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Trans-11 Vaccenic Acid Reduces Hepatic Lipogenesis and Chylomicron Secretion in JCR: LA-*cp* Rats[1–3]

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Abstract

Trans-11 vaccenic acid (VA) is the predominant *trans* isomer in ruminant fat and a major precursor to the endogenous synthesis of *cis9,trans11*-conjugated linoleic acid in humans and animals. We have previously shown that 3-wk VA supplementation has a triglyceride (TG)-lowering effect in a rat model of dyslipidemia, obesity, and metabolic syndrome (JCR:LA-*cp* rats). The objective of this study was to assess the chronic effect (16 wk) of VA on lipid homeostasis in both the liver and intestine in obese JCR:LA-*cp* rats. Plasma TG ($P < 0.001$), total cholesterol ($P < 0.001$), LDL cholesterol ($P < 0.01$), and nonesterified fatty acid concentrations, as well as the serum haptoglobin concentration, were all lower in obese rats fed the VA diet compared with obese controls ($P < 0.05$). In addition, there was a decrease in the postprandial plasma apolipoprotein (apo)B48 area under the curve ($P < 0.05$) for VA-treated obese rats compared with obese controls. The hepatic TG concentration and the relative abundance of fatty acid synthase and acetyl-CoA carboxylase proteins were all lower ($P < 0.05$) in the VA-treated group compared with obese controls. Following acute gastrointestinal infusion of a VA-triolein emulsion in obese rats that had been fed the control diet for 3 wk, the TG concentration was reduced by 40% ($P < 0.05$) and the number of chylomicron (CM) particles (apoB48) in nascent mesenteric lymph was reduced by 30% ($P < 0.01$) relative to rats infused with a triolein emulsion alone. In conclusion, chronic VA supplementation significantly improved dyslipidemia in both the food-deprived and postprandial state in JCR:LA-*cp* rats. The appreciable hypolipidemic benefits of VA may be attributed to a reduction in both intestinal CM and hepatic de novo lipogenesis pathways. J. Nutr. 139: 2049–2054, 2009.

Introduction

The potential deleterious cardiovascular properties of *trans*-fats [from partially hydrogenated vegetable oils (PHVO)][6] have received considerable attention over the past few years (1–6). However, there has been a void in the understanding of the different metabolic properties of naturally derived *trans* fats, such as vaccenic acid [VA; *trans*-11 18:1(n-9)], compared with those *trans* fats derived from PHVO and produced by industrial means. VA is a monounsaturated fatty acid (MUFA) and the predominant isomer accounting for ~70% of the total *trans* fatty acids (TFA) found in ruminant-derived lipids (7,8). VA is also the primary precursor for endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in animals and humans (9–11). Clinical evidence indicates that relative to industrially derived TFA, natural sources of TFA (such as VA-enriched butter) in the diet can elicit either neutral or beneficial health effects on blood lipid variables among healthy individuals (12,13). More specifically, it has been reported that feeding VA-enriched dairy foods lowers atherogenic lipoproteins and inhibits adipose tissue lipogenesis in several animal models (14–17). We have recently reported novel plasma triglyceride (TG)-lowering properties of pure VA supplementation in hyperlipidemic JCR:LA-*cp* rats (18). However, the hypolipidemic benefit of VA supplementation has not been demonstrated under chronic feeding conditions, nor have the corresponding putative mechanisms been elucidated. Consequently, in this study, we hypothesized that long-term supplementation of VA in JCR:LA-*cp* rats would result in a persistent

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[3] Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
[6] Abbreviations used: ACC-1, acetyl-CoA carboxylase-1; apo, apolipoprotein; AUC, area under the curve; CD, control diet; CM, chylomicron; CVD, cardiovascular disease; FAS, fatty acid synthase; IL, interleukin; MUFA, monounsaturated fatty acids; NEFA, nonesterified fatty acid; PHVO, partially hydrogenated vegetable oil; SREBP-1, sterol regulatory element binding protein-1; TC, total cholesterol; TFA, *trans* fatty acid; TG, triglyceride; VA, *trans*-11 vaccenic acid.
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improvement in dyslipidemia associated with effects on lipogenic pathways involved in whole body lipid homeostasis.

