

Co-Administration of Conjugated Linoleic Acid and Rosiglitazone Increases Atherogenic Co-Efficient and Alters Isoprenaline-Induced Vasodilatation in Rats Fed High Fat Diet

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Summary

The cis(c)-9, trans(t)-11 (c9,t11) and t10,c12 isomers of conjugated linoleic acid (CLA) have been reported as agonists of peroxisome proliferator-activated receptor (PPAR) and beneficial in lipidemia and glycemia. However, it is unclear whether CLA isomers enhance or antagonize effects of conventional drugs targeting PPAR. Male Sprague-Dawley rats were fed high fat diet (HFD) for 8 weeks and treated without or with CLA, rosiglitazone or both for 4 weeks. Oral glucose tolerance and surrogate markers of insulin resistance were not significantly different for all treatments compared to untreated normal diet (ND) or HFD group, except lipoprotein levels. The combination of CLA and rosiglitazone had suppressed levels of low and high density lipoproteins (46 % and 25 %, respectively), compared to HFD-alone. Conversely, the atherogenic co-efficient of the animals received HFD or HFD+rosiglitazone+CLA was 2-folds higher than ND, HFD+rosiglitazone or HFD+CLA. Isolated aortic rings from the combined CLA and rosiglitazone treated animals were less sensitive to isoprenaline-induced relaxation among endothelium-denuded aortas with a decreased efficacy and potency ($R_{max}=53\pm4.7\%$; $pEC50=6\pm0.2$) compared to endothelium-intact aortas ($R_{max}=100\pm9.9\%$; $pEC50=7\pm0.2$). Our findings illustrate that the combination of CLA and rosiglitazone precede the atherogenic state with impaired endothelium-independent vasodilatation before the onset of HFD-induced insulin resistance.

Key words

PPAR agonist • High fat diet • High density lipoprotein • Rosiglitazone • Atherogenic

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Introduction

Type 2 diabetes mellitus (T2DM) is one of the major global health burdens and have reached worldwide epidemic. T2DM is accompanied by major hallmarks such as atherogenic dyslipidemia and endothelial dysfunction (Mooradian 2009, Bakker *et al.* 2009). Together, these hallmarks predispose T2DM patients to cardiovascular diseases. The current options available for treatment and management of T2DM are relatively limited, having problems with non-negligible side effects, and/or often require multiple combinations of drugs that mostly potentiate the adverse effects.

Thiazolidinediones (TZD) or glitazones are oral anti-hyperglycemic drugs commonly referred as “insulin sensitizers”. The use of these glitazones, including rosiglitazone, have been cautioned for increased risk of cardiovascular diseases in T2DM patients (Hiatt *et al.*

2013, Nissen and Wolski 2007, Nissen and Wolski 2010). This prompts the search for alternative drugs with a better clinical safety profile or suitable supplements to combat the adverse effects of TZD without altering its insulin sensitizing potential.

Conjugated linoleic acids (CLA) refer to a group of positional and geometric isomers of octadecadienoate (C18:2) with conjugated double bonds. Specifically two isomers of CLA, cis-9, trans-11 (c9,t11) and trans-10, cis-12 (t10,c12) isomers exist in abundance in dairy products and ruminant meats (Pariza *et al.* 1999) and garnered immense interest for their biological activity (Dilzer and Park 2012, Mohankumar *et al.* 2011, Shokryzadan *et al.* 2017, Taylor and Zahradka 2004). Unlike TZDs, dietary supplementation of CLA has been shown to promote weight loss in obese animals and weight gain in lean animals, prevent cardiac hypertrophy and blood pressure elevation in spontaneously hypertensive rats (Alibin *et al.* 2008, DeClercq *et al.* 2012b), and decrease LDL cholesterol in obese insulin-resistant rats and healthy human subjects (Derakhshande-Rishehri *et al.* 2015, Noone *et al.* 2002, Wanders *et al.* 2010). These distinct properties of CLA compared to TZDs suggest that there is a possibility that CLA may be used as an alternative supplement or substitute to combat the adverse effects of TZD.

In regards, a previous study conducted in mice fed a high fat diet demonstrated that supplementation with CLA prevented weight gain in rosiglitazone-treated mice without affecting the insulin sensitizing effects of rosiglitazone (Liu *et al.* 2007). However, it is not clear whether or not co-administration of CLA and TZD alter lipoproteins level and vascular functions. The present study therefore examined the effects of CLA isomers and rosiglitazone either alone or in combination on surrogate markers of insulin resistance, including lipoproteins, and on vascular contractility in rats fed high fat diet.

Methods

Chemicals

Rosiglitazone was purchased from LKT Laboratories (R5773), USA. Conjugated linoleic acid (1:1 ratio of c9,t11 and t10,c12) was purchased from Nu Chek Prep (UC-59-AX), USA. (R)-(-)-Phenylephrine hydrochloride and isoproterenol hydrochloride (isoprenaline) were purchased from Tocris Bioscience, UK.

Animals and diet

Thirty male Sprague-Dawley (SD) rats (6 weeks old) were received (Monash University Malaysia Campus, Malaysia) and acclimatized with normal rodent chow diet (Altromin, 1324, Germany) for two weeks. We selectively used male rats as they are known to be more susceptible to metabolic disorder than female rats and to avoid the estrous cycle mediated interference on metabolic parameters in female rats. The experimental protocol was approved by the Faculty of Medicine Institutional Animal Care and Use Committee, University of Malaya (Approval number: 2014-03-05/PHAR/R/DDM) and the Animal Welfare and Ethical Review Body, University of Nottingham. Food and water were provided *ad libitum*.

The rats were divided into groups fed with normal diet (ND; 4.10 % total energy from fat; 6 rats) and high fat diet (24 rats; 60 % total energy from fat; Research Diets, D12492, USA). After completion of 8 weeks of HFD feeding, rats were divided into the following groups and treated for 4 weeks: HFD control (HFD), HFD treated with rosiglitazone (HFD+RGZ), HFD treated with CLA (HFD+CLA) and HFD treated with rosiglitazone and CLA (HFD+RGZ+CLA). CLA was dissolved in 5 % w/w fat free milk and administered at 0.2 g per animal per day by oral gavage. The dose of CLA is based on the human consumption equivalent, which amounts to 1-2 % of dietary weight per day. On average, a rat consumes 20 g of food per day. With this assumption, we calculate that each rat should be given 0.2 g of CLA per day. The control rats received only 5 % w/w fat free milk. Rosiglitazone was administered daily by intraperitoneal injection at 2 mg/kg body weight and control rats received only saline injection. The dose of rosiglitazone is based on the previously published study (Zhao *et al.* 2012).

Body weight, feed intake and blood glucose (Accu-Check blood glucose monitor) were recorded weekly, except terminal blood glucose. A week before the sacrifice, rats were fasted overnight and undergone oral glucose tolerance test (OGTT) as previously described (Noto *et al.* 2006). At the end of the study, all rats were fasted overnight, asphyxiated with CO₂ and their blood and organs were collected. The blood samples were centrifuged at 2,000 rpm for 10 min and the serum separated were stored at -80 °C until further analyses. The thoracic aortas were isolated for vascular contractility studies.

