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Polyunsaturated fatty acids do not activate AMP-activated protein kinase in mouse tissues ☆

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Abstract

Stearoyl-CoA desaturase 1 (SCD1) deficiency partitions fatty acids away from lipid synthesis towards fatty acid oxidation in liver and skeletal muscle in part due to activation of AMP-activated protein kinase (AMPK) pathway. The mechanism of AMPK activation by SCD1 mutation is unknown, however since SCD1−/− animals have increased relative amounts of polyunsaturated fatty acids (PUFA), we hypothesized that the increased levels of PUFA might be responsible for the activation of AMPK in SCD1 deficient mice. Therefore, the present study was undertaken to analyze the effect of PUFA on AMPK in liver, skeletal muscle, and heart. We fed mice ad libitum for 14 days with diet supplemented with fish oil (5% fat). As expected, fish oil supplementation significantly increased $n-3$ PUFA content in each of the analyzed tissues. Hepatic mRNA levels of fatty acid synthase and acyl-CoA oxidase decreased by 92% and increased by 60%, respectively, consistent with known PUFA effects. However, after 14 days of PUFA feeding, we did not find any changes in AMPK phosphorylation and protein content in mouse liver, skeletal muscle, and heart. The data suggest that PUFA are not involved in AMPK activation in mouse tissues and that the increased activity of AMPK in SCD1−/− mice is probably PUFA-independent.
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Keywords: PUFA; AMPK; SCD; Skeletal muscle; Liver; Heart

AMP-activated protein kinase (AMPK), a heterotrimeric complex composed of a catalytic α-subunit and regulatory β- and γ-subunits, is a major sensor of cellular energy status [1]. Activation of AMPK downregulates biosynthetic pathways such as fatty acid and cholesterol synthesis, yet switches on catabolic pathways that generate ATP, such as fatty acid oxidation, glucose uptake, and glycolysis. It achieves this through direct phosphorylation of metabolic enzymes (i.e., acetyl-CoA carboxylase) and through effects on gene expression, such as upregulation of glucose transporter-4 and genes of mitochondrial enzymes in muscle, and downregulation of genes encoding enzymes of fatty acid synthesis and gluconeogenesis in liver (reviewed in [2]). In vivo AMPK is activated by any stress that causes a rise in the cellular AMP:ATP ratio, including heat shock and metabolic poisoning in hepatocytes, and hypoxia and ischemia in the heart (reviewed in [3]). Low glucose levels activate AMPK in pancreatic β-cells, whereas exercise or contraction enhances AMPK activity in skeletal muscle. AMPK is allosterically inhibited by phosphocreatinine and high glycogen content represses AMPK activation. Also, hormones (leptin, adiponectin) and drugs (metformin, thiazolidinediones) regulate the AMPK pathway (reviewed in [3]).

☆ *Abbreviations:* AMPK, AMP-activated protein kinase; PUFA, polyunsaturated fatty acids; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; AOX, acyl-CoA oxidase; SCD, stearoyl-CoA desaturase.

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Because AMPK plays a role in partitioning of long chain fatty acids (FA) between degradative and biosynthetic fates, it has been hypothesized that AMPK may also be regulated directly by FA and/or their acyl-CoA esters. In support of this notion, incubation of primary rat hepatocytes with 150 μM palmitate caused a 2-fold increase in AMPK activity [4]. Perfusion of rat heart with 0.5 mM palmitate or 0.5 mM oleate has also been shown to increase AMPK activity, whereas heart perfusion with lower palmitate concentration (0.075 mM) significantly decreased AMPK activity [5]. Long chain acyl-CoA esters have been shown to inhibit the AMPK kinase (AMPKK) activity and thereby downregulate AMPK signaling cascade in rat liver [6]. Furthermore, incubation of L6 myocytes with fatty acids with various chain lengths showed that phosphorylation of AMPK was significantly higher in the presence of shorter chain (12:0 and 14:0) fatty acids and inhibited in the presence of longer chain (16:0, 18:0, and 20:0) fatty acids [7].

Polyunsaturated fatty acids (PUFA), particularly those of the $n-3$ family, play pivotal roles as "fuel partitioners" in that they direct FA away from triglyceride storage towards oxidation in liver, skeletal muscle, and potentially in cardiomyocytes and β-cells [8]. PUFA exert their effect by upregulating the expression of genes of fatty acid oxidation while simultaneously downregulating genes of lipid synthesis (reviewed in [9]).

