British Journal of Nutrition (2013), **109**, 1628–1636
doi:10.1017/S000711451200339X

Dietary supplementation of *trans*-11-vaccenic acid reduces adipocyte size but neither aggravates nor attenuates obesity-mediated metabolic abnormalities in *fa/fa* Zucker rats

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(Submitted 25 January 2012 – Final revision received 8 June 2012 – Accepted 2 July 2012 – First published online 23 August 2012)

Abstract

Conjugated linoleic acid (CLA) present in dairy and ruminant fat has beneficial effects on metabolic syndrome characteristics in humans and some rodent models. Production practices to increase the milk content of CLA are also substantially elevating *trans*-11-vaccenic acid (VA). Questions are being raised whether VA has the same beneficial actions as CLA or has adverse biological effects similar to industrially produced *trans*-fatty acids. The present study examined the effects of dietary supplementation of either 0 or 1·5 % (w/w) VA for 8 weeks on lipidaemia, glycaemia, blood pressure, hepatic steatosis, adipocyte size and molecular markers of inflammation and insulin signalling in *fa/fa* Zucker rats. Dietary supplementation of VA did not alter feed intake, weight gain, blood pressure or organ:body weight (BW) ratios, except the epididymal fat:BW ratio which was lower in the VA group compared with the control group. The total liver lipid concentration as an indicator of hepatic steatosis was not different between the groups. Likewise, there were no changes in fasting lipidaemia, glycaemia or oral glucose tolerance. Although there were no physiological differences observed between the groups, animals supplemented with VA had smaller adipocytes (approximately 7 % smaller than the controls). The VA group also had higher adipophilin and IL-10 protein levels in epididymal adipose tissue (1·7- and 1·4-fold higher than the controls, respectively); however, there were no changes observed in critical nodes of insulin signalling. The present study provides evidence that supplementation with VA, a naturally produced *trans*-fat, has some positive effects on adipose tissue and did not exacerbate obesity-mediated metabolic abnormalities.

Key words: ***Trans*-fat: Insulin resistance: Adipocyte size: *fa/fa* rats**

The metabolic syndrome (MetS) refers to a cluster of risk factors associated with the onset of two major chronic and lethal diseases, type 2 diabetes mellitus (T2DM) and CVD[(1)]. Although the origins of the MetS remain largely unknown, accumulating evidence indicates that obesity and insulin resistance (IR) are the two critical components of the MetS that are positively associated with the rising prevalence of T2DM and CVD[(1–3)]. Despite significant advancements in treatment options, lifestyle interventions comprising a combination of diet and physical activity have gained widespread recognition as a means of combating obesity and IR (reviewed in Kimokoti & Brown[(4)]). Consequently, these trends necessitate better understanding of the beneficial or adverse effects of diet and its constituents on the MetS. A plethora of both epidemiological and interventional studies have suggested that dietary fats influence metabolic function[(5,6)]. However, due to the large functional and structural complexity of dietary fats, our current understanding of how and which dietary fat can beneficially modulate metabolic functions remains limited.

Conjugated linoleic acid (CLA; 18 : 2*n*-7) is one of the dietary fatty acids (FA) present in dairy products and ruminant meats

Abbreviations: AT, angiotensin II receptor type; BW, body weight; *c*9,*t*11-CLA, *cis*-9, *trans*-11-conjugated linoleic acid; CLA, conjugated linoleic acid; FA, fatty acid; HOMA-IR, homeostasis model assessment of insulin resistance; IPTF, industrially produced *trans*-fat; IR, insulin resistance; MAPK, mitogen-activated protein kinase; MetS, metabolic syndrome; PL, phospholipid; P:S, PUFA: SFA; SAPK/JNK, stress-activated protein kinase/c-Jun NH_2-terminal kinase; T2DM, type 2 diabetes mellitus; TF-R, *trans*-fats from ruminant sources; VA, *trans*-11-vaccenic acid.

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that has been proposed to beneficially influence the characteristics of the MetS[7–20]. However, differences in the metabolic actions of CLA have been linked to the genotype, phenotype and CLA isomer[21–24]. While these discrepancies require further investigation, research into production practices that enrich the milk content of *cis*-9,*trans*-11 (*c*9,*t*11)-CLA has resulted in a concurrent elevation of *trans*-vaccenic acid (VA)[25].

VA (*trans*-11-18 : 1*n*-7) is a positional and geometric isomer of oleic acid (18 : 1*n*-9)[26]. Biohydrogenation of linoleic acid (18 : 2*n*-6) and α-linolenic acid (18 : 3*n*-3) by microbes in the rumen produces VA[26]. Further metabolism of VA produces either stearic acid (18 : 0) by saturation or *c*9,*t*11-CLA by desaturation. The bioconversion of VA to *c*9,*t*11-CLA also occurs in mammalian tissues, and is estimated to be 5–12 % in rats[27] and 19–30 % in humans[28].

