Is palmitoleic acid a plausible non-pharmacological strategy to prevent or control chronic metabolic and inflammatory disorders?

Running title: Palmitoleic acid and metabolic and inflammatory disorders

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Abstract

Although dietary fatty acids can modulate metabolic and immune responses, the effects of palmitoleic acid (16:1n-7) remain unclear. Since this monounsaturated fatty acid was described as a lipokine, studies with cell culture and rodent models have suggested it enhances whole body insulin sensitivity, stimulates insulin secretion by β-cells, increases hepatic fatty acid oxidation, improves the blood lipid profile, and alters macrophage differentiation. However, human studies report elevated blood levels of palmitoleic acid in people with obesity and metabolic syndrome. These findings might be reflection of the level or activity of stearoyl-CoA desaturase-1, which synthesizes palmitoleate and is enhanced in liver and adipose tissue of obese patients. In this review we aimed to describe the immune-metabolic effects of palmitoleic acid observed in cell culture, animal models, and humans to answer the question of whether palmitoleic acid is a plausible non-pharmacological strategy to prevent, control, or ameliorate chronic metabolic and inflammatory disorders. Despite the beneficial effects observed in cell culture and in animal studies, there are insuficient human intervention studies to fully understand the physiological effects of palmitoleic acid. Therefore more human-based research is needed in order to identify whether palmitoleic acid meets the promising therapeutic potential suggested by the pre-clinical research.

Keywords: monounsaturated fatty acid, lipokine, vaccenic acid, palmitoleic acid, metabolic syndrome, non-alcoholic fatty liver disease, atherosclerosis, inflammation

Introduction

Palmitoleic acid is a 16 carbon, monounsaturated, omega-7 fatty acid. Its shorthand nomenclature is 16:1n-7; the double bond in the acyl chain is in the *cis* configuration and the systematic name of palmitoleic acid is *cis*-9 heptadecaenoic acid. Palmitoleic acid can be obtained directly from the diet: good sources are macadamia (*Macadamia integrifolia*) nuts, macadamia oil, and sea buckthorn (*Hippophae rhamnoides*) oil. Many oily fish and fish oils contain appreciable amounts of palmitoleic acid. Palmitoleic acid can be synthesized by the desaturation of palmitic acid (16:0) in a reaction catalyzed by delta-9 desaturase, often called stearoyl-CoA desaturase-1 (SCD1) (Figure 1). It is likely that the principal sites of SCD activity are the liver and adipose tissue. There are significant stores of palmitoleic acid in human adipose tissue, where it typically contributes over 5% of the fatty acids present [1]. In common with other fatty acids, palmitoleic acid circulates in the bloodstream as a component of complex lipids (triglycerides, phospholipids, cholesteryl esters) within lipoproteins. There is also some non-esterified ("free") palmitoleic acid in the bloodstream [2]. Palmitoleic acid is also a component of cell membranes. Table 1 shows some reported absolute and relative (% of total fatty acids) palmitoleic acid concentrations in blood, blood cells, and adipose tissue in humans [1, 2].

Palmitoleic acid has been described as being produced and released by adipocytes and acting as a lipokine modulating several metabolic processes in other tissues [3]. For example, several studies observed that palmitoleic acid enhanced whole body glucose disposal in rodents [3-5], attenuated hepatic steatosis in high-fat-fed and diabetic mice [3, 6], protected pancreatic β-cells from death induced by palmitic acid [7-9], and improved the circulating lipid profile in mice [3] and humans [10]. Furthermore, palmitoleic acid supplementation was seen to reduce liver inflammation in mice with non-alcoholic fatty liver disease (NAFLD) [4, 11], and to slow the progression of atherosclerosis in obese mice [12]. In cell culture, adipocytes incubated with palmitoleic acid showed lower expression of genes related to toll-like receptor (TLR) pathways [13] while macrophages exposed to palmitoleic acid showed reduced inflammatory

gene expression, a lower production of inflammatory cytokines [12, 14], and higher differentiation to an anti-inflammatory phenotype [14, 15].

In this review, we will describe studies that have investigated the immuno-metabolic effects of palmitoleic acid relevant to chronic metabolic diseases such as obesity, insulin resistance, NAFLD, and atherosclerosis and we summarize findings from cell culture, animal model, and human studies to conclude whether palmitoleic acid plays an important role in preventing, controlling, or ameliorating those conditions.

Palmitoleic acid and the role of SCD in metabolic disease

Palmitoleic acid can be synthesized by the desaturation of palmitic acid (16:0) in a reaction catalyzed by SCD [16]. Four isoforms of SCD have been identified in mice (SCD-1 to 4) and two in humans (SCD-1 and SCD-5). SCD-1 is conserved among mammalian species [17]. It is modulated by dietary components: high levels of palmitoleic acid inhibit SCD-1 expression and activity [3, 6], while saturated fatty acids, sucrose, fructose and alcohol are known inducers and activators of this enzyme [18]. SCD activity has been estimated by the ratio of palmitoleic to palmitic acids (16:1n-7/16:0) in plasma [19].

Dietary intake of palmitoleic acid is thought to be very low in those on a Western diet [20]. Thus, palmitoleic acid levels largely reflect its endogenous synthesis from palmitic acid by SCD-1. For this reason, the ratio of palmitoleic to palmitic acids, which reflects SCD-1 activity, is considered to be a marker of lipogenesis [19]. SCD-1 is mainly expressed in lipogenic tissues such as liver and adipose tissue [19] where its biological importance is considered to be the prevention of proposed harmful effects from saturated fatty acid accumulation in tissues and the bloodstream [16]. However, it has been observed that SCD-1-deficient mice were protected against some features of the metabolic syndrome [21, 22]; on the basis of such observations a link between SCD-1 activity and development of the metabolic syndrome and type-2 diabetes has been suggested [16, 23]. In fact, the inhibition of SCD-1 seems to prevent diet-induced obesity, NAFLD, glucose-tolerance and insulin intolerance in rodents [21, 22]. Consequently inhibitors of SCD-1 have been proposed for the treatment of obesity and metabolic diseases [16].

