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ARTICLE

Increased systolic blood pressure associated with hypertriglyceridemia in female Sprague-Dawley rats

Conrad Mogane, Lebogang P. Mokotedi, Aletta M.E. Millen, and Frederic S. Michel

Abstract: The effect of hyperlipidemia on the cardiovascular system is uncertain in females. The aim of the present study was to determine whether administration of a lipogenic diet alters cardiovascular parameters in female rats. Fifty female Sprague-Dawley rats were assigned into 2 groups of rats receiving a standard or a high-fat, high-sucrose diet (HFHS) for 6 weeks (n = 25 per group). Body mass, blood lipids concentrations, triglycerides clearance, blood pressures (BPs), systolic and diastolic functions, as well as vascular reactivity were assessed at the end of the diet intervention. At termination, body mass was similar between the 2 groups. Fasting blood triglycerides concentration (BTG) was greater in the HFHS group. Triglycerides clearance was impaired in the HFHS group. High-density lipoprotein (HDL) cholesterol concentration was lower in the HFHS group. The early-to-late diastolic filling velocity ratio (E/A) was lower in the HFHS group and negatively associated with BTG. The sensitivity (EC_{50}) of mesenteric arteries to phenylephrine was greater in HFHS and was negatively associated with BTG, but not HDL. Systolic BP was higher in the HFHS group and was positively associated with BTG and HDL. The association between systolic BP and BTG was independent of other lipids measured. In conclusion, hypertriglyceridemia may have increased resistance arteries responsiveness to alpha-agonist and systolic BP in female rats.

Key words: female, lipogenic diet, hypertriglyceridemia, HDL cholesterol, blood pressure, diastolic function, vascular reactivity.

Résumé : On ne connaît pas bien l'effet de l'hyperlipidémie sur le système cardiovasculaire chez les sujets de sexe féminin. La présente étude avait pour but d'établir si l'administration d'un régime alimentaire lipogène entraîne des modifications dans les paramètres cardiovasculaires chez la rate. Nous avons réparti 50 rates Sprague-Dawley dans 2 groupes avec régime alimentaire standard ou à teneur élevée en matières grasses et en saccharose (TEMGS) pendant 6 semaines (n = 25 par groupe). À la fin de l'intervention diététique, nous avons évalué la masse corporelle, les taux de lipides sanguins, la clairance des triglycérides, les tensions artérielles (TA), les fonctions systolique et diastolique, ainsi que la réactivité vasculaire. À la fin de l'étude, la masse corporelle était similaire dans les 2 groupes. Les concentrations sanguines de triglycérides à jeun (TGS) étaient plus élevées dans le groupe TEMGS. La clairance des triglycérides était moins élevée dans le groupe TEMGS. Les taux de cholestérol-HDL (pour « high-density lipoprotein ») étaient moins élevés dans le groupe TEMGS. Le rapport entre la vitesse de remplissage diastolique précoce et tardif (P/T) était moins élevé dans le groupe TEMGS et inversement proportionnel aux TGS. La sensibilité (EC_{50}) des artères mésentériques à la phényléphrine était plus importante dans le groupe TEMGS et inversement proportionnelle aux TGS, mais pas aux HDL. La TA systolique était plus élevée dans le groupe TEMGS et proportionnelle aux TGS et aux HDL. L'association entre la TA systolique et les TGS était indépendante des autres taux de lipides mesurés. En conclusion, l'hypertriglycéridémie pourrait entraîner une augmentation de la réactivité des artères résistives à un agoniste alpha et à la TA systolique chez la rate. [Traduit par la Rédaction]

Mots-clés : femelle, régime alimentaire lipogène, hypertriglycéridémie, cholestérol HDL, tension artérielle, fonction diastolique, réactivité vasculaire.

Introduction

The consumption of a Western diet, rich in saturated fat and carbohydrate, is associated with an increased incidence of metabolic syndrome (Grundy 2016; Rodríguez-Monforte et al. 2017). Clinically, metabolic syndrome is diagnosed by the presence of 3 of the following factors: abdominal obesity, hyperglycemia, dyslipidemia, and increased blood pressure (Ginsberg et al. 2006; Grundy 2016). Hypertriglyceridemia and reduced high-density lipoprotein (HDL) cholesterol concentration compose the atherogenic dyslipidemia characteristic of metabolic syndrome. (Grundy 2016). Metabolic syndrome is also defined as a cluster of risk factors that increase the prevalence of cardiovascular diseases (CVD) and diabetes mellitus type 2 (Grundy 2016). Each component of the metabolic syndrome has been identified as an independent risk factor for CVD (Goldberg 2001; Ginsberg et al. 2006; Grundy 2016; Koliaki et al. 2019). In addition, their co-existence increases the severity of CVD (Goldberg 2001; Ginsberg et al. 2006; Grundy 2016). However, the interaction between these conditions is not fully understood. In this regard, it is important to identify the contribution of the different components of the metabolic syndrome in the pathogenesis of CVD.

The most controversial feature of the metabolic syndrome remains the increased triglycerides concentration. Growing evidence has suggested that hypertriglyceridemia is a significant predictor of CVD (Hokanson and Austin 1996; Austin et al. 1998; Sarwar et al. 2007; Langsted et al. 2011). However, the effects of hypertriglyceridemia on the development to CVD may be depen-

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dent of those of other plasma lipids and, in particular, the associated reduced HDL cholesterol concentration. In this regard, although CVD risk reductions with fenofibrate monotherapy have been shown mainly in patients with hypertriglyceridemia (Scott et al. 2009), CVD risk reductions remain similar with simvastatin monotherapy compared with the combination of fenofibrate and simvastatin (ACCORD Study Group et al. 2010). On the other hand, experimental studies have primarily assessed the effect of diet-induced metabolic syndrome on the cardiovascular system, preventing conclusions on the effect of hypertriglyceridemia per se (Barnard et al. 1993, 1998; Naderali et al. 2001; Roberts et al. 2005; Pérez-Torres et al. 2008; Bourgoin et al. 2008; Panchal et al. 2011; Sweazea and Walker 2011; Wong et al. 2012, 2017; Alam et al. 2013; Smith et al. 2016; Vileigas et al. 2016; Battault et al. 2018; Qin et al. 2018). Few experimental human and animal studies have focused their interests on the impact of increased triglycerides concentrations on the cardiovascular system, but similarly the conclusions may still be biased by other components of the metabolic syndrome such as the presence of obesity or insulin resistance (Štolba et al. 1992; Kusterer et al. 1999; Lewis et al. 1999; Bartus et al. 2005; Torok et al. 2007; Sotnikova et al. 2012; Mak et al. 2013). Whether the effect of hypertriglyceridemia on the cardiovascular system occurs independent of other metabolic syndrome parameters is still uncertain.

