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Trans-11 Vaccenic Acid Dietary Supplementation Induces Hypolipidemic Effects in JCR:LA-*cp* Rats[1,2]

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Abstract

Trans-11 vaccenic acid [VA; 18:1(n-9)] is a positional and geometric isomer of oleic acid and is the precursor to conjugated linoleic acid (CLA) in humans. Despite VA being the predominant *trans* monoene in ruminant-derived lipids, very little is known about its nutritional bioactivity, particularly in conditions of chronic metabolic disorders, including obesity, insulin resistance, and/or dyslipidemia. The aim of this study was to assess the potential of VA to improve dyslipidemia, insulin sensitivity, or inflammatory status in obese and insulin-resistant JCR:LA-*cp* rats. The obese rats and age-matched lean littermates were fed a control diet or a control diet supplemented with 1.5% (wt:wt) VA for a period of 3 wk. The incorporation of VA and subsequent conversion to CLA in triglyceride was measured in adipose tissue. Glucose and insulin metabolism were assessed via a conscious adapted meal tolerance test procedure. Plasma lipids as well as serum inflammatory cytokine concentrations were measured by commercially available assays. VA supplementation did not result in any observable adverse health effects in either lean or obese JCR:LA-*cp* rats. After 3 wk of feeding, body weight, food intake, and glucose/insulin metabolism did not differ between VA-supplemented and control groups. The incorporation of VA and CLA into adipose triglycerides in obese rats fed VA increased by 1.5-fold and 6.5-fold, respectively, compared with obese rats fed the control diet. The most striking effect was a 40% decrease ($P < 0.05$) in fasting triglyceride concentrations in VA-treated obese rats relative to obese controls. Serum Il-10 concentration was decreased by VA, regardless of genotype ($P < 0.05$). In conclusion, short-term dietary supplementation of 1.5% VA did not result in any detrimental metabolic effects in JCR:LA-*cp* rats. In contrast, dietary VA had substantial hypo-triglyceridemic effects, suggesting a new bioactivity of this fatty acid that is typically found in ruminant-derived food products. J. Nutr. 138: 2117–2122, 2008.

Introduction

Trans-11 vaccenic acid [VA;[5] *trans*-11 18:1(n-9)] is the predominant *trans* monoene in ruminant fat, which is produced naturally during the partial biohydrogenation of linoleic acid (LA) [18:2(n-6)] and α-linolenic acid (ALA) [18:3(n-3)] (1,2). VA acts as a precursor for the endogenous synthesis of *cis*9, *trans*11-conjugated linoleic acid (CLA) via the action of the △9 desaturase enzyme in both humans and animals (1,3). The rate of the conversion of VA to CLA has been estimated to range from 5 to 12% in rodents to 19 to 30% in humans (3). Recent nutritional studies have provided insight into the beneficial health effects of dietary-derived CLA in redistributing visceral fat stores (in both animals and humans), protecting against several types of cancer, as well as improving dyslipidemia (4–7). Interestingly, whereas the dairy industry has made efforts to increase the content of CLA in foods to take advantage of these beneficial properties, recent reports have shown that these production processes also increase VA by up to 10-fold more than the CLA (8).

Nutritional recommendations in North America have further highlighted that *trans* fatty acids (particularly from commercial hydrogenated vegetable oils, e.g. elaidic acid) are linked to increased risk of cardiovascular disease (CVD) (9–12). The existing literature relates industrial *trans* fatty acids to decreased insulin sensitivity in adipose tissue (13), increased total and LDL cholesterol concentrations, systemic inflammation, and endothelial dysfunction (14–24). Unfortunately, the literature to date has not differentiated between the detrimental effect of industrial-hydrogenated *trans*-fat vegetable oils and the effect of naturally

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[5] Abbreviations used: ALA, α-linolenic acid; AUC, area under the curve; CLA, conjugated linoleic acid; CVD, cardiovascular disease; LA, linoleic acid; MTT, meal tolerance test; VA, *trans*-11 vaccenic acid.
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occurring *trans* fats, including CLA and VA. In addition, we know very little about the potential bioactivity of VA on blood lipids or inflammation. The availability and/or expense of purified VA have been primary factors limiting the characterization of VA's potential metabolic actions. However, it is interesting to note that there is some evidence, albeit limited, supporting the hypothesis that VA per se may not be detrimental to health (25–29).