JCR:LA-*cp* rats, when homozygous for the *cp* trait (*cp*/*cp*), develop leptin receptor deficiency (19). Rats from this strain exhibit symptoms of metabolic syndrome and prediabetes, which include obesity, insulin resistance, hepatic steatosis, hypertriglyceridemia, as well as exacerbated production of hepatic VLDL and intestinal chylomicrons (CM) (20–26). Hepatic lipogenic enzymes including acetyl-CoA carboxylase-1 (ACC-1) and fatty acid synthase (FAS) are 2 key components involved in the production of long-chain fatty acids, which are subsequently used for TG synthesis and VLDL assembly. The transcriptional factor sterol regulatory element binding protein-1 (SREBP-1) regulates associated lipogenic pathways. The abundance of SREBP-1, ACC-1, and FAS mRNA in JCR:LA-*cp* rats is elevated (20) and thus represents a potential regulatory mechanism by which VA supplementation may improve hepatic fatty acid and TG de novo synthesis. Complementary studies have also demonstrated postprandial dyslipidemia and intestinal CM oversecretion in JCR:LA-*cp* rats (26,27). Therefore, we proposed that VA may improve circulating TG concentration in JCR:LA-*cp* rats by lowering hepatic and intestinal de novo lipogenesis and/or intestinal CM secretion.

Materials and Methods

Rat model and experimental protocol. Male rats of the corpulent JCR:LA-*cp* strain, both obese (*cp*/*cp*) ($n = 16$) and lean (+/?) ($n = 8$), were raised in our established breeding colony at the University of Alberta, as previously described (28). At 8 wk of age, obese rats were randomized into 2 groups and were fed for 16 wk either a lipid balanced control diet (CD) or an isocaloric lipid balanced diet containing 1.0% (wt:wt) purified VA. Lean littermates were fed the CD for the same period of time as normolipidemic controls. Rat care and experimental protocols were conducted in accordance with the Canadian Council on Animal Care and approved by the University of Alberta Animal Care and Use Committee. Food consumption and body weight were recorded throughout the study. At 23 wk of age, an oral fat challenge test, as previously described (26), was performed in 4 randomly chosen rats from each obese group. At 24 wk of age, rats were food deprived overnight and killed the following morning under isofluorane anesthesia. Plasma and serum samples were collected from the left ventricle. Liver as well as perirenal, inguinal, and epididymal fat pads were weighed and snap frozen in liquid N_2 at −80°C for further analysis. Jejunum was rinsed with PBS for mucosal enterocyte collection. Adipose fatty acid composition was measured on the epididymal fat pad as previously described (18).

Diet preparation. A lipid-balanced CD was designed to resemble the Western diet as previously described for similar studies exploring the effects of fatty acid bioactivity (29). The CD was composed (wt:wt) of 1% cholesterol, 43.1% carbohydrate, 28% protein, 8% fiber, and 15% lipid (wt:wt) with a PUFA:SFA ratio of 0.6 and (n-6):(n-3) PUFA ratio of 10 (**Supplemental Table 1**). The VA diet was prepared by adjusting the lipid composition of the CD to provide 1.0% (wt:wt) of VA while maintaining the PUFA:SFA and (n-6):(n-3) PUFA ratio. Purified VA was produced by chemical alkali isomerization from linoleic acid-rich vegetable oil (30). The amount of VA in the diet was chosen based on previous findings (18) while still maintaining normal dietary fatty acid ratios. The diet mixture was extruded into pellets, dried at room temperature, and stored at 4°C in air-tight containers. GC analysis was performed on lipid blend samples to confirm fatty acid composition (**Supplemental Table 2**).

Plasma biochemical components and serum cytokines. The concentrations of select biochemical variables in plasma from lean and obese rats obtained in the food-deprived and postprandial states were assessed using commercially available homogenous, enzymatic kits, including plasma TG, total cholesterol (TC), LDL cholesterol, and serum inflammatory markers [haptoglobin and interleukin (IL)-10] as previously described (18). Plasma nonesterified fatty acids (NEFA) were measured using an enzymatic colorimetric assay (HR Series NEFA-HR, catalog no. 999–34691, Wako Diagnostics). Samples were analyzed using assay kits from a single lot and performed in one batch. Samples were measured in triplicate, except cytokines, which were measured in duplicate, with an intra-assay CV at <5%. ApoB48 was quantified by western blot coupled with enhanced chemiluminescence on plasma obtained from rats in food-deprived and postprandial states (26).