Finally, sexual dimorphism in the development of CVD has been well documented (Cauwenberghs and Kuznetsova 2018; Palmisano et al. 2018). Estrogen, the female sex hormone, has been described as the causal factor for the observed disparities in CVD risk between sexes (Palmisano et al. 2018). However, in contrast to the reported cardioprotective effect of estrogen, an intriguing question remains on whether sex difference exists on the effect of hypertriglyceridemia on the cardiovascular system (Palmisano et al. 2018). Indeed, whether women with hypertriglyceridemia are at increased risk of developing CVD is still controversial (Hokanson and Austin 1996; Austin et al. 1998; Sarwar et al. 2007). Moreover, a sexual dismorphsim on the effect of hypertriglyceridemia on the cardiovascular system has been shown in a rodent model (Sotnikova et al. 2012). However, a limited number of experimental studies have investigated the effect of hypertriglyceridemia on cardiovascular parameters in females (Barnard et al. 1993, 1998; Roberts et al. 2005; Sotnikova et al. 2012). Because of the paucity of evidence on the effect of hyperlipidemia per se on cardiovascular parameters in females, the pathophysiology of CVD resulting from diet-induced hypertriglyceridemia is poorly understood in females. The aim of the present study was then to determine whether administration of a lipogenic diet inducing hypertriglyceridemia alters cardiovascular parameters in female rats.

Methods

Animals and study design

The present study was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Approval No. 2017/03/02/C) and was conducted in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*. The experiments were conducted on 50 10-week-old female Sprague-Dawley rats. Rats were housed individually under conditions of controlled ambient temperature with a light–dark cycle of 12 h. Rats were given access to drinking water ad libitum. The standard laboratory animal chow diet (Labchef, Johannesburg, South Africa) was composed (in % of dry mass) of 57% carbohydrates, 24% protein, 5% fat, 4% fiber, as well as 10% ash, minerals, and vitamins with an estimated total energy provided of 369 kcal/100 g. Animals were allowed to habituate for 2 weeks to the standard rat chow reconstituted with 5% water and dried again. Rats were then randomly divided into 2 groups (n = 25 per group) that received either a reconstituted standard (STD) diet or a high-fat, high-sucrose (HFHS) diet for 6 weeks. The HFHS diet was composed of 35% lecithinated fat powder (BergaFat HPL-106; Berg+Schmidt, Hamburg, Germany), 30% sucrose sugar (Selati, Malalane, South Africa), and 35% standard rat chow with an estimated total energy provided of 564 kcal/100 g.

Tail-cuff blood pressure measurements

The systolic and diastolic blood pressures (SBP and DBP, respectively) were measured before termination using a noninvasive tail-cuff technique, which employs noninvasive blood pressure amplifiers (Biopac Systems, Santa Barbara, California, USA). A built-in pump automatically inflates the tail cuff to occlude the vessel in the tail of the rat and then slowly deflate the cuff when the inflation point is reached, providing a linear drop in pressure. Each rat was placed in a restrainer with a cuff attached to a heated tail. Rats were placed once a week from the beginning of the diet intervention into a restrainer with a cuff attached to the heated tail to allow them to adapt to the procedure and habituate them before the measurement at termination. Measurements were taken at midday to avoid diurnal variation.

Fasting blood glucose and triglycerides concentrations

The fasting blood glucose and triglycerides concentrations were measured before termination. The animals were fasted overnight before the blood glucose and triglycerides concentrations measurement. Fasting blood glucose and triglycerides concentrations were measured using a drop of blood obtained by a pin prick into the tail vein by the use of a calibrated glucometer (Contour plus; Bayer, Germany) and triglyceride meter (Accutrend Triglycerides; Roche Diagnostics, Germany).

Triglyceride clearance test

Two days before termination, 20 rats (n = 10 per group) were subjected to a triglyceride clearance test. The animals were fasted overnight. Extra virgin olive oil was administered by oral gavaging at a dose of 10 mL/kg. Using a drop of blood obtained by a pin prick into the tail vein, serial blood triglycerides concentrations were measured using a triglyceride meter (Accutrend Triglycerides; Roche diagnostics, Germany) at 0, 30, 60, 90, 120, 240, 360, and 480 min after administration of the extra virgin olive oil.