To address the void in the current literature, we chose an established animal model of metabolic syndrome, the JCR:LA-*cp* rat, to assess the health benefits of increased dietary VA (30). The JCR:LA-*cp* rat is a unique strain that has a complete absence of the leptin receptor in the plasma membrane (30,31). It spontaneously develops symptoms associated with metabolic syndrome and the prediabetic state in humans, including obesity, insulin resistance, hyperlipidemia, and inflammatory dysregulation (30–34). Thus, the objective of this study was to investigate the potential for dietary VA supplementation to improve dyslipidemia, insulin resistance, and/or inflammatory status associated with metabolic syndrome in JCR:LA-*cp* rats.

Materials and Methods

Rats and experimental protocol. Male rats of the JCR:LA-*cp* strain, both obese (*cp*/*cp*) ($n = 20$) and lean (+/?) ($n = 20$), were raised in our established breeding colony at the University of Alberta, as previously described (35). At 3 wk of age, rats were transferred from the isolated breeding colony areas to a state-of-the-art individually ventilated caging environment (Tecniplast). At 6 wk of age, rats had access to a standard rat nonpurified diet (5001, PMI Nutrition International) for 2 wk. Rats of the same genotype were randomly divided into 2 groups and were fed either a lipid-balanced control diet or an isocaloric lipid-balanced control diet containing 1.5% (wt:wt) purified VA (Sigma, catalog no. 693–72–1). Animal care and experimental protocols were conducted in accordance with the Canadian Council on Animal Care and approved by the University of Alberta Animal Ethics Committee. Food consumption and body weight were recorded throughout the study. At 11 wk of age, rats were food deprived overnight and killed the following morning under isofluorane anesthesia. Plasma and sera were collected from the left ventricle and the epididymal fat pads removed and snap-frozen until analyzed for lipids.

Diet preparation. A lipid-balanced control diet (control) was designed to resemble the Western diet as previously described (36). The control diet 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 acid ratio of 10 (**Tables 1** and **2**). The VA diet was prepared by adjusting the lipid composition of the control diet to provide 1.5% (wt:wt) of VA while maintaining PUFA:SFA and (n-6):(n-3) PUFA ratios (Tables 1 and 2). The amount of VA in the diet was chosen based on previously published studies allowing for metabolic sufficiency while maintaining a normal dietary fatty acid proportion (25,26). The diet mixture was extruded into pellets, dried at room temperature, and stored at 4°C in air-tight containers. Automated GC analysis was performed on fat blend samples to confirm fatty acid composition (Table 2).

Meal tolerance test. At 10 wk of age, plasma glucose and insulin concentrations were measured in samples from conscious, unrestrained rats after they consumed a standardized test meal to mimic a clinical oral tolerance test in humans (37). After overnight food deprivation, rats ($n = 4$, randomly chosen from each group) were kept warm on a heated table to ensure vasodilatation of the tails and 0.5 mL of blood was taken from the tip of the tail as T = 0 min. Rats were then replaced in their cages, with the test meal given 30 min after the beginning of the dark phase (37). Timing was started when 50% of the test meal had been consumed, and 3 additional samples of blood were taken at time points T = 30 min and T = 60 min following the initial consumption of the food pellet meal. Area under the curve (AUC) analysis was used to calculate the total postprandial excursion of both glucose and insulin (Graph Pad Prizm 4.0).