The observational and interventional studies concerning the intake of *trans*-fats from ruminant sources (TF-R) and the effects on human health are limited. The majority of studies investigating the intake of *trans*-fats have focused on industrially produced (hydrogenated) *trans*-fat (IPTF) and have consistently been shown to adversely affect the risk factors of heart disease[29–31]. Conversely, epidemiological studies have suggested that dietary intake of TF-R has no association with the risk factors of cardiovascular health[32–34]. Likewise, a recent meta-analysis of cohort studies has indicated that, unlike IPTF, TF-R does not affect the risk factors of CHD[35]. However, the number of studies available to reach a firm conclusion on the potential effects of TF-R, including VA, on human health is limited.

On the other hand, studies conducted in experimental animals have demonstrated that dietary supplementation with VA has beneficial effects on lipidaemia[36–38] and atherosclerosis[39]. Both short-term (3-week) and long-term (16-week) dietary supplementation with VA in JCR:LA-*cp* rats, a model of dyslipidaemia and IR, increased VA in adipose TAG and its bioconversion to *c*9,*t*11-CLA, and improved lipidaemia without altering glycaemia[36,37]. If the biological effects of VA are due to its conversion to *c*9,*t*11-CLA, then the beneficial effects on the components of the MetS would be expected based on the fact that dietary supplementation with *c*9,*t*11-CLA attenuates IR and associated metabolic disorders in other rodent models of IR[7,13,17,18,20]. Hence, it remains unclear whether the effects of VA are direct or indirect, and/or specific to the metabolic characteristics of the experimental model. Given the evidence for bioconversion of VA to *c*9,*t*11-CLA[28,36,37] and our recent finding that dietary *c*9,*t*11-CLA (0·4 %, w/w) attenuates blood pressure in *fa*/*fa* Zucker rats[20], we examined the effects of VA on various MetS characteristics in *fa*/*fa* Zucker rats, an animal model that has been well characterised for obesity-mediated IR and metabolic abnormalities[40,41].

Experimental methods

Animals and diet

For the present experiment, 5-week-old *fa*/*fa* Zucker male rats (total *n* 20; Harlan Laboratories) were acclimatised for a week and randomly assigned to the VA group (1·5 % (w/w) *trans*-11-VA) or the control group (0 % VA). VA (98 % purity as determined by GC) was synthesised by the method of Duffy *et al.*[42]. The 1·5 % (w/w) dose of VA was based on the study by Wang *et al.*[37]. The diet formulation is shown in Table 1. Both the VA and control diets contained a total of 8·5 % (w/w) fat. Feed intake (corrected for spillage) and weekly body weights (BW) were recorded. The experimental protocol was approved by the University of Manitoba Protocol Management and Review Committee and conducted according to the Canadian Council on Animal Care Guidelines.

Table 1. Diet formulation

Ingredients (g/kg)	Control	VA
Maize starch*	363	363
Maltodextrin*	132	132
Sucrose*	100	100
Egg white*	212·5	212·5
Cellulose*	50	50
Mineral mix (AIN-93G)*	35	35
Vitamin mix (AIN-93)*	10	10
Choline bitartrate*	2·5	2·5
Biotin mix*†	10	10
t-Butylhydroquinone‡	0·014	0·014
Soyabean oil§	85	69·7
VA‖	0	15·3

VA, *trans*-11-vaccenic acid; AIN, American Institute of Nutrition.
* Dyets, Inc.
† 200 mg/kg biotin in maize starch as egg white was the protein source.
‡ Sigma-Aldrich.
§ Bunge Canada.
‖ Synthesised by the method of Duffy *et al.*[42]; 98 % purity by GC.

Blood pressure

At the beginning of week 8 of the dietary intervention, blood pressure was measured in conscious rats by the indirect tail cuff method (IITC Life Sciences blood pressure monitoring system) as described previously[43].

Oral glucose tolerance test

Assessment of glucose responsiveness was determined during the middle of week 8 using a standard method[14]. The area under the curve (AUC) for glucose was calculated using the method of Brouns *et al.*[44] and used as an indicator of glucose tolerance:

$$\text{Glucose AUC} = \left(\left(\frac{(G_{15}+G_0)}{2}\times T_{15}\right)+\left(\frac{G_{30}+G_{15}}{2}\times T_{15}\right)+\left(\frac{(G_{60}+G_{30})}{2}\times T_{30}\right)+\left(\frac{(G_{120}+G_{60})}{2}\times T_{60}\right)\right),$$

where G_0, G_{15}…G_{120} are glucose concentrations (mmol/l) and T_0, T_1…T_{120} indicate time (min).