Liver homogenization, hepatic TG, and relative protein abundance of lipogenic factors. Rat liver (0.5 g) was homogenized in 200 μL lysis buffer [PBS (pH 7.4) with 1.5% Triton X-100 and 1% protease inhibitor cocktail (Sigma)]. The concentration of TG was measured by ELISA using an aliquot of the whole homogenate and adjusted by the protein concentration of the homogenate (18). The remainder of the homogenate was centrifuged at 700 *g* for 15 min, the supernatant collected and stored at −80°C. Hepatic ACC-1, FAS, and SREBP-1 proteins were determined by western blot analysis as previously described (31). Western blot membranes were incubated with primary antibodies raised against ACC-1 (catalog no. 3662, Cell Signaling), FAS (catalog no. sc-55580, Santa Cruz Biotechnology), SREBP-1 (catalog no. sc-366, Santa Cruz Biotechnology), and β-actin (internal control) (catalog no. A5441, Sigma-Aldrich). Adherent antibodies were visualized by chemiluminescence using ECL Advance Western Blotting Detection kit (catalog no. RPN2135, GE Health). Protein bands were quantified using Image J (version 1.41) software developed by NIH. The final value of FAS and ACC-1 relative protein abundance was normalized based on the respective β-actin protein mass.

Mucosal enterocyte collection, protein extraction, and relative protein abundance of lipogenic factors. The jejunum was excised and enterocyte fractions (1–10) were collected using the Weiser method (32). Samples (50 μL) from each fraction were pooled and SREBP-1, ACC1, and FAS relative protein abundances were assessed on the pooled enterocyte protein extract as described above for liver homogenates.

Mesenteric lymph duct cannulation. To determine the direct effect of VA on the secretion of CM in mesenteric lymph, the superior mesenteric lymph duct of 9-wk-old obese JCR:LA-*cp* rats ($n = 8$) were cannulated following consumption of the CD for 3 wk (33). Rats were randomized to receive either a pure triolein emulsion or a triolein emulsion containing VA.

Triolein emulsion preparation and nascent lymph collection. VA oil (7.5 μg) was dissolved in 100 μL chloroform and vortexed with 500 μL glyceryl trioleate (Sigma, catalog no. T7140) and the chloroform evaporated under N_2 as described previously (33). The emulsion was flushed with N_2, sealed with parafilm, and protected from exposure to light. The prepared emulsion was then infused to rats via the gastric cannula, which was flushed just prior with saline (1 mL). The triolein emulsion was prepared identical to that described for the VA emulsion with the exception of VA. Mesenteric lymph was collected into EDTA-coated vacutainers for 5 h following the emulsion infusion. TG, TC, and apoB48 concentrations were all measured as described above for plasma.

Statistical analysis. All results are expressed as mean ± SEM. Data were tested for normal distribution and differences among lean CD, obese CD, and obese VA groups for most variables and were analyzed using 1-way ANOVA followed by Tukey's post hoc tests. Postprandial lipemia was assessed by area under the curve (AUC) analysis. Differences in postprandial and lymphatic lipid profile between groups were analyzed using an unpaired *t* test. The level of significance was set at $P < 0.05$ (Graph Pad Prism 5.0).

Results

Food intake, body weight, and tissue/organ weights. At the end of the dietary intervention (16 wk), food consumption and

body weight did not differ between obese rats fed the CD or VA diet (**Table 1**). Epididymal, inguinal, and perirenal adipose tissue weight and jejunum weight did not differ between the obese groups. However, the VA-fed obese rats had a lower liver weight than the CD-fed obese rats ($P < 0.05$).