Echocardiography

Twenty female Sprague-Dawley rats (n = 10 per group) were used to determine the acute effect of cumulative intraperitoneal injections of isoprenaline (a nonselective β-adrenoreceptor agonist; 0.001 to 0.06 mg/kg) on systolic and diastolic functions (Michel et al. 2017). Because of the isoprenaline injection, no blood was drawn after termination. On the day of the experiment, rats were anaesthetized using ketamine (75 mg/kg) and xylazine (15 mg/kg). Left ventricular (LV) systolic and diastolic function were assessed in vivo using an Acuson Cypress SC2000 Ultrasound echocardiograph and a 7.0 MHz transducer (Siemens Medical Division, USA). LV internal dimensions were measured using two-dimensional targeted M-mode echocardiography according to the American Society for Echocardiography's leading-edge method (Sahn et al. 1978). LV endocardial (FS_{end}) and midwall (FS_{mid}) fractional shortening were determined as previously described (Michel et al. 2017). LV FS_{end} was determined from the formula {[LV end diastolic diameter (EDD) − LV end systolic diameter (ESD)]/EDD} × 100 and was employed as a load-dependent index of chamber systolic function. Left ventricular FS_{mid} was determined from {[(LVEDD + LVED PWT) − (LVESD + LVES PWT)]/(LVEDD + LVED PWT)} × 100 (where PWT is the posterior wall thickness) and was used as a load-dependent index of myocardial systolic function. LV diastolic function was assessed using pulsed-wave Doppler and Tissue Doppler Imaging. Trans-mitral flow velocities were sampled by pulsed-wave Doppler at the level of mitral valve leaflet tips. Peak flow velocities of early diastole (E) and late diastole (A)

were used to calculate early-to-late diastolic filling velocity ratio (E/A), an index of diastolic function. Tissue Doppler Imaging was used to measure the myocardial lengthening in apical 4-chamber view. The lateral (Lat e′) wall myocardial tissue lengthening which is at the mitral annulus was used to calculate the ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e′), an index of LV filling pressure.

Vascular reactivity

Thirty female Sprague-Dawley rats (n = 15 per group) were used to determine mesenteric and renal vascular reactivity as previously described (Mokotedi et al. 2018). Rats were anaesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg) and euthanized by thoracotomy. The intestine and the kidneys were excised and placed in a cold modified Krebs-Ringer bicarbonate (control) solution (in mmol/L: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11.1 glucose). Second branches of mesenteric arteries and the main branches of renal arteries were isolated. Rings of arteries (2 mm long) were cleaned of fat and connective tissue and suspended into a wire myograph (model 610M; Danish Myo Technology, Aahrus, Denmark). The rings were suspended between 2 stainless steel wires with a diameter of 40 μm in an organ chamber filled with control solution kept at 37 °C and aerated with 95% O_2 and 5% CO_2. One of the wires was connected to a movable holder supporting a tension transducer (Grass Instrument Co., Quincy, Massachusetts, USA) to collect the isometric force measurements by a data acquisition system (PowerLab 4SP; ADInstruments, USA). The rings were placed under an optimal resting tension according to a previously described method (Mokotedi et al. 2018). After a 60 min stabilisation period, the arteries were exposed to potassium chloride (80 mM), which produced a maximal contraction response to potassium and served as the reference contraction. Preparations were exposed to increasing concentration of potassium chloride (1–120 mM) or phenylephrine (1 nM – 0.1 mM (in a half-log increments)). Changes in isometric tension under baseline tension were recorded as absolute values in mN. The relaxation responses induced by acetylcholine, an endothelium-dependent vasodilator (0.1 nM – 100 μM), or sodium nitroprusside, an endothelium-independent vasodilator (1 nM – 100 μM), were assessed in a half-log increment during phenylephrine (30 μM)-induced contractions. Phenylephrine (30 μM)-induced contractions were similar between the groups in rings of renal artery (STD: 18.3 ± 1.9 mN; HFHS: 19.4 ± 1.5 mN) and rings of mesenteric arteries (STD: 22.5 ± 2.1 mN; HFHS: 20.1 ± 1.7 mN). The relaxation caused by acetylcholine and sodium nitroprusside were expressed as a percentage of the tension during contractions induced by phenylephrine. In renal arteries, acetylcholine (3 μM – 100 μM) induced an increase in tension that may be related to acetylcholine-induced release of endothelium-derived contracting factor (Michel et al. 2008).

Serum insulin concentration

Serum insulin was measured in 30 female Sprague-Dawley rats (n = 15 per group), using a solid phase 2-site enzyme immunoassay (DRG Ultra-Sensitive Rat Insulin ELISA; DRG, USA). The ultrasensitive rat insulin ELISA is based on the direct sandwich technique in which 2 monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule.

Lipid profile

Serum total and HDL cholesterol were measured in 30 female Sprague-Dawley rats (n = 15 per group) by enzymatic colorimetric method (Cobas 6000; Roche Diagnostic, Germany).

Statistical analysis

Data are expressed as means ± SEM. The concentrations that produced 50% of the maximal contractile response (EC_{50}) and the maximal responses (E_{max}) were determined from regression analysis logistic sigmoid function curves (GraphPad Software Inc., San Diego, California, USA). Data analysis was performed using SAS software version 9.4 (SAS institute Inc., Cary, North Carolina). An unpaired t test was performed to determine differences between the 2 experimental groups. Associations were determined by Pearson's correlation coefficients. A P value <0.05 was considered statistically significant.

Table 1. Morphologic and metabolic parameters of female Sprague-Dawley rats receiving a standard (STD) or a high-fat, high-sucrose (HFHS) diet for 6 weeks.

	STD diet	HFHS diet
Morphologic parameters[a]		
Body mass (g)	284±20	276±15
Tibial length (cm)	4.1±0.2	4.2±0.1
Food intake (g/day)	19±3	16±3*
Visceral fat mass (g)	3.2±1.7	3.0±1.5
Liver mass (g)	10.1±1.0	9.4±1.2*
Left ventricular mass (g)	0.55±0.08	0.51±0.09
Metabolic parameters		
Fasting blood glucose concentrations (mmol/L)[a]	3.71±0.69	3.37±0.60*
Serum insulin concentration (μg/L)[b]	0.044±0.002	0.044±0.002
Serum total cholesterol concentration (mmol/L)[b]	1.52±0.24	1.73±0.27*
Serum HDL cholesterol concentration (mmol/L)[b]	1.01±0.15	0.78±0.13*
Fasting blood TG concentrations (mmol/L)[a]	1.39±0.25	2.53±0.69*
Peak TG clearance (mmol/L)[c]	2.84±0.77	6.38±0.89*
TG clearance (AUC)[c]	12.4±2.2	24.9±5.2*

Note: Data expressed as means ± SD. AUC, area under the curve; HDL, high-density lipoprotein; TG, triglycerides.