Blood and tissue collection

After 8 weeks on the diet, the rats were fasted overnight, and asphyxiated with CO_2 before collection of blood and tissues. Blood samples were collected and centrifuged at 1500 ***g*** for 15 min at 4°C. The serum layer was collected, aliquoted and

stored at −80°C for biochemical analyses. Various organs including adipose tissue (epididymal, perirenal and mesenteric fat pads), liver, heart, kidneys and pancreas were dissected and weighed. A small portion of epididymal adipose tissue was placed in Cryogel embedding media (Instrumedics, Inc.) and frozen immediately in a dry ice–ethanol bath, and then stored at −80°C. Tissues were flash-frozen in liquid N_2 and stored at −80°C for various analyses.

Serum biochemistry

Enzymatic colorimetric kits were used to quantify glucose, TAG and cholesterol (Genzyme Diagnostics P.E.I. Inc.). Insulin was quantified using an ELISA kit (Alpco Diagnostics). The end points of these assays were detected at the designated wavelength and quantified using a microplate reader (FLUOstar Omega; BMG Labtech). Fasting glucose and insulin values were used to calculate the homeostatic model of assessment of insulin resistance (HOMA-IR), an index of IR:

$$\text{HOMA-IR} = (\text{insulin}\,(\mu\text{U/ml}) \times \text{glucose}\,(\text{mmol/l}))/22{\cdot}5.$$

Fatty acid composition of liver and epididymal fat

To verify the presence of VA in the liver and epididymal fat tissue, and to determine whether the bioconversion in the body is contributing to more *c*9,*t*11-CLA, the FA composition of liver and epididymal fat was analysed by GC as described previously[10]. Briefly, lipid extracts were separated by TLC to obtain TAG and phospholipid (PL) fractions. Moreover, two methylating agents (sodium methoxide for CLA isomers and methanolic hydrochloric acid for all other FA) were used to prevent isomerisation of double bonds in the FA with conjugated bonds and because no single methylating agent provides adequate methylation of CLA and all other FA[10]. Thus, samples were split for the two methylation procedures and the results combined based on the internal standards (TAG: triheptadecanoin (Nu-Chek Prep, Inc.); PL: 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine (Avanti)) added to the samples[10]. The methylated samples were analysed by GC using a Varian WCOT Fused Silica CP-SELECT FAME column (length 100 m, diameter 0·25 mm and film thickness 0·25 μm; Varian Canada, Inc.) and a Varian 450 GC with a flame ionisation detector. The column was operated at 100°C for 2 min and then the temperature was raised to 175°C at 25°C/min, held for 30 min, raised again to 220°C at 15°C/min, held for 10 min, raised again to 240°C at 20°C/min and held for 11 min. The total run time was 60 min and the samples were run with a 10:1 split ratio and a flow rate of 1·8 ml/min. The identity of peaks was verified with standards: Nu-Chek Prep #463 GLC Standard (Nu-Chek Prep, Inc.) and NIST #2377 (National Institute of Standards and Technology) for *c*9-*t*11-CLA and *t*10,*c*12-CLA isomers.

Adipocyte size

Cryosections (10 μm) of epididymal adipose tissue in Cryogel were fixed with ice-cold acetone for 10 min, rinsed with Tris-buffered saline (50 mM-Tris–HCl, pH 7·4, 150 mM-NaCl) and dehydrated with ethanol and xylene washes. Coverslips were mounted using AquaMount aqueous mounting medium (Lerner Laboratories). Digital images were captured with a light microscope fitted with a camera (Olympus IX81 microscope, Olympus IX2-UCB camera; Olympus America Inc.) at 200× magnification using Infinity Analyze software (Release 5.0.2; Lumenera Corporation). The cell area (μm^2) of approximately 250 adipocytes per rat was measured with ImageJ (National Institutes of Health) as described previously[15].

Western blotting

Proteins were extracted from frozen tissue and quantified as described previously[15]. Western blotting was performed by separating proteins (10–20 μg) by SDS–PAGE, transferring to a polyvinylidene fluoride membrane and probing with primary (1:1000 dilution) and horseradish peroxidase-conjugated secondary antibodies (1:10 000 dilution). Quantification of band intensities was carried out using a FluorChem®Q gel scanning system with a charge-coupled device camera (Proteinsimple) and AlphaView®Software (version 1.3.0.6; Alpha Innotech Corporation). Data are expressed as arbitrary units relative to the loading control. Western blot analysis was performed using the following antibodies: Akt, phospho-Akt-Ser^{473}, p42/44 mitogen-activated protein kinase (MAPK), phospho-MAPK-Thr^{202}/Tyr^{204}, β-tubulin, endothelial NO synthase, phospho-endothelial NO synthase-Ser^{1177}, NF-κB p65, phospho-NF-κB p65-Ser^{536}, stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK), phospho-SAPK/JNK-Thr^{183}/Tyr^{185}, angiotensin II receptor type (AT) 1, AT2 (Cell Signaling Technologies); angiotensinogen (Fitzgerald); IL-10 (Biosource); adiponectin (Calbiochem); adipophilin (Progen).