Palmitoleic acid has been described as a powerful blocker (of activity and expression) of SCD-1, reducing insulin resistance and NAFLD in mice [3, 6]. The inhibitory effect of palmitoleic acid on SCD-1 expression depends on the tissue, being less pronounced in adipose tissue and greater in liver of FABP knockout mice, which presented high levels of palmitoleic acid, lower *de novo* hepatic lipogenesis, and resistance to deleterious effects of high fat diet [3]. Similar inhibition of SCD-1 was observed in adipose tissue of type-2 diabetic mice treated with palmitoleic acid, in which this monounsaturated fatty acid increased insulin sensitivity and controlled NAFLD progression[6]. Thus, in murine models of metabolic disease, the modulation of SCD-1 in adipose tissue promoted by palmitoleic acid may be crucial to the metabolic improvements seen.

Palmitoleic acid elongation product in metabolic disease

Besides being linked to SCD-1 expression and activity, palmitoleic acid reduced *de novo* fatty acid synthesis by inhibition of the expression of the lipogenic genes fatty acid synthase (FASN) and elongation of very long chain fatty acids protein 6 (ELOVL6) in cultured adipocytes [24-26] and hepatocytes [3, 11]. Furthermore, palmitoleic acid-treated cells showed an increase of *cis*-vaccenic acid (VA, 18:1n-7) [25, 27], which itself induces several immune-metabolic effects [28-30]. Studies with *cis*-VA are summarized in Table 2.

A study performed by Green et al. [31], in which mammalian cells were targeted to knockdown or overexpression of elongation of very long chain fatty acids elongase protein 5 (ELOVL5) or ELOVL6, suggested that ELOVL6 is responsible for the elongation of palmitic acid (16:0) to stearic acid (18:0), and that ELOVL6, but mainly ELOVL5, elongates palmitoleic acid (16:1n-7) to *cis*-VA. In support of the latter, other studies described elongation of palmitoleic acid to *cis*-VA in adipocytes with an inhibition of ELOVL6 expression but only if ELOVL5 expression was not altered [25, 27].

The transcription of both ELOVL5 and 6 is activated by PPARα [32], which is described as a target of palmitoleic acid in adipose tissue [24]. The deletion of ELOVL6 in mice increased the levels of palmitoleic acid and the ratio of C16:1n-7 to C16:0 in liver, an effect suggested to

prevent the development of diet-induced insulin resistance, without amelioration of obesity or NAFLD [33]. Although increased in ELOVL6 knockout mice, as the substrate for the elongation to *cis*-VA [25, 27], palmitoleic acid seems to increase the elongation capacity itself by increasing the transcription of the genes encoding elongase enzymes, especially ELOVL5 [32]. In agreement, our group observed in endothelial cells exposed to several concentrations of palmitoleic acid, that the elongation to *cis*-VA was increased in a dose-dependent manner (Souza et al., in press).

Epidemiological studies have attempted to correlate circulating levels and/or intake of *cis*-VA to cardiovascular disease risk without convincing association [28]. However, clinical trials suggest the opposite and indicate *cis*-VA as a protective factor towards cardiovascular diseases [29] (Table 2). Similarly, animal studies with spontaneously hypertensive rats [34] and LDLr$^{(-/-)}$ mice [35] showed the beneficial effects of *cis*-VA in lowering blood pressure and lowering blood cholesterol levels.

Furthermore, in obese rats, a diet rich in *cis*-VA decreased the NAFLD score and the expression of lipogenic factors such as sterol receptor element binding protein 1 (SREBP-1) and FASN, controlling the progression of NAFLD and increasing the whole-body glucose tolerance and insulin sensitivity [36]. Unfortunately, there are not sufficient human studies to support the association of *cis*-VA reducing insulin resistance and NAFLD, as observed *in vitro* and in animal studies. A large prospective cohort study (1992-2005) performed with 3004 participants free of diabetes showed that *cis*-VA levels were inversely associated with diabetes [37] (Table 2).

Studies also indicate *cis*-VA supplementation as a promising alternative to control inflammation [28, 30]. Lean and obese rats fed with *cis*-VA presented lower proportions of T-cells and production of interleukin (IL)-2 and tumor necrosis factor (TNF)-α in their mesenteric lymph nodes [30]. Similarly, obese JCR-rats fed a high *cis*-VA diet showed a reduction in hepatic ectopic lipid accumulation and a lower expression of TNF-α and IL-1β in the intestinal mucosa [38]. There are insufficient human studies to support an anti-inflammatory role of *cis*-VA. However, similar to animal model data [30], a study with 1373 healthy men associated high levels

of *cis*-VA in erythrocyte membranes with high concentrations of the anti-inflammatory peptide adiponectin [39] (Table 2).

Although the human data for *cis*-VA are insufficient to prove its efficacy against the harmful alterations of metabolic syndrome, inflammation, and NAFLD, this fatty acid could be an important non-pharmacologic alternative for co-morbidities of obesity, and akin to its precursor, palmitoleic acid, the therapeutic effects of *cis*-VA should be further investigated.

Is palmitoleic acid a plausible non-pharmacological strategy to prevent, control, or treat metabolic and inflammatory disorders?