Serum biochemical analysis

Terminal fasting blood samples were analyzed for glucose (Cayman, 10009582, Ann Arbor, MI, USA), total cholesterol (Cayman, 10007640, USA), total triglycerides (Zen-Bio, STG-1-NC, USA), high density lipoprotein (HDL) and LDL/vLDL (BioVision, K613-100, Milpitas, CA, USA) and insulin levels (Alpco Diagnostics, 80-INSRT-E01, Salem, NH, USA) according to manufacturer's instructions. The colorimetric and fluorimetric end points were measured in Varioskan Flash Multimode Reader (Thermo Scientific, USA).

In vitro organ bath experiments

This procedure was performed as previously described (Loong *et al.* 2015). Briefly, thoracic aortae were immediately excised and transferred into cold Krebs-Ringer bicarbonate solution upon sacrifice. In an aerated tissue bath, the aortic rings (4 mm) were left

in the bath for 30 min to equilibrate before the application of 2 g weight tension. Endothelium denuded rings were prepared by gently rubbing the intimal surface using the blunt tip of stainless steel forceps. Successful denudation was confirmed if the tissue produced less than 30 % relaxation in the presence of 1×10^{-6} M carbachol in the pre-constricted rings. All aortic rings were pre-contracted to at least 70 % of maximal contraction with 0.1 μ M phenylephrine before the addition of isoprenaline (1×10^{-10} – 3×10^{-5} M). Maximal relaxation (R_{max}) and pEC₅₀ values were calculated using Prism version 6.0 (GraphPad Software, USA) in which EC₅₀ is the concentration of isoprenaline that produces 50 % of maximal relaxation and pEC₅₀ is taken as the negative common logarithm of EC₅₀.

Calculations

Weekly energy intake was calculated with the following formula:

$$\text{Energy intake (KJ g}^{-1}\text{)} = \frac{\text{Metabolisable energy per gram of food (KJ g}^{-1}\text{)} \times \text{Average food consumed per rat (g)}}{\text{Average body weight per rat (g)}}$$

Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated by taking the product of blood glucose in mg/dl and serum insulin in mU/l, divided by 405, as described previously (Mohankumar *et al.* 2013).

Lipoprotein ratios were obtained by using serum total, LDL and HDL cholesterol readings in mmol/l. Non-HDL cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol (Martirosyan *et al.* 2007). Ratios of LDL:HDL, total cholesterol (TC):HDL and non-HDL:HDL were obtained.

Statistics

Results are shown as means \pm standard error of the mean for the number of rats tested. Statistical analyses were performed using unpaired Student's t-test to compare two groups and one-way ANOVA with Tukey's *post hoc* tests to compare three or more groups. Repeated measure one-way ANOVA was performed for concentration-responses and time responses. Analyses were done on SPSS version 22 (IBM, USA) and graphics prepared using Prism version 6.0 (GraphPad Software, USA). A probability of less than 0.05 ($p < 0.05$) was considered to be statistically significant. Diet specific differences were compared with ND and treatment specific differences were compared with HFD.

Results

Physical characteristics and energy intake

First, we analyzed the effect of diet and treatment on body weight, organ weight and food intake. From week 4 onwards, all groups fed high fat diet (HFD, HFD+RGZ, HFD+CLA, HFD+RGZ+CLA) were ~20 % heavier than normal diet (ND) (Fig. 1A). Organ weights were similar in all groups except fat pads including epididymal, perirenal and mesenteric fats, the weight of groups fed high fat diet were 1.3 to 2.7 folds higher than normal diet (Table 1). However, no treatment specific difference was observed ($p > 0.05$). Energy intakes, in kilojoules measured weekly, were similar across all groups (Fig. 1B).

Blood glucose and insulin

Since hyperglycemia and insulin resistance are major hallmarks of T2DM and metabolic syndrome (Kaur 2014), we measured the fasting blood glucose and insulin levels to determine the effect of the diet and/or treatment of glycemia.

The fasting blood glucose levels were found to be similar across all groups (Fig. 2A). All rats were found to have similar glucose clearance with respect to OGTT (Fig. 2B). In addition, whilst rats belonging to

HFD+RGZ, HFD+CLA and HFD+CLA+RGZ groups geared towards improved insulin sensitivity as shown by homeostatic model assessment of insulin resistance

(HOMA-IR) index (Fig. 2C), they were not statistically different from ND or HFD control groups.

Table 1. Effects of diet and treatment on organ weights.

	ND	HFD	HFD+RGZ	HFD+CLA	HFD+RGZ+CLA
Heart (g)	1.28 ± 0.07	1.47 ± 0.05	1.52 ± 0.10	1.51 ± 0.04	1.43 ± 0.04
Liver (g)	10.67 ± 0.06	10.33 ± 0.46	11.00 ± 0.11	11.19 ± 0.35	10.00 ± 0.66
<i>Fat pads</i>					
Epididymal fat (g)	4.24 ± 0.45	8.15 ± 0.96*	7.90 ± 0.14*	9.06 ± 0.99*	7.50 ± 0.97*
Perirenal fat (g)	3.8 ± 0.46	8.64 ± 1.4*	9.81 ± 1.16*	10.54 ± 0.89*	8.87 ± 1.20*
Mesenteric fat (g)	2.14 ± 0.26	3.39 ± 0.45*	3.78 ± 0.32*	3.60 ± 0.23*	2.92 ± 0.48*
Pancreas	1.03 ± 0.14	1.45 ± 0.18	1.30 ± 0.18	1.61 ± 0.15	1.50 ± 0.18
<i>Kidneys</i>					
Right kidney (g)	1.46 ± 0.11	1.54 ± 0.08	1.62 ± 0.13	1.69 ± 0.08	1.60 ± 0.06
Left kidney (g)	1.44 ± 0.11	1.50 ± 0.08	1.56 ± 0.14	1.67 ± 0.04	1.57 ± 0.08
Gastrocnemius muscle (g)	2.23 ± 0.36	2.33 ± 0.18	3.00 ± 0.50	2.21 ± 0.10	2.16 ± 0.16
Soleus muscle (g)	0.16 ± 0.01	0.20 ± 0.02	0.19 ± 0.01	0.20 ± 0.07	0.20 ± 0.02

Data were presented as mean ± SEM, n=6 for each group. * p<0.05 compared to ND.