TABLE 1 Compositions of control and VA diets

Ingredient	VA diet	Control diet
	g/kg	
Casein	266.7	266.7
L-Methionine	2.4	2.4
Dextrose, monohydrate	231.3	231.3
Corn starch	221.8	221.8
Cellulose	49.4	49.4
Sodium selenite	0.4	0.4
Manganese sulfate ($MnSO_4 \cdot H_2O$)	0.3	0.3
Mineral mix, Bemhart-Tomarelli (170750)	50.2	50.2
Vitamin mix, A.O.A.C. (no. 40055)	9.9	9.9
Inositol	6.2	6.2
Choline chloride	1.3	1.3
Cholesterol	10.0	10.0
Sunflower oil	65.7	65.0
Flaxseed oil	6.4	6.1
Soy tallow	62.9	58.6
Olive oil	0.0	20.3
VA	15.0	0.0

Adipose fatty acids. Lipid was isolated from epididymal fat pad tissue in a 4:1 mixture of $CaCl_2$ to $ChCl_3$:CH_3OH (2:1) as previously described (38). Total triglycerides were separated on silica G plates and visualized with 8-anilino-1-naphthalenesulfonic acid under UV light and compared with the appropriate standards (39). Triglyceride FAME were prepared from the scraped silica band using the base-catalyzed method with sodium methoxide. Prepared FAME were flushed with N_2 and stored at −35°C until analysis by GC. Fatty acids were separated by automated GLC (Varian 3800, Varian Instruments) using a 100-m CP-Sil 88 fused capillary column (Varian Instruments) (40).

Plasma biochemical components and serum cytokines. The concentrations of select biochemical variables in either fasting and/or postprandial plasma from lean and obese groups were assessed using commercially available homogenous, enzymatic colorimetric assays. Triglyceride (Wako Pure Chemical Industries, catalog no. 998–40391,

TABLE 2 Fatty acid compositions of control and VA diets

Fatty acid	VA diet	Control diet
	g/100 g total fatty acids	
12:0	0.00	0.03
14:0	0.13	0.14
16:0	10.71	12.86
16:1	0.00	0.10
17:0	0.00	0.19
17:1	0.10	0.00
18:0	37.00	35.47
18:1 (*cis*-9)	11.76	20.94
18:1 (*trans*-11, VA)	9.14	0.40
18:2(n-6) (LA)	28.22	26.01
18:3(n-3) (ALA)	1.98	2.56
20:0	0.37	0.29
20:1	0.00	0.68
20:2	0.00	0.04
20:5(n-3) (EPA)	0.00	0.00
22:0	0.34	0.24
22:2	0.08	0.00
22:5(n-6) (DPA)	0.16	0.00
24:0	0.00	0.04

0.01 mmol/L minimum), total cholesterol (Wako Pure Chemical Industries Industries, catalog no. 993–00404, 0.002 mmol/L minimum), LDL cholesterol (Wako Pure Chemical Industries Industries, catalog no. 993–00404, 0.03–10.4 mmol/L) and HDL cholesterol (Diagnostic Chemical, catalog no. 258–20, 0.05–3.9 mmol/L) were measured using direct colorimetric chemical enzymatic reactions. Haptoglobin was assayed by an enzymatic procedure (Tridelta Development, TP 801). Plasma glucose was measured as per the glucose oxidase method (Diagnostic Chemical, catalog no. 220–32, 0.03–33.3 mmol/L). Plasma insulin (Ultrasensitive rat insulin ELISA, Mercodia, catalog no. 80-INSRTU-E01, 0.03–1.0 pmol/L) was determined using commercially available enzymatic immunoassays for rodents. Serum interleukin (IL)-6 and IL-10 were measured using BD OptEIA ELISA kits [BD Biosciences, PharMingen, 550319 (IL-6) and 555134 (IL-10); 0.2 nmol/L minimum]. Samples were analyzed using assay kits from a single lot and performed in 1 batch. Samples were measured in triplicate, except cytokines, which were measured in duplicate, with an intra-assay CV of <5%.

Statistical analysis. All results are expressed as means ± SEM. Data were tested for normal distribution and differences between lean and obese groups as well as between control and VA treatment groups were analyzed using 2-way ANOVA followed by Bonferroni post hoc tests. The MMT procedure was assessed by AUC analysis. The level of significance was set at $P < 0.05$ (Graph Pad Prism 4.0).