Statistical analysis

Experimental data were analysed using SAS statistical software (SAS Institute, Inc.). Student's *t* test was used to compare the

Table 2. Physical characteristics of *fa/fa* Zucker rats fed 1·5 % *trans*-11-vaccenic acid (VA) for 8 weeks

(Mean values with their standard errors; *n* 10 rats/group)

	Control		VA	
	Mean	SE	Mean	SE
Body weight (g)	629	14	636	19
Weight gain (g)	318	55	312	51
Total feed intake (g)	2047	40	2054	37
Body length (cm)	24·4	0·2	24·6	0·2
Organ weight (g/100 g body weight)				
Liver	3·93	0·17	3·73	0·08
Visceral fat†	9·80	0·24	9·23	0·23
Epididymal fat	3·31	0·12	2·86*	0·11
Mesenteric fat	1·37	0·04	1·31	0·07
Perirenal fat	5·12	0·13	5·06	0·13
Heart	0·210	0·003	0·204	0·002
Kidneys	0·60	0·02	0·56	0·01
Pancreas	0·15	0·01	0·15	0·01

* Mean value was significantly different from that of the control group ($P<0{\cdot}05$).
† Visceral fat = epididymal fat + mesenteric fat + perirenal fat.

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Table 3. Metabolic parameters of *fa/fa* Zucker rats fed 1·5 % *trans*-11-vaccenic acid (VA) for 8 weeks

(Mean values with their standard errors; *n* 6–10 rats/group)

	Control		VA	
	Mean	SE	Mean	SE
Glycaemia				
Fasting glucose (mmol/l)	13·9	0·6	12·5	0·5
Fasting insulin (μU/ml)	394	59	368	61
HOMA-IR (mmol/l × μU/ml)	231	40	199	32
OGTT glucose AUC (min × mmol/l)	714	104	637	87
Lipidaemia				
Fasting TAG (mmol/l)	3·1	0·7	2·7	0·4
Fasting cholesterol (mmol/l)	10·8	1·2	8·5	0·9
Hepatic steatosis				
Liver lipids (g/100 g liver)	10·8	0·9	9·5	0·9
Blood pressure				
Systolic (mmHg)	140	5	141	3
Diastolic (mmHg)	87	4	94	3

HOMA-IR, homeostatic model assessment of insulin resistance; OGTT, oral glucose tolerance test AUC, area under the curve.

two groups (VA *v.* control) for end-point data; the χ^2 test was used for the adipocyte size distribution. All results are reported as mean values with their standard errors. Differences were considered statistically significant at $P<0·05$.

Results

Physical characteristics

Dietary supplementation of VA for 8 weeks did not alter physical measures including final BW, weight gain, total feed intake and body length (Table 2). Likewise, the organ:BW ratios were not different between the VA and control groups for various organs, including liver, visceral fat (sum of epididymal, mesenteric and perirenal fat pads), individual fat pads (mesenteric and perirenal), heart, kidneys and pancreas. However, the VA group had a 14 % lower epididymal:BW ratio than the control group.

Metabolic parameters

Various indicators of glycaemia/IR (fasting glucose and insulin concentrations, and HOMA-IR) and glucose tolerance (AUC for glucose) at the whole body level were not significantly different from the control group (Table 3). Lipidaemia as assessed by fasting TAG and cholesterol was unchanged. Likewise, there was no difference in total liver lipid concentration, as an indicator of hepatic steatosis. Furthermore, there were no significant differences in both systolic and diastolic blood pressure between the groups.

Fatty acid composition of liver

The effect of dietary VA on the liver FA composition of both TAG (intracellular storage) and PL (pool of membrane signalling molecules) in obese IR rats is summarised in Table 4. In both TAG and PL, the total SFA, PUFA, *n*-3, *n*-6, *n*-7 and *n*-9, and PUFA: SFA (P:S) ratio did not differ between the groups. Notably, PL MUFA was 20 % higher in the VA-fed group compared with the control group. In contrast, the TAG composition of MUFA remained unchanged between the groups. VA in TAG was not different between the dietary groups. VA in PL of the VA-fed group was 50 % less than VA in TAG, while rats fed the control diet had non-detectable levels of VA in PL. In contrast, *c*9,*t*11-CLA, a biometabolite of VA, was approximately 2-fold higher in TAG of the VA group compared with the control, whereas there was no difference in PL *c*9,*t*11-CLA between the groups.

Fatty acid composition of epididymal fat

To investigate whether dietary supplementation of VA has any preferential incorporation or bioconversion into other endocrine tissues, we examined the TAG and PL FA composition of epididymal fat (Table 5). The total SFA, MUFA, PUFA, *n*-3, *n*-6 and *n*-9, and P:S ratio in both TAG and PL did not differ between the groups. However, *n*-7 in TAG was found to be approximately 1·2-fold higher in the VA-fed group than the control group, whereas PL *n*-7 was not different between the groups. The VA-fed group had higher VA in both TAG (approximately 3-fold) and PL (approximately 17-fold) compared with the control group. The bioconversion of VA to *c*9,*t*11-CLA was indicated by the approximately 24-fold elevation of *c*9,*t*11-CLA in TAG and the presence of *c*9,*t*11-CLA in PL of the VA-fed group compared with the control group. *c*9,*t*11-CLA in PL of the VA-fed group was approximately 2-fold higher than *c*9,*t*11-CLA in TAG while the control group had non-detectable *c*9,*t*11-CLA in PL.

