Feature

Lipid-lowering and anti-inflammatory effects of palmitoleic acid: Evidence from preclinical and epidemiological studies

Nancy Morse

N.M. B. Sc. (Hons), CNPA, NWS, is a Scientific Consultant, 9 Horsburgh Dr., Berwick, Nova Scotia, Canada B0P 1E0. E-mail: nancy.morse@eastlink.ca

Summary

In preclinical and human epidemiological studies, the monounsaturated fatty acid, cis-palmitoleic acid, has shown anti-inflammatory and lipid lowering effects [1] linked to prevention of metabolic syndrome including cardiovascular disease and insulin resistance associated with diabetes and obesity. Randomised, double-blind, placebo-controlled trials are needed to clarify its role in these conditions.

Introduction

Palmitoleic acid (POA), C16:1n-7, is limited in plant and marine based foods [2, 3], but it is particularly concentrated in macadamia nut (Macadamia integrifolia) and sea buckthorn (Hippophae rhamnoides) oils, where it accounts for roughly 17% and up to 29% respectively of fatty acids [3]. Although our dietary intake of POA accounts for less than 4% of total energy, it is the second most abundant monounsaturated fatty acid in most blood lipid pools and is abundant in adipose tissue. This clear compartmentalization and tissue-specific formation and/or storage indicates its content in lipid pools is influenced mostly by endogenous synthesis, and may be the cause or result of its reported divergent effects in various tissues [1].

POA is a key substrate for the formation of triglycerides [4], but is also found in phospholipids, wax esters, cholesteryl esters and free fatty acids in mammalian tissues. It primarily originates via de novo synthesis from surplus dietary carbohydrate [1], or through desaturation of palmitic acid via steroyl-coenzyme A desaturases (SCDs) [3], including SCD1 primarily found in adipose tissue and liver and SCD5 observed in brain and pancreas [4]. SCD1 and its products are important contributing factors in obesity and therefore its activity is often measured and reported as a desaturation index where the ratios of POA to palmitic acid and of oleic acid to stearic acid are quoted (Table 1) [4]. The intricate role of POA between fat and carbohydrate metabolism suggests its importance in homeostasis.

In vitro studies

Results of in vitro studies are currently the most consistent measure of POA action.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Formula</th>
<th>IUPAC nomenclature</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitoleic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇COOH</td>
<td>(9Z)-hexadec-9-enoic acid</td>
<td>9-cis-hexadecenoic acid, 16:1Δ9, 16:1 n-7, 16:1ω7</td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>CH₃(CH₂)₁₄COOH</td>
<td>hexadecanoic acid</td>
<td>16:0</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>CH₃(CH₂)₇CH=CH(CH₂)₇COOH</td>
<td>(9Z)-octadec-9-enoic acid</td>
<td>cis-Δ9-octadecenoic acid, 18:1ω9</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>CH₃(CH₂)₁₆COOH</td>
<td>octadecanoic acid</td>
<td>18:0</td>
</tr>
</tbody>
</table>

Anti-inflammatory action

Chronic exposure of skeletal muscles to saturated fatty acids such as palmitic acid enhances pro-inflammatory signaling that is potently repressed by POA, which has the same carbon length as palmitic acid but the addition of one double bond. POA also increases neutral lipid storage and enhances cellular oxidative capacity while protecting against palmitate-induced mitochondrial dysfunction [5].

Anti-diabetic action

One of the most significant beneficial effects of POA identified through in vitro studies is its impact on β-cell proliferation and apoptosis. β-cells located in the pancreas islets of Langerhans, excrete insulin in response to elevated blood glucose concentration. Their optimal response is dependent on the total β-cell number and their functional activity. They are capable of adapting to prolonged excess glucose exposure by increasing in number and size, followed by apoptosis when glucose levels moderate over an extended period. Type 2 diabetes may occur in genetically susceptible people when these long-term feedback adaptations fail.

