Biochimica et Biophysica Acta 1861 (2016) 119–129

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Lack of phosphatidylethanolamine *N*-methyltransferase in mice does not promote fatty acid oxidation in skeletal muscle

Guergana Tasseva [a,b], Jelske N. van der Veen [a,c], Susanne Lingrell [a,c], René L. Jacobs [a,d], Dennis E. Vance [a,c], Jean E. Vance [a,b,*]

[a] Group on Molecular and Cell Biology of Lipids, University of Alberta, Edmonton, Canada
[b] Department of Medicine, University of Alberta, Edmonton, Canada
[c] Department of Biochemistry, University of Alberta, Edmonton, Canada
[d] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 8 October 2015
Accepted 16 November 2015
Available online 18 November 2015

Keywords:
Phosphatidylcholine
Mitochondria
High-fat diet
Myotubes
Fatty acid oxidation
Malonyl-CoA

ABSTRACT

Phosphatidylethanolamine *N*-methyltransferase (PEMT) converts phosphatidylethanolamine (PE) to phosphatidylcholine (PC) in the liver. Mice lacking PEMT are protected from high-fat diet-induced obesity and insulin resistance, and exhibit increased whole-body energy expenditure and oxygen consumption. Since skeletal muscle is a major site of fatty acid oxidation and energy utilization, we determined if rates of fatty acid oxidation/oxygen consumption in muscle are higher in *Pemt*$^{-/-}$ mice than in *Pemt*$^{+/+}$ mice. Although PEMT is abundant in the liver, PEMT protein and activity were undetectable in four types of skeletal muscle. Moreover, amounts of PC and PE in the skeletal muscle were not altered by PEMT deficiency. Thus, we concluded that any influence of PEMT deficiency on skeletal muscle would be an indirect consequence of lack of PEMT in liver. Neither the *in vivo* rate of fatty acid uptake by muscle nor the rate of fatty acid oxidation in muscle explants and cultured myocytes depended upon *Pemt* genotype. Nor did PEMT deficiency increase oxygen consumption or respiratory function in skeletal muscle mitochondria. Thus, the increased whole body oxygen consumption in *Pemt*$^{-/-}$ mice, and resistance of these mice to diet-induced weight gain, are not primarily due to increased capacity of skeletal muscle for utilization of fatty acids as an energy source.

1. Introduction

The mechanisms underlying obesity and type-2 diabetes are complex and incompletely understood. For example, the regulation of whole body energy metabolism involves multiple tissues including the skeletal muscle, liver, adipose tissue and brain [1]. Recently, the metabolism of phosphatidylcholine (PC), the most abundant membrane phospholipid in mammalian cells, has been implicated in the development of obesity and insulin resistance ([2–4]; reviewed in [5]).

In mammalian cells, PC is synthesized from choline via the CDP-choline pathway [6]. In addition, in the liver, PC is also made by the methylation of phosphatidylethanolamine (PE) catalyzed by phosphatidylethanolamine *N*-methyltransferase (PEMT) ([7,8]; reviewed in [5]). Approximately 70% of hepatic PC is generated from the CDP-choline pathway and ~30% from the PEMT pathway [9]. The liver contains significant PEMT enzymatic activity whereas in other tissues PEMT activity is less than 1% of that in the liver [5,10]. Nevertheless, the low level of PEMT in white adipose tissue appears to have some physiological relevance during adipocyte differentiation [11,12].

Pemt$^{-/-}$ mice appear phenotypically normal when fed a chow diet [2,13]. Body weight gain, oxygen consumption and glucose tolerance, as well as the amounts of hepatic PC and triacylglycerols (TG), are equivalent in chow-fed *Pemt*$^{-/-}$ and *Pemt*$^{+/+}$ mice [2,13,14]. However, *Pemt*$^{-/-}$ mice succumb to liver failure when fed a choline-deficient diet for three days [14,15]. Moreover, the lack of PEMT reduces the secretion of very low density lipoproteins by hepatocytes and also reduces plasma lipoprotein levels [16–18]. Correspondingly, elimination of PEMT in two mouse models of atherosclerosis (i.e. mice lacking the low density lipoprotein receptor, and mice lacking apolipoprotein E) decreases atherosclerotic lesion area by 85% [19] and ~30% [20], respectively. As expected, when *Pemt*$^{+/+}$ mice were fed a high-fat (HF) diet (containing 60% of calories as fat) for 10 weeks, they gained weight and developed insulin resistance. Remarkably, however, *Pemt*$^{-/-}$ mice were strikingly protected against diet-induced weight gain and insulin resistance [2]. The HF-fed *Pemt*$^{-/-}$ mice also exhibited 50% higher whole-body energy expenditure/oxygen consumption and accumulated ~90% less white

Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; HF, high-fat; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine *N*-methyltransferase; SREBP, sterol-regulatory element binding protein; TG, triacylglycerols; UCP, uncoupling protein; VDAC, voltage-dependent anion channel.