Inflammation is a central process that links co-morbidities associated with metabolic syndrome (MetS) [40]. In response to hypoxia generated by adipocyte hypertrophy and other factors, such as metabolic stress, lipotoxicity or endoplasmic reticulum stress, several pro-inflammatory cytokines and adipokines are produced and released into the circulation, raising the systemic levels of these inflammatory markers in obesity [41]. High concentrations of the cytokines TNFα, IL-1β, and IL-6, and the chemokine MCP-1 are found in adipose tissue (and blood) of obese patients, suggesting that MetS and its co-morbidities have an inflammatory component [42, 43]. Therefore, anti-inflammatory strategies have been evaluated as a possible treatment of MetS co-morbidities. Pharmacological approaches have been used to target inflammatory pathways or to inhibit the effects of inflammatory mediators [42]. Salicylates, which block the NF-κB pathway [44] and monoclonal antibodies against TNF-α [45], IL-1β [46] or MCP-1 [47] have yielded promising results to control MetS co-morbidities. However, some non-pharmacological components such as fatty acids, an important class of nutrients able to modulate inflammatory response in different cell types and by several pathways, have been shown to promote similar effects [48].

The ability of palmitoleic acid to promote anti-inflammatory effects has been described [3, 4, 6, 11, 14, 15, 49]. In cell cultures, palmitoleic acid was shown to modulate the phenotype of macrophages, reduce expression and production of pro-inflammatory factors, and block some pro-inflammatory effects of palmitic acid and lipopolysaccharide (LPS) [12, 14, 15, 50] (Figure 2).

Similarly, supplementation with palmitoleic acid or palmitoleic acid-enriched oils reduced inflammation in adipose tissue [26] and liver [4, 11] of obese mice fed a high-fat diet. Such anti-inflammatory properties might be linked to the beneficial effects of palmitoleic acid on some features of MetS and atherosclerosis. In addition to influencing the inflammatory response, palmitoleic acid treatment (300 mg/kg/day) for 10 to 28 days improved glucose tolerance and insulin resistance [3-5], modulated some metabolic functions in liver and adipose tissue [4, 24], increased fatty acid oxidation and attenuated the adverse effects of saturated fatty acids [3, 10], and improved NAFLD [3, 6, 51], and pancreatic β-cell function [7-9]. More details regarding the metabolic effects of palmitoleic acid on obesity and metabolic syndrome abnormalities will be presented in the next section.

Obesity and dyslipidemia

Obese individuals have a greater risk for developing chronic diseases such as hypercholesterolemia, insulin resistance, hepatic steatosis, hypertension, and cardiovascular diseases such as atherosclerosis [52]. Palmitoleic acid supplementation seems to promote important effects to control obesity. For example, Yang et al. [49] showed that palmitoleic acid induces satiety in mice, while studies with obese high-fat diet fed mice have shown that daily administration of palmitoleic acid (300 mg/kg) for 10 days increased whole body insulin sensitivity [4, 51] and enhanced glucose uptake into adipose tissue by modulation of GLUT-4 content and AMPK phosphorylation [5]. Palmitoleic acid also increased expression of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [24] (Figure 2). This higher expression and activity of lipases was mediated by activation of PPARα [24], a transcription factor that targets and stimulates the transcription of genes involved in several metabolic pathways, especially those related to lipid metabolism, including uptake and transport of fatty acids, and mitochondrial and peroxisomal fatty acid oxidation [53]. Similarly, dietary supplementation with macadamia nut oil (2 g/kg of body weight, 3 times per week, for 12 weeks), which is rich in palmitoleic acid (19% of fatty acids), had beneficial effects, reducing hypertrophy of adipose tissue in mice fed a high-fat diet [26], while reducing levels of

inflammatory markers in the adipose tissue [26]. It is important to mention that besides being rich in palmitoleic acid (19%) macadamia oil has a high percentage of oleic acid (56%) [26]. However, most of the animal studies presented here [4, 5, 24, 51], compared the treatment of palmitoleic acid to that of oleic acid (e.g., at 300 mg/kg, respectively), showing a higher oxidative, lipid lowering, and insulin sensitization with palmitoleic acid.

Although we have described many positive effects of palmitoleic acid in animal models, human data are inconsistent and frequently demonstrate different effects from those seen in the animal models (Table 3, Table 4, Table 5). A positive correlation of plasma palmitoleic acid and insulin sensitivity has been described [54]. Several studies considered higher levels of palmitoleic acid in plasma (as free fatty acid) or in red blood cell membranes to be a risk factor for the development of MetS and type-2 diabetes [55-58], and associated high amounts of palmitoleic acid in adipose tissue with obesity [59]. Similarly, plasma levels of palmitoleic acid have been positively correlated with the progression of MetS in obese children [23, 60] and adults [61-63] (Table 3). High plasma levels of palmitoleic acid (as a NEFA or in phospholipids) have been associated with the progression of other metabolic diseases such as NAFLD [64, 65] and coronary heart disease [29, 66] (Table 3). Thus, data presented so far suggest that high levels of palmitoleic acid are associated with the progression of obesity, metabolic syndrome, NAFLD, and coronary heart disease (Table 3). However, some investigators have reported no correlation of palmitoleic acid as free fatty acid, in plasma phospholipids, or in red blood cell membranes with obesity, insulin resistance [67], or cardiovascular diseases [68].

It is important to note that these human studies are association studies and not intervention trials, so cause and effect cannot be firmly established [20]. In fact, several human intervention trials that used macadamia nut oil (40-90 g/day) as the source of palmitoleic acid showed that, even in a fairly short time period (3 weeks) macadamia oil as a source of palmitoleic acid promoted significant reduction of total cholesterol, LDL-cholesterol and body weight of Japanese women (n=71) compared to upplementation with coconut oil or butter (neither contain much palmitoleic acid) [69]. Similarly, It was observed that 4 weeks macadamia oil supplementation reduced total cholesterol and LDL-cholesterol and increased HDL-

cholesterol in hypercholesterolemic men (n=17), at the same time palmitoleic (C16:1n-7), vaccenic (18:1n-7) and eicoseinoic (C20:1n-9) acid levels were enhanced [70]. It is important to mention that, although macadamia oil or amacadamia rich diet has been observed to be an efficient non-pharmacological treatment of hypercholesterolemia [69-72], the effects have been attributed to palmitoleic acid rather than other fatty acid components. , A randomized, crossover, controlled feeding study which compared the fatty acid content and effects of a macadamia rich diet and an average American diet observed that while palmitoleic acid amounts increased 6-times with the macadamia rich diet, the others MUFAs were very similar between the diets. At same time, macadamia rich diet decreased total cholesterol, LDL and non-HDL cholesterol, also increasing the plasma levels of palmitoleic acid in hypercholesterolemic patients [72] (Table 3).