Fatty acid composition of adipose tissue. The composition of total SFA, MUFA, and PUFA in epididymal adipose tissue did not differ between the obese groups (**Table 2**). However, there was an 80-fold increase in the amount of VA ($P < 0.001$) in the VA-fed obese rats compared with the CD-fed obese rats. The amount of *cis*9,*trans*11-conjugated linoleic acid was also elevated 20-fold in VA-fed obese rats compared with obese rats fed the CD ($P < 0.001$), likely due to the in vivo conversion from VA. Interestingly, obese rats fed the VA diet had lower proportions of myristic acid (14:0), α-linolenic acid [18:3(n-3)], and arachidonic acid [20:4(n-6)] and a higher proportion of stearic acid (18:0) in adipose tissue compared with the CD-fed obese rats.

Plasma lipids and serum inflammatory markers. Obese rats fed the CD had higher plasma TG, TC, LDL-cholesterol, and NEFA concentrations compared with lean rats fed the CD (**Table 3**; $P < 0.05$). Dietary VA supplementation reduced plasma TG ($P < 0.001$), TC ($P < 0.001$), LDL cholesterol ($P < 0.01$), and NEFA ($P < 0.05$) concentrations compared with CD-fed obese rats (Table 3). Surprisingly, the LDL cholesterol concentration did not differ between lean rats fed the CD and obese rats fed the VA diet, suggesting a normalizing effect of dietary VA.

Chronic dietary supplementation of VA also normalized the serum concentration of haptoglobin in obese rats to levels comparable with lean rats fed the CD (Table 3; $P < 0.001$). Supplementation of VA in obese rats did not affect the concentration of IL-10 (Table 3).

Hepatic TG and lipogenic factors. Hepatic TG was higher in obese rats fed the CD compared with lean rats fed the CD ($P < 0.001$) (Table 3). Further, the relative protein abundance of both FAS (**Fig. 1***A*,*B*) and ACC (Fig. 1*A*,*C*) was higher in obese rats fed the CD ($P < 0.001$) relative to lean rats fed the CD. In contrast, VA supplementation in obese rats resulted in a lower (49%) hepatic TG concentration compared with obese rats fed the CD ($P < 0.001$). In addition, VA supplementation reduced the relative abundance of FAS and ACC enzymes by ~40% ($P < 0.05$) compared with obese rats fed the CD. The relative protein mass of mature SREBP-1 in liver did not differ among groups (data not illustrated).

TABLE 1 Food intake, body weight, and tissue weight in lean and obese JCR:LA-*cp* rats fed a CD or VA-supplemented diet for 16 wk[1]

	Lean control	Obese control	Obese VA
		g	
Food intake, *per day*	19.7 ± 0.3[b]	32.4 ± 0.5[a]	33.2 ± 1.0 [a]
Body weight	383.9 ± 8.2[b]	646.0 ± 9.3[a]	659.4 ± 10.9[a]
Liver weight	9.1 ± 0.4[c]	23.1 ± 0.7[a]	18.9 ± 1.0[b]
Jejunum weight	3.4 ± 0.1[a]	4.7 ± 1.0[a]	5.6 ± 0.84[a]
Epidydimal fat pad weight	2.0 ± 0.2[b]	11.5 ± 0.3[a]	11.9 ± 0.3[a]
Pararenal fat pad weight	1.3 ± 0.1[b]	7.4 ± 0.4[a]	7.1 ± 0.4[a]
Inguinal fat pad weight	1.4 ± 0.2[b]	17.9 ± 1.1[a]	18.6 ± 1.0[a]

[1] Values are means ± SEM, $n = 8$. Means in a row without a common letter differ, $P < 0.05$.