Table 4. Liver fatty acid composition (g/100 g fatty acids) of *fa/fa* Zucker rats fed 1·5 % *trans*-11-vaccenic acid (VA) for 8 weeks

(Mean values with their standard errors; *n* 5 rats/group)

	Control		VA	
	Mean	SE	Mean	SE
TAG				
SFA	41·9	1·8	44·5	0·8
MUFA	42·0	2·7	43·7	0·6
PUFA	16·1	3·8	11·8	1·0
P:S	0·27	0·03	0·4	0·11
n-3	2·38	0·96	1·48	0·24
n-6	13·6	2·9	10·7	0·8
n-7	11·5	1·1	13·1	0·3
n-9	30·2	1·8	30·1	0·5
VA	0·14	0·02	0·14	0·02
*c*9,*t*11-CLA	0·14	0·05	0·32*	0·02
Phospholipids				
SFA	45·9	0·7	45·1	0·5
MUFA	5·84	0·39	6·97*	0·14
PUFA	47·3	0·4	46·8	0·5
P:S	1·03	0·02	1·04	0·02
n-3	11·5	0·5	11·5	0·3
n-6	35·8	0·8	36·3	0·6
n-7	3·11	0·32	3·44	0·14
n-9	3·46	0·16	3·5	0·1
VA	ND	–	0·07	–
*c*9,*t*11-CLA	0·39	0·11	0·34	0·12

*c*9,*t*11-CLA, *cis*-9, *trans*-11-conjugated linoleic acid; ND, not detectable.
* Mean value was significantly different from that of the control group ($P<0·05$).

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Table 5. Epididymal fat fatty acid composition (g/100 g fatty acids) of *fa/fa* Zucker rats fed 1·5 % *trans*-11-vaccenic acid (VA) for 8 weeks
(Mean values with their standard errors; *n* 5 rats/group)

	Control		VA	
	Mean	SE	Mean	SE
TAG				
SFA	35·8	1·5	34·1	1·2
MUFA	40·1	0·8	42·7	1·0
PUFA	24·1	0·8	23·3	0·4
P:S	0·68	0·05	0·69	0·03
n-3	2·78	0·08	2·7	0·08
n-6	21·0	0·8	19·8	0·3
n-7	10·4	0·2	12·5*	0·4
n-9	29·2	0·6	29·3	0·6
VA	0·29	0·01	0·94*	0·19
*c*9,*t*11-CLA	0·03	0·01	0·64*	0·04
Phospholipids				
SFA	36·3	0·7	36·9	1·8
MUFA	33·7	1·8	33·0	4·1
PUFA	30·0	1·3	29·8	2·4
P:S	0·83	0·03	0·81	0·06
n-3	3·67	0·15	3·5	0·26
n-6	25·3	1·0	24·6	1·5
n-7	7·64	0·66	9·14	1·52
n-9	24·1	1·4	21·2	3·7
VA	0·07	0·06	1·19*	0·07
*c*9,*t*11-CLA	ND	–	0·35	–

*c*9,*t*11-CLA, *cis*-9, *trans*-11-conjugated linoleic acid; ND, not detectable.
* Mean value was significantly different from that of the control group ($P<0·05$).

Adipocyte size

Adipose tissue function including adipocyte plasticity and expandability has been suggested as one of the firm determinants of obesity-mediated metabolic impairments[45–47]. Thus, we examined the effect of VA on adipocyte size. As illustrated in Fig. 1(a), the animals receiving dietary VA had 7 % smaller adipocytes compared with the control group. Based on the size distribution graph (Fig. 1(b)), 66 % of adipocytes in the VA group were $<2500\,\mu m^2$, whereas only 56 % of cells in the control group were in this range. Conversely, the number of adipocytes of $>2500\,\mu m^2$ was approximately 20 % lower in the VA group compared with the control group (34 *v.* 43 %, respectively).