Noteworthy, the only clinical trial published to date that supplemented subjects (patients with dyslipidemia and evidence of mild systemic inflammation, n = 60) with a palmitoleic acid concentrate described effects similar to those seen with macadamia nut oil: a decrease in triglyceride and LDL-cholesterol, increase in HDL cholesterol, and a reduction of the inflammation marker C-reactive protein (Table 4) (Figure 2)[73]. These observations suggest that palmitoleic acid may be a non-pharmacologic alternative to control some features of obesity. However, more human studies are needed in order to better understand its effects on insulin resistance, NAFLD, and atherosclerosis. The following sections provide greater detail on cell culture and animal studies performed with palmitoleic acid; they indicate important and beneficial effects of this monounsaturated fatty acid on these metabolic abnormalities.

Insulin resistance

Since being described as a lipokine, the effects of palmitoleic acid on insulin resistance have been investigated. Cao et al. [3] showed that AP2 knockout mice, which presented high levels of palmitoleic acid in plasma non-esterified fatty acids and adipose tissue, did not develop

insulin-resistance when fed with a high-fat diet. These knockout animals had higher phosphorylation of several proteins from the insulin signaling pathway (e.g., insulin receptor, insulin receptor substrate, protein kinase B) in both muscle and liver [3]. In contrast, it was observed that GPR-120 knockout mice have a reduced SCD-1 expression in adipose tissue and lower levels of palmitoleic acid in plasma and adipose tissue, and, as a consequence, those animals presented lower insulin sensitivity, with disrupted phosphorylation of insulin receptor, insulin receptor substrate, and protein kinase B in muscle, liver and adipose tissue [74]. In addition, human primary adipocytes extracted from the gluteofemoral depot showed higher production and release of palmitoleic acid compared to those from the abdominal depot, which was correlated with better insulin sensitivity and higher SCD-1 expression [75] (Table 5).

Others studies have shown improvement in insulin signaling and metabolic effects with palmitoleic acid incubation or supplementation. *In vitro*, when incubated with palmitoleic acid, 3T3L1 adipocytes stimulated with insulin showed greater glucose uptake, increased rates of aerobic and anaerobic glycolysis, and inhibition of *de novo* lipogenesis [5] (Figure 2). Similarly, higher uptake and oxidation of glucose was observed in cultured L6 myotubes incubated with palmitoleic acid [76] and in muscle isolated from mice fed a high-fat diet and treated with palmitoleic acid [4] (Figure 2). Moreover, palmitoleic acid supplementation improved insulin sensitivity in high-fat diet-induced obese mice [4, 11], as well as in genetic type-2 diabetic mice [6] (Table 5) (Figure 2).

Although palmitoleic acid has beneficial effects on insulin resistance in *in vitro* and animal studies, correlation studies in humans do not show a clear connection between circulating palmitoleic acid levels and insulin resistance in humans. For example, higher palmitoleic acid levels in plasma and VLDL were not associated with insulin resistance in skeletal muscle, liver, or adipose tissue in obese patients [67]. In a eutrophic weight Japanese population, high serum levels of palmitoleic acid and SCD-1 activity were associated with insulin resistance [58], while in a Caucasian population with high risk of type-2 diabetes, the serum levels of palmitoleic acid were positively correlated with better insulin sensitivity [54] (Table 3).

Increased levels of SCD-1 were found in adipose tissue of type-2 diabetic patients treated by rosiglitazone [77, 78] and in healthy human subjects treated by pioglitazone [79], two common antidiabetic thiazolidinedione (TZD) drugs. Likewise, rosiglitazone supplementation promoted SCD-1 expression in mice fed a Western-type diet, and palmitoleic acid levels in liver and adipose tissue were consequently increased [80]. In conjunction with increases in palmitoleic acid, the TZDs also improved glycemic control [80], indicating that palmitoleic acid synthesis could benefit the insulin-sensitizing effect of TZD treatment [77].

In addition to being correlated with greater insulin sensitivity, palmitoleic acid has been observed to modulate insulin production [51] (Figure 2). In fact, palmitoleic acid improved β-cell secretory function that was impaired by palmitic acid and high glucose [7]. Similarly, islets of Langerhans from humans and rats exposed to several concentrations of glucose and incubated with palmitoleic acid showed higher insulin secretion then those incubated with glucose alone [9] (Table 5) (Figure 2). While a direct effect of palmitoleic acid on insulin secretion is suggested, it is important to mention that the mechanism by which palmitoleic acid influences insulin response and/or secretion is not fully understood.

Palmitoleic acid seems to be able to switch metabolism from storing energy to using it, an effect that may occur by activation of AMPK. Adipocyte and macrophage cultures showed higher phosphorylation of AMPK when exposed to palmitoleic acid, as well as an increase in oxidative metabolism [5, 15] (Table 5). AMPK can be phosphorylated and activated by conditions that change the AMP/ATP ratio, calcium concentration, or by the action of hormones, cytokines, and adipokines [81]. In general, activation of AMPK increases processes that generate or preserve cellular ATP, including glycolysis, oxidative phosphorylation, β-oxidation of fatty acids, and autophagy, while inhibiting processes that consume ATP such as protein translation, ribosome assembly, and lipid synthesis [82]. Similar to *in vitro* studies, intravenous palmitoleic acid infusion (10 mg/kg body weight/day) up-regulated AMPK expression in liver, subcutaneous adipose tissue, and muscle of obese sheep [83]. In addition, palmitoleic acid supplementation (300 mg/kg/day) increased the phosphorylation of AMPK in liver of obese mice [51], an effect that depends on the activation of the pro-oxidative transcription factor, PPARα (Table 5).