[a] n = 25 per group.

[b] n = 15 per group.

[c] n = 20 per group.

*P < 0.05 versus STD diet.

Results

Morphologic and metabolic parameters

Baseline body mass was similar between the HFHS diet groups and the STD diet groups (274 ± 19 g and 279 ± 17 g, respectively). After the 6 weeks of dietary intervention, body mass was similar between the 2 experimental groups (Table 1). Food intake was significantly lower in the HFHS diet groups compared with the STD diet groups (P = 0.003; Table 1). Tibial length, visceral adipose tissue, and LV masses were not different between the 2 experimental groups (Table 1). Liver mass was significantly lower in rats receiving the HFHS diet compared with those receiving the STD diet (P = 0.015; Table 1). The appearance of the liver was similar in rats receiving the HFHS diet compared with those receiving the STD diet (data not shown). After 6 weeks of dietary intervention, fasting blood glucose concentration was significantly lower in the HFHS diet groups compared with the STD diet groups (P = 0.05; Table 1). Serum insulin concentration was similar between the 2 experimental groups. Fasting blood triglycerides concentration was significantly greater in the HFHS diet groups compared with the STD diet groups (P <0.0001; Table 1). Triglycerides clearance was significantly impaired in HFHS diet group compared with the STD diet group (P < 0.0001; Table 1). Serum total cholesterol concentration was significantly greater in HFHS diet group compared with the STD diet group (P < 0.019; Table 1). Serum HDL-cholesterol concentration was significantly lower in HFHS diet group compared with the STD diet group (P < 0.0001; Table 1).

Blood pressures

After 6 weeks of dietary intervention, SBP was significantly greater in the HFHS diet group compared with the STD diet group

Table 2. Blood pressures and left ventricular (LV) dimensions in female Sprague-Dawley rats receiving a standard (STD) or a high-fat, high-sucrose (HFHS) diet for 6 weeks.

	STD diet	HFHS diet
Systolic blood pressure (mm Hg)[a]	138±6	146±11*
Diastolic blood pressure (mm Hg)[a]	119±10	118±10
LV EDD (mm)[b]	5.42±0.66	4.60±0.59*
LV ESD (mm)[b]	2.10±1.01	1.76±0.63
LVED PWT (mm)[b]	2.17±0.47	2.53±0.54
LVED PWT (mm)[b]	3.13±0.54	3.31±0.53

Note: Data expressed as means ± SD. EDD, end diastolic diameter; ESD, end systolic diameter; PWT, posterior wall thickness.

[a] $n = 25$ per group.

[b] $n = 10$ per group.

*$P < 0.05$ versus STD diet.

($P < 0.001$; Table 2). DBP was similar between the 2 experimental groups (Table 2).

Echocardiography

Baseline LVEDD was significantly lower in the HFHS diet group compared with the STD diet group ($P = 0.006$; Table 2). LVESD as well as the LVES and LVED PWT were similar between the 2 experimental groups (Table 2). Baseline E/A was significantly lower in rats receiving the HFHS diet compared with those receiving the STD diet ($P = 0.01$; Fig. 1). Baseline FS_{end}, FS_{mid}, and E/e′ were similar between the 2 experimental groups (Fig. 1). The EC_{50} and E_{max} of isoprenaline-induced changes in systolic function, indexed by FS_{end} and FS_{mid}, and changes in diastolic function, indexed by E/A and E/e′, were similar between the 2 experimental groups (Fig. 1).

Vascular reactivity

The EC_{50} of phenylephrine-induced contractions were significantly lower in mesenteric arteries, but not in renal arteries, of rats receiving the HFHS diet compared with those receiving the STD diet ($P = 0.001$; Fig. 2). The E_{max} of phenylephrine-induced contraction in mesenteric and renal arteries were similar between the 2 experimental groups (Fig. 2). The EC_{50} and E_{max} of KCl-induced contractions or Ach- and SNP-induced relaxations in mesenteric and renal arteries were similar between the 2 experimental groups (Fig. 3).

Correlation between metabolic and cardiovascular parameters

Lower liver mass was significantly associated with greater triglycerides concentration (Fig. 4A) and lower HDL cholesterol concentration ($r = -0.35$; $P = 0.033$; $n = 30$). Liver mass was not correlated with total cholesterol concentration and SBP. LVEDD was not associated with either SBP, blood triglycerides, HDL and total cholesterol concentrations, or baseline E/A. Lower EC_{50} of phenylephrine-induced contractions and baseline E/A were significantly associated with greater blood triglycerides concentration (Figs. 4B and 4C). EC_{50} of phenylephrine-induced contraction and baseline E/A were not correlated with HDL and total cholesterol concentrations as well as SBP. Greater SBP was significantly associated with greater fasting blood triglycerides concentration (Fig. 4D) and greater impaired triglycerides clearance ($r = 0.45$, $P = 0.026$; $n = 20$). Greater SBP was significantly associated with lower HDL cholesterol concentration ($r = -0.35$; $P = 0.031$; $n = 30$). In a multivariate regression analysis, the relationship between SBP and blood triglycerides concentrations remains significant after the inclusion of HDL cholesterol and total cholesterol concentrations as confounding variables.

Discussion

The main findings of the current study are as follows. A lipogenic diet impaired the LV diastolic function, increased the sensitivity of resistance arteries to an alpha-receptor agonist, and increased SBP in female rats. Moreover, these cardiovascular disturbances were associated with increased fasting blood triglycerides concentration. Importantly, SBP was still positively associated with blood triglycerides concentration even after adjustment for HDL and total cholesterol concentrations. In response to the lack of evidence on the effect of hypertriglyceridemia on the cardiovascular system in females, these results may ameliorate our understanding of the pathogenesis of CVD risk factors in women.