In vitro exposure of β-cells to palmitic acid reduces β-cell proliferation [2] and induces cell death upon exposure to normo-glucose concentrations [6]. Conversely, POA has no effect on apoptosis, promotes β-cell division, possibly through a metabolite, and when administered concurrently with palmitic acid, counters its toxic effects [6]. Preventing β-cell apoptosis and instead promoting proliferation may be a way of improving diabetic control [2]. Furthermore, POA increases islet insulin content and glucose-stimulated insulin secretion, and prevents the opposite effects caused by palmitic acid exposure [6].
Anti-obesity action

Saturated fatty acids are known to induce inflammation in adipose tissue while monounsaturates have the opposite effect [2]. For example, palmitic acid induces the toll-like receptor (TLR) signaling pathway in pre-adipocytes while POA has the opposite effect.

In adipose tissue, POA increases lipolysis and the content of major lipases [7], and regulates lipogenic gene expression and rates of lipogenesis [3]. Although it stimulates the formation of triglycerides [7], an effect that one would expect to increase fat accumulation, its overall impact has the opposite outcome due to its influence within adipocytes as follows:

- **Reduces**
  - de novo fatty acid synthesis from glucose and acetate [7]
  - activity of the lipogenic enzymes glucose-6- phosphatase dehydrogenase and adenosine triphosphate (ATP)-citrate lyase [7]
  - messenger ribonucleic acid (mRNA) for SCD1, fatty acid synthase (FAS), and elongase protein 6 (ELOVL6) which decreases lipogenesis [3]
  - desaturation rate [3]

- **Increases**
  - basal and insulin-stimulated glucose uptake [7]
  - mRNA for glucose transporter type 4 (GLUT4) [7]
  - protein content [7]
  - aerobic and anaerobic glycolysis [7]
  - dose dependent gene expression of carnitine palmitoyltransferase 1A (CPT1A), which increases β-oxidation [3]

POA can be elongated to cis-vaccenic acid [(Z)-11-octadecenoic acid, 18:1 cis-11] that also reduces lipogenesis [3].

Animal studies

Animal studies are helping to clarifying the mechanisms whereby POA impacts a variety of metabolic events.

Anti-inflammatory action

Chronic administration of POA to genetically diabetic/obese mice reduces mRNA levels of pro-inflammatory adipocytokines that influence insulin sensitivity [2]. Its treatment in peroxisome proliferator activated receptor α (PPARα)1 knock out mice on high fat diets, significantly reduces hepatic levels of pro-inflammatory interleukin (IL)1-β and IL-12, and causes a trend towards reduced levels of IL-8, tumor necrosis factor (TNF)-α, TLR4, and the phosphorylation of a protein complex that controls transcription. It also dramatically increases IL-1Ra mRNA expression which inhibits the pro-inflammatory effects of IL-1-β [8].

In wild type mice on high fat diets, POA treatment tends to reduce hepatic expression of pro-inflammatory TLR-4 and TNF-α and reverses the high fat diet induced decrease in IL-1Ra mRNA expression.

These anti-inflammatory effects warrant further investigation to clarify the overall metabolic consequences of POA treatment on inflammation.

Lipid-lowering effects

Macadamia oil-fed hamsters have lower blood non-HDL cholesterol and triglycerides than those fed palm and coconut oils (containing mostly saturates) and higher HDL-cholesterol than those fed coconut, canola (mostly monounsaturated 18:1) and safflower (mostly polyunsaturated 18:2) oils [2]. Aortic cholesterol is not affected by any of these diets [2].

The effects of POA on fat accumulation in the liver is varied. The previous study suggests it increases liver cholesterol [2] while another shows it increases fat deposition [8]. No effect is observed in intravenous infused sheep [3] or in PPARα knock out mice or wild type mice fed high fat diets although serum levels of the liver damage marker, aspartate transaminase (AST) are normalised in the wild types [8]. However, chronic administration of POA to genetically diabetic/obese mice significantly reduces plasma triglycerides, and liver weight, triglycerides, and lipids [2].