NAFLD

Although cell culture and animal studies indicate that palmitoleic acid plays an important role in improving insulin sensitivity, increasing fatty acid oxidation, and reducing inflammation, its effects on NAFLD remain unclear.

Cao et al. [3] observed that the expression of several genes involved in *de novo* lipogenesis (SCD-1, FAS, ELOVL6) was modulated differently by palmitoleic acid, according to tissue; increased in adipose tissue and decreased in the liver of FABP knockout mice which had higher plasma levels of palmitoleic acid and a lower degree of steatosis [3]. On other hand, following the lower production of palmitoleic acid, animals with GPR-120 dysfunction showed a downregulation of SCD-1, SREBP, PPARγ in adipose tissue while an upregulation of SCD-1 and high ectopic accumulation of triglycerides in their liver occurred when they were fed a high fat diet [74]. Administration of palmitoleic acid to genetic type-2 diabetic mice reduced lipid accumulation and lipogenic gene expression (SREBP-1, FAS, and SCD-1) in the liver [6] (Table 5). However, studies performed with wild-type animals or PPARα knockout mice were unable to show beneficial effects of palmitoleic acid on the degree of steatosis [4, 11]. Guo et al. [11] observed upregulation of the lipogenic genes FAS and SREBP-1c by palmitoleic acid, while Souza et al. [4] observed that the higher content of triacylglycerol in the liver of PPARα knockout mice fed a high-fat diet was not modulated by palmitoleic acid. Despite the lack of effect on the hepatic accumulation of fat, both studies showed that palmitoleic acid improved the insulin response and metabolic functions in the liver; effects that may be related to the anti-inflammatory properties of palmitoleic acid [4, 11] (Figure 2). However, it was recently reported that improvement of liver metabolic functions, downregulation of SREBP-1 and up-regulation of glucokinase, a key factor for liver uptake of glucose, are dependent on activation of the PPARα-AMPK axis induced by palmitoleic acid in obese mice [51] (Table 5) (Figure 2).

Opposing observations have been made in correlation studies. High levels of plasma palmitoleic acid have been described as a marker of MetS and liver fat accumulation [64, 65, 84, 85] (Table 3). Puri et al. [85] observed that plasma palmitoleic acid levels in patients with liver

disease increased according to the grade of damage, with higher levels in patients with non-alcoholic steatohepatitis than in those with NAFLD [85] (Table 3). However, these studies evaluated only the plasma palmitoleic acid concentrations, which reflect SCD-1 activity in the liver. As previously stated, SCD-1 is a lipogenic enzyme associated with higher risk of obesity [16, 21] and is modulated by high intake of carbohydrate [59]. While palmitoleic acid was increased in the plasma of obese patients, the association between adipose tissue palmitoleic acid concentrations and obesity was attenuated by low carbohydrate intake [59].

Atherosclerosis

Atherosclerosis is highly correlated with obesity [86] and it is also described as an inflammatory disease [12, 87, 88]. In particular, elevated plasma levels of non-esterified fatty acids (NEFAs) are frequently observed in patients with metabolic syndrome and visceral obesity [52]; NEFAs may promote endothelial dysfunction, the first step of atherosclerosis, by immune activation and upstream regulation of the nuclear transcription factor–kappa B (NFκB) [87].

By triggering NFκB [89], saturated fatty acids such as palmitate (16:0) and stearate (18:0), promote crucial events in the initial steps of atherogenesis [90]. They enhance the adherence and transmigration of monocytes and lymphocytes by increasing the expression of cell adhesion molecules (ICAM-1, VCAM-1 and E-selectin), up-regulating the expression of inflammatory cytokines (TNF-α, IL-6 and IL-8), and promoting apoptosis of endothelial cells [90, 91]. The effects of palmitoleic acid on endothelial cell dysfunction and atherosclerosis are unknown and underexplored. Palmitoleic acid seems to promote anti-inflammatory effects in primary macrophages [15], lowering levels of pro-inflammatory cytokines produced by macrophages stimulated with LPS [14], and lowering the levels of pro-inflammatory cytokines and the phosphorylation of NFκB (p65) in liver of mice fed a high-fat diet [4] (Figure 2).

In endothelial cells, Staiger et al. [52] showed that palmitoleic acid reduced the pro-apoptotic effects of palmitate and stearate. We observed that palmitoleic acid reduced the production of cytokines and chemokines (IL-6, IL-8, MCP-1) and the cell-surface content of ICAM-1 in endothelial cells stimulated with TNF-α (Souza et al., in press) (Figure 2). Consistent

with these observations, a positive correlation of serum palmitoleic acid levels and endothelial function was observed in healthy subjects [92] and in patients with high cardiometabolic risk [64]. Recently, it was observed that palmitoleic acid treatment decreased atherosclerotic lesion formation in the aorta of ApoE$^{-/-}$ mice (atherosclerosis murine model) on a hypercholesterolemic diet, reducing foam cell plaques and necrotic areas by preventing the inflammasome activation [12] (Table 5).

In summary, current understanding of the effect of palmitoleic acid in the health of the vascular system is lacking, but the anti-inflammatory properties of palmitoleic acid illuminate a potential new approach in atherosclerosis prevention and treatment.

