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Feature

Are some health benefits of palmitoleic acid supplementation due to its effects on 5′ adenosine monophosphate-activated protein kinase (AMPK)?

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Summary

In preclinical and human epidemiological and intervention trials, palmitoleic acid (POA) has shown anti-inflammatory and lipid lowering effects linked to prevention of metabolic syndrome including cardiovascular disease and insulin resistance associated with diabetes and obesity. Similar benefits have been reported following AMPK activation suggesting a convergent mode of action. A comparison of POA supplementation metabolic effects and AMPK activation impact reveals a multitude of similarities. This observation suggests that POA could be exerting at least some of its beneficial effects through AMPK activation. Preclinical studies have reported increased cellular AMPK transcription and activated AMPK levels and activity following POA treatment. A plausible explanation of how POA may activate AMPK has been reported. Further research will establish the role of POA in AMPK activation and its effects on specific biochemical activities, overall metabolism and health.

Introduction

Preclinical and human epidemiological study outcomes suggest that palmitoleic acid, the naturally occurring omega-7 monounsaturated fatty acid [(9Z)-hexadec-9-enoic acid, POA], has potential as a therapeutic agent against metabolic syndrome, insulin resistance, and diabetes [1]. In vitro study results strongly indicate that POA enhances insulin production and secretion, increases fat breakdown, and reduces fat synthesis and storage. Animal studies describe significant anti-inflammatory action that may reduce liver fat accumulation and consistently reduce blood insulin levels, increase insulin sensitivity, and prevent diet-induced weight gain. Human epidemiological studies assessing the impact of POA on metabolic syndrome risk factors provide less definitive results [1] while human intervention trials are lacking but provide enticing yet weak evidence of potential benefits, imploring the need for more robust investigations [2].

The quantity of POA is limited in plant and marine based foods, 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 [1]. Although we typically eat only about 2 g of POA daily, it is the second most abundant MUFA in most blood lipids and is a key substrate for triglyceride synthesis [2], hence it is concentrated in fat tissue. Within our bodies, it is predominantly made from surplus dietary carbohydrate, or through desaturation of palmitic acid via steroyl-coenzyme A desaturases (SCDs), including SCD1 primarily found in fat and liver [1]. 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 [1]. This intricate role of POA between carbohydrate and fat metabolism suggests its importance in body homeostasis including weight management.

How POA produces health benefits in humans is only now being elucidated and proving to be exceedingly complex [1, 2]. That's partly because most POA within the body is de novo synthesized from carbohydrate rather than originating from direct dietary intake, and additionally because POA has been measured in various lipid fractions from different tissues or blood lipids, and the mechanisms responsible for its presence in those fractions are poorly understood. Some epidemiological studies report that higher blood POA is associated with metabolic syndrome, type 2 diabetes and cardiovascular disease [1]. However, since POA accumulation in certain tissues may result from excess carbohydrate intake, this may be a consequence rather than a contributing factor to obesity and associated disease states. If so, the health benefits of *dietary POA* observed in animal models, particularly those related to glucose metabolism and weight management, nonetheless warrant further investigation in humans. To date, although few researchers have attempted to explain the mechanism of action of POA in this regard, similar benefits observed following AMPK activation suggest that POA may be acting through its effects on AMPK.

The role of AMPK

AMPK, an enzyme found in various tissues including liver, brain and skeletal muscle, functions in cellular energy homeostasis at both the cellular and whole body level. It coordinates multiple metabolic pathways bringing energy resources in line with energy requirements and responds to nutritional environmental variation [4]. It does this by activating energy- producing pathways [such as catabolic pathways generating adenosine triphosphate (ATP), including fatty acid oxidation and glycolysis], while inhibiting energy consuming ones (such as transcription and fatty acid, triglyceride, cholesterol, and protein synthesis) in response to reduced energy status [i.e. decrease in intracellular ATP and corresponding increase in

the de-energized form of ATP, adenosine monophosphate (AMP)] [3]. This efficient control of ATP consuming/ATP-generating processes maintains energy homeostasis under stress conditions such as hypoxia, myocardial ischemia, heat shock, oxidative stress, starvation and exercise [3].