In the present study, SBP was increased in young female normotensive rats presenting with hypertriglyceridemia induced by a diet rich in carbohydrate and saturated fat. The increase in SBP was present without increases in body mass or adipose tissue, hyperglycemia or increased insulin concentration, as well as fatty liver, suggesting the absence of other components of the metabolic syndrome. Finally, the strong relationship between SBP and blood triglycerides concentration was independent of other blood lipids measured and, in particular, HDL cholesterol concentration. Hypertriglyceridemia per se may have increased SBP in normotensive females. Studies using different rodent models of diet-induced metabolic syndrome have described increased SBP in the presence of increased blood triglycerides concentration in males (Bourgoin et al. 2008; Pérez-Torres et al. 2008; Panchal et al. 2011; Sweazea and Walker 2011; Wong et al. 2012, 2017; Alam et al. 2013; Vileigas et al. 2016; Qin et al. 2018) and females (Barnard et al. 1993, 1998). However, the absence of changes in SBP has also been shown in diet-induced metabolic syndrome with increased blood triglycerides concentration (Bartus et al. 2005; Smith et al. 2016; Battault et al. 2018), whereas increased in SBP has been demonstrated in diet-induced metabolic syndrome without increased blood triglycerides concentration (Poudyal et al. 2012). Moreover, different treatments have decreased SBP without decreasing blood triglycerides concentration (Wong et al. 2012, 2017; Alam et al. 2013). Therefore, in diet-induced metabolic syndrome models, hypertriglyceridemia is at least not the sole determinant of increased SBP. The presence of other metabolic disturbances such as visceral adiposity or insulin resistance may have predisposed to the increase in SBP (Barnard et al. 1993, 1998; Bourgoin et al. 2008; Pérez-Torres et al. 2008; Panchal et al. 2011; Sweazea and Walker 2011; Wong et al. 2012, 2017; Alam et al. 2013; Vileigas et al. 2016; Qin et al. 2018). In this regard, obesity and insulin resistance are important risk factors for the development of hypertension (Barnard et al. 1993; Goldberg 2001; Ginsberg et al. 2006; Grundy 2016). In genetically induced hypertriglyceridemic rats, hypertension is present in males (Štolba et al. 1992; Kusterer et al. 1999; Torok et al. 2007) and females (Sotnikova et al. 2012). Moreover, BP is positively associated with the triglycerides concentration (Štolba et al. 1992). However, rats develop dyslipidemia, hyperglycemia, and insulin-resistance in parallel, which may also be at the origin of hypertension (Goldberg 2001; Ginsberg et al. 2006; Grundy 2016). To the best of our knowledge, the present study is the first evidence in female rats of a positive association between hypertriglyceridemia and SBP independent of other lipids measured.

In addition to increased SBP, the baseline diastolic function was impaired with the lipogenic diet in the present study. Moreover, the diastolic function was associated with blood triglycerides concentration, suggesting that hypertriglyceridemia was, at least, in part responsible of the impaired diastolic function. Baseline LVEDD was decreased and the systolic function was preserved in the current study. The diastolic and systolic cardiac performances following beta-adrenergic stimulation were also preserved. Results on diastolic function in diet-induced models of metabolic syndrome with increased blood triglycerides concentration are controversial. Indeed, preserved or impaired diastolic function have been reported in these models (Panchal et al. 2011; Wong et al. 2012, 2017; Alam et al. 2013; Smith et al. 2016; Qin et al. 2018). Treatments may ameliorate diastolic function, but without reduc-

Fig. 1. Isoprenaline-induced changes in (A) endocardial fractional shortening (FS_{end}), (B) midwall fractional shortening (FS_{mid}), (C) early-to-late diastolic filling velocity ratio (E/A), and (D) early mitral inflow velocity and mitral annular early diastolic velocity (E/e′) in left ventricle of female Sprague-Dawley rats receiving a standard (open circles) or a high-fat, high-sucrose (black squares) diet. Data are expressed as means ± SEM, $n = 10$ per group. *$P < 0.05$ compared with rats receiving a standard diet.