* Corresponding author at: 328 HMRC, University of Alberta, Edmonton AB T6G 2S2, Canada.
E-mail address: jean.vance@ualberta.ca (J.E. Vance).

http://dx.doi.org/10.1016/j.bbalip.2015.11.008
1388-1981/

adipose tissue mass than did their *Pemt*$^{+/+}$ counterparts despite equal, or even higher, food consumption [2]. The resistance of *Pemt*$^{-/-}$ mice to weight gain was not due to higher plasma levels of leptin since leptin levels were 85% *lower* in *Pemt*$^{-/-}$ mice than in *Pemt*$^{+/+}$ mice. Moreover, the concentration of unesterified fatty acids and TG in plasma was 60% and 77%, respectively, lower in HF-fed *Pemt*$^{-/-}$ mice than *Pemt*$^{+/+}$ mice [2], consistent with the attenuated secretion of very low density lipoproteins [16,17]. The HF-fed *Pemt*$^{-/-}$, but not *Pemt*$^{+/+}$, mice developed severe hepatic steatosis with a 10-fold elevation in plasma alanine aminotransferase activity [2]. The hepatic accumulation of TG and the profound depletion of white adipose tissue mass in PEMT-deficient mice suggested that TG storage might have been diverted from adipose tissue to liver, and possibly to muscle. However, the amount of TG in skeletal muscle of *Pemt*$^{-/-}$ mice was ~60% lower than in *Pemt*$^{+/+}$ mice [2].

The mechanisms underlying the protection of HF-fed *Pemt*$^{-/-}$ mice from weight gain have not yet been completely defined. The ~25% lower level of PC in livers of HF-fed *Pemt*$^{-/-}$ mice compared to *Pemt*$^{+/+}$ mice is unlikely to confer the protection against obesity since liver-specific elimination of CTP:phosphocholine cytidylyltransferase (the rate-limiting enzyme of the CDP-choline pathway for PC synthesis) also reduced hepatic PC by ~20% in HF-fed mice [21] but did not protect the mice from weight gain [2]. On the other hand, PEMT is the only endogenous source of choline in animals [2,5] since choline is released from PEMT-derived PC by phospholipases, whereas degradation of PC derived from the CDP-choline pathway does not result in a net production of choline. Importantly, when *Pemt*$^{-/-}$ mice were fed the HF diet containing additional choline, their resistance to weight gain and insulin resistance were both abolished [2,3,22]. These data indicate that choline availability is an important regulator of energy metabolism and that choline availability in *Pemt*$^{-/-}$ mice is insufficient for a normal response to the HF diet.

Muscle is the largest tissue in the human body, comprising ~40% of body mass. Skeletal muscles are rich in mitochondria and the majority of fatty acid oxidation in animals occurs in skeletal muscle [23–26]. We, therefore, tested the hypothesis that an increased rate of fatty acid oxidation in skeletal muscle is a key factor in protecting HF-fed *Pemt*$^{-/-}$ mice from diet-induced weight gain. In contrast to our hypothesis, however, our data demonstrate that the capacity of skeletal muscle for fatty acid oxidation is not enhanced by PEMT deficiency.

