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RESEARCH ARTICLE

The intestinal bioavailability of vaccenic acid and activation of peroxisome proliferator-activated receptor-α and -γ in a rodent model of dyslipidemia and the metabolic syndrome

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Scope: Evidence suggests a neutral to beneficial role of certain *trans* fatty acids (TFA) from natural ruminant sources. *Trans*11–18:1 (vaccenic acid, VA), the most predominant ruminant TFA and a precursor to conjugated linoleic acid, has been shown to improve atherogenic dyslipidemia and symptoms of hepatic steatosis in animal models. The objective of this study was to assess the intestinal bioavailability of various VA sources including synthetic free fatty acid (FFA) and natural ruminant triglyceride forms, as well as the mechanistic pathways that mediate VA's bioactivity.

Methods and results: VA acts as a partial agonist to both peroxisome proliferator-activated receptors (PPAR)-α and PPAR-γ in vitro, with similar affinity compared to commonly known PPAR agonists. It was further confirmed that VA at 30 and 100 μM concentrations suppressed cardiomyocyte hypertrophy vitro in a PPAR-α- and PPAR-γ-dependent manner. In vivo, feeding of VA (1%, w/w) resulted in increased mRNA and protein expression of PPAR-γ in the mucosa of JCR:LA-*cp* rats, a model of the metabolic syndrome ($p < 0.01$ and $p < 0.05$, respectively) compared to control. In addition, VA from a triglyceride source had greater intestinal bioavailability in vivo compared to VA provided in an FFA form ($p < 0.01$).

Conclusion: The activation of PPAR-α- and PPAR-γ-dependent pathways provides a mechanistic explanation of how VA improves blood lipids and related metabolic disorders during conditions of hyperlipidemia. This report also supports the consideration of differential reporting of industrially produced versus natural TFA on food nutrient labels.

Keywords:
Conjugated linoleic acid / Dyslipidemia / Metabolic syndrome / Peroxisome proliferator-activated receptor / Vaccenic acid

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Abbreviations: **ABC**, ATP binding cassette; **ACC**, acetyl-CoA carboxylase; **ALA**, α-linolenic acid; **CLA**, conjugated linoleic acid; **CVD**, cardiovascular disease; **DHA**, docosahexaenoic acid; **EC_{50}**, half-maximal effective concentration; **ET-1**, endothelin-1; **FAS**, fatty acid synthase; **FAT/CD36**, fatty acid translocase/cluster determinant 36; **FATP**, fatty acid transfer protein; **FDA**, Food and Drug Administration; **FFA**, free fatty acid; **GAPDH**, glyceraldehyde-3-

1 Introduction

Cardiovascular disease (CVD) has become the leading cause of death worldwide, accounting for approximately 30% of all mortalities [1]. Over the past few decades, epidemiological and biochemical evidence has suggested that *trans* fatty acid (TFA) consumption (particularly from partially hydrogenated vegetable oils or PHVOs), through its effects on dyslipidemia and inflammation, is causatively related to increased CVD and a higher prevalence of obesity and the metabolic syndrome (MetS) [2]. In contrast, specific TFA from natural ruminant products such as butter, beef, and dairy have been shown to display neutral to beneficial health effects, suggesting that natural TFA have differential bioactivity compared to industrially produced TFA in PHVO [3,4]. Conjugated linoleic acid (CLA) was the first ruminant TFA (rTFA) to be recognized as having anticarcinogenic and lipid-lowering effects, and was consequently excluded from most TFA-related legislation in European countries such as Denmark, as well as North America [5]. Notably, *trans*11–18:1 (vaccenic acid or VA) is the most prominent rTFA in ruminant-based foods and is the precursor to CLA; however in recent years, the bioactive effects of this natural fatty acid has received limited scientific or public attention. Unlike CLA, VA continues to be categorized with other TFA on food labels, despite VA's potential health benefits.

Emerging studies in animal models have suggested that chronic dietary supplementation with synthetic VA can exert effects, which improve hyperlipidemia, hepatic steatosis, postprandial lipemia, and inflammatory dysregulation [6–9]. A plasma cholesterol-lowering effect of dietary VA has also been reported in the hamster model [10]. Despite these reports on the hypolipidemic effects of VA, the molecular mechanism of VA's bioactivity remains unclear. However, the improvement in plasma lipid profile and inflammatory regulation observed in these studies [7–9] resembles those reported for fenofibrate and thiazolidinedione (TZD) during conditions of MetS [11]. Fibrates and TZD are chemically synthesized compounds that have been widely used in clinical practice to treat patients with two components of MetS: dyslipidemia and insulin resistance. TG-lowering fibrates (e.g., fenofibric acid) bind and activate peroxisome proliferator-activated receptor (PPAR)-α and enhance fatty acid catabolism in the liver and peripheral tissues. In contrast, the insulin-sensitizing TZD family (e.g., pioglitazone, rosiglitazone) is a class of potent PPAR-γ activators, which improve whole-body insulin sensitivity and inflammatory regulation [11]. Fatty acids such as docosahexaenoic acid (DHA) and *cis*9,*trans*11-CLA function as natural PPAR agonists, thereby regulating lipid metabolism [12–14]. In addition to improving major lipid risk factors for CVD, CLA isomers suppress endothelin-1 (ET-1) induced cardiomyocyte hypertrophy due to robust PPAR-α and PPAR-γ but not PPAR-β/δ activation [15]. Given the known mechanisms of action for CLA, fibrates, and the TZD family, it has been proposed that VA may also act as a potential PPAR ligand mediating the hypolipidemic and cardioprotective effects observed in animal models [6,7].

A number of clinical intervention trials have explored the potential health benefits of dietary VA-enriched ruminant fat, but these studies have failed to show consistent improvements in dyslipidemia [16–18]. It was proposed that the hypolipidemic bioactivity of rTFA may be limited to study participants that have severe hyperlipidemia. On the other hand, the divergent bioavailability of VA from different forms [triglyceride (TG) versus free fatty acid (FFA)] may have caused the inconsistencies observed in clinical trials.

In order to address these questions, the objectives of this study were (i) to determine the interaction of VA and PPAR-α/γ in in vitro using competitive binding assays, coactivator recruitment assays and a cell model of cardiomyocyte hypertrophy; (ii) to assess the effect of VA in vivo on regulating intestinal mRNA and protein abundance of PPAR and other lipogenic target proteins (iii) to compare the intestinal bioavailability of VA provided as an FFA and a TG source in vivo, using an established animal model of MetS, the JCR:LA-*cp* rat [19].

2 Materials and methods

2.1 Vaccenic acid sources

Purified VA in the form of FFA was synthesized by Dr.'s Shen and Reaney (University of Saskatchewan) using the Wittig reaction of methyl ester yilid and heptanal under phase transfer catalysis conditions [20]. Beef meat fat was used as a source of natural ruminant VA in TG, and was derived from pooled sources of beef meat high in VA (5.8% of total fatty acids, Table 1) kindly provided by Dr. Shannon Scott (Agriculture and Agri-Food Canada Brandon Research Center).

2.2 Animal model and dietary study design

Male rats of the JCR:LA-*cp* strain, the control lean (+/?) or the obese MetS (*cp/cp*) phenotype, were raised in the established breeding colony at the University of Alberta as previously described [21]. 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

phosphate dehydrogenase; **IC_{50}**, half-maximal inhibitory concentration; **I-FABP**, intestine-type fatty acid binding protein; **LA**, linoleic acid; **LDL-r**, low-density lipoprotein receptor; **L-FABP**, liver-type fatty acid binding protein; **MetS**, metabolic syndrome; **MTP**, microsomal triglyceride transfer protein; **NPC1L1**, Nieman Pick C 1-like protein 1; **OA**, oleic acid; **PHVO**, partially hydrogenated vegetable oil; **PPAR**, peroxisome proliferator-activated receptor; **rTFA**, ruminant *trans* fatty acid; **SREBP**, sterol response element binding protein; **SR-BI**, scavenger receptor B-type I; **TFA**, *trans* fatty acid; **TG**, triglyceride; **TR-FRET**, time-resolved fluorescence resonance energy transfer; **TZD**, thiazolidinedione; **VA**, vaccenic acid

Table 1. Fatty acid composition of the vaccenic acid enriched beef meat fat

	Percentage of total fatty acids
14:0	2.6
16:0	22.2
*cis*9–16:1	3.2
18:0	19.2
VA	5.8
*cis*9–18:1	39.2
*cis*11–18:1	1.7
20:0	2.3
*cis*9,*trans*11-CLA	0.8

Table 2. Ingredients of the control and the VA diet

Ingredient	Control diet (g/kg)	VA 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)[a)]	50.2	50.2
Vitamin mix, A.O.A.C. (no. 40055)[a)]	9.9	9.9
Inositol	6.2	6.2
Choline chloride	1.3	1.3
Cholesterol	10.0	10.0
Sunflower oil	55.0	56.0
Flaxseed oil	4.5	4.5
Soy tallow	76.2	76.5
Olive oil	14.3	0.0
VA oil	0.0	13.0

a) Composition of diets have been previously reported [7].

and Use Committee (PROCTOR-001). At 8 weeks of age, MetS rats ($n = 16$) were randomized to either a control diet with no VA (MetS) or a semipurified VA diet containing 1.0%, w/w VA in the FFA form (MetS-VA) for 16 weeks. Lean littermates ($n = 8$) were fed the control diet (Control) for the same period of time. The ingredients and fatty acid composition of diets are shown in Tables 2 and 3. Relevant food intake and body weight data have been previously reported [7]. At the end of the feeding period rats were euthanized; the jejunum and liver of each rat were harvested.