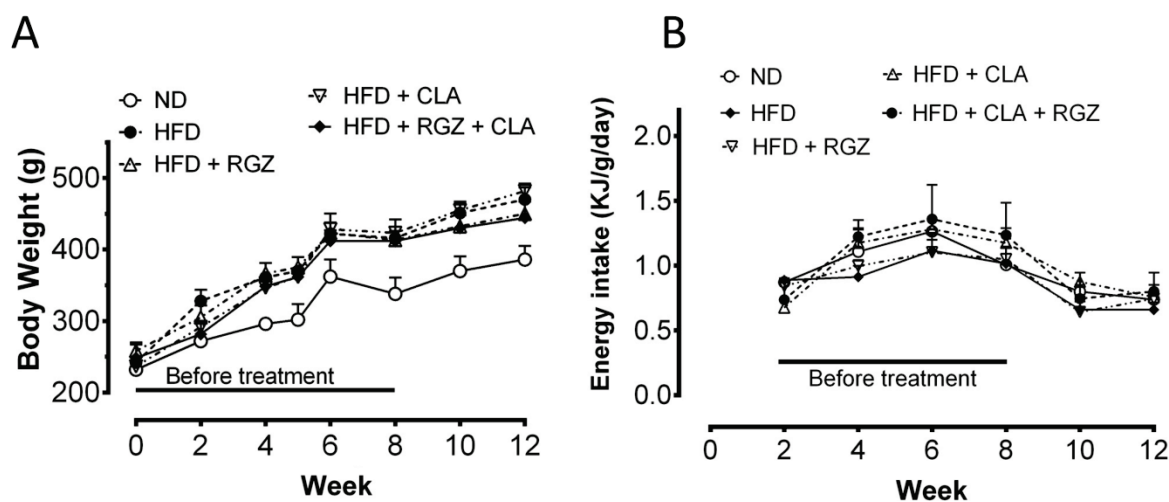


Fig. 1. Effect of diet and treatments on body weight and energy intake. (A) Body weight of Sprague-Dawley rats throughout the study. (B) Average energy intake of Sprague-Dawley rats throughout the study. Data is presented as mean ± SEM, n=6 in all groups. # p<0.05 compared to ND.

Serum lipoprotein levels and ratios

Atherogenic dyslipidemia is one of the critical metabolic characteristic of T2DM (Mooradian 2009), hence various serum lipid markers of dyslipidemia were measured. As depicted in Figures 3A and 3D, neither diet nor treatments have altered the serum TC and triglyceride levels. The serum LDL level was 46 % reduced in HFD+CLA+RGZ group in comparison to HFD alone (p<0.05, Fig. 3B). Conversely, animals from HFD,

HFD+RGZ and HFD+CLA have shown 1.3 folds elevated levels of HDL compared to ND (p<0.05), whereas HFD+RGZ+CLA have 25 % suppressed serum HDL than HFD group and the level was similar to the level measured in ND group (Fig. 3C). A plethora of published studies reported that cholesterol ratios, especially LDL:HDL, TC:HDL and non-HDL:HDL ratios have been recognized as the critical markers of atherogenicity and are widely used to assess

cardiovascular risk (van Deventer *et al.* 2011, Hermans *et al.* 2007, Lu *et al.* 2003). As shown in Figures 4A and 4B, the TC:HDL and non-HDL:HDL ratios in the animals received HFD or HFD+RGZ+CLA was 2-folds higher than ND ($p < 0.05$), whereas no significant changes

observed for HFD+RGZ and HFD+CLA. Although the LDL:HDL ratio was lower in treatment groups (Fig. 4C), they were not significantly different compared to ND and HFD, respectively ($p > 0.05$).

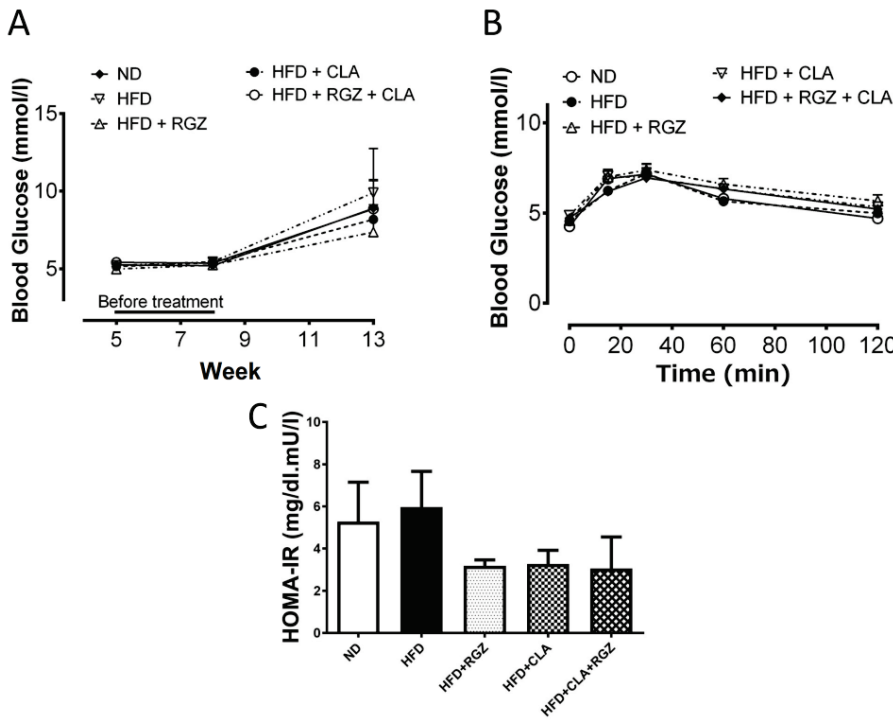


Fig. 2. Effect of diet and treatment on glycemia (A) Measurement of blood glucose level. Blood glucose on week 5 and week 8 measured using tail prick method; terminal blood glucose on week 13 measured using glucose assay kit. Data is presented as mean \pm SEM, $n=6$ in all groups. (B) Oral glucose tolerance test (OGTT). A day before the 12 week, rats were fasted overnight before undergoing oral glucose tolerance test. (C) HOMA-IR of rats at termination. Data were presented as mean \pm SEM, $n=6$ in all groups.

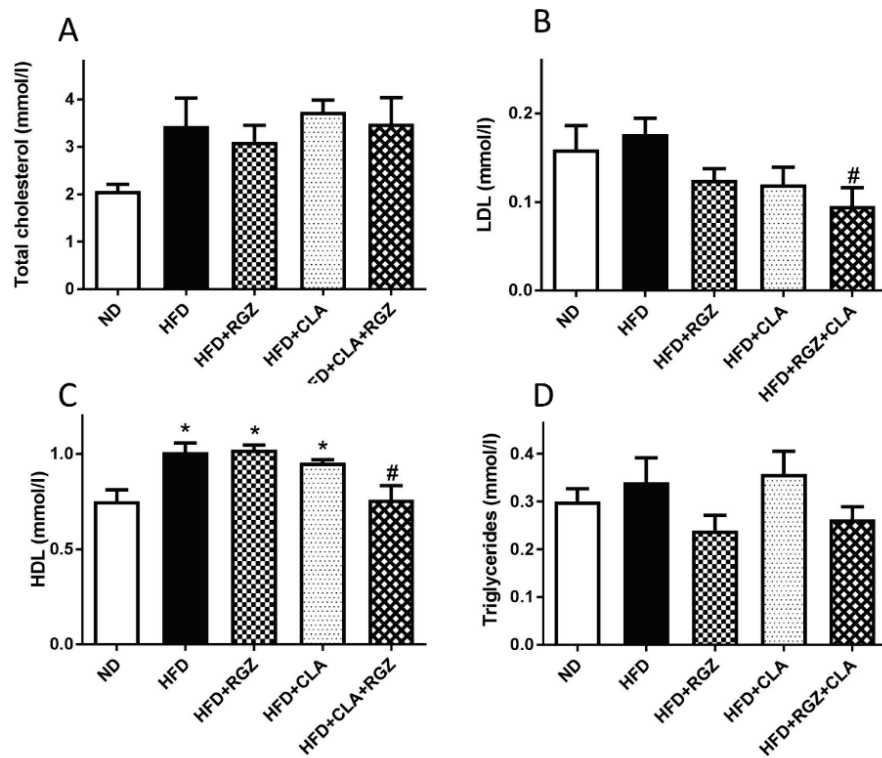


Fig. 3. Effect of diet and treatments on lipid profiles. (A) Total cholesterol. (B) LDL-cholesterol. (C) HDL-cholesterol. (D) Triglycerides. Data were presented as mean \pm SEM, $n=6$ in all groups. * $p < 0.05$ compared to ND. # $p < 0.05$ compared to HFD.