Molecular analyses of adipose

Various molecular effectors in adipose tissue participate in IR, inflammation and hypertension. Specific adipose tissue targets of IR and inflammation that were unaffected by VA supplementation were Akt, p44/42 MAPK, endothelial NO synthase, SAPK/JNK and NF-κB p65 (phosphorylated and total protein for each; data not shown). Conversely, protein levels of IL-10, an anti-inflammatory cytokine, were increased in adipose tissue of the VA group compared with the control group (Fig. 2(a) and (b)) while adiponectin, a cardioprotective and anti-inflammatory adipokine, was unchanged (data not shown). With regard to blood pressure regulation, the VA group had higher levels of the AT2 receptor compared with the control group (Fig. 2(a) and (b)), while the AT1 receptor and angiotensinogen (data not shown) were unchanged. To determine whether the reductions in the epididymal:BW ratio and adipocyte size in response to the VA diet were due do changes in proteins that regulate lipid storage in the adipose, we quantified the abundance of the lipid droplet protein, adipophilin, by Western blotting. Interestingly, the abundance of adipophilin in epididymal adipose tissue was elevated in the VA group (Fig. 2(a) and (b)), indicating a difference in lipid droplet packaging between the two groups.

Discussion

The main finding of the present study was that dietary VA supplementation reduced the epididymal fat:BW ratio and adipocyte size and modulated components of the local renin–angiotensin system (AT2 receptor) and inflammation (IL-10); however, these tissue/molecular-level changes were insufficient to improve adipose function or positively alter the whole-body response in terms of IR and hypertension. However, it is also noteworthy that VA, a TF-R present in dairy products, did not exacerbate MetS characteristics including obesity, IR, lipidaemia, hypertension, inflammation and hepatic steatosis.

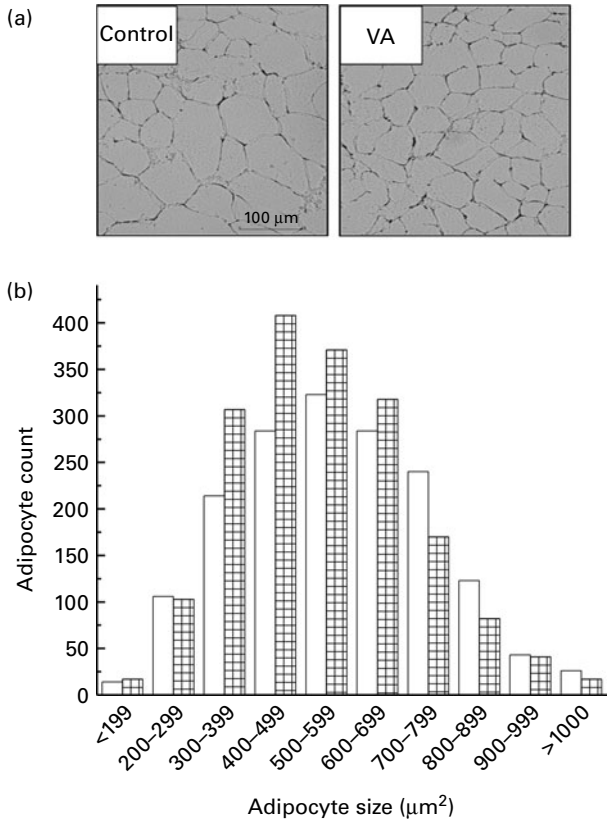

Fig. 1. (a) Size and (b) distribution of adipocytes of *fa/fa* Zucker rats fed the control or *trans*-11-vaccenic acid (VA) diet for 8 weeks. (a) Representative images with the overall mean cell areas (*n* 10 rats/group) with their standard errors: control 2449 (SE 32) μm^2; VA 2284 (SE 22) μm^2. Mean value of the VA group was significantly different from that of the control group ($P<0·05$). (b) Size distribution pattern for six rats per group. ⊏, Control diet-fed rats; ⊞, VA diet-fed rats. The distribution of the VA group was significantly different from that of the control group ($P<0·05$; χ^2 test).