Cellular AMPK is present in both the nucleus and the cytoplasma and therefore enables both rapid energy regulating changes within cytoplasma through phosphorylation of regulatory proteins as well as gradual adjustments of metabolic processes that require changes in gene transcription within the nucleus [3, 4]. Aside from these effects on individual cells, AMPK also regulates food intake and energy expenditure within the whole body, in particular by mediating the effects of the insulin sensitizing adipokines, including leptin, adiponectin and resistin [4]. At the same time, its activity is regulated by a number of hormones and cytokines (adipokines) including leptin, interleukin-6, resistin, ghrelin and adiponectin [3].

In normal everyday living, muscle contraction is the main trigger for AMPK activation within the body. As muscles contract, energy expenditure dramatically increases the ratio of AMP:ATP leading to activation of AMPK. Various natural substances and numerous drug products are also known to activate AMPK [3].

Impact on metabolism

AMPK impacts metabolism of carbohydrates, lipids, proteins and overall cellular function through various biochemical processes (**Table 1**) [3]. Although it is best known for its cellular and whole body energy management, it is involved in many other functions including the regulation of cell growth and proliferation, cell polarity, apoptosis, autophagy, synthesis of mitochondria (the cellular power generator), and regulation within the endocrine system [3]. Its overall regulatory activity impacts glucose uptake by peripheral tissues, glucose production within the liver [3], sugar and fat burning in skeletal muscles [3, 4], appetite/food intake, fat metabolism in the liver and β-cell insulin secretion [4].

Potential health benefits of AMPK activation

Recently, AMPK has been recognized as a probable link in biochemical activities underlying progression to metabolic syndrome [4] where loss of its activity or attenuation of its expression appears to be associated with development of type 2 diabetes, obesity and cancer [3]. Health benefits of AMPK activation include lipid lowering and anti-obesity effects [3, 4], type 2 diabetes and metabolic syndrome improvements [4], prevention of non-alcoholic fatty liver disease [3, 4], and cardio-protective, neuro-protective and anti-cancer influence [3].

Potential impact of POA on AMPK activity

In recent years, many naturally occurring compounds that are AMPK activators capable of preventing and treating diseases have been identified including flavonoids and a diverse array of unrelated compounds including biguanides, berberine, salidroside, D-xylose, capsaicin and curcumin [3]. A comparison of the metabolic effects of AMPK activation and POA supplementation reveals a multitude of similarities and suggests that POA could also be exerting at least some of its beneficial effects through AMPK activation (**Table 2**). As well, results of recent preclinical and human intervention trials support this proposal as follows:

Table 1. Impact of AMPK activation on metabolism [3, 4].

Carbohydrate metabolism
• Increases glucose uptake
• Increases glycolysis
• Decreases gluconeogenesis
• Decreases glycogen synthesis
Lipid metabolism
• Decreases triglyceride and phospholipid synthesis
• Decreases fatty acid synthesis
• Increases fatty acid oxidation
• Increases fatty acid uptake
• Decreases transcription of lipogenic genes
• Decreases isoprenoid synthesis
• Decreases lipolysis
Protein metabolism
• Decreases protein synthesis
Overall cellular function
• Increases transcription of genes involved in oxidative stress response
• Increases cell cycle arrest autophagy
• Increases mitochondrial bioneogenesis

In vitro Studies

Certain fatty acids can affect rates of glucose uptake through the insulin stimulated intracellular signaling pathway according to their carbon chain length and number of double-bonds. Specifically, saturated fatty acids including palmitic (16 : 0) and stearic acid (18 : 0) impair insulin stimulated glucose uptake while POA (16 : 1n-7) improves its absorption. However, glucose uptake by adipocytes within white adipose tissue can also be carried out independent of insulin by glucose transporter type (GLUT) proteins. This process is enabled in adipocytes by activated AMPK that increases GLUT4 transcription and protein levels and causes GLUT4 translocation to the plasma membrane [6].