Isoprenaline-induced vasorelaxation

A set of preliminary experiments were conducted to optimize the experimental conditions for isoprenaline-induced relaxation (data not shown). The degree of initial contraction induced by 0.1 μ M phenylephrine for both control and treatment groups

either without or with endothelium were not statistically significant ($p > 0.05$; data not shown). A representative trace of isoprenaline-induced relaxation in aortic rings excised from SD rats fed normal rodent chow diet is shown in Figure 5A.

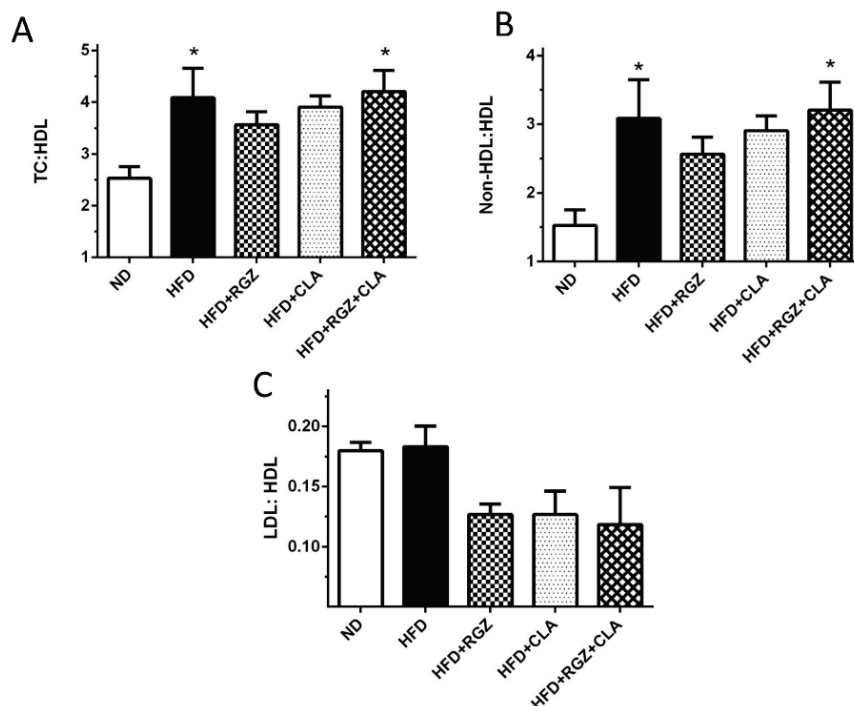


Fig. 4. Effect of diet and treatments on lipoprotein ratios. **(A)** TC:HDL ratio. **(B)** non-HDL:HDL ratio. **(C)** LDL:HDL ratio. Data were presented as mean \pm SEM, $n=6$ for each group. * $p < 0.05$ compared to ND.

The effect of diet and treatment on isoprenaline-induced vascular relaxation in isolated aortic rings excised from the rats was examined. The endothelial layer was removed on some of the aortic rings to check if the endothelium has been affected by the various treatments. All isolated aortic rings exhibited concentration-dependent vasodilatation in response to isoprenaline as the vasodilator. Repeated measures analysis indicated that the isoprenaline-induced vasorelaxation was significantly different between ND, HFD and HFD+RGZ+CLA regardless of endothelial presence (Figs 5B and 5C).

Among endothelium intact rings (Fig. 5B), all rings fed HFD, regardless of treatments were more responsive than ND. HFD+RGZ+CLA was the most responsive to isoprenaline. This was followed by HFD. The responsiveness of HFD+RGZ is intermediate of HFD and HFD+RGZ+CLA, while HFD+CLA is intermediate of HFD and ND. All groups exhibited similar pEC_{50} and R_{max} (Table 2 and Fig. 5B). However, the trend was reversed in endothelium denuded rings (Table 2 and Fig. 5C). Removal of endothelium worsened the

isoprenaline-induced vasorelaxation in all HFD and treatment groups. The relaxation was severely affected in aortic rings obtained from HFD+RGZ+CLA treated animals ($pEC_{50}=6 \pm 0.2$, $R_{max}=53.6 \pm 4.7\%$) compared to ND ($pEC_{50}=7 \pm 0.2$, $R_{max}=113 \pm 22\%$). Likewise, aortic rings from both HFD and HFD+CLA had diminished response to isoprenaline compared to ND ($pEC_{50}=6.6 \pm 0.1$, $R_{max}=93 \pm 24.2\%$ and $pEC_{50}=6.4 \pm 0.4$, $R_{max}=77.70 \pm 22.8\%$, respectively). Notably, the efficacy and potency of isoprenaline was significantly reduced in endothelium denuded aortic rings in comparison to endothelium intact aortic rings of HFD+RGZ+CLA treated rats (Endothelium intact: $pEC_{50}=7 \pm 0.2$, $R_{max}=100 \pm 9.9\%$ vs. endothelium denuded: $pEC_{50}=6 \pm 0.2$, $R_{max}=53 \pm 4.7\%$) (Figs 5B and 5C), indicating the critical role of endothelium in protecting the PPAR agonists-mediated atherogenic risks.

Discussion

Our key findings of this study were that the co-administration of CLA and rosiglitazone to SD rats

fed HFD (60 % energy from fat) had elevated TC:HDL and non HDL:HDL ratio, suppressed LDL and HDL level, and a marked decrease in endothelium-independent aortic relaxation, suggesting that CLA and rosiglitazone precede the atherogenic state and impaired endothelium-independent vasodilatation despite the reduction of HFD-induced insulin resistance.

There have been previous efforts to examine feeding rosiglitazone and CLA alone in rodent models fed with HFD. Dietary supplementation with CLA for seven weeks, regardless of isomer composition, led to an improvement in glucose tolerance, insulin resistance and serum triglycerides (Choi *et al.* 2004). Likewise, treatment with rosiglitazone improved insulin sensitivity with a side effect of increasing serum LDL (Zhao *et al.*

2012). It is noteworthy that in this study SD rats were treated for four weeks to induce acute, instead of chronic effects as showed previously (Lessard *et al.* 2007, Murthy *et al.* 2005, Umrani *et al.* 2002). The onset of insulin resistance in animals can be influenced by a number of factors such as feeding period, type of fat in the diet and genotype of animals. We observed neither change in glucose tolerance nor obvious insulin resistance following 12 weeks of HFD. We reason that the rats used in our study possibly mimic the obesity resistant phenotype, since we did not observe significant impairments in glucose tolerance following HFD supplementation for 12 weeks, whilst rats gained marginal rise in body and fat pad weights compared to their normal diet counterparts (Dake and Oltman 2015).