Results

Food intake and body weight gain. At the end of the 3-wk study, food consumption and body weight gain were lower in lean rats (17.0 ± 0.7 g/d and 54 ± 2.3 g, respectively) than in obese JCR:LA-*cp* rats (36.7 ± 1.3 g/d and 119.9 ± 3.9 g, respectively) ($P < 0.0001$). Increased dietary VA did not significantly affect food intake or weight gain of rats irrespective of genotype.

Fatty acid in epididymal adipose tissue. The amount of *cis*9, *trans*11-CLA in adipose tissue triglyceride was 6.5-fold higher in both lean and obese rats fed VA than in control diet groups (from 0.04 ± 0.01 to 0.3 ± 0.04 and from 0.04 ± 0.02 to 0.3 ± 0.01, respectively) (**Table 3**). Moreover, lean rats had a higher concentration of VA in adipose tissue (8-fold increase from 0.4 ± 0.01 to 3.6 ± 0.2) than obese rats (1.5-fold increase from 0.4 ± 0.02 to 1.0 ± 0.3) when fed the VA diet. Thus, the ratio of VA:CLA in triglycerides from adipose tissue was identical (10.0) for both lean and obese rats from each of the control diet groups. However, the VA diet resulted in a lower ratio of VA:CLA in triglycerides from adipose tissue in obese rats compared with lean rats (3.3 and 12.0, respectively). Interestingly, adipose tissue from lean control rats had less oleic acid ($P < 0.001$) and more LA ($P < 0.001$) and ALA ($P < 0.05$) compared with adipose tissue from obese control rats. Further, oleic acid in adipose tissue from lean rats fed the VA diet was lower ($P < 0.01$), whereas LA was greater ($P < 0.001$) compared with the lean rats fed the control diet.

TABLE 3 Fatty acid composition of adipose tissue triglycerides in lean and obese JCR:LA-*cp* rats fed control or VA diet for 3 wk[1]

	Lean control diet	Lean VA diet	Obese control diet	Obese VA diet
		% total fatty acids		
VA	0.4 ± 0.01	3.6 ± 0.2***	0.4 ± 0.02	1.0 ± 0.3[c]***
18:1(n-9)*c*	31.6 ± 0.3	26.3 ± 0.4***	35.3 ± 0.2[c]	33.7 ± 0.4[c]
18:2(n-6)	29.4 ± 0.2	32.6 ± 0.4***	16.5 ± 0.4[c]	16.2 ± 0.5[c]
18:3(n-3)	2.0 ± 0.1	2.3 ± 0.1	1.4 ± 0.3[a]	1.4 ± 0.04[b]
18:2,9*c*11*t*	0.04 ± 0.01	0.3 ± 0.04***	0.04 ± 0.02	0.3 ± 0.01***

[1] Values are means ± SEM, $n = 10$. Superscript letters indicate different from the corresponding lean group: [a]$P < 0.05$, [b] $P < 0.01$, [c]$P < 0.001$. Asterisks indicate different from the corresponding control diet group: ***$P < 0.001$.

Fasting plasma lipid and serum inflammatory markers. The fasting plasma triglyceride concentration in obese rats (*cp*/*cp*) supplemented with 1.5% VA was ~40% lower than in obese rats fed the control diet (**Table 4**; $P < 0.05$). Plasma lipids were not affected by dietary VA in lean rats. Serum IL-6 concentration did not differ between the VA-treated and control dietary groups, whereas serum Il-10 concentration decreased by VA treatment regardless of genotype ($P = 0.02$). Haptoglobin concentration was higher in obese rats than in lean rats ($P < 0.05$), but dietary VA had no effect.

Glucose and insulin metabolism. Dietary VA did not significantly affect fasting plasma glucose or insulin concentrations (Table 4). The metabolism of glucose or insulin was not altered, as assessed by AUC throughout the meal tolerance test (MTT). Euglycemia was maintained with no change in insulin postprandial excursion.