A study with differentiated 3T3-Li cells (mouse embryonic fibroblast cells programmed to become adipocytes) was carried out to see if certain fatty acids could also differentially impact glucose uptake by GLUTs and if the mechanism included AMPK activation [6]. Cells treated with POA, but not palmitic acid, had increased:

- Non-insulin stimulated glucose uptake by 51%
- Insulin- stimulated glucose uptake by 36%
- GLUT 4 transcription by 34%
- GLUT4 protein levels by 78%

Table 2. Similar metabolic effects of AMPK activation and POA supplementation.

General effects	Specific Effects
Anti-diabetic and anti-obesity action [1, 3]	Regulation of lipogenic gene expression [1, 3]
Suppression of fat synthesis [1, 3]	Increased glucose uptake [1, 3]
Enhanced energy expenditure [1, 3]	Reduces hepatic glucose production [1, 3]
Reduced fat storage [1, 3]	Increased transcription of genes for synthesis of GLUT4 [1, 4]
Improved glucose metabolism [1, 3]	Increased fatty acid oxidation [1, 3]
Reduced blood LDL-cholesterol [1, 5] and triglycerides [1, 2, 4, 5]	Improved glucose transport into skeletal muscles [1, 4]
Reduced liver weight and lipid content [1, 4]	Reduced body weight [1, 7] or weight gain [1, 7]

Treatment with POA, but not palmitic acid, also significantly increased AMPK activity as evidenced by a higher concentration of the activated form of AMPK, pThr172AMPKα. As well, pharmacological inhibition of AMPK activation by compound C, prevented an increase in GLUT4 concentration and ultimately glucose uptake. These results suggest that POA is an AMPK activator and AMPK activation was responsible for the increased GLUT4 concentration and resulting glucose uptake [6].

In vivo Studies

A study akin to that described above but using adipocytes from mice treated with POA (16 : 1n-7) or oleic acid (18:1n-9) for 10 days had similar results where cells from mice treated with POA, but not oleic acid, had increased:

- Non-insulin stimulated glucose uptake by 3-fold relative to water treated control mice
- Insulin- stimulated glucose uptake by 1.8-fold relative to water treated control mice
- GLUT4 transcription by 86%

POA also enhanced adipocyte glucose metabolism through energy-producing instead of energy-storing pathways based on increased aerobic and anaerobic glycolysis and inhibition of de novo lipogenesis. At least part of the POA actions seemed due to increased AMPK activity suggesting that POA, similar to exercise and adiponectin among others, is an important AMPK activator associated with stimulated glucose uptake [6].

Another study including obese sheep that were intravenously infused with an ethyl ester of PAO (Provinal®) for 28 days reported it specifically [8]:

- increased cellular levels of acetyl-CoA carboxylase, fatty acid elongase-6, AMPK transcription and activated AMPK levels in liver, adipose tissue and muscle
- increased production of GLUT4 and CPT-1 in adipose and liver
- reduced production of GLUT4 and CPT-1 in muscle

The overall effects were:

- reduced circulating insulin levels and improved insulin resistance through altering gene expression that regulated glucose uptake and fat burning
- reduced intramuscular fat cell size
- reduced total fat content within fat cells
- reduced weight gain by 77%, in a dose dependent manner as the blood concentration of POA increased

Previous studies have shown that AMPK activation in skeletal muscle is a critical determinant of fuel selection for energy production and can prevent or delay insulin resistance onset. Typically, *chronic AMPK activation* within muscle increases the expression of genes used to make GLUT4 [4] and directly phosphorylates and inactivates glycogen synthase, thereby inhibiting glycogen synthesis. The net effect is activation of glycolysis, a process where glucose is converted to energy rather than to glycogen, which is a storage carbohydrate within muscle [3]. AMPK activation also stimulates the creation of more mitochondria (biogenesis) within cells and enhances fatty acid oxidation (fat burning), thereby shifting the balance away from fat storage [4].

In the above described 28 day study in obese sheep, the production of GLUT 4 and CPT-1 in muscle following POA treatment was *reduced* and that would be expected to *reduce* glucose uptake and transfer of fatty acids into mitochondria for subsequent oxidation, respectively. The impact of this would *reduce* energy expenditure and would be expected to *increase* energy storage (fat accumulation). However, the observed whole animal net improvements including reduced circulating insulin levels, improved insulin resistance and reduced intramuscular adipocyte size and fat content suggests that the positive impact of POA on liver and adipocyte metabolism was greater than the differing response observed in muscle.