TABLE 2 Fatty acid composition of adipose tissue TG in lean and obese JCR:LA-*cp* rats fed a CD or VA-supplemented diet for 16 wk[1]

	Lean control	Obese control	Obese VA
		g/100 g fatty acids	
14:0	0.7 ± 0.04[c]	1.4 ± 0.03[a]	1.3 ± 0.01[b]
16:0	13.0 ± 0.4[b]	23.4 ± 0.1[a]	23.2 ± 0.2[a]
16:1(n-9)	0.7 ± 0.1[b]	3.8 ± 0.1[a]	3.8 ± 0.07[a]
18:0	14.4 ± 0.6[a]	6.4 ± 0.1[c]	7.3 ± 0.1[b]
VA	0.06 ± 0.01[b]	0.02 ± 0.01[c]	1.6 ± 0.04[a]
18:1(*cis*-9)	29.1 ± 0.2[b]	37.9 ± 0.6[a]	34.4 ± 2.3[a]
18:1(*cis*-11)	1.7 ± 0.05[b]	2.2 ± 0.03[a]	2.2 ± 0.02[a]
18:2(n-6)	36.0 ± 0.2[b]	20.5 ± 0.2[a]	20.0 ± 0.3[a]
18:3(n-3)	1.3 ± 0.03[a]	0.9 ± 0.01[b]	0.8 ± 0.02[c]
18:2(*cis*-9, *trans*-11)	0.03 ± 0.01[c]	0.04 ± 0.01[b]	0.8 ± 0.02[a]
20:4(n-6)	0.5 ± 0.02[a]	0.4 ± 0.02[b]	0.3 ± 0.01[c]
22:5(n-3)	0.2 ± 0.04[a]	0.1 ± 0.01[a]	0.1 ± 0.01[a]
22:6(n-3)	0.3 ± 0.07[a]	0.1 ± 0.01[a]	0.1 ± 0.01[a]
SFA	28.4 ± 0.3[b]	31.4 ± 0.3[a]	32.0 ± 0.3[a]
MUFA	31.6 ± 0.3[b]	44.4 ± 0.5[a]	42.4 ± 2.4[a]
PUFA	38.8 ± 0.1[a]	22.8 ± 0.2[b]	22.2 ± 0.3 [b]

[1] Values are means ± SEM, $n = 8$. Means in a row without a common letter differ, $P < 0.05$.

Postprandial assessment of plasma TG and apoB48. Obese rats supplemented with VA had a significantly lower postprandial TG (**Fig. 2***A*) and apoB48 (Fig. 2*B*) response following an oral lipid load compared with obese rats fed the CD. AUC analysis of postprandial plasma TG (obese control, 63.3 ± 10.5 mmol/L·h; obese VA, 40.9 ± 8.2 mmol/L·h; $P < 0.05$) and apoB48 (obese control, 37.0 ± 7.7 μmol/L·h; obese VA, 22.0 ± 3.3 μmol/L·h; $P < 0.05$) indicated improvements to postprandial dyslipidemia in obese rats fed dietary VA compared with those fed the CD.

Intestinal lipogenic factors. The abundance of mature SREBP-1 protein was lower in enterocytes isolated from obese rats fed the CD relative to lean rats fed the CD (**Fig. 3**; $P < 0.01$). Interestingly, VA supplementation in obese rats resulted in a small, yet significant increase (25%) in mature SREBP-1 compared with obese rats fed the CD ($P < 0.05$) to an extent not different from lean control rats (Fig. 3). The relative abundance of FAS and ACC-1 protein in enterocytes from obese rats fed VA did not differ from obese rats fed the CD (data not shown).

TABLE 3 Plasma lipid concentrations, serum inflammatory markers, and hepatic TG in lean and obese JCR:LA-*cp* rats fed a CD or VA-supplemented diet for 16 wk [1]

	Lean control	Obese control	Obese VA
TG, *mmol/L*	0.5 ± 0.1[c]	3.3 ± 1.1[a]	1.6 ± 0.6[b]
TC, *mmol/L*	2.3 ± 0.2[a]	5.8 ± 0.9[b]	4.0 ± 0.7[c]
LDL cholesterol, *mmol/L*	1.0 ± 0.2[b]	1.8 ± 0.4[a]	1.3 ± 0.5[b]
NEFA, *mmol*/L	0.3 ± 0.07[c]	0.5 ± 0.07[a]	0.4 ± 0.08[b]
IL-10, *nmol/L*	1.2 ± 0.5[a]	1.0 ± 0.5[b]	0.7 ± 0.3[b]
Haptoglobin, *pmol/L*	4.7 ± 1.7[b]	10.8 ± 2.5[a]	5.2 ± 1.4[b]
Liver TG, *mmol/g protein*	3.1 ± 0.5[c]	26.4 ± 3.0[a]	10.1 ± 0.6[b]

[1] Values are means ± SEM, $n = 8$. Means in a row without a common letter differ, $P < 0.05$.