Discussion

Increased intake of *trans* fatty acids from hydrogenated vegetable oils (e.g. elaidic acid) has consistently been shown to be related to increased coronary heart disease risk, incidence of myocardial infarction, elevated LDL cholesterol concentration, and small-dense LDL particles (16,17,22,23). However, few studies have drawn a consistent conclusion on the potential health effects of naturally derived *trans* fats, in particular VA, and its relationship with CVD risk. Bauchart et al. (27) have reported that VA/CLA-enriched butter had a neutral effect on the risk of atherogenesis related to plasma lipoprotein profile in hamsters, whereas *trans*-10 18:1 butter enhanced pro-atherogenicity. However, it is feasible that the VA diet described by Bauchart et al. may have been confounded by differences in either CLA and/or the amounts of hydrolyzed fat used. It is also curious that Meijer et al. (28) ascribed no beneficial effect to blood lipids in hamsters when comparing diets that contained 10% of energy originating from either VA or elaidic acid. In contrast, several other animal or human studies have indicated that feeding VA-enriched dairy products may have neutral health effects or may even improve plasma lipid profile related to reducing atherosclerotic risk. Lock et al. (29) showed that increased intake of VA (15% of total fat) was associated with a reduced risk of atherosclerosis by improving plasma lipoprotein profile in cholesterol-fed hamsters. Further, Tholstrup et al. (25) showed that feeding butter with a higher content of VA significantly lowered total cholesterol in healthy men. In very recent studies, 2 independent research groups both suggest that *trans* fatty acids from natural sources had neutral to beneficial effects on risk factors of CVD with modest consumption in humans (41,42). Whereas the evidence from these important clinical studies provided proof-of-concept, they may also be limited due to the potential discrepancy of other dietary bioactive fatty acids such as SFA, CLA, and oleic acid. It is also noteworthy that many of the clinical VA-related studies have included subjects with relatively normal blood lipid profiles, which may have limited the potential of VA to improve these variables.

In our current study, we have shown that, distinct from industrially produced *trans* fatty acid, a diet containing 1.5% (wt:wt) purified VA (10% of total fat) did not result in any adverse effects on body weight, food consumption, and/or inflammatory

TABLE 4 Plasma lipid concentrations from food-deprived rats, serum inflammatory markers, and glucose and insulin AUC after MTT in JCR:LA-*cp* rats treated with control or VA diet[1]

	Lean control diet	Lean VA diet	Obese control diet	Obese VA diet
Triglyceride, *mmol/L*	0.5 ± 0.04	0.6 ± 0.07	4.1 ± 0.6[a]	2.7 ± 0.3[a]*
Total cholesterol, *mmol/L*	2.4 ± 0.1	2.4 ± 0.1	5.8 ± 0.3[a]	5.1 ± 0.2[a]
LDL cholesterol, *mmol/L*	0.4 ± 0.03	0.6 ± 0.08	1.1 ± 0.1[a]	0.9 ± 0.1[a]
IL-6, *nmol/L*	2.8 ± 0.6	2.0 ± 0.6	2.3 ± 0.4	2.6 ± 0.4
IL-10, *nmol/L*	0.4 ± 0.09	0.4 ± 0.07	0.3 ± 0.08	0.1 ± 0.05*
Haptoglobin, *pmol/L*	8.4 ± 1.1	8.1 ± 0.5	21.0 ± 4.7[a]	17.0 ± 3.1[a]
Glucose, *mmol/L·h*	114.6 ± 6.4	123.9 ± 11.3	129.2 ± 5.2	134.8 ± 11.1
Insulin, *pmol/L·h*	11320 ± 1973	11107 ± 766.2	335381 ± 58081[a]	344827 ± 72513[a]

[1] Values are means ± SEM, $n = 10$. Superscript letters indicate different from the corresponding lean group: [a]$P < 0.001$. Asterisks indicate different from the corresponding control diet group: *$P < 0.05$.