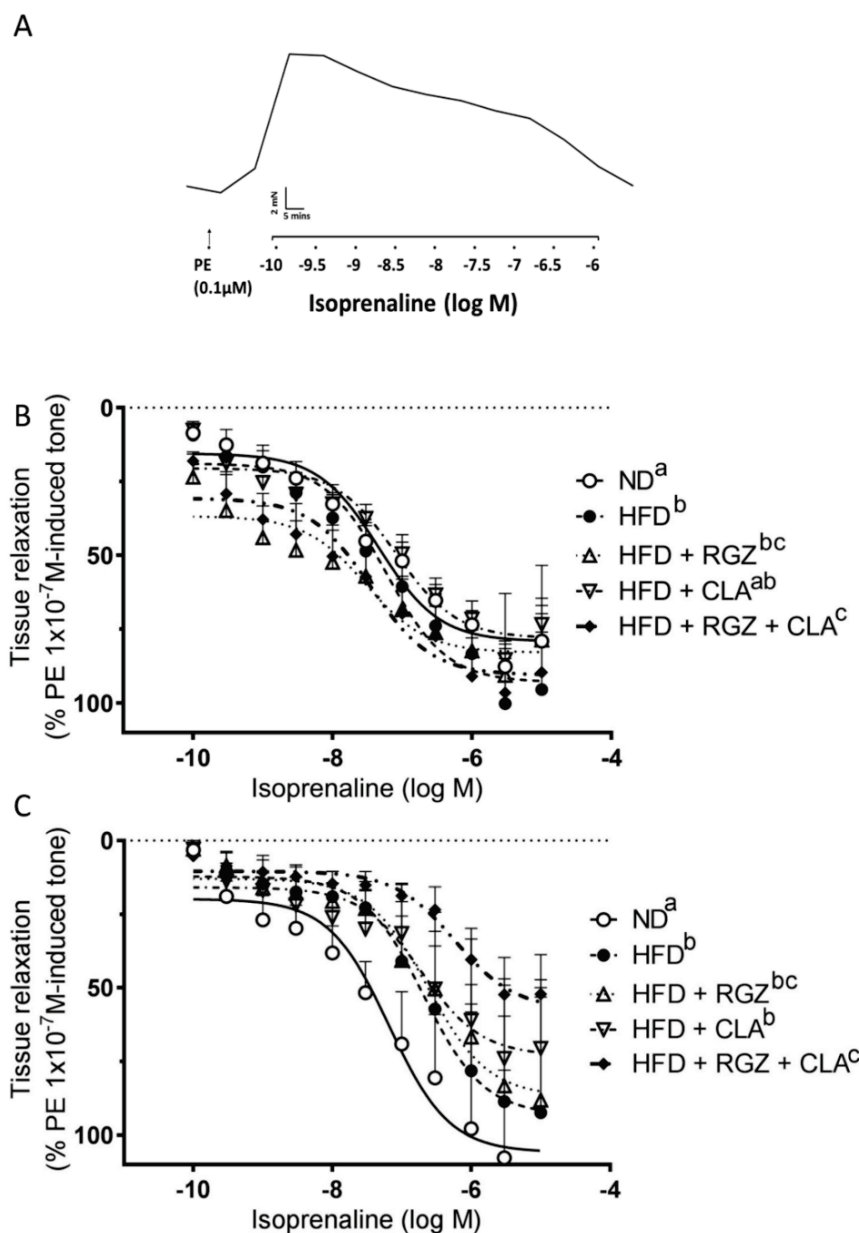


Fig. 5. Effect of diet and treatments on isoprenaline-induced aortic ring relaxation. **(A)** Representative trace of isoprenaline-induced aortic ring relaxation of normal SD rats. **(B)** Relaxation of endothelium-intact aortic rings. **(C)** Relaxation of endothelium-denuded aortic rings. Data were presented as mean \pm SEM, $n=4-6$ for endothelium intact and $n=3$ for endothelium denuded. For B and C, statistical significance is indicated by letters next to the legends, groups having the same letter(s) are not significant from each other.

Table 2. pEC50 and maximal relaxation (R_{max}) of aortic rings isolated from rats.

	ND	HFD	HFD+RGZ	HFD+CLA	HFD+RGZ+CLA
$pEC50$ E+	7.414 ± 0.236	7.271 ± 0.239	7.979 ± 0.453	7.128 ± 0.2414	7.453 ± 0.2088
$pEC50$ E-	7.105 ± 0.176	6.600 ± 0.127	6.583 ± 0.375*	6.398 ± 0.3754	6.133 ± 0.231*
R_{max} E+ (% PE induced tone)	89.75 ± 6.95	102.50 ± 13.18	91.00 ± 12.02	85.93 ± 6.16	100.2 ± 9.92
R_{max} E- (% PE induced tone)	113.4 ± 22.45	93.19 ± 24.15	90.60 ± 29.71	77.70 ± 22.81	53.58 ± 4.68*

Data were presented as mean ± SEM. * $p < 0.05$ compared to E+.

Alternatively, as observed in earlier studies (Pranprawit *et al.* 2013, Stark *et al.* 2000, Xu *et al.* 2010), the period of less than 12 weeks is too short to induce metabolic changes in obesity resistant rats. In contrast, Huang *et al.* (2004) reported reduction in glucose tolerance after 8 weeks of high fat diet feeding. It is apparent that no standardization of diet-induced obesity exists in literature despite using the common strain of SD rats. In a study that report the body weight reduction in SD rats with dietary supplementation of CLA, the animals have been fed with CLA from the beginning of the study together with HFD; moreover CLA elicits isomer-specific effects (Choi *et al.* 2004). Conversely, in another study dietary supplementation of CLA mixture for 6 weeks in rats showed no change in body weight (Moya-Camarena *et al.* 1999). Thus, our observation that the absence of weight reduction following oral administration of CLA mixture (0.2 g/day) for 4 weeks in SD rats pre fed with HFD for 8 weeks could be explained by the duration of CLA supplementation and isomer composition of CLA mixture.

Dyslipidemia is linked to vascular dysfunction in T2DM (Dixon *et al.* 1999). Generally, increased circulating concentration of HDL is associated with lowered risk of cardiovascular disease. HDL are lipoprotein particles that mediate reverse cholesterol transport (RCT) with apolipoprotein A as the major protein component. Beyond its role in RCT, HDL binds to scavenger receptor (SR)-B1 and SR-B2 and directly exerts vasoprotective effects on the endothelium (Calabresi 2003). As demonstrated previously by Hayek *et al.* (1993), the increase in HDL is typical in rodents fed HFD. Thus, the HDL rise in our study is possibly an adaptive response to maintain vascular tone to compensate the increased atherogenicity brought by HFD.