2.3 Jejunal enterocyte isolation and PPAR-α and PPAR-γ protein and mRNA analysis

Primary enterocytes were separated from frozen jejunum samples (fractions 1–10) for protein quantitation using the

Table 3. Fatty acid composition of the control and the VA diet[a)]

Fatty acid	Control diet (g/100 g total fatty acids)	VA diet (g/100 g total fatty acids)
16:0	9.1	8.9
16:1	0.1	0.1
18:0	47.0	47.1
18:1 (*cis*9)	17.3	9.0
18:1 (*trans*11-VA)	0.0	6.0
18:2 (n-6, LA)	23.4	24.7
18:2 (*cis*9,*trans*11-CLA)	0.0	0.0
18:3 (n-3)	1.6	1.9
20:0	0.4	0.3
20:2 (n-6)	0.1	0.1
SFA[b)]	57.6	57.3
MUFA	17.4	16.1
PUFA	25.0	26.7
P/S ratio[c)]	0.43	0.46
(n-6)/(n-3) PUFA ratio	14.9	13.4

a) Verified by GC after the oil mixture were prepared.
b) Saturated fatty acids.
c) PUFA/SFA ratio.

Weiser method [22], and mucosal tissue was frozen for mRNA extraction. To prepare primary enterocytes for protein analysis, samples (50 mL) from each enterocytic fraction were pooled and homogenized in 200 mL of lysis buffer [PBS (pH 7.4) with 1.5% Triton X-100 and 1% protease inhibitor cocktail (catalog no. P8340, Sigma-Aldrich, Oakville, ON, Canada)]. The homogenate was centrifuged at 700*g* for 15 min and the supernatant was collected and stored at −80°C for further analysis. PPAR-α and PPAR-γ protein abundance was determined by Western blot analysis as previously described [7]. In brief, Western blot membranes were incubated with primary antibodies raised against PPAR-α (1:1000 dilution, catalog no. sc-9000, Santa Cruz Biotechnology, Santa Cruz, CA) and PPAR-γ (1:1000 dilution, catalog no. sc-7196, Santa Cruz Biotechnology) and β-actin (1:20 000 dilution, internal control, catalog no. A5441, Sigma-Aldrich). Adherent antibodies were visualized by chemiluminescence using ECL Advance Western Blotting Detection kit (catalog no. RPN2135, GE Healthcare, Buckinghamshire, UK). Protein bands were quantified using Image J (National Institute of Health, Bethesda, MD, USA, version 1.41). The final value of PPAR-α and PPAR-γ relative protein abundance was normalized based on the respective β-actin protein mass.

Total RNA was extracted from jejuna mucosa using the RNeasy Mini kit (catalog no. 74104, Qiagen, Valencia, CA) and reverse transcribed to cDNA per manufacturer's instructions (high capacity cDNA reverse transcription kit, catalog no. 4368814, Applied Biosystems, Carlsbad, CA). Real-time PCR was performed using TaqMan Gene Expression Assays (Applied Biosystems). Relative mRNA expression of genes related to lipid metabolism were assessed, which included *PPAR*-α, *PPAR*-γ, sterol response element binding protein (*SREBP*)-*1*, *SREBP-2*, fatty acid synthase (*FAS*), acetyl-CoA

carboxylase (*ACC*)-*1*, microsomal TG transfer protein (*MTP*), apolipoprotein (*apo*) *B*, fatty acid translocase (*FAT/CD36*), fatty acid transport protein-4 (*FATP-4*), Nieman Pick C 1 like protein 1 (*NPC1L1*), liver-type fatty acid binding protein (*L-FABP*), intestine-type fatty acid binding protein (*I-FABP*), ATP-binding cassette (*ABC) A-1*, *ABCG-5*, *ABCG-8*, scavenger receptor B-type I (*SR-BI*), low-density lipoprotein receptor (*LDL-r*). Data were normalized to the expression of a housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and data is presented as fold change relative to the MetS group.

2.4 Hepatic mRNA quantification of PPARs and their target genes

Total RNA was isolated from frozen liver samples using Trizol™ reagents (catalog no. 15596-026, Invitrogen, Burlington, ON, Canada) as described in the manufacturer's protocol. Total RNA was reverse transcribed into cDNA with Moloney Murine Leukaemia Virus reverse transcriptase (catalog no. 28025-013, Invitrogen) for real-time PCR in a quantitative high-throughput system (Fluidigm corporation, San Francisco, CA). The expression of PPAR-α, PPAR-γ, as well as a number of PPAR-α target genes were measured including medium-chain acyl-CoA dehydrogenase (ACADM), long-chain acyl COA dehydrogenase (ACADL), peroxysomal enzyme acyl-CoA oxidase (ACOX1), and carnitine palmitoyltransferase 1(CPT1)-α and were normalized to GAPDH.

2.5 PPAR-α and PPAR-γ competitive binding assays

PPAR-α (catalog no. PV4892, Invitrogen, Carlsbad, CA) and PPAR-γ (catalog no. PV4894, Invitrogen) competitive binding assays were performed in vitro using LanthaScreen™ time-resolved fluorescence resonance energy transfer (TR-FRET) assays according to the manufacturers' instructions. Competitive ligand binding to the receptor was detected by a test compound's ability to displace the tracer from the receptor, resulting in a loss of fluorescent signal between the antibody and the tracer and quantified by TR-FRET ratio. Measurement was performed on a SpectroMax M5 microplate reader (Molecular Devices, Sunnyvale, CA) with instrument settings as recommended from LanthaScreen™ TR-FRET assays. The following ligand compounds were tested: VA, *cis*9,*trans*11-CLA (Lipid Nutrition, Channahon, IL), oleic acid (OA, catalog no. O1008, Sigma-Aldrich), DHA (catalog no. U-84-A, NuChek Prep, Inc. Elysian, MN), fenofibric acid (catalog no. F-06060, A.S. Chemical Laboratories Inc., ON, Canada), rosiglitazone (catalog no. 71740, Cayman Chemical, Ann Arbor, MI), and pioglitazone (catalog no. E6910, Sigma-Aldrich). Test compound solutions were prepared in serial dilutions from 0.1 nM to 100 mM (1% final DMSO concentration). GW7647 (a known PPAR-α agonist, catalog no. 1677, Tocris Bioscience, Ellisville, MS), GW1929 (a known PPAR-γ agonist, catalog no. 1664, Tocris Bioscience) are used as the positive control for PPAR-α and PPAR-γ assays, respectively. Assays were performed in duplicates and validated by determining the respective Z′-factors per manufacturer's instructions. The half-maximal inhibitory concentration (IC_{50}) value of each test compound was assessed using a sigmoidal dose–response equation (GraphPad Prism 5.0, GraphPad Software, La Jolla, CA, USA).

2.6 PPAR-α and -γ coactivator assays

Similar to the competitive binding assays, PPAR-α (catalog no. PV4684, Invitrogen) and PPAR-γ (catalog no. PV4548, Invitrogen) coactivator assays were performed in vitro using Invitrogen LanthaScreen™ TR-FRET assays according to the manufacturers' instructions. The TR-FRET ratios were assessed by a SpectroMax M5 microplate reader. Binding of a potential agonist to the ligand-binding domain of PPAR would cause a conformational change, resulting in higher affinity for the coactivator peptide (PPAR-γ coactivator-1-α used in this particular assay) and greater TR-FRET ratios. VA, fenofibric acid, GW7646 were tested in the PPAR-α coactivator assays whereas VA, rosiglitazone, pioglitazone, and GW1929 were tested in the PPAR-γ coactivator assays. All compounds tested were from the same batch as those in PPAR competitive binding assays. The half-maximal effective concentration (EC_{50}) value of each test compound was assessed using a sigmoidal dose–response equation (GraphPad Prism 5.0) per manufacturer's instructions.