status under either normolipidemic or hyperlipidemic conditions in JCR:LA-*cp* rats. Fasting levels of insulin or glucose, or corresponding postprandial metabolism in response to a MTT, were not affected by VA, consistent with recent findings by Tardy et al. (43). Interestingly, we have shown that VA significantly reduced plasma triglycerides in JCR:LA-*cp* rats, supporting a beneficial effect on CVD risk. We speculate that longer term feeding of VA may have a greater potential to influence cholesterol metabolism in this model. We are also aware that the VA diet in our study contained 50% less oleic acid than the control diet due to the substitution of VA. Consequently, the improvement in lipid profile could not be attributed to the presence of oleic acid alone, which has been previously shown to improve postprandial glucose response and lipid profiles (44). Similarly, the discrepancy between the amount of oleic acid in epididymal fat pads from the control and VA groups could be the result of differential dietary oleic acid as opposed to an effect of VA per se.

The bioconversion of VA to CLA has been calculated previously in different animal species. Early reports have shown that ~5–12% of VA is converted to CLA in rodents, which have been previously reviewed (3). In humans, conversion has been reported to be within the range of 19–30% (3). In our study, feeding VA for 3 wk led to 8.0- and 1.5-fold increases in VA incorporation into adipose tissue in lean and obese rats, respectively. In addition, we observed a 6.5-fold increase in CLA in triglycerides from adipose tissue compared with the control group. Interestingly, lean rats had a greater ratio of VA to CLA than the obese rats, which may reflect either a greater incorporation of VA or a lower conversion to CLA. One of the limitations of this study was that both VA and CLA concentrations were not monitored continuously throughout the study, which would have enabled us to evaluate the incorporation and conversion rate of VA (3). Although it is plausible that indirect endogenous production of CLA from dietary VA may have mediated the hypolipidemic effects observed in our study, we did not find any corresponding change in body weight, food intake, or insulin sensitivity, which are distinctive biological effects of dietary CLA supplementation in this animal model and others (45,46). Therefore, it is our contention that the substantial hypolipidemic benefits of VA treatment were not caused by indirect bioconversion to CLA but rather by the direct dietary supplementation of VA.

Because obesity and diabetes are associated with impaired inflammatory regulation, the concentrations of serum cytokines (e.g. IL-6, IL-10) and acute phase proteins such as haptoglobin are regarded as important proinflammatory biomarkers (47,48). Indeed, others have shown that CLA has antiinflammatory effects (49–51). In this study, feeding VA resulted in reduced serum IL-10 concentration, indicating a potential direct antiinflammatory effect of VA on inflammatory regulation, for which the mechanisms remain unclear (52).

When considering the potential mechanistic properties of VA, it is important to note that VA acts as a precursor for the endogenous synthesis of CLA in animals and humans. It is plausible that both these fatty acids may regulate similar hepatic or intestinal lipogenic pathways to mediate hypolipidemic effects. There is emerging evidence that CLA is one of the few known naturally occurring agonists of both PPARα and PPARγ (53–56). Therefore, it would seem reasonable to speculate that VA may play a role in regulating PPARα. Additionally, there is evidence to suggest that VA could inhibit the activity of acetyl-CoA carboxylase, fatty acid synthase, or both, and these are the subject of ongoing studies (57,58).

In conclusion, short-term feeding of 1.5% (wt:wt) VA did not result in any detrimental health effects in either lean or obese rats, and thus distinguishes this natural *trans* fatty acid from commercially hydrogenated sources of *trans* fats. Further, dietary VA supplementation leads to a significantly decreased circulating plasma triglyceride concentration in JCR:LA-*cp* rats, an established rodent model of metabolic syndrome. Consequently, we propose that VA may have substantial hypotriglyceridemic benefits under conditions of dyslipidemia. These observations contribute to the hypothesis that industrial and ruminant *trans* fatty acids have differential bioactivity that warrants further investigation.

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