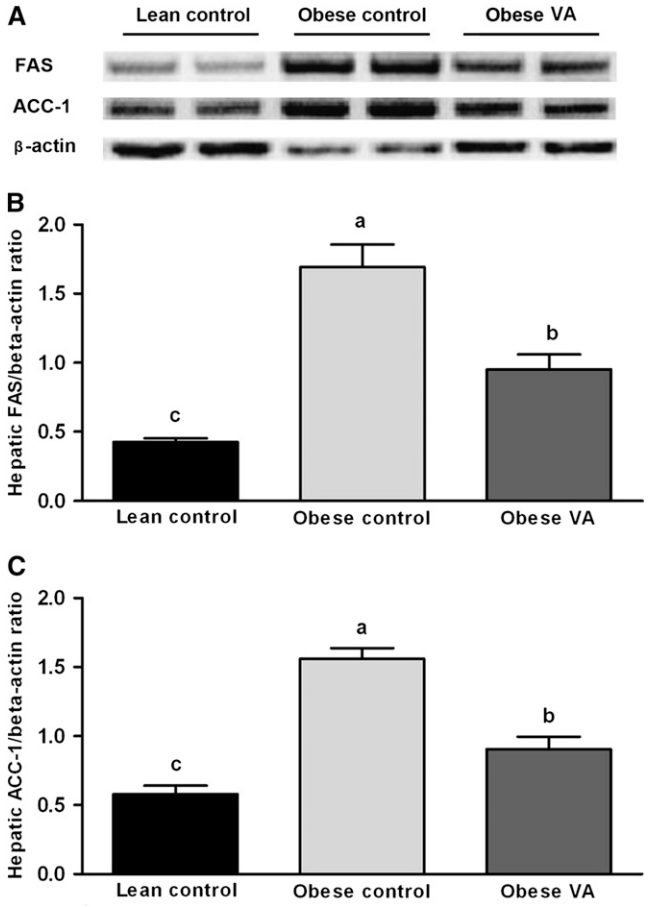

FIGURE 1 Western blots of hepatic lipogenic enzymes (*A*) and relative protein abundance of ACC-1 (*B*) and FAS (*C*) in the lean and obese JCR:LA-*cp* rats fed the CD or VA diet for 16 wk. Values are means ± SEM, $n = 8$. Means without a common differ; $P < 0.05$.

Mesenteric lymphatic CM secretion. Infusion of the VA-containing triolein emulsion in obese rats fed the CD resulted in a 40% reduction in the TG concentration of lymph ($P < 0.05$) and the number of CM particles (determined by apoB48 concentration) was reduced by 30% ($P < 0.01$) compared with those infused with the triolein-only emulsion (**Table 4**). The concentration of lymph TC did not differ between rats infused with triolein-VA or triolein alone.

Discussion

Hypertriglyceridemia and hypercholesterolemia are risk factors used for the diagnosis of metabolic syndrome and cardiovascular diseases (CVD) (34–38). Limited evidence to date has shown that feeding VA or VA-enriched dairy products may have neutral or even beneficial health effects in animal models of these 2 diseases (14–16,39). Several research groups have reported the potential for (acute) benefits of feeding ruminant *trans* fats on CVD risk markers in healthy human populations (12,13,40). We have shown previously that 3-wk feeding with a diet enriched in purified VA reduces plasma TG by ~40% without any concomitant change in TC, LDL cholesterol, or glucose/insulin metabolism in dyslipidemic JCR:LA-*cp* rats (18). These studies suggest differential properties of the natural ruminant-derived TFA, VA, compared with PHVO. More recently, Tyburczy et al. (17) also reported a hypocholesterolemic effect in hamsters following a