2.7 Cell culture of neonatal rat ventricular cardiomyocytes and cell-size assessment

Ventricular cardiomyocytes were isolated from 1-day-old neonatal Sprague Dawley rats by digestion of ventricles with several cycles of 0.05% trypsin and mechanical disruption as previously described [15]. Myocytes were cultured in DMEM containing 10% cosmic calf serum (Hyclone, catalog no. SH3008703) on glass cover slips (2×10^6 cells/cover slip), serum-deprived for 24 h, treated with vehicle or VA (0–100 μM; 1 h) before hypertrophy induced by ET-1 (0.1 μM, 24 h catalog no. 135842–15-8, Enzo Life Sciences, Farmingdale, NY). ET-1 is a potent vasoconstrictor peptide derived from vascular endothelial cells and cardiomyocytes. It induces cardiomyocyte hypertrophy through activating a number of pathways involving phospholipase C, protein kinase C, and extracellular signal-regulated kinase 1/2 [23], which could be suppressed by PPAR-α and/or PPAR-γ agonists [24, 25]. Two subgroups of myocytes were pretreated with either GW9662, a PPAR-γ-specific antagonist (1.0 μM, catalog no. M6191, Sigma-Aldrich) or MK886, a PPAR-α-specific antagonist (1.0 μM, catalog no. EI-266, Enzo Life Sciences)

Table 4. Major fatty acid composition of VA-meal emulsion and beef fat meal emulsion[a)]

	VA-meal emulsion	Beef fat meal emulsion
14:0	0.2	2.4
16:0	8.9	20.3
*cis*9–16:1	0.2	3.0
18:0	44.9	15.7
*cis*9–18:1	9.9	34.6
*cis*11–18:1	0.4	1.2
VA	6.3	3.2
*cis*9,*cis*12–18:2n-6	22.7	2.4
*cis*9,*trans*11–18:2n-6	0	0.8
18:3n-3	1.7	0.3

a) Values expressed as percentage of total fatty acids.

before the addition of vehicle or VA (30 μM; 1 h) and subsequent hypertrophy induction with ET-1. Cells were immunostained with anti-rat sarcomeric α-actinin, and visualized by fluorescence microscopy. Myocyte size was assessed by and computer-assisted planimetry as previously described [15]. Pixel values were converted to surface area (μm^2) by multiplying by scale factors of the *x*- and *y*-axes.

2.8 Lipid-meal emulsion preparation for bioavailability assessment

The following lipid or meal emulsions were prepare: (1) VA-triolein emulsion containing 7.5 mg synthetic VA in 500 μL triolein (catalog no. 44895-U, Sigma-Aldrich); (2) VA-meal emulsion containing the semipurified diet used above (1.0%, w/w synthetic VA); and (3) beef fat meal emulsion containing a semipurified diet supplemented with beef fat high in VA (Table 4). Both semipurified meal emulsions contained equivalent contents: 1% cholesterol, 43.1% carbohydrate, 28% protein, 8% fiber, and 15% lipid (w/w) with a PUFA:SFA ratio of 0.6 and (n-6):(n-3) PUFA ratio of 10, and the respective fatty acid composition was verified by GC as shown in Table 4. All emulsions were freshly prepared within 24 h of scheduled use for bioavailability studies, and were flushed with N_2, covered in foil and stored at 4°C to prevent light exposure.

2.9 Bioavailability and lymphatic-gastronomy surgical protocol

In order to elicit conditions reflective of a western dietary fat intake and intestinal lipid exposure for bioavailability studies, both lean and MetS rats were prefed for 3 weeks the control diet (Tables 2 and 3) before lymphatic-gastronomy surgery. Following an overnight fast, the gastroduodenal axis and the superior mesenteric lymph duct of each rat were cannulated as previously described [26]. After surgery and recovery, MetS rats ($n = 15$) were randomized to receive one of the three emulsions via a gastric cannula to compare the bioavailability of different forms of VA under MetS and dyslipidemic conditions. Control rats ($n = 5$) received the VA-triolein emulsion to determine the bioavailability of VA as an FFA in normolipidemic conditions. Each emulsion was warmed to approximately 37°C and infused into the gastric cannula. The emulsion container, syringe, and needle were weighed before and after infusion to accurately calculate the amount of emulsion delivered. Lymph was collected into EDTA-coated vacutainers for 10 h and was centrifuged at 400 × *g* at the end of the experiment to remove cell debris.

2.10 Fatty acid quantification in lymph and calculation of fatty acid bioavailability

Total lipid extraction from lymph was performed using adapted methods [27]. The fatty acid composition of the lipids extracted from diet emulsions and lymph samples was assessed by GC as previously described [28]. The bioavailability of VA refers to the proportion of dietary VA that was absorbed and incorporated into intestinal lymph chylomicron TG. The percentage intestinal bioavailability of VA and other fatty acids was calculated based on the absolute amount (μg) of each fatty acid quantitated in the lymph divided by the amount (μg) of fatty acid administered in the lipid-meal emulsion, and further expressed in terms of total mass of TG absorbed to adjust for interanimal differences in absorptive capacity.

2.11 Statistical analysis

All results are expressed as mean ± SEM. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test. The level of significance was set at $p < 0.05$ (Graph Pad Prism 5.0).

3 Results

3.1 Intestinal and hepatic PPAR-α and PPAR-γ expression

In MetS rats fed VA for 16 weeks, there was an 80 and 50% increase in PPAR-α and PPAR-γ relative mRNA abundance, respectively, in the jejunal mucosa (Fig. 1A) but not in the liver (Fig. 1B) compared to control and MetS rats fed the control diet. There was a consistent and significant increase in enterocytic protein abundance of PPAR-γ ($p < 0.05$, Fig. 2B) but only ascending trend for PPAR-α in VA-fed MetS rats relative to MetS control rats (Fig. 2A).

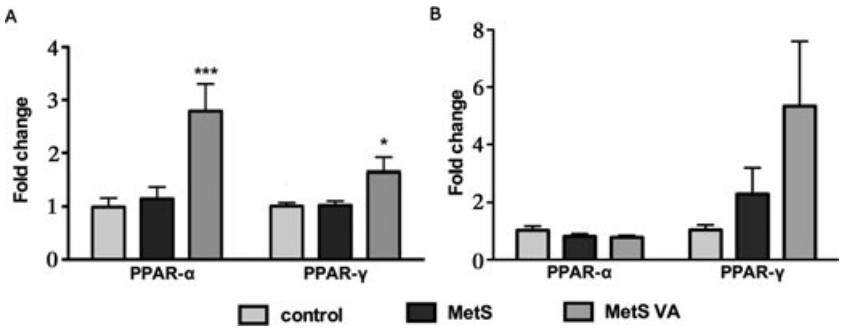

Figure 1. Jejunal mucosal mRNA expression of PPAR-α and PPAR-γ (A) in control and MetS JCR:LA-*cp* rats fed the control or VA diet. In a separate feeding trial with identical study design, control ($n = 8$) and MetS JCR:LA-*cp* rats ($n = 6$) were fed isocaloric control or VA diet, with same macronutrient composition and comparable VA content. Liver was collected after overnight fasting and hepatic mRNA expression of PPAR-α and PPAR-γ measured (B) as previously described in Section 2.3. Values are expressed as mean ± SEM and normalized to GAPDH ($n = 8$ rats per group). $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to both control and MetS groups.

3.2 Jejunal mucosal expression of lipid metabolism genes

Relative gene expression of *FAT/CD36*, *ACC-1*, *FAS*, and *SREBP-1* were elevated in the jejunal mucosa of MetS rats fed VA for 16 weeks compared to MetS rats fed the control diet (Figs. 3 and 4). No difference between MetS and MetS-VA groups was detected in the mRNA expression of genes involved in fatty acid transport: *L-FABP*, *I-FABP*, or *FATP-4* (Fig. 3), chylomicron and lipid synthesis: *MTP*, *apoB48*, or *SREBP-2* (Fig. 4) or cholesterol trafficking: *NPC1L1*, *ABCG5/G8*, *ABCA-1*, *SR-BI*, or *LDL-r* (Fig. 5).