Conclusion

The literature on palmitoleic acid and health is inconsistent. In cell culture models and experimental animals, palmitoleic acid has been frequently shown to improve glucose metabolism, restore glucose intolerance, induce oxidative metabolism, and reduce inflammation. On the other hand, the relatively higher levels of palmitoleic acid in the bloodstream of obese patients suggest an increased risk in the development of several abnormalities of MetS, such as dyslipidemia, insulin resistance, NAFLD, and atherosclerosis. However, some clinical trials show that palmitoleic acid can promote weight loss, reduce cholesterol levels, and manage inflammation. Clearly, there are insufficient human intervention studies with palmitoleic acid to understand the physiological effects of this unique monounsaturated fatty acid. The beneficial effects *in vitro* and in animal intervention studies indicate that palmitoleic acid may well be a non-pharmacologic alternative to control some features of obesity and other common conditions, showing that more research is needed to be clearer about this.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1. Palmitoleic concentrations in various lipid compartments in humans.

Population	Compartment	Palmitoleic acid (% of total fatty acids)	Palmitoleic acid (µmol/l)	Reference
Generally healthy men and women aged 18-45 years; UK	Plasma triglycerides	3.2	53.5	AL West and PC Calder, unpublished
Generally healthy men and women aged 18-45 years; UK	Plasma phospholipids	0.5	25.2	
Generally healthy men and women aged 18-45 years; UK	Plasma non-esterified fatty acids	2.5	9.6	
Generally healthy men and women aged 18-45 years; UK	Plasma cholesteryl esters	2.3	186.6	
Generally healthy men and women aged 20-80 years; UK	Plasma triglycerides	3.5	-	[2]
Generally healthy men and women aged 20-80 years; UK	Plasma phospholipids	0.7	-	
Generally healthy men and women aged 20-80 years; UK	Plasma cholesteryl esters	2.8	-	
Generally healthy men and women aged 20-80 years; UK	Blood mononuclear cells	1.1	-	
Generally healthy men and women aged 20-80 years; UK	Platelets	1.9	-	
Generally healthy men and women aged 20-80 years; UK	Red blood cells	0.4	-	
Generally healthy men and women aged 20-80 years; UK	Plasma non-esterified fatty acids	3.2	-	[1]
Generally healthy men and women aged 20-80 years; UK	Adipose tissue	5.2	-	

Table 2. Summary of the effects of *cis*-vaccenic acid on metabolic and inflammatory conditions in animal and human studies

	Reference	Experimental or clinical model	Major findings
Animal studies	[30]	Lean (2:1 mix of +/cp and +/+) and obese (cp/cp) JCR:LA-cp rats, fed 3-wk a control diet (0% VA) or a VA diet (1.5% w/w)	VA was ↑ in plasma phospholipids vs. control-fed rats. Lean VA-fed rats: ↓ proportions of CD45RC+ helper cells, and T-helper cells. ↓ IL-2, IL-10 and TNFα in spleen. Obese VA-fed rats: normalized IL-2 and TNFα in MLN to those seen in lean rats.
	[35]	$LDLr^{-/-}$ mice fed 14-wk with atherogenic diet (2% cholesterol) (CH) or 15% vaccenic butter (1.5% w/w VA) (VB)	CH+VB: ↓ serum cholesterol and triglyceride levels, ↓ atherosclerosis
	[34]	Spontaneously hypertensive ratss fed 7-wk with AMF (rich in VA and CLA), or AMF + sterculic oil (SO; which inhibits the conversion of VA into CLA)	AMF diet: ↓ insulin levels, ↓ HOMA, ↓ total cholesterol, ↓ HDL, ↓ TAG, ↑ adiponectin AMF + SO: ↓ systolic blood pressure, ↓↓insulin, ↓ HOMA, ↓↓ total cholesterol, ↓ HDL, ↓↓ TAG, ↑↑ adiponectin
	[38]	Obese JCR:LA-cp rats fed 8-wk with a control diet with or without VA (1% w/w), CLA (1% w/w) or VA+CLA (1% + 0.5% w/w)	VA: ↓ 2-arachidonoylglycerol (2-AG) in the liver and visceral adipose tissue (VAT), ↓ ectopic lipid accumulation. VA: ↑ jejunal concentrations of anandamide, oleoylethanolamide and palmitoylethanolamide, ↓ TNFα and IL1β mRNA.
Human Studies	[29]	Risk set sampling: 1,000 incident CHD events and 1,000 matched controls	Levels of VA in RBC membrane were inversely associated with CHD risk.
	[39]	1373 randomly selected men aged 45-70 y (Metsim study) in Eastern Finland	↑ RBC VA acid was positively associated with adiponectin.
	[37]	3004 participants free of diabetes, in which the plasma phospholipid fatty acids were measured in 1992, and incident diabetes identified in 2005	Plasma VA was inversely associated with diabetes.

AMF, anhydrous milk fat; CHD, coronary heart disease; CLA, conjugated linoleic acid; PA, palmitic acid; RBC, red blood cells; VA, Vaccenic acid.

Table 3. Human studies that correlate palmitoleic acid levels with metabolic alterations.