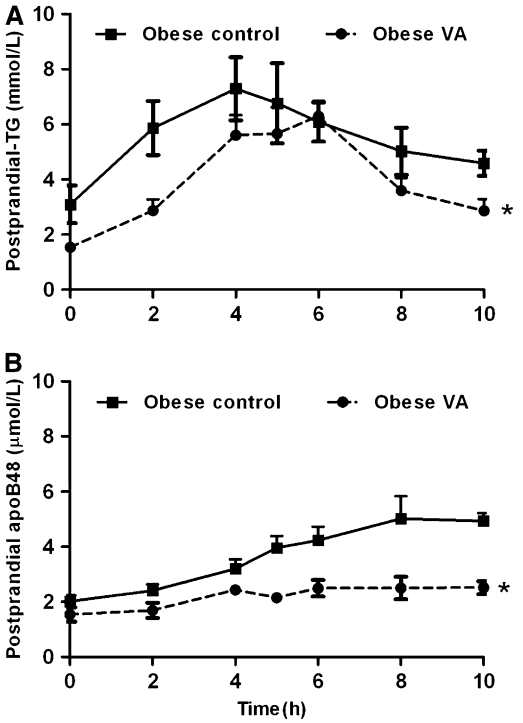

FIGURE 2 Postprandial response following an oral fat challenge in obese JCR:LA-*cp* rats fed the CD or VA diet for 16 wk. (*A*) Plasma TG response. (*B*) Plasma apoB48 response. Values are means ± SEM, $n = 4$. *AUC differ, $P < 0.05$.

4-wk dietary supplementation with VA compared with a diet containing PHVO.

The current study is consistent with previous results from a shorter-term study in JCR:LA-*cp* rats (18), in that chronic (16-wk) VA feeding did not significantly influence food intake, body weight, or glucose/insulin metabolism. Rather, chronic supplementation of VA resulted in a striking reduction in plasma TG (50%) as well as reductions in plasma TC, LDL cholesterol, and NEFA concentrations in JCR:LA-*cp* rats. Moreover, postprandial dyslipidemia improved during longer-term VA dietary

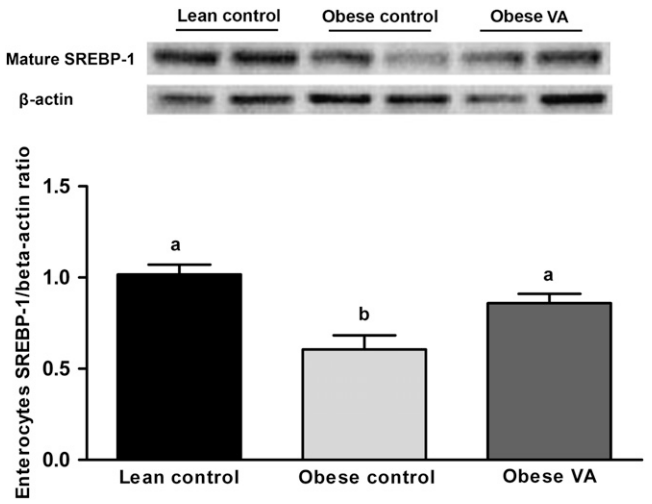

FIGURE 3 Mature SREBP-1 relative protein abundance in pooled enterocyte protein extracts of the lean and obese JCR:LA-*cp* rats fed the CD or VA diet for 16 wk. Values are means ± SEM, $n = 4$. Means without a common letter differ, $P < 0.05$.

TABLE 4 Mesenteric lymph concentrations of TG, TC, and apoB48 following gastrointestinal infusion of an emulsion containing triolein alone or triolein with VA in obese JCR:LA-*cp* rats previously fed the CD for 3 wk[1]

	Triolein infusion	VA+ triolein infusion
TG, *mmol/L*	40.0 ± 8.0	24.4 ± 8.6*
TC, *mmol/L*	5.1 ± 0.8	3.9 ± 0.9
apoB48, *μmol/L*	54.2 ± 2.2	37.3 ± 5.9**

[1] Values are means ± SEM, $n = 4$. Asterisks indicate different from the triolein-infused group: $^*P < 0.05$, $^{**}P < 0.01$.

supplementation. These results support our hypothesis that chronic VA intake may modulate lipogenic pathways under conditions of dyslipidemia.