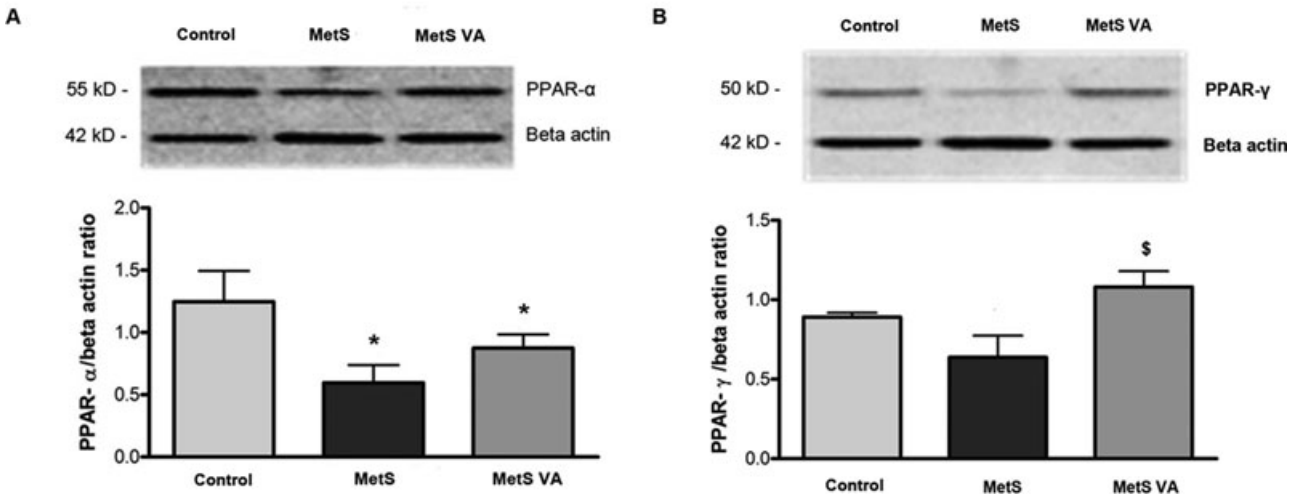

Figure 2. Relative intestinal protein abundance of PPAR-α (Panel A) and PPAR-γ (Panel B) in control and MetS JCR:LA-*cp* rats fed control or VA diet. Values are expressed as mean ± SEM and normalized to β-actin ($n = 4$ rats per group). $^{*}p < 0.05$ compared to the control; $^{\$}p < 0.01$ compared to the MetS group.

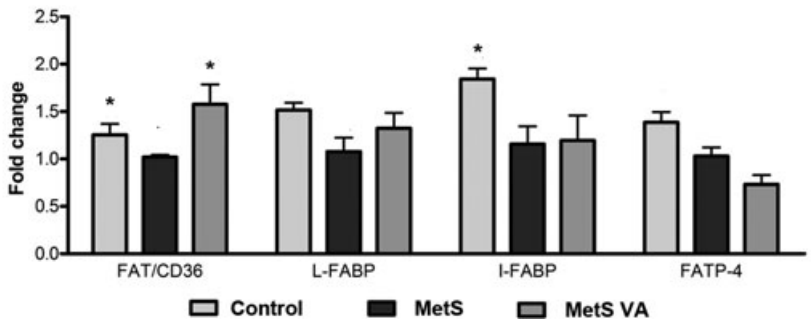

Figure 3. The mRNA expression of major lipid transporters in the jejunal mucosa of control and MetS JCR:LA-*cp* rats fed the control or VA diet. Values are expressed as mean ± SEM and normalized to GAPDH ($n = 8$ rats per group). $^{*}p < 0.05$ compared to the MetS.

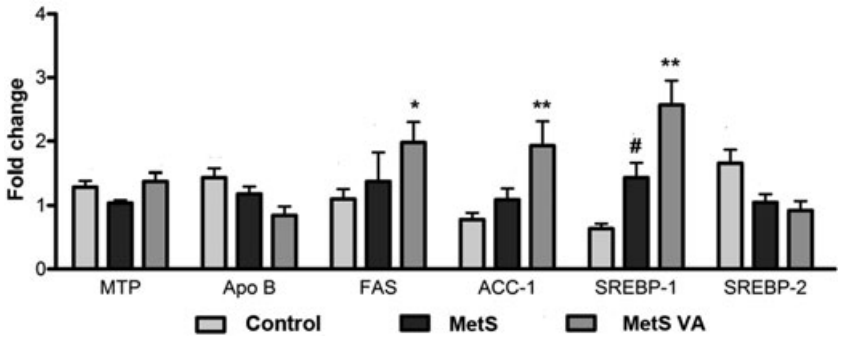

Figure 4. The mRNA expression of lipogenic genes in the jejunal mucosa of control and MetS JCR:LA-*cp* rats fed the control or VA diet. Values are expressed as mean ± SEM and normalized to GAPDH ($n = 8$ rats per group). $^{*}p < 0.05$ and $^{**}p < 0.01$compared to the MetS; $^{\#}p < 0.05$ compared to the control group.

3.3 Hepatic expression of PPAR-α target genes

The hepatic expression of a few genes involved in fatty acid oxidation pathways and regulated by PPAR-α was assessed, which include CPT1-α, ACADM, ACADL, and ACOX1. No difference was observed in these genes either between control and MetS rats, or in MetS rats fed with or without VA (Fig. 6).

3.4 Affinity of dietary and synthetic ligands to PPAR-α and PPAR-γ ligand-binding domain

The inhibition curves from TR-FRET competitive binding assay (Fig. 7A and B) indicate that VA is a potent ligand of the nuclear receptors PPAR-α and PPAR-γ in vitro. For PPAR-α, VA had an IC_{50} comparable to that of DHA, OA, *cis*9,*trans*11-CLA as well as a 1:1 mixture of VA and *cis*9,*trans*11-CLA (Table 5, Fig. 7A). Interestingly, the IC_{50} for fenofibric acid appeared to be five-fold higher than VA, suggesting that VA may bind much stronger to PPAR-α, at least in vitro, compared to this commonly known PPAR-α activator.

The affinity of different compounds for the PPAR-γ ligand-binding domain was also assessed (Fig. 7B). In contrast to the PPAR-α ligand-binding data, the IC_{50} of VA was double compared to DHA and 15% higher than OA for PPAR-γ. Interestingly, VA showed approximately 20% greater binding affinity to PPAR-γ compared to *cis*9,*trans*11-CLA and pioglitazone, the latter a potent PPAR-γ agonist and an insulin sensitizer previously prescribed in patients with type-2 diabetes [29].

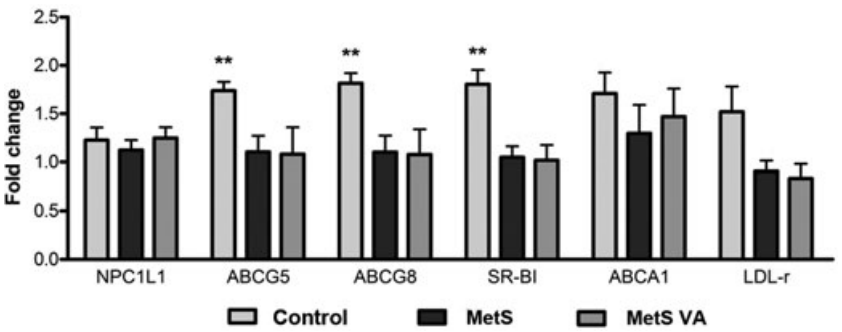

Figure 5. The mRNA expression of major lipid and cholesterol transporters in the jejunal mucosa of control and MetS JCR:LA-*cp* rats fed the control or VA diet. Values are expressed as mean ± SEM and normalized to GAPDH ($n = 8$ rats per group). $^{**}p < 0.01$ compared to the MetS control.

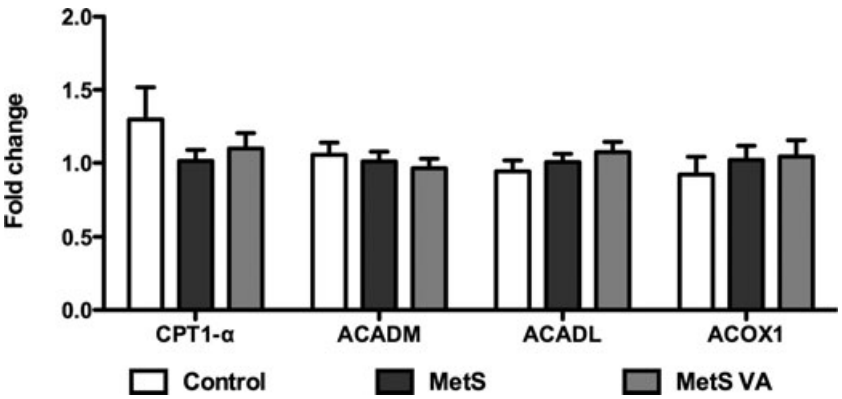

Figure 6. The mRNA expression of PPAR-α target genes involved in fatty acid oxidation in the liver of control and MetS JCR:LA-*cp* rats fed the control or VA diet. Values are expressed as mean ± SEM and normalized to GAPDH ($n = 8$ rats per group).