Reference	Subjects	Where palmitoleic acid measured	Findings
[60]	Healthy children (n=112), mean age 12 y	Plasma (% total FA)	PM +ly associated with abdominal obesity
[56]	Healthy men (n=128), age 28-70 y	Plasma (% total FA)	PM +ly associated with plasma TG
[85]	Patients with NAFLD (n = 25) and NASH (n = 50) compared with lean normal controls (n = 50)	Plasma (% total FA)	PM +ly associated with risk of NAFLD
[54]	100 subjects with increased risk of type-2 diabetes	Plasma (% total FA)	PM + ly associated with insulin sensitivity
[59]	Men and women (n=1926), mean age 58 y	AT biopsy (% total FA)	PM +ly associated with obesity
[58]	Men and women (n=437), age 21-67 y	Serum cholesteryl esters (ng/mL)	PM +ly associated with C-peptide
		Serum phospholipid (%total FA)	PM: No association with C-peptide
[57]	Men and women (n=3107) age 50-70 y from Nutrition and Health of Aging Population in China	RBC (% total FA)	PM +ly associated with MetS
[29]	Physicians' Health Study: 1000 incident CHD events and 1000 matched controls.	RBC membrane (% total FA)	PM +ly associated with CHD risk
[66]	Physicians' Health Study: 788 pairs	Plasma phospholipid (% total FA)	PM +ly associated with heart failure risk
[55]	Men and women (n=1176), age 50-70 y from Nutrition & Health of Aging Population in China	RBC (% total FA)	PM +ly associated with diabetes
[61]	Men and women (n=452), obese healthy vs obese with type-2 diabetes	NEFA (mcg/ml)	PM higher in obese with type-2 diabetes
[23]	Korean boys (n=131), mean age 10.5 y	Plasma phospholipid (% total FA)	PM higher in obese individuals
[62]	Men and women from PREDIMED study (n=427), age 55-80 y	Plasma (% total FA)	PM +ly associated to MetS
[65]	Overweight adults (n=24), elevated de novo lipogenesis and liver fat	(VLDL)-triacylglycerols (mol% total FA)	PM +ly associated with de novo lipogenesis, liver fat, and insulin resistance
[63]	Women's Health Initiative Memory Study (n=6379), 11 y follow-up (n=703), type-2 diabetes	RBC membrane (% total FA)	PM +ly associated with type-2 diabetes
[64]	Men and women (n=358), age 30-65 y at coronary artery disease risk	NEFA (μmol/L)	PM +ly association with NAFLD but not with atherosclerosis

PM, palmitoleic acid; FFA, free fatty acids; TG, triacylglycerol; RBC, red blood cells; MetS, metabolic syndrome; CHD, coronary heart disease; NAFLD, Non-alcoholic fatty liver disease, NASH, non-alcoholic steatohepatitis.

Table 4. Metabolic effects of macadamia nuts or nut oil (palmitoleic acid-rich) or palmitoleic acid supplementation in human clinical trials

Reference	Subjects	Measured	Main findings
[93]	Hypercholesterolemic men (n=34, mean age 49 y) fed (3-wk) with 3 test diet in random order containing palmitoleic acid (macadamia nut oil, 9% 16:0, 18% 16:1n-7, 50% 18:1n-9), oleic acid (sunflower oil, 9% 16:0, 0% 16:1n-7, 50% 18:1n-9) or palmitic acid (palm oil, 25% 16:0, 0% 16:1n-7, 50% 18:1n-9).	Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride	Palmitoleic acid vs oleic acid diet - palmitoleic acid diet: ↑ total cholesterol, ↑ LDL-cholesterol, ↓ HDL -cholesterol.
[71]	Subjects (n=30, age18-53 y) fed with 3 diets for 30-days in a random order: a "typical American" diet (12% MUFA); an American Heart Association Step 1 diet (15% MUFA); or a macadamia nut-based monounsaturated fat diet (20% MUFA) (randomized crossover trial).	Total cholesterol, HDL cholesterol, triglycerides.	Macadamia nut diet: ↓↓ total cholesterol, ↓ LDL-cholesterol, ↓ HDL-cholesterol levels vs. typical American diet.
[70]	Hypercholesterolemic men (n=17, mean age 54 y) received macadamia nuts (40-90 g/d), 4-wk.	Total cholesterol, LDL-cholesterol, HDL-cholesterol and fatty acid composition of plasma lipids, pre and post intervention.	Macadamia nut consumption: ↓ total cholesterol, ↓ LDL-cholesterol, ↑ HDL-cholesterol, ↑ Plasma 16:1n-7, 18:1n-7 and 20:1n-9.
[69]	Healthy Japanese women from the Mukogawa Women's University (age 19–23 y) (n=71) divided into 3 groups which received daily 20 g of macadamia nuts (3% 16:1n-7), coconut oil or butter (both 0.04% 16:1n-7) for 3-wk.	Total cholesterol, LDL-cholesterol and bodyweight.	Macadamia nut group: ↓ total cholesterol, ↓ LDL-cholesterol, ↓ bodyweight, ↓ BMI.
[72]	Mildly hypercholesterolemic (n = 25; 15 female, 10 male) subjects fed (5-wk) with a macadamia nut-rich diet (42.5 g/day, 14% 18:1n-9, 2.5% 16:1n-7) or Average American Diet (AAD, 10% 18:1n-9, 0.4% 16:1n-7) (randomized, crossover, controlled feeding study).	Total cholesterol, LDL-cholesterol, non HDL-cholesterol and plasma fatty acids	Macadamia nut diet *vs* AAD: ↓ total cholesterol (TC), ↓ LDL-cholesterol ↓ non-HDL-cholesterol (HDL-C) ↓ TC:HDL-C, ↓ LDL-C:HDL-C, ↑ Plasma 16:1n-7.
[73]	Adults with dyslipidemia and evidence of mild systemic inflammation (n = 60) received daily for 30-days a capsule with 220.5 mg of palmitoleic acid (n=30) or placebo (1000 mg of medium chain triglycerides, n=30) (randomized crossover trial).	Total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride and CRP.	Palmitoleic acid supplemented group: ↓ CRP, ↓ triglyceride, ↓ LDL-cholesterol, ↑ HDL-cholesterol.

BMI, body mass index; CRP, C-reactive protein.

