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Elevated tissue omega-3 fatty acid status prevents age-related glucose intolerance in fat-1 transgenic mice

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ABSTRACT

The objective of this study was to investigate the impact of elevated tissue omega-3 (n-3) polyunsaturated fatty acids (PUFA) status on age-related glucose intolerance utilizing the fat-1 transgenic mouse model, which can endogenously synthesize n-3 PUFA from omega-6 (n-6) PUFA. Fat-1 and wild-type mice, maintained on the same dietary regime of a 10% corn oil diet, were tested at two different ages (2 months old and 8 months old) for various glucose homeostasis parameters and related gene expression. The older wild-type mice exhibited significantly increased levels of blood insulin, fasting blood glucose, liver triglycerides, and glucose intolerance, compared to the younger mice, indicating an age-related impairment of glucose homeostasis. In contrast, these age-related changes in glucose metabolism were largely prevented in the older fat-1 mice. Compared to the older wild-type mice, the older fat-1 mice also displayed a lower capacity for gluconeogenesis, as measured by pyruvate tolerance testing (PTT) and hepatic gene expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6 phosphatase (G6Pase). Furthermore, the older fat-1 mice showed a significant decrease in body weight, epididymal fat mass, inflammatory activity (NFκ-B and p-IκB expression), and hepatic lipogenesis (acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) expression), as well as increased peroxisomal activity (70-kDa peroxisomal membrane protein (PMP70) and acyl-CoA oxidase1 (ACOX1) expression). Altogether, the older fat-1 mice exhibit improved glucose homeostasis in comparison to the older wild-type mice. These findings support the beneficial effects of elevated tissue n-3 fatty acid status in the prevention and treatment of age-related chronic metabolic diseases.

1. Introduction

The increasing number of elder people associated with sedentary lifestyle presents unprecedented public health and societal challenges. Several studies have demonstrated that glucose tolerance declines progressively with age in humans and other mammals [1,2]. The pathogenesis of age-related glucose intolerance has been associated with increased body fat mass and visceral fat content [3], physical inactivity, and changes in dietary patterns [4]. Impairments in both lipid metabolism and glucose homeostasis are often implicated in age-related disorders and reduced life expectancy [1,5,6].

There is interplay among lipid metabolism, inflammation, and insulin sensitivity. In adipose tissue, insulin resistance impairs the insulin-induced suppression of lipolysis [7], leading to triglyceride (TG) accumulation in tissues that typically do not specialize in fat storage, such as the liver, skeletal muscle, and pancreas. This ectopic fat accumulation may result in a low-grade inflammatory state, characterized by the increased production of pro-inflammatory molecules, such as tumor necrosis factor α (TNF-α), plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein (MCP)-1, as well as the activation of inflammatory signaling pathways, such as IKK, c-jun kinase (JNK) and nuclear factor-κB (NF-κB) [8,9]. Consequently, chronic inflammation may lead to impaired responses of peripheral tissues to insulin and thereby cause glucose intolerance.

The amount and type of dietary fat differentially affect glucose homeostasis; in particular, saturated fat is known to contribute to the development of glucose intolerance and insulin resistance [10–13]. In contrast, omega-3 polyunsaturated fatty acids (n-3 PUFA) have been shown to improve insulin sensitivity [14–16]. Omega-3 PUFA is a natural ligand for the transcription factor peroxisome proliferator-activated receptor-α (PPARα), which can increase the expression of enzymes involved in the beta-oxidation of fatty acids [17,18]. This increased oxidation of fatty acids in the liver may be due to the induction of

Abbreviations: n-3, omega-3; n-6, omega-6; PUFA, polyunsaturated fatty acids; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose 6 phosphatase; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; PMP-70, 70-kDa peroxisomal membrane protein; ACOX1, acyl-CoA oxidase1; TG, triglyceride; TNF-α, tumor necrosis factor α; PAI-1, plasminogen activator inhibitor-1; MCP-1, monocyte chemoattractant protein; IKK, IκB kinase; JNK, c-jun kinase; NF-κB, nuclear factor-κB; PPARα, peroxisome proliferator-activated receptor-α; WT, wild-type; GC, gas chromatography; GTT, glucose tolerance test; PTT, pyruvate tolerance test

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peroxisomes, through the activation of PPARα [17,18]. Together, these interactions can reduce fat deposition in the liver and plasma triglyceride levels. Furthermore, through the activation of PPAR transcription factors, n-3 PUFA can suppress inflammatory signaling pathways such as NF-κB, inhibiting the production of pro-inflammatory cytokines [19]. It is therefore possible that the lipogenesis-suppressing and anti-inflammatory properties of n-3 PUFA afford them the capability to prevent age-related glucose intolerance.

To eliminate the potential confounding factors of diet, we utilized the transgenic fat-1 mouse model, which expresses the *C. elegans* fat-1 gene and is capable of endogenously synthesizing n-3 PUFA from n-6 PUFA without the need for dietary supplementation [20,21]. In this study, we investigated the influence of elevated tissue n-3 PUFA status on age-related glucose tolerance.