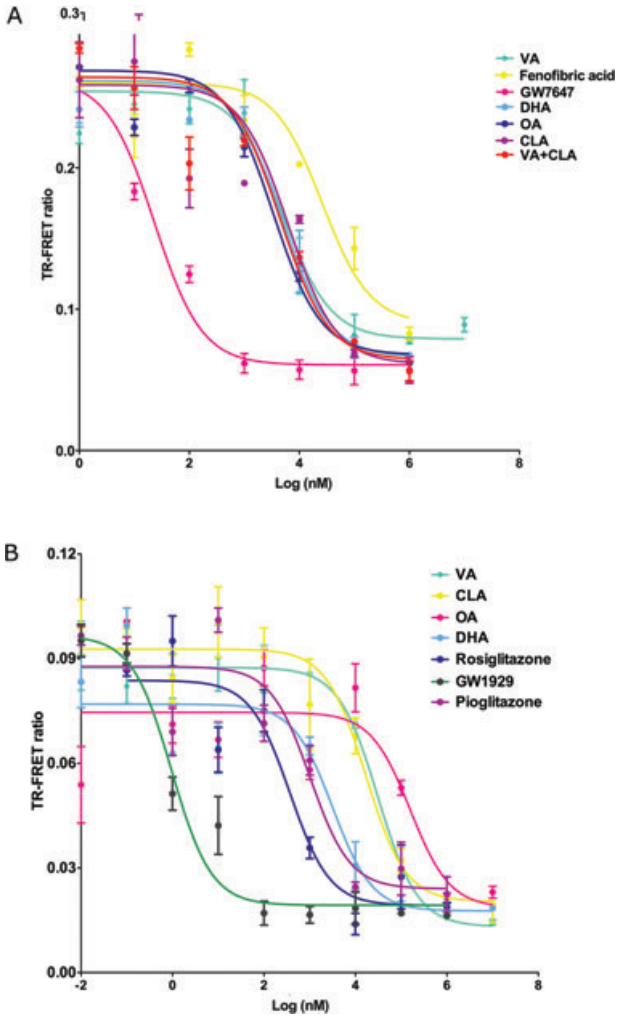

Figure 7. Competitive binding of test compounds to PPAR-α (A) and PPAR-γ (B) ligand-binding domain. *x*-Axis represents the natural logs of test compound concentrations in nM. The TR-FRET ratio in *y*-axis refers to the 520 nm/495 nm emission ratio.

3.5 Effect of VA on PPAR coactivator recruitment

Upon binding to the ligand-binding domains of PPAR-α and PPAR-γ, VA induced conformational change of the nuclear receptors similar to their respective agonists, and facilitated the subsequent recruitment of coactivator (i.e., PGC1-α; Fig. 8A and B). The EC_{50} of VA for PPAR-α is slightly lower than fenofibric acid and substantially higher than a full agonist GW7647 (Table 6). In addition, the maximal coactivator recruitment initiated by VA (as indicated by the plateau of the dose–response curve in Fig. 8A) was weaker than that by GW7647. Similarly for PPAR-γ, the EC_{50} of VA was lower than pioglitazone but significantly higher than two full agonists, rosiglitazone and GW1929 (Table 6, Fig. 8B). These data confirm that VA acts as a partial agonist for both PPAR-α and PPAR-γ in mediating its bioactivity.

Table 5. The IC_{50} values of PPAR-α and PPAR-γ ligands in the TR-FRET competitive binding assay[a)]

Ligand	PPAR-α (nM)	PPAR-γ (nM)
Rosiglitazone	-	368.8
Pioglitazone	-	1023
GW1929[b)]	-	0.9551
GW7647[c)]	31.41	-
Fenofibric acid	26 168	-
VA	4991	31 805
*cis*9,*trans*11-CLA	6241	16 854
VA + CLA mixture[d)]	4160	14 348
OA	3698	158 400
DHA	4845	3176

a) IC_{50} is the concentration of the fatty acid that produces 50% displacement of the tracer.
b) PPAR-γ agonist as the positive control.
c) PPAR- α agonist as the positive control.
d) VA + CLA mixture was composed of equal amounts of VA and *cis*9,*trans*11-CLA in the form of free fatty acids.

3.6 Effect of VA on cardiomyocyte hypertrophy

VA treatment at various concentrations had no effect on myocyte size before hypertrophy was induced (Fig. 9). ET-1

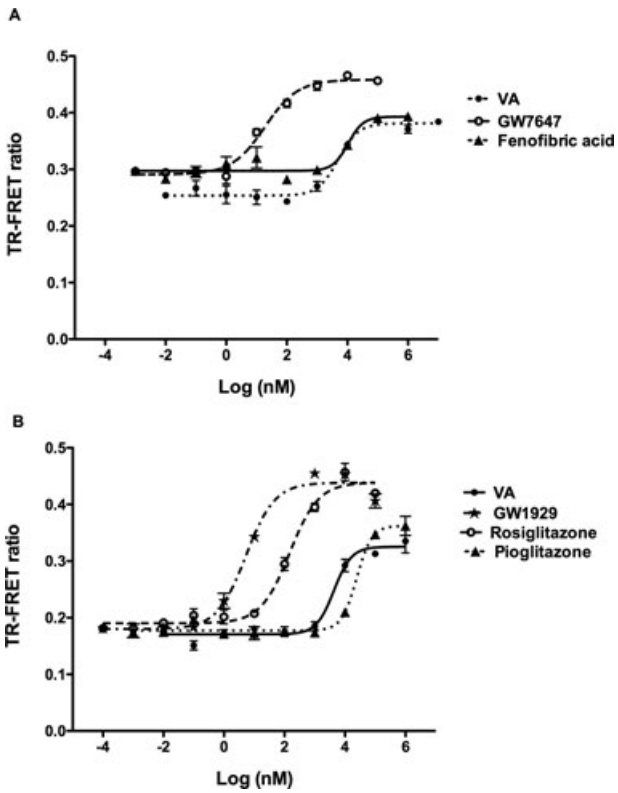

Figure 8. The PPAR-α (A) and PPAR-γ (B) coactivator recruitment assays using VA and known agonists for both nuclear receptors. *x*-Axis represents the natural logs of test compound concentrations in nM. The TR-FRET ratio in *y*-axis refers to the 520 nm/495 nm emission ratio.

Table 6. The EC_{50} values of PPAR-α and PPAR-γ ligands in the TR-FRET coactivator recruitment assay[a)]

Ligand	PPAR-α (nM)	PPAR-γ (nM)
Rosiglitazone	-	148.0
Pioglitazone	-	24 358
GW1929[b)]	-	5.312
GW7647[c)]	18.70	-
Fenofibric acid	10 506	-
VA	4924	4313

a) EC_{50} is the concentration of the fatty acid that produces 50% recruitment of the coactivator.
b) PPAR-γ agonist as the positive control.
c) PPAR-α agonist as the positive control.

treatment caused enlargement of myocytes ($p < 0.01$), whereas VA attenuated ET-1-induced cell-size augmentation at doses of 30 and 100 μM ($p < 0.01$, Fig. 9). Both MK886 and GW9662 (antagonists of PPAR-α and PPAR-γ, respectively) effectively abolished the antihypertrophic action of VA ($p < 0.01$, Fig. 9).

3.7 Fatty acid composition of mesenteric lymph

There was no difference observed in the proportion of VA in the lymph isolated from MetS or control rats infused with the VA-triolein emulsion, however we did observe greater myristic acid, palmitoleic acid, OA, eicosapentaenoic acid, and DHA ($p < 0.05$), and lower arachidonic acid ($p < 0.05$) between phenotypes (Table 7). Since only VA and OA were provided in the VA-triolein emulsion, other fatty acids incorporated into lymph TG may have been derived from the intracellular pool, de novo synthesis, or sloughed lumenal enterocyte membranes. Notably, the fatty acid composition of mesenteric lymph from the meal emulsion groups reflected the respective dietary fatty acid composition, with the beef fat meal group having higher OA and lower linoleic acid (LA) and α-linolenic acid (ALA; Table 7). Interestingly, the percentage of VA in the lymph was comparable between the VA-meal and beef fat meal emulsion groups despite a higher content of VA in the FFA form in the VA-meal emulsion, suggesting greater bioavailability of VA when given in the esterified from in TG. *Cis*9,*trans*11-CLA was not detected in control rats given the VA-triolein emulsion, which is likely reflective of a relatively lower intestinal stearoyl-CoA reductase activity compared to MetS rats (Okada 2005).