Elevated concentrations of circulating acute phase proteins (such as C-reactive protein, fibrinogen, and haptoglobin) have emerged as biomarkers of CVD risk and/or progression (41–43). Interestingly, Balestrieri et al. (44) and Cigliano et al. (45) have both reported that haptoglobin may inhibit the activation of lecithin-cholesterol acyltransferase activity by binding with apoAI and may attenuate HDL maturation. In our study, the serum haptoglobin concentration was reduced by 50% in obese rats fed VA, suggesting a mechanism by which VA may improve both the inflammatory regulation and reverse cholesterol transport during conditions of dyslipidemia.

It is well established that feeding PUFA, such as the (n-3) PUFA docosahexanoic acid confers TG-lowing properties, in part by downregulating genes involved in lipid biosynthesis, including ACC-1 and FAS (46,47). In this study, lower hepatic ACC-1 and FAS proteins in VA-fed obese rats suggests that VA may also regulate endogenous fatty acid synthesis during conditions of hypertriglyceridemia. Elam et al. (20) has previously shown that hepatic SREBP-1 mRNA is higher in obese JCR:LA-*cp* rats. However, in this study, the relative protein abundance of mature hepatic SREBP-1 did not differ among groups, suggesting a possible post-transcriptional and/or post-translational regulation of lipidogenic pathways following VA supplementation. We speculate that reduced hepatic fatty acid synthesis may contribute to lower total hepatic TG and reduced secretion of VLDL and thus decrease the overall contribution to plasma TG and/or LDL.

Nascent intestinal CM and hepatic VLDL are 2 predominant contributors to food-deprived and postprandial plasma TG. During insulin resistance, the regulation of postprandial lipemia becomes compromised, resulting in exaggerated secretion of apoB48-containing CM from the intestine and hepatic VLDL (20,27). Similarly, JCR:LA-*cp* rats have been shown to develop impaired postprandial lipemia with an associated increase in CM production (measured as apoB48) (21,26,27). In this study, VA-fed obese JCR:LA-*cp* rats had a substantial decrease in the postprandial apoB48 AUC after an oral fat challenge compared with obese control rats. We then hypothesized that improvements to postprandial dyslipidemia may result from either reduced intestinal nascent CM secretion or accelerated clearance of CM by the liver, or both. To partially address this, we sampled nascent lymph CM directly from the superior mesenteric lymph duct following infusion of a VA-containing emulsion. We observed a significant reduction in CM particle number and TG concentration in lymph isolated from obese rats infused with triolein-VA relative to triolein-alone. These results suggest that VA may mediate changes in CM and TG synthesis in the enterocytes. We propose that the obese corpulent phenotype may have a compensatory intestinal adaptation to downregulate lipidogenesis in response to hyperphagia (greater lipid intake) compared with lean controls. This is in part supported by the observation that obese rats had lower relative abundance of enterocyte mature SREBP-1 compared with their lean counterparts. Following VA supplementation, the abundance of mature SREBP-1 was increased to that comparable with lean controls, suggesting a VA-mediated normalization of lipogenic pathways involved in CM production. However, VA did not alter the relative protein abundance of FAS or ACC-1 in enterocytes. It is feasible that VA may play a specific role in the regulation of CM assembly rather than fatty acid de novo synthesis per se in the intestine. It may also lend support to the notion that lipid absorption transport pathways are preferentially downregulated in response to VA treatment. Lampen et al. (48) reported that VA enhances the expression of PPARα, β, and δ in a Caco-2 cell culture model, which may translate to changes in fatty acid transporter expression and long-chain fatty acid uptake into the enterocyte; however, these aspects of VA-mediated PPAR effects on lipid absorption require further investigation.

In conclusion, long-term dietary supplementation of VA (1.0%, wt:wt) in obese, dyslipidemic JCR:LA-*cp* rats results in a substantial improvement in the associated atherogenic plasma lipid profile and postprandial lipemia. Specifically, we have shown novel effects of dietary VA on de novo lipogenesis pathways, including suppression of hepatic fatty acid synthesis enzymes and intestinal CM secretion under conditions of dyslipidemia in a rat model of metabolic syndrome.

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