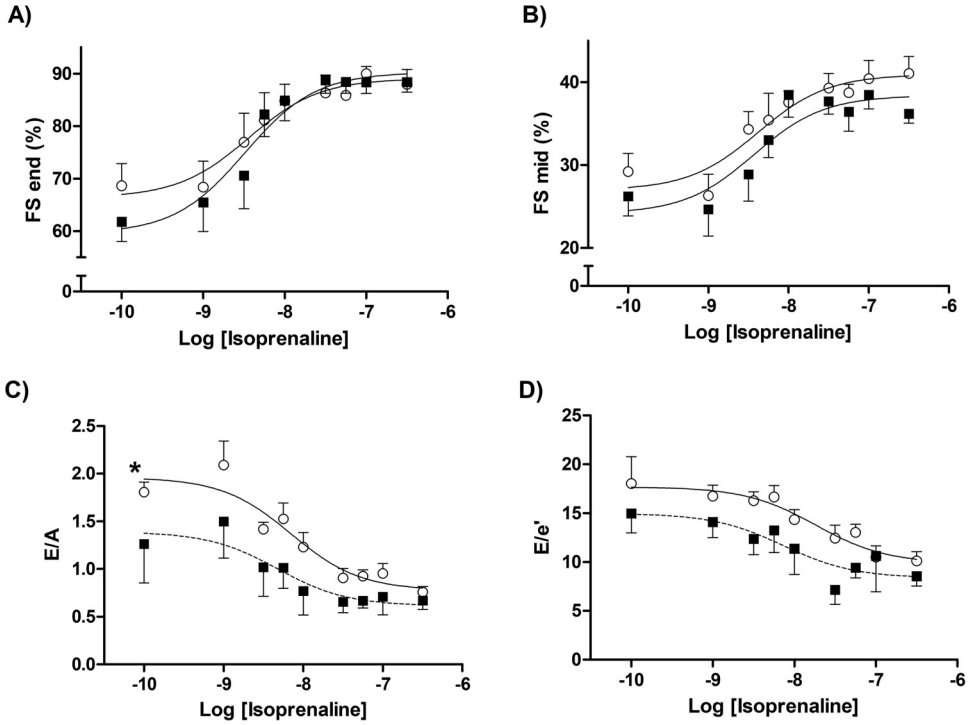
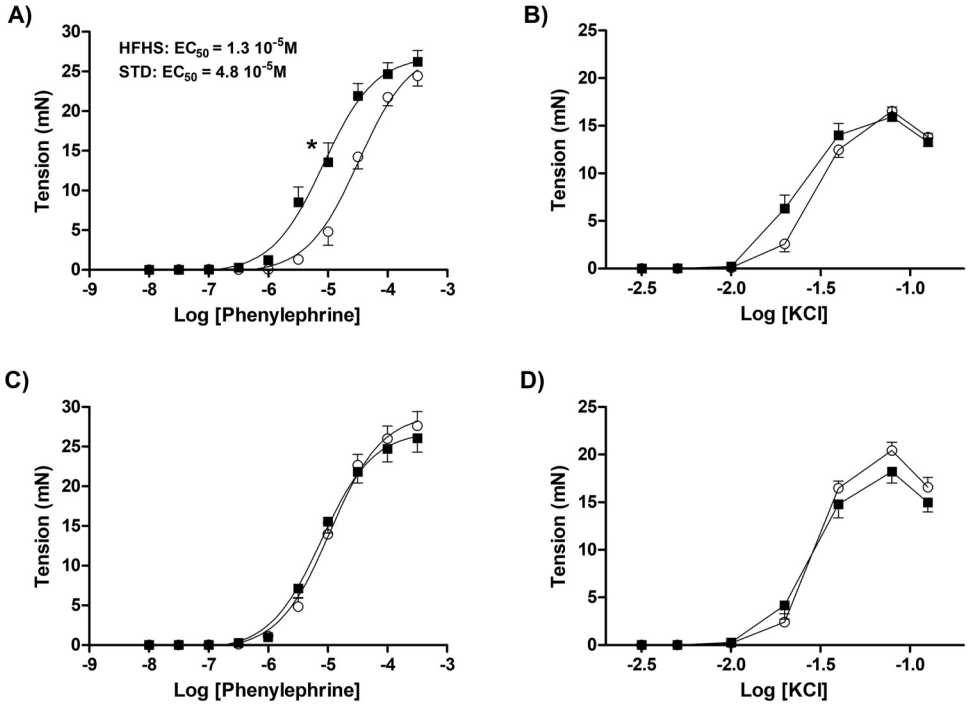


Fig. 2. Cumulative dose–response curves to (A and C) phenylephrine or (B and D) potassium chloride in second branch mesenteric arteries (top panels) or renal arteries (bottom panels) of female Sprague-Dawley rats receiving a standard (STD, open circles) or a high-fat, high-sucrose (HFHS, black squares) diet. Data are expressed as means ± SEM, $n = 15$ per group. *$P < 0.05$ compared with rats receiving a STD diet.



ing blood triglycerides concentration or with reduction of all the different components of the metabolic syndrome (Alam et al. 2013; Wong et al. 2017). Rats receiving anti-retrovirus have shown amelioration of the diastolic function following an antihyperlipidemic treatment (Mak et al. 2013). However, blood triglycerides concentration is as much reduced as other lipids concentration. Similarly, the present study cannot rule out the effect of other blood lipids on diastolic function.

Fig. 3. Cumulative dose–response curves to (A and C) acetylcholine or (B and D) sodium nitroprusside in second branch mesenteric arteries (top panels) or renal arteries (bottom panels) of female Sprague-Dawley rats receiving a standard (open circles) or a high-fat, high-sucrose (black squares) diet. Data are expressed as means ± SEM, n = 15 per group.