3.8 Intestinal bioavailability of VA

VA showed significantly higher bioavailability in MetS rats compared to control rats ($p < 0.01$, Fig. 10). In MetS rats, the bioavailability of the FFA form of VA in the VA-triolein emulsion was significantly greater compared to that found in the VA-meal emulsion ($p < 0.01$, Fig. 10), suggesting other dietary components in the meal emulsion may reduce the bioavailability of VA. Interestingly, VA in beef fat meal emulsion had approximately 200% and 40% greater absorption compared to the VA-meal and the VA-triolein

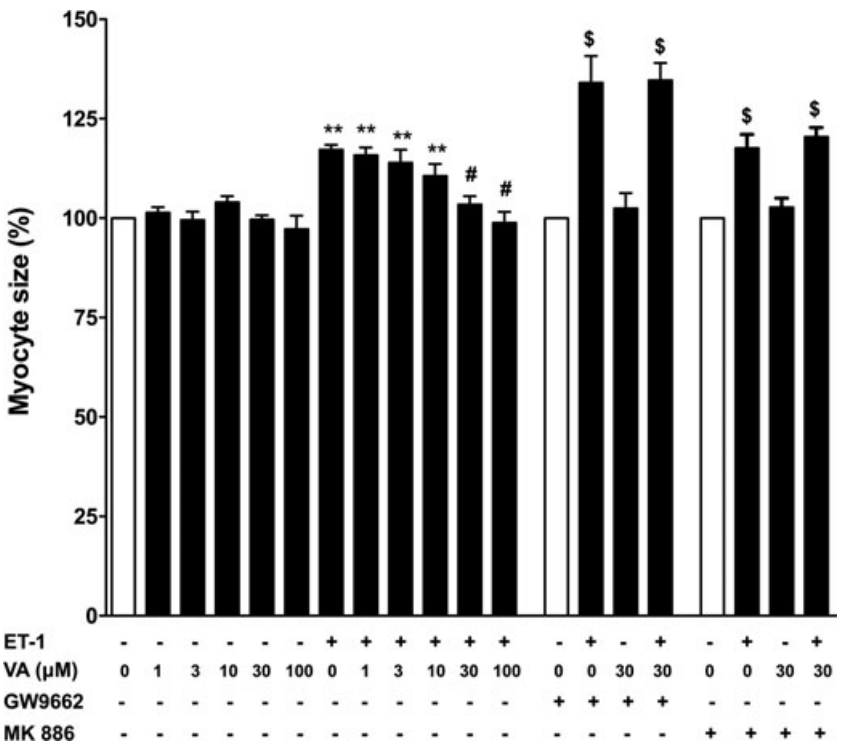

Figure 9. Effect of VA on myocardial hypertrophy with or without the presence of GW9662 (a PPAR-γ-specific antagonist) and MK886 (a PPAR-α-specific antagonist) in cultured rat ventricular myocytes ($n = 4$–8 sets of 30 cells) upon stimulation by ET-1. Results are presented as percent of myocyte size (μm^2) of vehicle-treated controls. $^{**}p < 0.01$ and $^{\$}p < 0.001$ versus respective control (open bars); $^{\#}p < 0.01$ versus the ET-1 only group.

Table 7. Fatty acid composition of mesenteric lymph following a lipid/meal emulsion infusion in control and MetS JCR:LA-*cp* rats

	Control VA-triolein	MetS VA-triolein	MetS VA-meal	MetS beef fat meal
14:0	0.3 ± 0.05	0.9 ± 0.06[b)]	0.6 ± 0.05[c)]	2.1 ± 0.04[d)]
16:0	16.6 ± 1.7	16.3 ± 2.3	13.9 ± 0.6	19.1 ± 0.3[d)]
*cis*9–16:1	0.4 ± 0.04	2.4 ± 0.2[b)]	1.1 ± 0.2[c)]	3.1 ± 0.04[d)]
18:0	14.1 ± 1.7	13.5 ± 0.9	17.6 ± 0.7[c)]	14.4 ± 0.3[d)]
VA	0.3 ± 0.09	0.4 ± 0.06	3.4 ± 0.3[c)]	3.4 ± 0.1
OA	24.7 ± 7.7	27.9 ± 5.2	16.9 ± 0.5[c)]	34.4 ± 0.7[d)]
*cis*11–18:1	1.4 ± 0.03	1.7 ± 0.2	1.6 ± 0.2	1.2 ± 0.3[d)]
LA	25.8 ± 3.0	21.5 ± 1.7	32.0 ± 0.8[c)]	10.3 ± 0.4[d)]
20:0	0.3 ± 0.03	0.3 ± 0.02	0.3 ± 0.01[c)]	0.2 ± 0.005
ALA	0.7 ± 0.1	1.2 ± 0.1[b)]	1.9 ± 0.07[c)]	0.8 ± 0.03[d)]
*cis*9,*trans*11-CLA	ND[a)]	0.2 ± 0.06[b)]	0.3 ± 0.06	0.5 ± 0.02[d)]
20:4n-6	10.0 ± 1.0	5.2 ± 0.6[b)]	4.0 ± 0.2[c)]	2.2 ± 0.1[d)]
20:5n-3	0.2 ± 0.03	0.5 ± 0.06[b)]	0.5 ± 0.04[c)]	0.4 ± 0.03
22:6n-3	1.3 ± 0.1	1.8 ± 0.2[b)]	1.4 ± 0.1[c)]	0.9 ± 0.05[d)]

Values are mean ± SEM expressed as percentage of total fatty acids. Means without a common letter differ, $p < 0.05$.
a) ND, not detected.
b) $p < 0.001$ as compared to the control VA-triolein group.
c) $p < 0.001$ as compared to the MetS VA-triolein group.
d) $p < 0.001$ as compared to the MetS VA-meal group.

emulsion, respectively, suggesting that VA has greater absorption associated with TG compared to the FFA form (Fig. 10).

4 Discussion

4.1 VA enhances intestinal expression of both PPAR-α and PPAR-γ

To our knowledge this is the first report to suggest that dietary supplementation of VA may enhance intestinal gene expression of both *PPAR*-α and *PPAR*-γ, as well as corresponding protein abundance of PPAR-γ in a rodent model of MetS. Data from these studies also suggest dietary VA supplementation may increase the mucosal expression of *FAT/CD36* in the JCR:LA-*cp* rodent. FAT/CD36 is actively involved in long-chain fatty acid and cholesterol uptake at the enterocytic brush boarder membrane as well as intracellular trafficking of lipids [30]. Other studies also suggest that mitochondrial FAT/CD36 may be required to upregulate mitochondrial fatty acid oxidation, which is one of the major metabolic pathways modulated by PPAR-α [31]. Furthermore, the neutral effect of VA on PPAR-α protein abundance may suggest its predominant role in modulating PPAR-α activity, rather than the expression level in the intestine. The relatively steady protein abundance may possibly be due to increased posttranslational degradation.

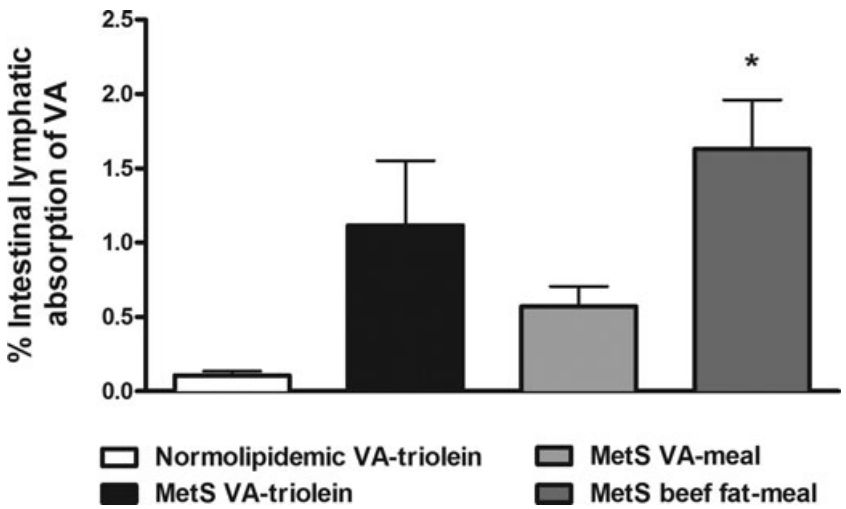

Figure 10. Intestinal lymphatic absorption of VA from triolein emulsions in control and MetS JCR:LA-*cp* rats infused with different emulsions. Values are expressed as mean ± SEM. $^{\&}p < 0.01$ compared to the MetS VA-triolein group, $\#p < 0.01$ compared to the MetS VA-meal group, $^{*}p < 0.05$ compared to the MetS VA-meal group, $^{\$}p < 0.05$ compared to the MetS VA-triolein group.