Table 5. Metabolic effects of macadamia nut oil or palmitoleic acid in cell culture and animal studies

Reference	Cell culture/animal model	Effect of palmitoleic acid
[8]	Rat pancreatic cells exposed to glucose	↑ β cell proliferation and function
[9]	Rat islets of Langerhans exposed to several glucose levels	Counteracted effect of high glucose
[7]	Human islet cells exposed to several glucose levels	↑ β cell proliferation; counteracted effect of high glucose
[76]	Rat L6 skeletal muscle cells	↑ glucose uptake, ↑ glucose oxidation, glycogen synthesis not attributed to insulin, ↑ GLUT 1 and 4
[52]	Endothelial cells treated with single or combined fatty acids	↓ stearate-induced apoptosis
[3]	Mice deficient in adipose tissue lipid chaperones aP2 and mal1, showing a high production of palmitoleic acid in adipose tissue, fed on HFD	Mouse resistant to the deleterious effects of high fat, ↑ AKT and IRS (muscle), ↑ insulin action (muscle), ↓ SCD-1, FAS, ELOV6 (liver), ↓ NAFLD
[94]	F1 hamster, 12 wks diet with 10% (wt:wt) of macadamia, palm, coconut or sunflower oil (n=8)	↓ non-HDL-cholesterol, ↓triglyceride, ↑ HDL-cholesterol
[6]	Obese, diabetic mice, fed palmitoleic acid (300 mg/kg/day), 4-wk	↓ hyperglycemia and hyperTG, ↑ insulin sensitivity, ↓ expression of TNF-α in AT and SREBP-1 and SCD1 in liver
[11]	Liver and primary hepatocytes of male rats fed on a high fat diet (HFD), fed palmitoleic acid (600 mg/kg/day), 4-wk	↓ insulin resistance, ↑ SREBP1c and FAS expression, ↓ macrophages/Kupffer cells, ↓ phosphorylation of NFkB (p65), ↓ expression of proinflammatory cytokines (TNF-α, IL-6)
[75]	Primary adipocytes extracted from periumbilical (ASAT) and upper buttock (GSAT) areas men and human (n=36), BMI 21-46 kg/m^2, incubated for 14 days with adipogenic cocktail.	GSAT: ↑ PM release, ↑ insulin sensitivity, ↑ SCD-1, sex independent.
[49]	Male rats, fasting, fed 3 doses of palmitoleic acid	↓ food intake, ↓ CCK in plasma, ↑ mRNA CCK (duodenum), ↑ PYY (ileum).
[24]	3T3-L1 cells from WT and PPARα KO mice fed palmitoleic acid (300 mg/kg/day), 10 days	↑ adipose lipolysis, adipose lipase, and hormone sensitive lipase; effects required PPARα
[5]	3T3-L1 cells	↑ glucose uptake, ↑ glucose oxidation and GLUT4 mRNA levels, ↓ DNL
	Epididymal AT from mice fed palmitoleic acid (300 mg/kg/day) for 10 days	↑ basal and insulin-stimulated glucose uptake
[4]	WT vs PPARα KO mice, on HFD, fed palmitoleic acid (300 mg/kg/day), 14 days	↓ insulin resistance, ↑ glucose uptake, ↓ liver inflammation (↓ IL-1β); all independent of PPARα
[83]	Obese sheep, i.v infusion of palmitoleic acid (10 mg/kg/day), 28 days	↓ weight gain, ↑ plasma cis-vaccenic acid, ↓ insulin, ↓ total lipid content (ST muscle, MAT), ↑ mRNA for ACC, ELOVL6 and AMPK in liver, sub AT and ST muscle, ↑ mRNA for GLUT4 and CPT1B (sub AT, liver)
[15]	Primary BMDM from HFD mice, Palmitic and/or palmitoleic acids 750 μM (6 h)	↓ NO, ↓ cytokine secretion, ↓ mRNA for CXCL1, IL-6, TNF, NOS2, ↑ mRNA MCP-1, TGFb1, IL-10, ↓ IκBα degradation and NFκB translocation; required AMPK.
[51]	Liver of mice on HFD, fed palmitoleic acid (300 mg/kg/day), 14 days	↓ gluconeogenesis, ↑ glucokinase, ↓ SREBP, ↑ AMPK, ↑ FGF21; required PPARα
[14]	Primary macrophages from WT, PPARα KO and PPARγ LoxP-LysCre mice incubated with LPS + palmitoleic acid (600 μM), 24 h	↓ TNF-α, IL-6 and IL-1β, ↓ mRNA for NFκB, IL-1β, TLR4 and HIF-1α, ↑ PPARγ, ↓ PPARβ; independent of PPARα, PPARγ and AMPK
[12]	Mice BMDM or human THP-1 and PBMC cells cotreated with LPS, palmitoleic and palmitic acid, 24 h	↓ mRNA and secretion of TNF-α and IL-1β, ↓ inflammassome NLRP3, not others (AIM2, NLRP1/4); requires ↑ MUFA/SFA
	APOE$^{-/-}$ HFD mice, fed palmitoleic acid (400 mg/kg/day), 28 days	↑ MUFA/SFA in aorta, ↓ atherosclerotic plaque and lesions, ↓ foam cells

ACC, acetyl-coA carboxilase; AKT, Protein kinase B; AMPK, AMP activated kinase; AT, adipose tissue; BMDM, bone marrow derived macrophage; CPT, carnitine palmitoyltransferase; CCK, colecystokinin; ELOV, Elongation of very long chain fatty acids; FAS, fatty acid synthase; FGF, fibroblast growth factor; GLUT, glucose transporter; HFD, high fat diet; HIF, hipoxia induced factor; IL, interleukin; IRS, insulin receptor substrate; KO, knockout; LPS, lipopolyssaccharide; MAT, mesenteric adipose tissue; NFκB, nuclear factor κB; NO, nitric oxide; NOS, nitric oxide synthase; PPAR, peroxisome proliferator-activated receptor; PYY, Peptide YY; SCD, stearoyl coA-desaturase; ST,skeletal muscle; SREBP, *Sterol regulatory element-binding proteins*.