There could be a number of explanations for these unanticipated results in muscle cells. It is possible that treatment for 28 days in sheep is too short to achieve the typical metabolic changes associated with *chronic* POA treatment in rodent muscle, although even short-term (24 hour) treatment with an AMPK activator has been shown to increase GLUT4 protein content in skeletal muscle in rats [9]. On the other hand, horses treated with an AMPK activator had deceased blood glucose along with increased activated AMPK in muscle with no change in GLUT4 expression [10]. This is similar to what was observed in the sheep study and could be indicative of a similar genetic tendency. The horses however, had increased expression of a novel GLUT isoform called GLUT8 which appeared to be transporting glucose into the muscles cells [10]. Perhaps a similar process also occurs in sheep.

Additionally, studies have shown that different AMPK activators can exert anti-diabetic effects even with opposing impacts on GLUT4. For example, chronic treatment with the anti-diabetic drug, metformin does not affect GLUT4 protein expression whereas AMPK activation by 5-aminoimidazole-4-carboxamide-1-β-D-ribofranoside (AICAR) increases GLUT4 expression [11]. Therefore, POA, similar to metformin, may be exerting its whole body beneficial effects independent of muscle cell GLUT4 involvement.

Human intervention trials

The lipid lowering effects of POA in human intervention trials are similar to those observed with AMPK activators. AMPK activation increases fatty acid oxidation and decreases fatty acid, cholesterol [4] and triglyceride [3] synthesis within the liver, which decreases blood lipids. Animal studies have shown that AMPK activators reduce plasma triglycerides [4] and lower blood pressure [4] while randomized, double-blind, placebo-controlled trials in humans report significantly reduce LDL-cholesterol [12]. Comparable lipid lowering effects were achieved in a randomized, double-blind, placebo-controlled trial including 60 adults with dyslipidemia and elevated C-reactive protein given 220.5 mg/day POA (Provinal®) for 30 days [5]. Compared to placebo, Provinal® reduced blood triglycerides by 15%, reduced LDL-cholesterol by 8% and increased HDL-cholesterol by 5%. These effects may have resulted from POA activation of AMPK.

Mechanism of action

Bolsoni-Lopes and associates proposed that POA may activate AMPK by reducing ATP levels. They refer to a study reporting a 25% reduction in cellular ATP levels following *lipolysis activation* which led to a significant increase in AMPK activation coupled with increased acyl-CoA synthase and triglyceride synthesis [6]. (Acyl-CoA is an enzyme responsible for fatty acid biosynthesis.) POA has been shown to increase both lipolysis and triglyceride synthesis [13]. Therefore, POA stimulated lipolysis may decrease ATP levels that cause activation of AMPK which then increases triglyceride synthesis. This increased triglyceride synthesis is asso-

ciated with an increased incorporation of glucose into the glycerol fraction of the triglyceride thereby generating higher glycerol-3-phosphate synthesis via glycolysis (the process whereby cells produce energy from glucose). Thus, POA through is effects on AMPK activation may stimulate conversion of storage fat triglycerides into molecules that can be used as fuel in energy producing metabolism.

Such increases in triglyceride-fatty acid cycling as described above have been shown not only to enhance cellular energy expenditure but also to increase the sensitivity of both triglyceride synthesis and lipolysis to neuro/hormonal control [13]. This may induce dramatic changes in energy production and utilization sufficient to modify whole body homeostasis and prevent or reverse metabolic disorders.

Conclusion

The mechanisms whereby POA supplementation improves cellular and whole body metabolism are only now being elucidated. Results of recent preclinical and human intervention trials indicate that at least some of its effects may be occurring through AMPK activation. Continued research is needed to establish the complex mechanisms whereby POA may manifest its health benefits and to better understand its role, efficacy and safety as a therapeutic agent for metabolic syndrome, cardiovascular disease, and diabetes.

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