The expression of several key factors in lipogenesis was also examined in the jejunal mucosa. *SREBP-1* is a transcription factor regulating genes involved in fatty acid and TG biosynthesis [32]. In this study, VA feeding increased *SREBP-1* mRNA abundance in the jejunal mucosa compared to controls, consistent with a previous report [7]. However, the protein concentration of FAS and ACC-1 did not parallel their mRNA changes [7], which could possibly be due to increased posttranslational degradation of these proteins. It is noteworthy that intestinal gene expression and respective protein abundance were measured following prolonged food deprivation, which only reflects the baseline metabolic activities. Follow-up studies on the expression profile of PPARs and their target genes during postprandial state may unfold new perspectives regarding intestinal metabolic dynamics in response to an exogenous lipid load.

4.2 VA as a potent PPAR-α and PPAR-γ agonist

It has been consistently shown in animal models that dietary supplementation with VA can effectively lower fasting and postprandial plasma TG, both of which are considered to be independent CVD risk factors [6, 7]. Improvements in plasma cholesterol profile, inflammatory regulation, and hepatic steatosis have also been observed [7, 9, 10], however to date, no mechanism to explain these findings has been elucidated. In this study we have shown that VA, similarly to other PUFA, acts as a ligand to PPAR-α and PPAR-γ in vitro using competitive binding assays. VA's affinity to PPAR-α appeared to be significantly higher than fenofibric acid, a drug approved by US Food and Drug Administration (FDA) for the treatment of hypertriglyceridemia (RLD 21656/21695) acting specifically as a PPAR-α agonist. In addition, VA also showed comparable affinity to PPAR-γ relative to pioglitazone, a member of the TZD family. Classic clinical outcomes of fibrates and TZD treatment [11] also largely agree with our earlier findings of improved circulating lipid profile and inflammatory markers following dietary VA supplementation [7, 33].

To further elucidate whether VA acts as an agonist or antagonist upon binding to these two nuclear receptors, we conducted a series of coactivator recruitment assays for both PPAR-α and PPAR-γ. The results provide direct evidence supporting the role of VA as a partial agonist for PPAR-α as well as PPAR-γ. Notably, common negative health outcomes such as hepatic steatosis and weight gain under obese and diabetic conditions in response to PPAR-γ full agonization (e.g., rosiglitazone) were not observed in MetS rats supplemented with VA [7, 34, 35]. On the contrary, VA effectively reduced lipid deposition in the liver, consistent with its role as a partial PPAR-α and PPAR-γ agonist. The robust activation of PPAR-α and PPAR-γ by VA was further supported by data from the induced myocardial hypertrophy model in which, specific PPAR-α and PPAR-γ antagonists completely blocked VA's antihypertrophic activity. It is noteworthy that the plasma concentrations of VA are in the μM range (approximately 20–30 μM without supplementation) [36]. Therefore, the dosage range of VA to effectively inhibit myocyte hypertrophy is relevant and physiologically achievable.

Fenofibric acid has been shown to reduce fasting and postprandial plasma TG concentrations in mice, consistent with our observations of dietary VA supplementation in a rodent model of MetS and the in vitro assessment of VA's potential in activating PPAR-α. It was proposed that the TG-lowering effect of fenofibric acid was partially mediated by increased mRNA expression of *acyl-CoA oxidase* and *uncoupling protein 2* in the enterocytes [37]. Therefore, it is plausible that VA may function in a similar manner to activate PPAR-α activity and upregulate target gene expression (e.g., FAT/CD36) in the enterocytes, leading to elevated baseline fatty acid oxidation during the fasted state. We did not observe any difference in mRNA abundance of either PPAR-α or several PPAR-α target genes involved in fatty acid oxidative pathways in the liver. However, recent findings have demonstrated upregulated hepatic citrate synthase activity in JCR:LA-*cp* rats fed a VA/CLA-supplemented diet [33]. Furthermore, reduced hepatic fatty acid de novo synthesis and concomitantly relieved feedback inhibition of CPT1 activity in VA-treated MetS rats may also facilitate fatty acid transport into mitochondria for oxidative degradation [38]. We therefore propose that VA may accelerate fatty acid turnover at functional levels but not necessarily via direct changes in gene transcription patterns in response to VA-induced PPAR-α activation in the liver per se. PPAR-α expression has been identified in other metabolically active tissues including intestine, heart, kidney, brown adipose tissue, and muscle, whereas PPAR-γ plays a key role in adipose tissue development, redistribution as well as related inflammatory regulations [39, 40]. Future investigation of the tissue-specific effect of VA on PPAR-α/γ-dependent metabolic pathways would prove valuable to advance our understanding of VA's bioactivity.

4.3 VA from beef fat TG has greater bioavailability compared to the FFA form in the MetS condition

Accumulating evidence from animal studies suggests ruminant *trans* fats have potential health benefits but these findings have to be translated to the clinical setting, especially among appropriate target population. In this study, we provide evidence of greater intestinal bioavailability of VA in MetS rats as compared to lean control rats. The difference between phenotypes may be indicative of morphological changes in the MetS phenotype, which has intestinal villus hypertrophy and subsequent greater absorptive capacity [41]. Therefore in individuals who are obese and dyslipidemic, VA absorption from the diet may be greater, leading to increased efficacy of VA's lipid-lowering benefits.

Interestingly, the intestinal bioavailability of VA in the FFA or esterified-TG form in the mixed meal emulsions differed markedly. Despite a higher concentration of VA in the

VA-meal emulsion in which VA was in an FFA form, VA had less absorption as shown by incorporation into the lymph when compared to VA sourced from beef fat TG (Fig. 10). When VA is delivered as an FFA rather than esterified to TG, there may be a limited supply of sn-2-monoacylglycerol and/or glycerol-3-phosphate to act as the backbone in TG synthesis. This may lower the incorporation of dietary VA into chylomicron TG resulting in lower concentrations in the lymph. Consistently, *cis*9,*trans*11-CLA from dairy fat also has been shown to be well absorbed, which is at the same sn-1/3 position on TG molecules as VA [17, 42]. It may also be possible that the alternative hepatic portal absorption of short- and medium-chain fatty acids present in the beef fat leads to more rapid incorporation of the remaining long-chain fatty acids into chylomicron TG and secretion into lymph. The fecal output of lipids was not measured in this particular experiment, making it difficult to calculate absolute lipid absorption. The relatively low absolute percentage of VA secreted into mesenteric lymph might also be associated with potential retention of lipids within the enterocytes and may be improved with extended lymph collection time. Regardless of its efficiency, the percentage of VA absorption was at the same order of magnitude compared to that of total lipids as well as other individual dietary fatty acids (e.g., OA).

In conclusion, VA appears to be a potent PPAR-α and PPAR-γ partial agonist and upregulates the intestinal expression of these nuclear receptors in conditions of dyslipidemia and MetS. These results suggest that the hypolipidemic and antihypertrophic bioactivity of VA is potentially mediated via PPAR-α/γ-dependent pathways. Furthermore, the health benefits and greater bioavailability of VA from natural ruminant fat sources provides impetus for national and international food labeling regulations to differentiate VA from industrially produced *trans* fats. The collective findings from these studies highlights the need for further investigations to explore the efficacy of VA, in particular the cardioprotective benefits in clinical conditions of MetS.

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5 References

[1] Lloyd-Jones, D. M., Cardiovascular risk prediction: basic concepts, current status, and future directions. *Circulation* 2010, *121*, 1768–1777.

[2] Micha, R., Mozaffarian, D., Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes. *Nat. Rev. Endocrinol.* 2009, *5*, 335–344.

[3] Field, C. J., Blewett, H. H., Proctor, S., Vine, D., Human health benefits of vaccenic acid. *Appl. Physiol. Nutrition Metab.* 2009, *34*, 979–991.

[4] Aro, A., Complexity of issue of dietary trans fatty acids. *Lancet* 2001, *357*, 732–733.

[5] Bhattacharya, A., Banu, J., Rahman, M., Causey, J. et al., Biological effects of conjugated linoleic acids in health and disease. *J. Nutr. Biochem.* 2006, *17*, 789–810.