CLA are known to be weak PPAR α agonists (Moya-Camarena *et al.* 1999). While studies of rosiglitazone and CLA are difficult to come by, our observation of HDL reduction in HFD+CLA+RGZ

treated rats is in agreement with the previous observations in case reports (Im *et al.* 2010, Normen *et al.* 2004, Schwing *et al.* 2010) and a clinical trial (Linz *et al.* 2014) in which rosiglitazone and fenofibrate, a commonly prescribed PPAR α agonist, were simultaneously administered to human patients. Whilst the biological actions of CLA on HDL and insulin sensitivity were complicated by isomer and composition dependence, our observation that a 1:1 mixture of c9,t11 and t10,c12-CLA increases HDL level is similar to a recent published study conducted in Wistar rats fed HFD (de Almeida *et al.* 2015). We have noted that HFD+RGZ+CLA brought about suppression of LDL and HDL without significant reduction in LDL:HDL ratio. Since fractions outside LDL and HDL are also involved in atherogenesis, we make use of the non-HDL:HDL ratio, also known as the atherogenic coefficient, that has been proposed to be better predictors of cardiovascular risks (Hermans *et al.* 2007, Kim *et al.* 2013). Another ratio, TC:HDL, is linked to non-HDL:HDL by a linear relationship. Both ratios were increased in HFD+RGZ+CLA group despite having similar HDL level with ND suggesting increased atherogenicity, that is corroborated with the decreased ability of aortic rings to respond to isoprenaline-mediated vasodilatation in HFD+RGZ+CLA treatment group.

It is well established that the changes in vascular function when fed an atherogenic diet precede the onset of insulin resistance and cardiovascular diseases. In agreement with these studies (Garcia-Prieto *et al.* 2015, Stark *et al.* 2000), we observed diminished endothelium-independent vasodilatation without significant changes in glycemia. The vascular endothelium forms the inner lining of blood vessels and plays important role in maintaining vessel physiology (Deanfield *et al.* 2007, Hadi *et al.* 2005). Under physiological conditions, blood vessels are kept quiescent and dilated by endothelial secretion such as nitric oxide (NO). However, endothelial denudation due to apoptotic loss of endothelium is known

to occur under the diabetic and insulin resistant state (Avogaro *et al.* 2011, Sena *et al.* 2013). The increased responsiveness in HFD-fed endothelium intact rings may be an acute adaptive response to high circulating lipoproteins. Similar observations were recorded in obese spontaneously hypertensive rats (Kagota *et al.* 2004).

Isoprenaline-induced vascular relaxation is dependent on β -adrenergic receptors present on both endothelium and vascular smooth muscle (VSM) (Akimoto *et al.* 2002). Endothelium independent relaxation of VSM is mediated by NO produced *in situ* (Bernhardt *et al.* 1991). Our observation of vascular impairment in rosiglitazone fed endothelium denuded rings agrees with a previous finding, which stemmed from the improvement in endothelial production of NO but impaired response of VSM in rats (Onuta *et al.* 2008). The decreased responsiveness of endothelium denuded rings can be explained by rosiglitazone, *via* PPAR γ , induced upregulation of CD36 in the VSM (Ballesteros *et al.* 2014, Kleinhenz *et al.* 2015), as CD36 is responsive to LDL and lead to inhibition of cGMP synthesis in the VSM (Isenberg *et al.* 2009). Conversely, it has been reported that t10,c12-CLA increases NO formation in human endothelium (DeClercq *et al.* 2012a), nonetheless our observations warrant further studies to elucidate the precise mechanisms by which CLA modulate endothelium-independent relaxation.

The ability of aorta to constrict in obesity has been shown to be impaired due to many factors, including overnutrition of dietary fats (Stapleton *et al.* 2008). It has been previously reported that under certain environmental stressors, including HFD, the VSM cells tends to lose its contractile ability due to the remodeling of VSM into synthetic phenotype (Beamish *et al.* 2010). Watts *et al.* (2012) have demonstrated the impairments in phenylephrine-mediated contraction in aorta excised from

SD rats fed HFD for 17 weeks. Conversely, we observed no differences with phenylephrine-induced contraction in both control and HFD diet groups. Whilst we reason that the differences could be due to the duration of HFD feeding, proportion of HFD and age of rats, the impairment in contractile ability of VSM in rodents fed HFD was shown to be more prominent in small mesenteric arteries than the thoracic aorta (Soares *et al.* 2017).

In summary, while glucose tolerance and HOMA-IR may be unchanged, simultaneous administration of CLA and rosiglitazone led to increase in atherogenic coefficient. The increase in atherogenicity is coupled with vascular resistance in the context of endothelial loss. This observation hints the complex interplay between nutrient-drug interactions and warrants further investigation on their molecular mechanisms. Whilst it is believed that the use of normal rodent phenotype is limited in studying lipoprotein metabolism due to higher level of circulating HDL compared to humans, our observations, the linkage of cholesterol ratios and vascular resistance in SD rats fed HFD provides a reasonable basis for revisiting the lipoprotein dynamics in rodents.

Conflict of Interest

There is no conflict of interest.

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References

- AKIMOTO Y, HORINOUCI T, SHIBANO M, MATSUSHITA M, YAMASHITA Y, OKAMOTO T, YAMAKI F, TANAKA Y, KOIKE K: Nitric oxide (NO) primarily accounts for endothelium-dependent component of beta-adrenoceptor-activated smooth muscle relaxation of mouse aorta in response to isoprenaline. *J Smooth Muscle Res* **38**: 87-99, 2002.
- ALIBIN CP, KOPILAS MA, ANDERSON HD: Suppression of cardiac myocyte hypertrophy by conjugated linoleic acid: role of peroxisome proliferator-activated receptors alpha and gamma. *J Biol Chem* **283**: 10707-10715, 2008.
- AVOGARO A, ALBIERO M, MENEGAZZO L, DE KREUTZENBERG S, FADINI GP: Endothelial dysfunction in diabetes: The role of reparatory mechanisms. *Diabetes Care* **34** (Suppl 2): S285-S290, 2011.
- BAKKER W, ERINGA EC, SIPKEMA P, VAN HINSBERGH VW: Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. *Cell Tissue Res* **335**: 165-189, 2009.