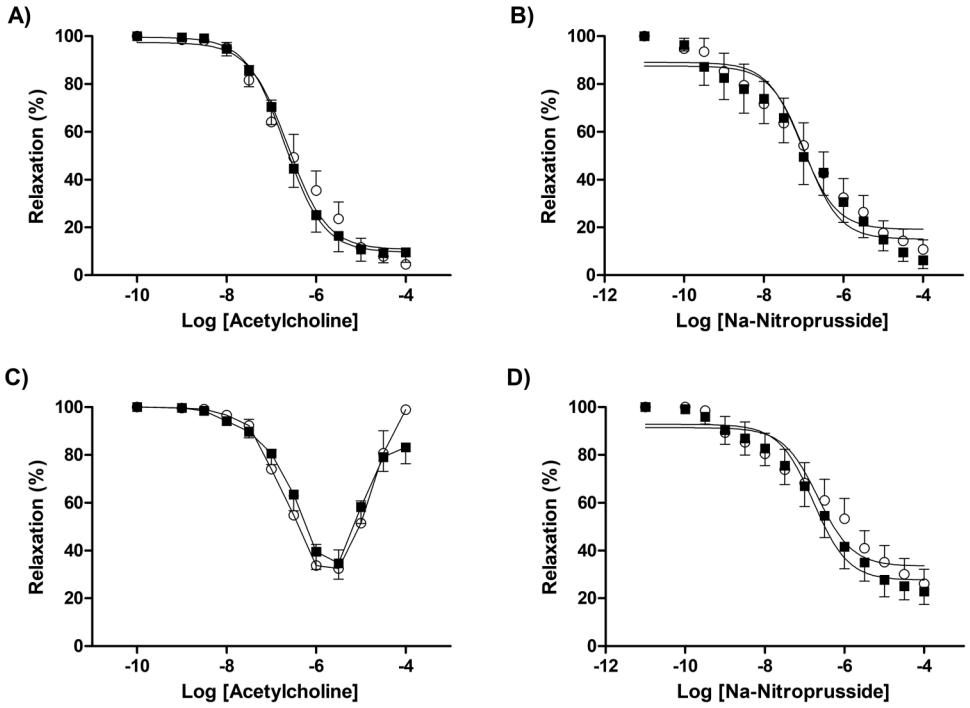
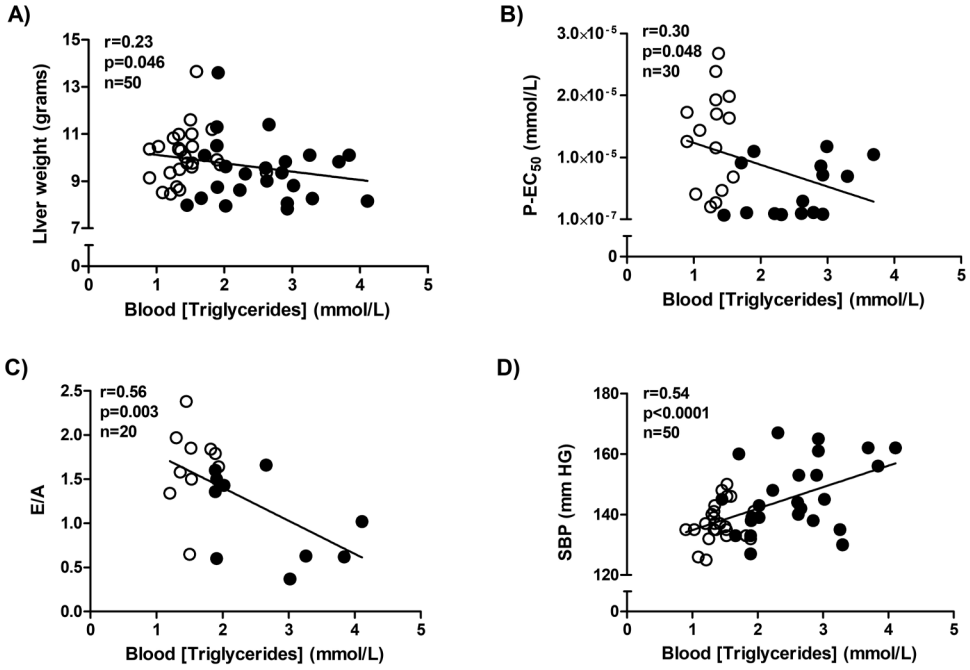


Fig. 4. Regression analysis illustrating the relationship between fasting blood triglycerides concentration and (A) liver mass, (B) the concentrations that produced 50% of the maximal contractile response to phenylephrine (P-EC_{50}) in the mesenteric arteries, (C) early-to-late diastolic filling velocity ratio (E/A), and (D) systolic blood pressure (SBP) of female Sprague-Dawley rats receiving a standard (open circles) or a high-fat, high-sucrose (filled circles) diet.



LV eccentric hypertrophy and systolic dysfunction have been often reported in diet-induced models of metabolic syndrome with and without increased blood triglycerides concentration (Panchal et al. 2011; Poudyal et al. 2012; Wong et al. 2012, 2017; Alam et al. 2013; Qin et al. 2018). The diet-induced increased in SBP and the subsequent increased wall stress leads to cardiac remodeling in these models. Moreover, no changes in LVEDD and systolic function as well as preserved myocardial performance have been described in similar metabolic syndrome models but without changes or slight changes in SBP (Carroll et al. 2006; Smith et al. 2016; Vileigas et al. 2016). Consistent with these findings, the present results suggest that the increased SBP is not pronounced

enough to induce eccentric remodeling with systolic dysfunction. Although not associated with E/A, the reduced LVEDD may result from the impaired diastolic function induced by the lipogenic diet. The lack of association between LVEDD and blood triglycerides concentration also reinforces the hypothesis involving other blood or tissue lipids in the development of diastolic dysfunction in the present study.

In relation to blood pressure changes, vascular responsiveness may also be affected by increased blood triglycerides concentration. However, as for BP, the effect of hypertriglyceridemia on vascular contractile function remains uncertain. Rodent models of diet-induced metabolic syndrome with increased triglycerides concentration often present with endothelial dysfunction in males (Bartus et al. 2005; Bourgoin et al. 2008; Qin et al. 2018) and females (Roberts et al. 2005). In contrast, endothelial dysfunction also occurs in rodent model of metabolic syndrome without a change in triglycerides concentration, suggesting that hypertriglyceridemia is not a cause of endothelial dysfunction in these models (Poudyal et al. 2012). Other studies have described rather an impaired smooth muscle cells relaxation than an endothelial dysfunction in diet-induced metabolic syndrome with increased triglycerides concentration (Naderali et al. 2001; Panchal et al. 2011; Wong et al. 2012). The different types of diet, durations of intervention, and strains of rat used may explain the controversial results. Endothelial dysfunction has also been described in obese hypertriglyceridemic subjects (Lewis et al. 1999) as well as in genetically induced hypertriglyceridemic rats (Kusterer et al. 1999; Torok et al. 2007; Sotnikova et al. 2012). Nevertheless, the presence of central obesity or insulin resistance may also be at the origin of the endothelial dysfunction (Poudyal et al. 2012; Oishi et al. 2018). In the present study and in contrast to the abovementioned studies, the absence of impairment of acetylcholine-induced vasodilation has been demonstrated in 2 different vascular beds. Hypertriglyceridemia without insulin resistance may not impair the endothelial response to acetylcholine. However, without the use of specific inhibitors, we cannot rule out a modification of the mechanisms responsible for acetylcholine-induced vasodilation in response to hypertriglyceridemia.