Suppressed lipid accumulation results from down regulated expression of lipogenic genes involved in de novo lipogenesis [2]. On the other hand, excess fat accumulation in the liver involves local inflammation associated with impaired ability of insulin to inhibit liver glucose production, leading to hyperglycemia and hyperinsulinemia [8]. Such hepatic lipid accumulation known as nonalcoholic fatty liver disease affects 20–30% of humans in Western countries and is linked to obesity, insulin resistance and Type 2 diabetes [2, 8, 9]. The positive effects observed in genetically diabetic/obese mice call for further investigations of varying dosages and dosage forms, modes of administration and genetic models to clarify the role of POA in liver steatosis and to enable practical application in humans.

Anti-diabetic action

How POA ameliorates insulin resistance in diabetic models has not been entirely elucidated [2], but the promising research to date demands a continued effort. POA, but not oleic acid treatment, reduces fasting glucose levels and insulin resistance in wild type and PPARα knock out mice on high fat diets [8]. In wild type mice fed a high fat diet, it increases glucose incorporation into muscle and improves glucose tolerance [8].

In genetically diabetic/obese mice, POA increases pancreas weight, decreases plasma glucose and insulin levels (Figure 1) and dramatically improves their diabetic condition [2]. It improves glucose homeostasis partly by reducing hepatic glucose production [7]. Unlike palmitic acid, it potentiates the insulin-signaling pathway thereby increasing glucose transport into skeletal muscles and improving glycemic control and insulin resistance, partly owing to suppression of lipogenic (Figure 1) and inflammatory gene expression [2].

Intravenous infusion of POA in obese sheep for 28 days reduces circulated insulin levels, and improves insulin resistance without altering circulating glucose levels, through alterations in gene expression for those regulating glucose uptake and fatty acid oxidation specifically within muscle [2]. However, short term (pulse) dose infusion causes immediate elevation in circulating glucose and insulin, indicating a different mechanism for acute versus chronic dosing [2, 3].

Anti-obesity action

The same treatment in sheep mentioned above reduces weight gain by 77%, intramuscular adipocyte size and total lipid content while POA and vaccenic acid increase in serum total lipids in a dose-de-
dependent manner [3]. Chronic administration of POA to genetically diabetic/obese mice reduces food intake and body weight [2]. Oral gavage of either POA or its triglyceride form, tripalmitolein, significantly decreases food intake and increases the satiety hormone cholecystokinin compared to palmitic acid, oleic acid or a control vehicle in male rats [3]. However, exactly how POA affects food intake remains unresolved [2] and requires further investigation.

High SCD1 activity is related to obesity in animal models and SCD1-deficient mice are protected against diet-induced obesity [4]. POA produced and released by adipose tissue mediates communication between adipose tissue and other tissues and inhibits SCD1 activity in the liver [4]. This prevents diet-induced obesity and enhances insulin sensitivity [4]. Infusing tripalmitolein into dietary-induced obese mice with homozygous mutations for fatty acid binding proteins, decreases not only SCD1, but also FAS and ELOVL6 expression thereby reducing production of storage fat while tripalmitin does not [3].

Human epidemiological studies

In a broad context, macadamia nut-based diets rich in POA provide health benefits since they reduce serum total cholesterol, LDL-cholesterol, body weight and body mass index in most studies, although these benefits may not be ascribed specifically to POA since other nutrients are also present [1]. However, the role of POA in human cardiovascular and homeostatic health is only now being elucidated and proving to be exceedingly complex, where seemingly opposing effects require more in depth investigation to unravel their meaning.

Part of this complexity stems from the role of POA in carbohydrate metabolism, where it is one of the main de novo synthesized fatty acids following excess carbohydrate consumption, and tends to accumulate in blood lipid fractions. As a consequence, a large body of evidence indicates that higher proportions of blood POA are associated with metabolic syndrome, type 2 diabetes and cardiovascular disease [10], although the significance of its presence in specific lipid pools has not be fully addressed. Nor has the potential for divergent metabolic response to de novo synthesised versus diet derived POA. For example, since POA is a product of SCD1 activities, could dietary POA, that may be present in specific blood lipid pools (i.e. plasma triglycerides or free fatty acids), inhibit any one or a combination of these or possibly other enzymes through a negative feedback mechanism? Could that inhibition, perhaps within a particular tissue (i.e. liver, pancreas, adipose, brain), initiate a cascade of metabolic events positively impacting insulin sensitivity? Could de novo synthesised POA, which may accumulate in different lipid fractions or tissues, have a divergent effect? To date, only one study had made an attempt to address such issues [4] while most have reported only correlations that may or may not be a definitive measure of cause and effect.