2. Materials and methods

2.1. Materials

Dulbecco's-modified Eagle's medium (DMEM), Glutamax™, fetal bovine serum, penicillin–streptomycin, recombinant human basic fibroblast growth factor and Geltrex™ LDEV-free basement membrane matrix were purchased from Invitrogen (Gibco-Life Technologies). Amphotericin B, essentially fatty acid-free bovine serum albumin (BSA), L-carnitine, phosphate-buffered saline with Mg^{2+} and Ca^{2+}, glucose, HEPES and sodium palmitate were from Sigma. Sodium palmitate (6 mM) was bound to BSA (2.4 mM) (palmitate:BSA = 6:1); only optically clear solutions were used. [1-^{14}C]Palmitic acid and [9,10-^{3}H]oleate were from Perkin Elmer. The rabbit anti-rat PEMT polyclonal antibody was raised against the C-terminal dodecapeptide of rat PEMT and has been extensively characterized in our laboratories [13,27]. The rabbit polyclonal anti-uncoupling protein-3 (UCP3) antibody (catalog #Ab3477) was from Abcam, the anti-rodent total OXPHOS antibody mixture (for immunoblotting electron transport chain proteins; catalog #MS604) was from Mitosciences, and the mouse monoclonal anti-outer mitochondrial membrane voltage-dependent anion channel protein-1 (VDAC1, catalog #Ab14734) antibody was from Mitosciences. The rabbit polyclonal anti-calnexin antibody was from BD Transduction. Horseradish peroxidase-conjugated secondary antibodies and the chemiluminescence reagent were from Pierce.

2.2. Animals

Male C57Bl/6 *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice, backcrossed for >7 generations, were maintained in a breeding colony at the University of Alberta [2,4,13]. Upon weaning, the mice were fed a chow diet (diet #5001 from LabDiet) *ad libitum* with free access to water. At 8 weeks of age the mice were fed a high-fat (HF) diet containing 60% of calories as fat (BioServ #F3282) for 10 weeks (unless otherwise indicated) [2]. All procedures with mice were approved by the University of Alberta Institutional Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care.

2.3. Immunoblotting

For immunoblotting of PEMT (molecular mass ~ 20 kDa), livers and four types of skeletal muscle (gastrocnemius, soleus, quadriceps and diaphragm) were removed from 3- to 4-month-old chow-fed *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice. The fat was scraped from the muscles and the muscles were homogenized. Homogenate proteins were electrophoresed on 12.5% polyacrylamide gels containing 0.1% SDS and subsequently transferred to polyvinylidene difluoride membranes for immunoblotting with polyclonal anti-rat PEMT antibodies (0.3 μg/ml) [13,27]. Secondary antibodies (dilution 1:5000; Pierce) conjugated to horseradish peroxidase were used for detection of immunoreactive proteins by enhanced chemiluminescence.

Amounts of mitochondrial proteins in homogenates of myotubes and in mitochondria isolated from gastrocnemius muscle and heart were examined by immunoblotting. The proteins, in buffer containing 62.5 mM Tris–HCl (pH 6.8), 10% glycerol, 2% SDS and 5% β-mercaptoethanol, were electrophoresed on 10% polyacrylamide gels containing 0.1% SDS. The proteins were subsequently transferred to polyvinylidene difluoride membranes which were incubated in 10 mM Tris–HCl (pH 7.4), 100 mM NaCl, 0.1% Tween 20 for 1 h at room temperature with the following primary antibodies: anti-UCP3 antibodies (dilution 1:1000), total OXPHOS antibodies raised against electron transport chain complexes (dilution 1:2000), anti-VDAC1 antibodies (dilution 1:1000), and anti-calnexin antibodies (dilution 1:1000). Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (dilution 1:5000; Pierce) for 1 h at room temperature and immunoreactivity was detected by enhanced chemiluminescence (Pierce).

2.4. PEMT enzymatic activity

PEMT enzymatic activity was measured for 30 min in 25 μg homogenate protein using phosphatidylmonomethylethanolamine (Avanti Polar Lipids) and [^{3}H-*methyl*]-*S*-adenosylmethionine as substrates. The activity of PEMT was linear over time and protein concentration at this time point and for this concentration of protein [13,28].

2.5. Measurement of phospholipid mass

Phospholipids were extracted from homogenates of skeletal muscles (gastrocnemius, quadriceps, soleus, diaphragm) [29] and separated by thin-layer chromatography in the solvent system chloroform:methanol:acetic acid:formic acid:water (70:30:12:4:1, v:v). PC and PE were quantified as inorganic phosphate (nmol/mg protein) [30] and the molar ratio of PC/PE was calculated.

2.6. Plasma content of non-esterified fatty acids

Amounts of non-esterified fatty acids in mouse plasma were measured using a commercially-available kit (HR series) from Wako Pure Chemicals Inc. according to manufacturer's instructions.