2. Methods

2.1. Animal model

Transgenic fat-1 mice were generated as previously described previously [20] and backcrossed onto a C57BL/6 background. Male fat-1 transgenic mice and non-transgenic littermate controls were divided in four study groups: 2-month-old wild-type (WT-2M), 2-month-old fat-1 (FAT1–2M), 8-month-old WT (WT-8M), and 8-month-old fat-1 (FAT1–8M). The phenotype of the fat-1 transgenic mice (as indicated by increased tissue n-3 PUFA) was confirmed by fatty acid analysis using gas chromatography (GC). WT and fat-1 animals were maintained on a 10% corn oil diet (AIN-76A, 10% corn oil, w/w) *ad libitum* and housed in temperature- and humidity-controlled conditions with a 12-h light/dark cycle. All animal procedures were carried out in accordance with the guidelines set by the Massachusetts General Hospital Animal Committee and with IACUC approval.

2.2. Determination of food intake

Food intake was measured twice a week during the last month before the sacrifice. Mice were food deprived for 6 hours (from 1 pm to 7 pm) and the food ingestion was determined over the next 12 hours.

2.3. Tissue sample collection

At the end of the experimental period, all mice were sacrificed after 6 hours of fasting and blood samples were collected and centrifuged. Serum aliquots were used to measure insulin (Millipore #EZRMI-13K), triglycerides (Abcam #Ab65336), and cholesterol (Abcam #Ab65390). The epididymal fat pad was removed and weighed. Other tissues and organs were harvested and frozen for future analysis.

2.4. Fatty acid analysis

The fatty acid profiles of liver and tail samples were determined by gas chromatography, as described previously [22]. In brief, tissue (~10 mg) was ground into powder under liquid nitrogen and subjected to fatty acid methylation by 14% boron trifluoride (BF3)-methanol reagent (Sigma-Aldrich) at 100 °C for 1 h. Fatty acid methyl esters were analyzed with the Agilent HP6890N gas chromatography system equipped with a flame ionization detector (Agilent Technologies, Palo Alto, CA). The fatty acid peaks were identified by comparing their relative retention times with the commercial mixed standards (Nu-Chek Prep, Elysian, MN), and the area percentages for all resolved peaks were analyzed using GC Chemstation software.

2.5. Measurement of hepatic triglyceride content

Frozen tissues (200 mg) were homogenized in 1.5 mL of phosphate-buffered saline. The protein concentration of homogenate was determined and an aliquot of 300 mL was extracted with 5 mL of chloroform/methanol (2:1) and 0.5 mL of 0.1% sulfuric acid. An aliquot of the organic phase was collected, dried under nitrogen and re-suspended in 2% Triton X-100. TG content was determined using a Triglyceride Quantification Kit (BioVision, Mountain View, CA) following the manufacturer's protocol.

2.6. Intraperitoneal glucose tolerance test

The intraperitoneal glucose tolerance test (GTT) was performed after 6 hours of fasting. After collection of an unchallenged sample (time 0), 20% glucose (2.0 g/kg body weight) was administered into the peritoneal cavity. Through a small cut at the tail tip, blood samples were collected at 15, 30, 45, 60, 75 and 90 minutes after the injection of glucose for the determination of glycemia.

2.7. Intraperitoneal pyruvate tolerance test

The intraperitoneal pyruvate tolerance test (PTT) was performed after 6 hours of fasting. After collection of an unchallenged sample (time 0), the pyruvate solution (2.0 g/kg body weight) was administered into the peritoneal cavity. Tail blood samples were collected at 15, 30, 45, 60, 75 and 90 minutes for the determination of glucose concentration in the blood.

2.8. Real-time PCR analysis

The mRNA content of PMP70 and ACOX1 were measured in liver samples, as previously described [23]. Real-time PCR data were analyzed using the Stratagene MX3005P qPCR System (Agilent Technologies, Palo Alto, CA).

2.9. Immunoblot analysis

The liver was homogenized and used for immunoblot analysis, as previously described [24]. Fifty micrograms of protein was loaded onto a 12% acrylamide gel, subjected to SDS-PAGE, and then transferred onto nitrocellulose membranes. Membranes were then blocked and probed with the appropriate antibodies. Antibodies for p-ACC, ACC, NFκB, p-Iκb and β-actin were obtained from Cell Signaling Technology (Danvers, MA) and antibodies for PEPCK, G6Pase and FAS were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Band intensities were quantified by optical densitometry (Scion Image, Frederick, MD).

2.10. Statistical analysis

Results are presented as means ± SEM. All results were analyzed by two-way ANOVA. When necessary, these analyses were complemented by the Bonferroni test to determine the significance of individual differences. The level of significance was set at $p < 0.05$.

3. Results

3.1. Changes in metabolic parameters with aging

The genotypes of the wild-type and fat-1 transgenic mice were confirmed by GC analysis of fatty acid composition. The fat-1 mice used in this study exhibited more than twice the hepatic levels of n-3 PUFA, mainly DHA, compared to the WT mice (6.58 ± 0.67 vs 2.91 ± 0.36, $p < 0.05$), while the n-6/n-3 PUFA ratio was significantly reduced from 13.30 ± 0.62 in WT mice to 5.69 ± 0.43 in fat-1 mice. Both the two-month-old and eight-month-old groups exhibited similar fatty acid profiles.

A number of metabolic parameters changed in the WT mice with aging. Specifically, body weight, epididymal fat pad mass, blood glucose, blood insulin, plasma triglycerides, and plasma cholesterol levels were