- BALLESTEROS I, CUARTERO MI, PRADILLO JM, DE LA PARRA J, PÉREZ-RUIZ A, CORBÍ A, RICOTE M, HAMILTON J A, SOBRADO M, VIVANCOS J, NOMBELA F, LIZASOAIN I, MORO MA: Rosiglitazone-induced CD36 up-regulation resolves inflammation by PPAR γ and 5-LO-dependent pathways. *J Leukoc Biol* **95**: 587-598, 2014.
- BEAMISH JA, HE P, KOTTKE-MARCHANT K, MARCHANT RE: Molecular regulation of contractile smooth muscle cell phenotype: implications for vascular tissue engineering. *Tissue Eng Part B Rev* **16**: 467-491, 2010.
- BERNHARDT J, TSCHUDI MR, DOHI Y, GUT I, URWYLER B, BÜHLER FR, LÜSCHER TF: Release of nitric oxide from human vascular smooth muscle cells. *Biochem Biophys Res Commun* **180**: 907-912, 1991.
- CALABRESI L: Endothelial protection by high-density lipoproteins: from bench to bedside. *Arterioscler Thromb Vasc Biol* **23**: 1724-1731, 2003.
- CHOI JS, JUNG MH, PARK HS, SONG J: Effect of conjugated linoleic acid isomers on insulin resistance and mRNA levels of genes regulating energy metabolism in high-fat-fed rats. *Nutrition* **20**: 1008-1017, 2004.
- DAKE BL, OLTMAN CL: Cardiovascular, metabolic, and coronary dysfunction in high-fat-fed obesity-resistant/prone rats. *Obesity* **23**: 623-629, 2015.
- DE ALMEIDA MM, DE SOUZA YO, DUTRA LUQUETTI SC, SABARENSE CM, DO AMARAL CORRÊA JO, DA CONCEIÇÃO EP, LISBOA PC, DE MOURA EG, ANDRADE SOARES SM, MOURA GUALBERTO AC, GAMEIRO J, DA GAMA MA, FERRAZ LOPES FC, GONZÁLEZ GARCIA RM: Cis-9, trans-11 and trans-10, cis-12 CLA mixture does not change body composition, induces insulin resistance and increases serum HDL cholesterol level in rats. *J Oleo Sci* **64**: 539-551, 2015.
- DEANFIELD JE, HALCOX JP, RABELINK TJ: Endothelial function and dysfunction: testing and clinical relevance. *Circulation* **115**: 1285-1295, 2007.
- DECLERCQ V, TAYLOR CG, WIGLE J, WRIGHT B, TWOREK L, ZAHRADKA P: Conjugated linoleic acid improves blood pressure by increasing adiponectin and endothelial nitric oxide synthase activity. *J Nutr Biochem* **23**: 487-493, 2012a.
- DECLERCQ V, TAYLOR CG, ZAHRADKA P: Isomer-specific effects of conjugated linoleic acid on blood pressure, adipocyte size and function. *Br J Nutr* **107**: 1413-1421, 2012b.
- DERAKHSHANDE-RISHEHRI SM, MANSOURIAN M, KELISHADI R, HEIDARI-BENI M: Association of foods enriched in conjugated linoleic acid (CLA) and CLA supplements with lipid profile in human studies: a systematic review and meta-analysis. *Public Health Nutr* **18**: 2041-2054, 2015.
- DILZER A, PARK Y: Implication of conjugated linoleic acid (CLA) in human health. *Crit Rev Food Sci Nutr* **52**: 488-513, 2012.
- DIXON JL, STOOBS JD, PARKER JL, LAUGHLIN MH, WEISMAN GA, STUREK M: Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. *Arterioscler Thromb Vasc Biol* **19**: 2981-2992, 1999.
- GARCÍA-PRIETO CF, HERNÁNDEZ-NUÑO F, RIO DD, RUIZ-HURTADO G, ARÁNGUEZ I, RUIZ-GAYO M, SOMOZA B, FERNÁNDEZ-ALFONSO MS: High-fat diet induces endothelial dysfunction through a down-regulation of the endothelial AMPK-PI3K-Akt-eNOS pathway. *Mol Nutr Food Res* **59**: 520-532, 2015.
- HADI HA, CARR CS, AL SUWAIDI J: Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. *Vasc Health Risk Manag* **1**: 183-198, 2005.
- HAYEK T, ITO Y, AZROLAN N, VERDERY RB, AALTO-SETÄLÄ K, WALSH A, BRESLOW JL: Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (Apo) A-I. Presentation of a new animal model and mechanistic stud. *J Clin Invest* **91**: 1665-1671, 1993.
- HERMANS MP, AHN SA, ROUSSEAU MF: The non-HDL-C/HDL-C ratio provides cardiovascular risk stratification similar to the ApoB/ApoA1 ratio in diabetics: comparison with reference lipid markers. *Diabetes Metab Syndr Clin Res Rev* **1**: 23-28, 2007.
- HIATT WR, KAUL S, SMITH RJ: The cardiovascular safety of diabetes drugs--insights from the rosiglitazone experience. *N Engl J Med* **369**: 1287-1289, 2013.
- HUANG BW, CHIANG MT, YAO HT, CHIANG W: The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. *Diabetes Obes Metab* **6**: 120-126, 2004.

- IM M, KIM M, LEE JK, CHANG YH, LEE DY, HONG S-I, LEE YY, HONG YJ: HDL cholesterol reduction during rosiglitazone and fenofibrate treatment in a type 2 diabetes mellitus patient with dyslipidemia (in Korean). *Korean J Lab Med* **30**: 17-19, 2010.
- ISENBERG JS, MARTIN-MANSO G, MAXHIMER JB, ROBERTS DD: Regulation of nitric oxide signalling by thrombospondin 1: implications for anti-angiogenic therapies. *Nat Rev Cancer* **9**: 182-194, 2009.
- KAGOTA S, TANAKA N, KUBOTA Y, YAMAGUCHI Y, NAKAMURA K, KUNITOMO M, SHINOZUKA K: Characteristics of vasorelaxation responses in a rat model of metabolic syndrome. *Clin Exp Pharmacol Physiol* **31** (Suppl 2): S54-S56, 2004.
- KAUR J: A comprehensive review on metabolic syndrome. *Cardiol Res Pract* **2014**: 943162, 2014.
- KIM SW, JEE JH, KIM HJ, JIN SM, SUH S, BAE JC, KIM SW, CHUNG JH, MIN YK, LEE MS, LEE MK, KIM KW, KIM JH: Non-HDL-cholesterol/HDL-cholesterol is a better predictor of metabolic syndrome and insulin resistance than apolipoprotein B/apolipoprotein A1. *Int J Cardiol* **168**: 2678-2683, 2013.
- KLEINHENZ JM, MURPHY TC, POKUTTA-PASKALEVA AP, GLEASON RL, LYLE AN, TAYLOR WR, BLOUNT MA, CHENG J, YANG Q, SUTLIFF RL, HART CM: Smooth muscle-targeted overexpression of peroxisome proliferator activated receptor- γ disrupts vascular wall structure and function. *PLoS One* **10**: 1-25, 2015.
- LESSARD SJ, RIVAS DA, CHEN ZP, BONEN A, FEBBRAIO MA, REEDER DW, KEMP BE, YASPELKIS BB, HAWLEY JA: Tissue-specific effects of rosiglitazone and exercise in the treatment of lipid-induced insulin resistance. *Diabetes* **56**: 1856-1864, 2007.
- LINZ PE, LOVATO LC, BYINGTON RP, O'CONNOR PJ, LEITER LA, WEISS D, FORCE RW, CROUSE JR, ISMAIL-BEIGI F, SIMMONS DL, PAPADEMETRIOU V, GINSBERG HN, ELAM MB: Paradoxical reduction in HDL-C with fenofibrate and thiazolidinedione therapy in type 2 diabetes: the ACCORD lipid trial. *Diabetes Care* **37**: 686-693, 2014.
- LIU LF, PURUSHOTHAM A, WENDEL AA, BELURY MA: Combined effects of rosiglitazone and conjugated linoleic acid on adiposity, insulin sensitivity, and hepatic steatosis in high-fat-fed mice. *Am J Physiol Gastrointest Liver Physiol* **292**: G1671-G1682, 2007.
- LOONG BJ, TAN JH, LIM KH, MBAKI Y, TING KN: Contractile function of smooth muscle retained after overnight storage. *Naunyn Schmiedebergs Arch Pharmacol* **388**: 1061-1067, 2015.
- LU W, RESNICK HE, JABLONSKI KA, JONES KL, JAIN AK, HOWARD WJ, ROBBINS DC, HOWARD BV: Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes: the strong heart study. *Diabetes Care* **26**: 16-23, 2003.
- MARTIROSYAN DM, MIROSHNICHENKO LA, KULAKOVA SN, POGOJEVA AV, ZOLOEDOV VI: Amaranth oil application for coronary heart disease and hypertension. *Lipids Health Dis* **6**: 1, 2007.
- MOHANKUMAR SK, ZAHRADKA P, TAYLOR C: Does conjugated linoleic acid boost or battle metabolic syndrome? *Clin Lipidol* **6**: 501-505, 2011.
- MOHANKUMAR SK, HANKE D, SIEMENS L, CATTINI A, ENNS J, SHEN J, REANEY M, ZAHRADKA P, TAYLOR CG: Dietary supplementation of trans-11-vaccenic acid reduces adipocyte size but neither aggravates nor attenuates obesity-mediated metabolic abnormalities in fa/fa Zucker rats. *Br J Nutr* **109**: 1628-1636, 2013.
- MOORADIAN A: Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract Endocrinol Metab* **5**: 150-159, 2009.
- MOYA-CAMARENA SY, VANDEN HEUVEL JP, BLANCHARD SG, LEESNITZER LA, BELURY MA: Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPAR α . *J Lipid Res* **40**: 1426-1433, 1999.
- MURTHY SN, OBREGON DF, CHATTERGOON NN, FONSECA NA, MONDAL D, DUNNE JB, DIEZ JG, JETER JR JR, KADOWITZ PJ, AGRAWAL KC, MCNAMARA DB, FONSECA VA: Rosiglitazone reduces serum homocysteine levels, smooth muscle proliferation, and intimal hyperplasia in Sprague-Dawley rats fed a high methionine diet. *Metabolism* **54**: 645-652, 2005.
- NISSEN SE, WOLSKI K: Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* **356**: 2457-2471, 2007.