Lipid-lowering effects

The association of circulating POA with plasma lipid profiles deserves notice. Various studies report positive correlations of plasma POA with triglyceridemia [1, 4] and increased risk of cardiovascular disease [10]. However, in 849 Swedish men and women with plasma triglycerides ranging from 0.3 to 20 mmol/L (normal is
<1.7 mmol/L), the desaturation index based on the plasma total lipid 18:1/18:0 ratio was positively correlated with triglyceride levels (Figure 2A) and to a lesser extent, correlated negatively with HDL-cholesterol (Figure 2B) and accounted for 53% of the variation in plasma triglyceride and 17% of the variance in HDL [4, 11]. However, there was no significant correlation between the plasma total lipid 16:1/16:0 ratio and plasma triglycerides (Figure 2C) or HDL cholesterol (Figure 2D) [4, 11]. Multivariate analysis shows that higher POA is independently associated with lower LDL cholesterol, higher HDL cholesterol, lower total:HDL-cholesterol ratio and lower fibrinogen [1].

Anti-diabetic action

Elevated POA in erythrocytes, and plasma phospholipids and cholesteryl esters is reported to predict type 2 diabetes [10]. Contrary to this, in the Cardiovascular Health Study of 3630 men and women from the United States, higher plasma phospholipid POA is not significantly associated with the incidence of diabetes, and is associated with greater insulin resistance in men only [1]. In the plasma free fatty acid fraction, independent of age, gender and body fat, it strongly predicts insulin sensitivity as shown in 100 subjects at risk for type 2 diabetes that underwent 9 months of lifestyle intervention [9]. In addition, higher baseline POA predicts a greater increase in insulin sensitivity [9]. POA clearly plays an important role in the pathophysiology of insulin resistance in humans, although the mechanisms are still unclear [9].

Anti-obesity action

Lifestyle factors including body mass index, carbohydrate intake, and alcohol use are each independently associated with higher POA concentration in plasma phospholipids [1]. Studies report a positive association between POA status in various other tissues and abdominal adiposity, obesity [4] and markers for metabolic syndrome [10]. For example, in a study of 134 healthy men, abdominal adiposity was positively associated with the plasma concentration of POA [4]. In a similar study of 849 Swedish men and women, the desaturation index (16:1/16:0) derived from plasma total lipids was associated with the prevalence of obesity [4, 11]. However, in 1926 adults living in Costa Rica, the association between adipose tissue POA concentration and obesity was less in people with low carbohydrate intake [4] and a subsequent study confirmed that the quantity of POA in plasma triglyceride and cholesteryl esters significantly and uniformly drops as carbohydrate intake decreases, and then gradually increases as dietary carbohydrate is re-introduced [10]. This implies that POA accumulation in certain tissues is a result of excess carbohydrate intake rather than a...
contributing factor to obesity on its own. Consequently, any anti-obesity action that dietary POA may have should not be discounted. To date, it has not been possible to distinguish between adipose-derived and liver-derived POA within adipose tissue, or between liver and adipose tissue SCD1 activity [4]. Future development of such methods is needed to more accurately evaluate the involvement and impact of POA on obesity and metabolic syndrome risk.

Conclusion

In vitro studies provide strong evidence that POA enhances insulin production and secretion, increases fat breakdown, and reduces fat synthesis and storage. Animal studies reveal its significant anti-inflammatory effects that may reduce lipid accumulation in liver and undeniably reduce circulating insulin levels, increase insulin sensitivity, and prevent diet-induced weight gain. Human epidemiological studies reveal varying results thereby imploring the need to decipher mechanisms of action and to conduct randomised, double-blind, placebo-controlled trials to establish its role, efficacy and safety in the fight against metabolic syndrome, insulin resistance, and diabetes.

References