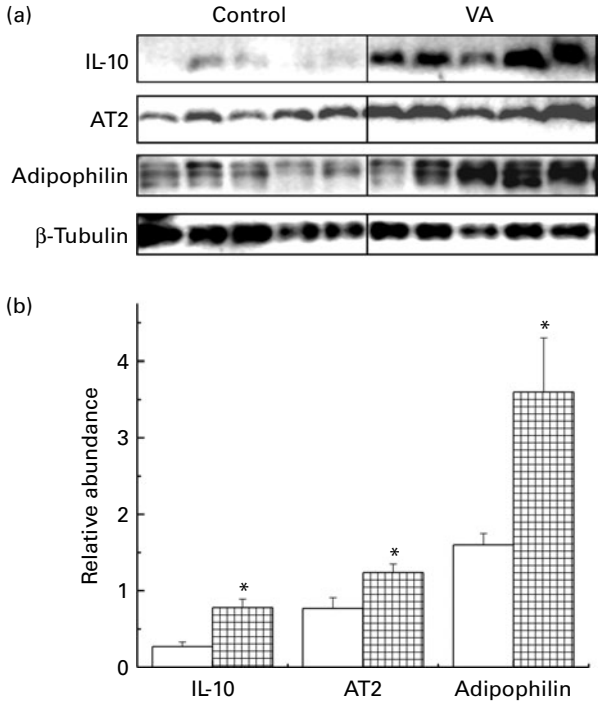

Fig. 2. (a) Representative Western blots for protein levels of IL-10, angiotensin II receptor type 2 (AT2) receptor, adipophilin and β-tubulin in the epididymal adipose tissue of *fa/fa* Zucker rats fed the control or *trans*-11-vaccenic acid (VA) diet for 8 weeks. (b) The relative Western blot band intensities were quantified and normalised to those of β-tubulin. ⊏, Control diet-fed rats; ⊞, VA diet-fed rats. Quantification of band intensities for adipophilin was based on bands at 48–52 kDa. Values are means (*n* 9–10 rats/group), with their standard errors represented by vertical bars. * Mean values were significantly different from those of the control group ($P<0·05$).

During the last several years, dietary intake of TF-R has remained fairly constant while there has been a substantial decline in IPTF[48,49]. The amount of VA in ruminant-based foods varies due to factors that have an impact on dairy and beef production, including geographical location, composition of cow feed, season, etc.[50]. Dietary TF-R is primarily (60–80 %) VA, and TF-R in human diets varies from low (0·8 % of energy; 2·2 g/d) to moderate (1·5 % of energy; 4·2 g/d) to higher (5 % of energy; 11–12 g/d) intakes[35]. The amount of VA (1·5 %, w/w) used in the present study is the same as used in the studies with JCR:LA-*cp* rats[36,37]; however, it is unlikely that this level of VA could be attained in the human diet. The present result that VA supplementation has null effects on obesity-mediated metabolic abnormalities parallels an intervention study with TF-R that found no changes in blood lipids and insulin sensitivity in overweight women[51], and epidemiological studies have reported neutral health outcomes for VA intake in human subjects[32–34]. To date, there are no published studies of VA intervention in human subjects with the MetS.

On the other hand, an increasing number of studies have indicated that CLA (a biometabolite of VA) has favourable effects on MetS parameters in human subjects and several rodent models, including genetic and high-fat diet-induced IR[7–20]. Consequently, questions are being raised whether TF-R, including VA, have the same biological effects as CLA or, instead, exert the adverse biological effects of IPTF. In the present study, 8-week dietary VA supplementation elevated *c*9,*t*11-CLA in the liver and adipose TAG, thus confirming that VA undergoes endogenous bioconversion as reported previously[28,36–38]. These results raise the possibility that both the physiological and molecular effects of VA could be explained by the indirect effects of CLA given that dietary supplementation with *c*9,*t*11-CLA attenuates IR, hypertension and other metabolic abnormalities in rodent models[7,13,17,18,20]. Recently, Wang *et al.*[36,37] reported in two different studies that dietary supplementation of VA in JCR:LA-*cp* rats for 3 or 16 weeks increased *c*9,*t*11-CLA in the adipose tissue and favourably modified lipidaemia and hepatic steatosis. However, we did not observe any changes in lipidaemia, hepatic steatosis or the liver lipid profile (except MUFA in PL) of *fa/fa* Zucker rats fed VA for 8 weeks, despite the elevation of *c*9,*t*11-CLA in the liver and epididymal fat TAG. Perhaps the JCR:LA-*cp* model and Western-type background diet (1 % cholesterol and 15 % lipid (w/w); P:S ratio of 0·6) used by Wang *et al.*[36,37] may be better suited to studying the hypolipidaemic effects of VA compared with the present study with *fa/fa* Zucker rats and a lower-fat diet (8·5 % lipid, w/w) containing soyabean oil (P:S ratio 4:1). Although the VA-fed group had higher MUFA in hepatic PL, there were no differences in specific MUFA or in the calculated indices for stearoyl-CoA desaturase activity (data not shown) in the present study.

The liver is a major organ for FA metabolism, including elongation and desaturation, and esterification of FA for the synthesis of TAG and their transport in VLDL to peripheral tissues. Perhaps the lack of response on metabolic parameters in the present study is due to insufficient tissue levels of *c*9,*t*11-CLA, as in our previous research with dietary supplementation of a CLA mixture, the *c*9,*t*11-CLA isomer was 5-fold higher in liver TAG and 2·5-fold higher in adipose TAG compared with the present study[14]. Also, we did not observe a consistent elevation of *c*9,*t*11-CLA in PL of the liver and adipose tissue of VA-fed *fa/fa* rats. Perhaps these results reflect the length of the dietary intervention and differences in tissue metabolism and kinetics for the endogenous production of CLA from VA and its partitioning and incorporation into TAG *v.* PL pools. Furthermore, it is not clear whether CLA in the adipose tissue is from endogenous conversion and/or transport from the liver.