A recent study by Suchankova et al. [10] suggests that PUFA activate AMPK, and their beneficial effect could also be mediated by the AMPK pathway. Stearoyl-CoA desaturase (SCD) is the major enzyme catalyzing desaturation of saturated fatty acyl-CoAs. We have shown that SCD1 deficiency activates AMPK in mouse liver [11] and skeletal muscles [12], however, the mechanism of AMPK activation by SCD1 mutation is currently unknown. Because SCD1−/− mice have changes in fatty acid composition, with a decrease in the content of monounsaturated fatty acids and the PUFA content tending to be increased [12,13], we hypothesized that the increased levels of PUFA are responsible for the activation of AMPK we observe in the SCD1 deficient mice. Therefore, the present study was undertaken to analyze the effect of dietary PUFA on AMPK in liver, skeletal muscle, and heart. We fed mice ad libitum for 14 days with diet supplemented with fish oil. Fish oil supplementation significantly increased $n-3$ PUFA content in each of the analyzed tissues, and as expected, decreased hepatic fatty acid synthase (FAS) and increased acyl-CoA oxidase (AOX) gene expression in liver. However, fish oil did not effect AMPK phosphorylation and protein content. The data suggest that PUFA are not involved in AMPK activation in mouse tissues and the increased activity of the AMPK observed in the SCD1−/− mice is probably PUFA-independent.

Materials and methods

Animals and diets. The generation of SCD1−/− mice has been previously described [14]. Twelve-week-old purebred homozygous (SCD1−/−) and wild type male mice on SV-129 background were used. Mice were housed in a pathogen-free animal facility of the Department of Biochemistry of the University of Wisconsin-Madison, operating at a 12-h light/12-h dark cycle. The breeding of the animals was in accordance with the protocols approved by the Animal Care Research Committee of the University of Wisconsin-Madison. At 8 weeks of age, the mice were fed ad libitum for 14 days semi-purified diet (TD 99252; Harlen Teklad, Madison, WI) containing 5% fat in the form of either triolein (control group) or fish oil. The semi-purified diet (TD 99252) contained, by weight, 54% sucrose, 21% casein, 14% maltodextrin, 5.2% cellulose, 3.6% mineral mix (AIN-93G-MX), 1.1% vitamin mix (AIN-93G-MX), 0.32% L-cysteine, and 0.26% choline bitartrate. The fish oil (Sigma Chemical, St. Louis, MO) contained about 25% $n-3$ fatty acids (eicosapentaenoic and docosahexsaenoic acids). Mice were sacrificed and the heart, soleus muscle, and liver were extracted, snap frozen in liquid nitrogen, and stored at −80 °C.

Materials. [α-^{32}P]dCTP was purchased from Perkin–Elmer Life Sciences (Boston, MA). Anti-AMPK α1 and α2, and anti-phospho-AMPK (Thr 172) antibodies were obtained from Dr. D. Grahame Hardie (Dundee University, Dundee, United Kingdom). Lipid standards were from Nu-Chek Prep (Newark, DE). All other chemicals were purchased either from Sigma (St. Louis, MO) or from Fisher Scientific (Pittsburgh, PA).

Measurement of lipids. Lipids were extracted from muscle, liver, and heart by the method of Bligh and Dyer [15] and measured as described [12]. Fatty acids were then transmethylated in the presence of 14% boron trifluoride in methanol. The resulting methyl esters were extracted with hexane and analyzed by gas–liquid chromatography [16].

Western blot analysis. Tissues were homogenized in ice-cold 50 mM Hepes buffer (pH 7.4) containing 150 mM NaCl, protease inhibitors, and 10% glycerol and centrifuged at 10,000*g* for 10 min. Proteins were separated on a 9% SDS–PAGE gel, then transferred, and immobilized on nitrocellulose membrane. The membranes were immunoblotted with antibody against phosphopeptides based on the amino acid sequence surrounding Thr-172 of the α subunit of AMPK. Protein levels of α1- and α2-subunits of AMPK were determined using specific antibodies. The proteins were visualized using ECL (Amersham Biosciences) and quantified by densitometry.

Isolation and analysis of RNA. Total RNA was isolated from liver using trizol reagent. FAS and AOX gene expression was analyzed by Northern blotting. Twenty micrograms of total RNA was fractionated on 1% agarose–2.2 M formaldehyde gels and transferred to Hybond N^+ nylon membranes. After UV cross-linking, the membrane was hybridized with ^{32}P-labeled cDNA probes for FAS and AOX as previously described [17]. After washing, the membranes were exposed to X-ray film at −80 °C and signals were quantified by densitometry. The pAL15 mRNA was used as an internal control.

Protein content. The protein concentration was determined with Bio-Rad protein assay (Hercules, CA) using bovine serum albumin as a standard.

Statistical analysis. Results were analyzed by using Student's *t* test. A difference of $P < 0.05$ was considered significant. Values are presented as means ± SD ($n = 6$ mice per group).

Results

SCD1−/− mice have increased relative amount of PUFA and enhanced AMPK phosphorylation in liver and skeletal muscle

Lack of SCD1 function affects fatty acid composition of free fatty acids and phospholipids [12,13]. When we

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