[6] Wang, Y., Lu, J., Ruth, M. R., Goruk, S. D. et al., Trans-11 vaccenic acid dietary supplementation induces hypolipidemic effects in JCR:LA-cp rats. *J. Nutr.* 2008, *138*, 2117–2122.

[7] Wang, Y., Jacome-Sosa, M. M., Ruth, M. R., Goruk, S. D. et al., Trans-11 vaccenic acid reduces hepatic lipogenesis and chylomicron secretion in JCR:LA-cp rats. *J. Nutr.* 2009, *139*, 2049–2054.

[8] Blewett, H. J., Gerdung, C. A., Ruth, M. R., Proctor, S. D. et al., Vaccenic acid favourably alters immune function in obese JCR:LA-cp rats. *Br. J. Nutr.* 2009, *102*, 526–536.

[9] Ruth, M. R., Wang, Y., Yu, H. M., Goruk, S. D. et al., Vaccenic and elaidic acid modify plasma and splenocyte membrane phospholipids and mitogen-stimulated cytokine production in obese insulin resistant JCR:LA-cp rats. *Nutrients* 2010, *2*, 17.

[10] Tyburczy, C., Major, C., Lock, A. L., Destaillats, F. et al., Individual trans octadecenoic acids and partially hydrogenated vegetable oil differentially affect hepatic lipid and lipoprotein metabolism in golden Syrian hamsters. *J. Nutr.* 2009, *139*, 257–263.

[11] Brown, J. D., Plutzky, J., Peroxisome proliferator-activated receptors as transcriptional nodal points and therapeutic targets. *Circulation* 2007, *115*, 518–533.

[12] Harris, W. S., Miller, M., Tighe, A. P., Davidson, M. H. et al., Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis* 2008, *197*, 12–24.

[13] Gani, O. A., Sylte, I., Molecular recognition of docosahexaenoic acid by peroxisome proliferator-activated receptors and retinoid-X receptor alpha. *J. Mol. Graph Model* 2008, *27*, 217–224.

[14] Taylor, C. G., Zahradka, P., Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. *Am. J. Clin. Nutr.* 2004, *79*, 1164S–1168S.

[15] Alibin, C. P., Kopilas, M. A., Anderson, H. D., Suppression of cardiac myocyte hypertrophy by conjugated linoleic acid: role of peroxisome proliferator-activated receptors alpha and gamma. *J. Biol. Chem.* 2008, *283*, 10707–10715.

[16] Motard-Belanger, A., Charest, A., Grenier, G., Paquin, P. et al., Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am. J. Clin. Nutr.* 2008, *87*, 593–599.

[17] Chardigny, J. M., Masson, E., Sergiel, J. P., Darbois, M. et al., The position of rumenic acid on triacylglycerols alters its bioavailability in rats. *J.Nutr.* 2003, *133*, 4212–4214.

[18] Tricon, S., Burdge, G. C., Jones, E. L., Russell, J. J. et al., Effects of dairy products naturally enriched with cis-9, trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. *Am. J. Clin. Nutr.* 2006, *83*, 744–753.

[19] Russell, J. C., Proctor, S. D., Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovasc. Pathol.* 2006, *15*, 318–330.

[20] Mouloungui, Z., Candy, L., in: Destaillats, F. (Ed.), *Trans Fatty Acids in Human Nutrition*, The Oily Press, High Wycombe, UK, 2009, p. 77.

[21] Russell, J. C., Amy, R. M., Graham, S. E., Dolphin, P. J. et al., Inhibition of atherosclerosis and myocardial lesions in the JCR:LA-cp rat by beta, beta'-tetramethylhexadecanedioic acid (MEDICA 16). *Arterioscler. Thromb. Vasc. Biol.* 1995, *15*, 918–923.

[22] Weiser, M. M., Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J. Biol. Chem.* 1973, *248*, 2536–2541.

[23] Suzuki, T., Hoshi, H., Mitsui, Y., Endothelin stimulates hypertrophy and contractility of neonatal rat cardiac myocytes in a serum-free medium. *FEBS Lett.* 1990, *268*, 149–151.

[24] Irukayama-Tomobe, Y., Miyauchi, T., Sakai, S., Kasuya, Y. et al., Endothelin-1-induced cardiac hypertrophy is inhibited by activation of peroxisome proliferator-activated receptor-alpha partly via blockade of c-Jun NH2-terminal kinase pathway. *Circulation* 2004, *109*, 904–910.

[25] Huang, Y., Zhang, H., Shao, Z., O'Hara, K. A. et al., Suppression of endothelin-1-induced cardiac myocyte hypertrophy by PPAR agonists: role of diacylglycerol kinase zeta. *Cardiovasc. Res.* 2011, *90*, 267–275.

[26] Vine, D. F., Croft, K. D., Beilin, L. J., Mamo, J. C., Absorption of dietary cholesterol oxidation products and incorporation into rat lymph chylomicrons. *Lipids* 1997, *32*, 887–893.

[27] Folch, J., Lees, M., Sloane Stanley, G. H., A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 1957, *226*, 497–509.

[28] Cruz-Hernandez, C., Kramer, J. K., Kennelly, J. J., Glimm, D. R. et al., Evaluating the conjugated linoleic acid and trans 18:1 isomers in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. *J. Dairy Sci.* 2007, *90*, 3786–3801.

[29] Schmidt, M. V., Brune, B., von Knethen, A., The nuclear hormone receptor PPARgamma as a therapeutic target in major diseases. *Sci. World J.* 2010, *10*, 2181–2197.

[30] Bonen, A., Holloway, G. P., Tandon, N. N., Han, X. X. et al., Cardiac and skeletal muscle fatty acid transport and transporters and triacylglycerol and fatty acid oxidation in lean and Zucker diabetic fatty rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2009, *297*, R1202–R1212.

[31] Holloway, G. P., Jain, S. S., Bezaire, V., Han, X. X. et al., FAT/CD36-null mice reveal that mitochondrial FAT/CD36 is required to upregulate mitochondrial fatty acid oxidation in contracting muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2009, *297*, R960–R967.

[32] Chen, G., Liang, G., Ou, J., Goldstein, J. L. et al., Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. *Proc. Natl. Acad. Sci. USA* 2004, *101*, 11245–11250.

[33] Jacome-Sosa, M. M., Lu, J., Wang, Y., Ruth, M. R. et al., Increased hypolipidemic benefits of cis-9, trans-11 conjugated linoleic acid in combination with trans-11 vaccenic acid in a rodent model of the metabolic syndrome, the JCR:LA-cp rat. *Nutr. Metab. (Lond.)* 2010, *7*, 60–70.

[34] Pan, H. J., Lin, Y., Chen, Y. E., Vance, D. E. et al., Adverse hepatic and cardiac responses to rosiglitazone in a new mouse model of type 2 diabetes: relation to dysregulated phosphatidylcholine metabolism. *Vasc. Pharmacol.* 2006, *45*, 65–71.

[35] Loke, Y. K., Kwok, C. S., Singh, S., Comparative cardiovascular effects of thiazolidinediones: systematic review and meta-analysis of observational studies. *BMJ* 2011, *342*, d1309–1317.

[36] Adlof, R. O., Duval, S., Emken, E. A., Biosynthesis of conjugated linoleic acid in humans. *Lipids* 2000, *35*, 131–135.

[37] Dietz, W. H., Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics* 1998, *101*, 518–525.

[38] Schreurs, M., Kuiper, F., van de Leij, F. R., Regulatory enzymes of mitochondrial beta-oxidation as targets for treatment of the metabolic syndrome. *Obes. Res.* 2010, *11*, 380–388.

[39] Braissant, O., Foufelle, F., Scotto, C., Dauca, M., Wahli, W., Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996, *137*, 354–366.

[40] Pyper, S. R., Viswakarma, N., Yu, S., Reddy, J. K., PPARalpha: energy combustion, hypolipidemia, inflammation and cancer. *Nucl. Recept. Signal* 2010, *8*, e002.

[41] Hassanali, Z., England, A., O'Neill, D., MacNaughton, K. et al., Intestinal villi hypertrophy is associated with overproduction of lymphatic chylomicrons during conditions of obesity and hyperinsulinemia in the JCR:LA-cp rodent. *Atherosc. Suppl.* 2008, *9*, 90–91.

[42] Mottram, H. R., Crossman, Z. M., Evershed, R. P., Regiospecific characterisation of the triacylglycerols in animal fats using high performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. *Analyst* 2001, *126*, 1018–1024.