Interestingly, VA in TAG and PL and *c*9,*t*11-CLA in TAG were higher in the epididymal fat than in the liver of VA-fed rats, indicating the preferential incorporation of these FA in the adipose tissue of *fa/fa* rats. This may be linked to the positive effects observed in the adipose tissue. Accumulating evidence indicates that epididymal fat pad mass and adipocyte size are critical components of obesity, T2DM and other metabolic dysfunctions (reviewed in Fruhbeck[52]). The present results that dietary supplementation of VA reduced the epididymal fat pad mass in *fa/fa* Zucker rats was not observed in the studies conducted with JCR:LA-*cp* rats[36,37], although the incorporation of VA and the bioconversion of CLA in the adipose tissue was similar, suggesting model-specific effects.

The architecture of adipose tissue is gaining attention, since adipocyte size is positively correlated with the onset of the MetS[45–47]. Azain *et al.*[53] reported that dietary supplementation of CLA reduces the fad pad mass by reducing the adipose cell size in female Sprague–Dawley rats. We have previously observed reductions in adipocyte size and positive effects on obesity-associated metabolic abnormalities with CLA supplementation in *fa/fa* rats despite no changes in BW or adiposity[15,20,43,54]. Within this context, the reduction of epididymal fat pad mass and the presence of smaller adipocytes with higher adipophilin protein levels in the adipose tissue of rats fed VA indicate a positive effect. Given that changes in adipocyte size would influence adipokine status[55,56], examining the protein biomarkers that are involved in IR and inflammation was relevant to the present study. Although there were no major changes in IR as reflected in the serum glycaemic profile, oral glucose tolerance or critical nodes of insulin signalling (Akt, p42/44 MAPK), IL-10, an anti-inflammatory molecule[57], was found to be elevated in the adipose tissue with the VA diet.

It has been suggested that the pathophysiology of obesity and hypertension is highly interconnected and, together, accelerate the progression of T2DM and CVD[58]. In this context, we have previously demonstrated that dietary supplementation with *c*9,*t*11-CLA (0·4 %, w/w) for 8 weeks attenuates obesity-mediated hypertension in the absence of changes in the renin–angiotensin system in adipose tissue[54]. Thus, given the positive results with *c*9,*t*11-CLA, we felt it was reasonable to expect VA would similarly reduce blood pressure. Furthermore, to our knowledge, no studies have investigated the effects of dietary VA on obesity-mediated hypertension. Unlike *c*9,*t*11-CLA, dietary supplementation of VA for 8 weeks failed to reduce blood pressure in obese IR rats. These results paralleled the absence of changes in components of the local renin–angiotensin system, except for the elevation in protein levels of the AT2 receptor. The AT2 receptor has been linked to obesity[59], and it appears to modulate blood pressure via the AT1 receptor[60]. Collectively, these molecular changes in adipose tissue indicate that VA positively influences some molecular parameters associated with IR.

The present results that there were no changes in glycaemia and insulinaemia of *fa/fa* Zucker rats fed VA are in accord with previous studies conducted with JCR:LA-*cp* rats[36,37]. It is noteworthy that *fa/fa* Zucker rats and JCR:LA-*cp* rats display an inherent IR and have 'sturdy' pancreatic β-cells capable of maintaining insulin-secreting activity and glycaemia throughout life[61]. Intriguingly, VA-fed *fa/fa* Zucker rats had smaller adipocytes and molecular changes in adipose tissue but no reduction in hepatic steatosis, whereas VA-fed JCR:LA-*cp* rats had beneficial changes in the liver[36,37], suggesting the existence of model-specific effects. Although there were no physiological changes observed in terms of IR, these interesting molecular changes and reduction of adipocyte size may require longer duration (greater than 8 weeks) to produce physiological effects.

In summary, the present study using an obese IR model provides evidence that VA, a dietary *trans*-FA present in dairy products and ruminant meats, does not exacerbate obesity-mediated IR and metabolic abnormalities. In fact, there were some positive changes in VA-fed rats as reflected by the smaller adipocyte size and higher levels of IL-10, an anti-inflammatory protein. Future studies need to continue to delineate the effects of naturally *v.* industrially produced *trans*-FA on MetS parameters and the development of CVD and T2DM.

Acknowledgements

We thank Leslie Rech, Alana Lyons and Jennifer Young for their help with the animal care. We would like to acknowledge Dennis Labossiere for his technical assistance with GC. We also thank St Boniface Hospital Research Foundation for the generous infrastructure support. This study was funded by competitive research grants (to C. G. T. and P. Z.) from the Dairy Farmers of Canada and the Natural Sciences and Engineering Research Council of Canada. The authors' contributions are as follows: S. K. M. participated in the blood pressure data collection, Western blotting, data interpretation and manuscript preparation; D. H. performed the FA composition and serum biochemistry data collection and analysis; L. S. carried out the Western blotting; A. C. and J. E. conducted the adipose tissue morphometry; J. S. and M. R. were involved in the synthesis and analysis of VA for the animal study; P. Z. and C. G. T. contributed to the funding, design, supervision and manuscript editing. The authors have no conflicts of interest to disclose.

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