- NISSEN SE, WOLSKI K: Rosiglitazone revisited: an updated meta-analysis of risk for myocardial infarction and cardiovascular mortality. *Arch Intern Med* **170**: 1191-1201, 2010.
- NOONE EJ, ROCHE HM, NUGENT AP, GIBNEY MJ: The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. *Br J Nutr* **88**: 243-251, 2002.
- NORMEN L, FROHLICH J, MONTANER J, HARRIS M, ELLIOTT T, BONDY G: Combination therapy with fenofibrate and rosiglitazone paradoxically lowers serum HDL cholesterol. *Diabetes Care* **27**: 2241-2242, 2004.
- NOTO A, ZAHRADKA P, YURKOVA N, XIE X, NITSCHMANN E, OGBORN M, TAYLOR CG: Conjugated linoleic acid reduces hepatic steatosis, improves liver function, and favorably modifies lipid metabolism in obese insulin-resistant rats. *Lipids* **41**: 179-188, 2006.
- ONUTA G, HILLEBRANDS JL, RIENSTRA H, BOER MW, KLATTER FA, NAVIS G, ROZING J, ROKS AJ: Dichotomous effects of rosiglitazone in transplantation-induced systemic vasodilator dysfunction in rats. *Transplantation* **85**: 582-588, 2008.
- PARIZA MW, PARK Y, COOK ME: Conjugated linoleic acid and the control of cancer and obesity. *Toxicol Sci* **52**: 107-110, 1999.
- PRANPRAWIT A, WOLBER FM, HEYES JA, MOLAN AL, KRUGER MC: Short-term and long-term effects of excessive consumption of saturated fats and/or sucrose on metabolic variables in Sprague Dawley rats: a pilot study. *J Sci Food Agric* **93**: 3191-3197, 2013.
- SCHWING W, HUSTAK L, TAYLOR H: Paradoxical severe decrease in high-density lipoprotein cholesterol due to rosiglitazone-fenofibrate interaction. *Endocr Pract* **16**: 382-388, 2010.
- SENA CM, PEREIRA AM, SEIÇA R: Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta* **1832**: 2216-2231, 2013.
- SHOKRYZADAN P, RAJION MA, MENG GY, BOO LJ, EBRAHIMI M, ROYAN M, SAHEBI M, AZIZI P, ABIRI R, JAHROMI MF: Conjugated linoleic acid: a potent fatty acid linked to animal and human health. *Crit Rev Food Sci Nutr* **57**: 2737-2748, 2017.
- SOARES AG, DE CARVALHO MHC, AKAMINE E: Obesity induces artery-specific alterations: evaluation of vascular function and inflammatory and smooth muscle phenotypic markers. *Biomed Res Int* **2017**: 5038602, 2017.
- STAPLETON PA, JAMES ME, GOODWILL AG, FRISBEE JC: Obesity and vascular dysfunction. *Pathophysiology* **15**: 79-89, 2008.
- STARK AH, TIMAR B, MADAR Z: Adaptation of Sprague Dawley rats to long-term feeding of high fat or high fructose diets. *Eur J Nutr* **39**: 229-234, 2000.
- TAYLOR CG, ZAHRADKA P: Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. *Am J Clin Nutr* **79** (6 Suppl): 1164S-1168S, 2004.
- UMRANI DN, BANDAY AA, HUSSAIN T, LOKHANDWALA MF: Rosiglitazone treatment restores renal dopamine receptor function in obese Zucker rats. *Hypertension* **40**: 880-885, 2002.
- VAN DEVENTER HE, MILLER WG, MYERS GL, SAKURABAYASHI I, BACHMANN LM, CAUDILL SP, DZIEKONSKI A, EDWARDS S, KIMBERLY MM, KORZUN WJ, LEARY ET, NAKAJIMA K, NAKAMURA M, SHAMBUREK RD, VETROVEC GW, WARNICK GR, REMALEY AT: Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. *Clin Chem* **57**: 490-501, 2011.
- WANDERS AJ, BROUWER IA, SIEBELINK E, KATAN MB: Effect of a high intake of conjugated linoleic acid on lipoprotein levels in healthy human subjects. *PLoS One* **5**: e9000, 2010.
- WATTS SW, McCLAIN JL, DORRANCE AM: Perivascular fat impairs contraction in aorta from obese but not lean adult rats. *FASEB J* **26** (Suppl 1): 1115.4, 2012.
- XU ZJ, FAN JG, DING XD, QIAO L, WANG GL: Characterization of high-fat, diet-induced, non-alcoholic steatohepatitis with fibrosis in rats. *Dig Dis Sci* **55**: 931-940, 2010.
- ZHAO D, McCULLY BH, BROOKS VL: Rosiglitazone improves insulin sensitivity and baroreflex gain in rats with diet-induced obesity. *J Pharmacol Exp Ther* **343**: 206-213, 2012.