Contractile dysfunction has also been reported with diet-induced metabolic syndrome and in the presence of increased blood triglycerides concentration. Indeed, phenylephrine-induced contractions have been shown to be impaired in the aorta (Panchal et al. 2011; Battault al. 2017; Wong et al. 2017) and may be related to an increased peripheral sympathetic outflow (Battault al. 2017). However, sympathetic nervous system overactivation may not be present in all models or affecting smaller vessels than the aorta (Naderali et al. 2001; Bourgoin et al. 2008; Sweazea and Walker 2011). On the other hand, hypercontractility has been described with diet-induced metabolic syndrome (Boustany-Kari et al. 2007; Qin et al. 2018). Similarly, the present study reported an increased sensitivity of the phenylephrine-induced contractions (but not KCl) with the lipogenic diet in the mesenteric arteries only. Moreover, the sensitivity of the phenylephrine-induced contractions was negatively associated with blood triglycerides concentration but not with total and HDL cholesterol concentrations. Hypertriglyceridemia may have increased the sensitivity of resistance arteries to an alpha-receptor agonist. Finally, changes in vascular responsiveness may significantly account for the changes in SBP (Mokotedi et al. 2018; Oishi et al. 2018). However, in the present study, the sensitivity of the phenylephrine-induced contractions was not associated with SBP, suggesting that increased contractions in response to an alpha-agonist does not directly translate into increase in SBP. The increased sensitivity to an alpha-agonist in resistance arteries may not be the only cause of the increase in blood pressure in the present study.

The experimental evidence of cardiovascular parameters changes in relation to hypertriglyceridemia is scarce in females. Only few experimental studies in female rats have investigated the response of the cardiovascular system to an increased blood triglycerides concentration, and also in addition to other components of the metabolic syndrome (Barnard et al. 1993, 1998; Roberts et al. 2005; Sotnikova et al. 2012). An increased SBP has been described after at least a year of diet intervention (Barnard et al. 1993, 1998). Insulin resistance was associated with the increased SBP and endothelial dysfunction preceded the increased SBP (Barnard et al. 1993; Roberts et al. 2005). Another study using 12-month-old hypertriglyceridemic rats showed a greater endothelial dysfunction in females compared with males despite similar blood pressure (Sotnikova et al. 2012). Although not measured, the advanced age of rats may coincide with a decreased of estrogen production. Therefore, the authors explained their results by a decrease protective action of estrogen. In the present study, insulin resistance is not present and a decreased estrogen production in young female rats is most probably not at the origin of the cardiovascular parameter changes. The type of fat used may explain the results of the present study. Trimethylamine N-oxide, which blood concentration increases after ingestion of lecithinated fat, has been shown to aggravate angiotensin-II-induced increase in blood pressure (Ufnal et al. 2014). However, blood pressure is not increased by trimethylamine N-oxide in normotensive rats (Ufnal et al. 2014). Another hypothesis is that, because of the very rich nature in saturated palmitic acid of the fat used in the present study, an increased blood concentration of palmitic acid may have resulted in an increase in blood pressure (Grimsgaard et al. 1999).

Finally, it is well known that estrogen confers a cardioprotective effect to women (Palmisano et al. 2018). However, sex difference has been noted in the cardiac maladaptation to hypertension (Cauwenberghs and Kuznetsova 2018). Sex difference in the complication of CVD related to metabolic disturbances has also been observed. Indeed, diabetes mellitus contribute to an increased incidence of CVD in women compared with men, suggesting that diabetic women are no longer more protected to CVD (Palmisano et al. 2018). Another intriguing observation concerns a possible greater risk of developing CVD with hypertriglyceridemia in women compared with men (Hokanson and Austin 1996; Austin et al. 1998). Despite these observations on sex-specific differences in the development of CVD, the pathophysiology of CVD remains barely studied in females. In the present study, young female rats develop a lipogenic diet-induced increased blood pressure, despite a probable physiological level of estrogen. Estrogen may not protect young females against the effect of a lipogenic diet on the cardiovascular system resulting in an increased blood pressure. In certain metabolic disturbances conditions, estrogen may not confer its protective effect, which may result in the development of CVD.

The current study presents certain limitations. First, measurements may have been confounded by experimental factors. Despite continuous habituation, stress may have resulted in the relatively high values of SBP and DBP. Indeed, the values of the noninvasive measurement of blood pressure in the present study is higher compared with state-of-art measurement of blood pressure using telemetry (Ufnal et al. 2014). Moreover, the use of anaesthetics may have affected the measurement of cardiac dimensions and functions. Indeed, ketamine may increase blood pressure and xylazine may induce hypotension, bradycardia, and ventricular arrhythmias (Saha et al. 2007). A combination of these agents may therefore induce depression in heart rate and LV systolic function. Without excluding the influence of these experimental factors, the group comparison in the present study may have overcome these biases. Second, because of the higher caloric intake, rats reduced their food intake with the HFHS diet, which may have diminished the minerals and vitamins intake. It is well known that vitamin D, for instance, may be responsible of essential hypertension (Chen et al. 2015). Third, hypertriglyceridemia promotes atherosclerosis (Palmisano et al. 2018). Despite using a lipogenic diet that induces atherogenic dyslipidemia in the pres-

ent study, rat is not a species prone to develop atherosclerosis (Bartus et al. 2005). Finally, the mechanisms of how hypertriglyceridemia increases blood pressure has not been explored in the present study. In this regard, to confirm our hypothesis, the blood concentration of saturated palmitic acid has to be measured and the possible association with blood pressure has to be determined. Moreover, inflammation and reactive oxygen species production may also be critical in the development of increased blood pressure.

In conclusion, the present study demonstrated an increased SBP associated with hypertriglyceridemia in young normotensive female rats. An impaired diastolic function is also associated with hypertriglyceridemia. However, the role of other lipids in the development of diastolic dysfunction cannot be ruled out in the present study. The impaired diastolic function is most probably not resulting in the increased blood pressure. Similarly, the sensitivity of resistance arteries to an alpha-receptor agonist is associated with blood triglycerides concentration and not with HDL cholesterol concentration. However, the lack of association between the sensitivity and SBP suggest that hypercontractility of resistance arteries to the sympathetic nervous system is not the only cause of increased SBP. Because of the importance of hypertension as a CVD risk factor, exploring the mechanism linking hypertriglyceridemia to increased SBP in female rats warrants further investigation.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

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