sympathetic and vagal outflow in the central nervous system and improving the afferent or efferent arms of the cardiovascular autonomic reflexes. These autonomic pleiotropic effects of statins may account for patient outcomes and require further characterization.

CONCLUSION

The results of the present study demonstrate that fructose overload in female rats induces increases in the AP and the cardiac sympathetic response, which are associated with insulin resistance. These findings reinforce the role of autonomic dysfunction in the development of early cardiometabolic disorders that are induced by a high fructose diet in female rats. Importantly, we demonstrated that a short-term simvastatin treatment may improve insulin sensitivity and cardiac autonomic control in an experimental model of MS in female rats. These effects were independent of improvements in the classical plasma lipid profile and of reductions in the AP, reinforcing the hypothesis that statins reduce the cardiometabolic risk in females with MS. However, additional studies are needed to confirm the pleiotropic effects of long-term statin treatment on autonomic dysfunction and on the outcome of women with MS.

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