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Transgenic ω-3 PUFA enrichment alters morphology and gene expression profile in adipose tissue of obese mice: Potential role for protectins

Phillip J. White[a], *Patricia L. Mitchell*[a], *Michael Schwab*[a], *Jocelyn Trottier*[b], *Jing X. Kang*[c], *Olivier Barbier*[b], *André Marette*[a,*]

[a] *Department of Medicine, Québec Heart and Lung Institute, Université Laval, Ste-Foy, Québec, Canada and Laval University Hospital Research Center, Metabolism, Vascular and Renal Health Axis, Ste-Foy, Québec, Canada*

[b] *Laboratory of Molecular Pharmacology, CHU-Québec Research Centre and the Faculty of Pharmacy, Laval University, Québec, Canada*

[c] *Laboratory for Lipid Medicine and Technology, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA*

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ABSTRACT

Objective. Dietary administration of ω-3 polyunsaturated fatty acids (PUFA) is often associated with altered adipose tissue (AT) morphology and/or function in obese mice. Yet, it is unclear whether this is an indirect consequence of reduced weight gain or results from direct actions of ω-3 PUFA. Here we studied the AT of high fat (HF)-fed *fat-1* transgenic mice that convert endogenous ω-6 to ω-3 PUFA while maintaining equivalent fat accretion as their wild-type (WT) counterparts.

Materials and methods. Adipocyte size profiling, Affymetrix microarray pathway analysis, qPCR and protectin identification and analysis were performed in epididymal AT from hemizygous *fat-1*(+/–) mice and their wild type littermates that had been fed a HF diet for 8 weeks from 6 weeks of age.

Results. Despite equivalent fat pad mass, we found that epididymal AT from HF-fed transgenic animals possesses fewer large and very large but more mid-size adipocytes compared to WT mice. In order to better understand the underlying mechanisms contributing to the observed alteration in adipocyte size we performed an Affymetrix microarray. Pathway analysis of these data highlighted adipogenesis, cholesterol biosynthesis, insulin signaling, prostaglandin synthesis/regulation and small ligand GPCRs as points where differentially expressed genes were significantly overrepresented. Observed changes were confirmed for four candidate genes: *Cnr1*, *Cnr2*, *Faah* and *Pparg* by qPCR. Finally we demonstrated that protectin DX is present in AT and that protectin DX and protectin D1 promote comparable PPARγ transcriptional activity.

Conclusions. These data provide unprecedented evidence that ω-3 PUFA coordinately regulate AT gene expression programs in a manner that is independent of restriction of weight gain or fat accrual and highlight an important influence of ω-3 PUFA on

Abbreviations: AT, adipose tissue; Cnr1, cannabinoid receptor 1; Cnr2, cannabinoid receptor 2; CE, collision energy; DP, declustering potential; EP, entrance potential; Faah, fatty acid amide hydrolase; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; GCPR, G-coupled protein receptor; Pparg, peroxisome proliferator activated receptor gamma; PUFA, polyunsaturated fatty acid; RMA, robust multi-array average; SPE, solid phase extraction; WT, wild type.

* *Corresponding author at:* Centre de recherche de l'Institut universitaire de cardiologie et pneumologie de Québec, Hôpital Laval, Pavillon Marguerite d'Youville, Bureau Y4340, Ste-Foy, Québec, Canada, G1V 4G5. Tel.: +1 418 656 8711x3781; fax: +1 418 656 4749.
E-mail address: Andre.Marette@crchul.ulaval.ca (A. Marette).

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adipogenesis. Furthermore we provide primary evidence suggesting that protectins likely contribute to these effects via their influence on PPARγ.

1. Introduction

Adipose tissue (AT) plays an important role in the maintenance of metabolic homeostasis due to its dual role as a repository for surplus nutrients and a prominent endocrine tissue. It is well appreciated that obesity-related alterations in AT function particularly in the visceral depots have dramatic consequences for systemic glucose and lipid metabolism [1]. For instance, the onset of inflammation in visceral AT is thought to be fundamental to the development of obesity-related metabolic complications [2]. Interestingly, adipocyte size in visceral AT is positively correlated with insulin resistance [3], glucose intolerance [4], inflammation [5] and circulating triglycerides and cholesterol [6,7] suggesting that proper adipose function is intimately linked to cell size .

Administration of marine oils containing the ω-3 polyunsaturated fatty acids (ω-3 PUFA) eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3) has been reported to improve both glucose [8–10] and lipid metabolism [11–13] in obesity. Interestingly, these beneficial effects of ω-3 PUFA have been associated with reduced adipose inflammation [14–17], increased expression and secretion of the insulin sensitizing adipokine adiponectin [18], raised adipose mitochondrial activity [19], and inhibition of high fat (HF) diet-induced remodeling of AT [20]. Furthermore, ω-3 PUFA have been identified as potential endogenous ligands for the peroxisome proliferator activated receptor (PPAR) family of nuclear receptors that play a vital role in adipocyte differentiation and lipid metabolism [18,21,22]. Together these reports appear to suggest that direct actions of ω-3 PUFA in AT are likely responsible for at least part of the beneficial effects of these essential fatty acids.

However the interpretation of many of the aforementioned studies is complicated by the fact that a common effect of ω-3 PUFA supplementation in rodent diets is reduced weight gain and fat accretion [23,24]. Although this is likely attributable to altered palatability of the experimental diet there is growing reference in the literature to the anti-obesity effects of ω-3 PUFA [25–27]. Thus it is not clear whether the reported actions of ω-3 PUFA in AT arise as a secondary result of reduced weight gain and limited adipose expansion or are indeed direct actions of ω-3 PUFA.

To alleviate this issue we have employed the *fat-1* transgenic mouse which has been genetically engineered to ubiquitously express the *fat-1* ω-3 fatty acid desaturase from *C. elegans*. This enzyme, not found in mammals, efficiently converts endogenous ω-6 to ω-3 PUFA such that in *fat-1* transgenic mice fed a diet rich in ω-6 and deficient in ω-3 PUFA the tissue ω-6: ω-3 ratio is approximately 1:1 compared to 50:1 in wild type (WT) animals [28]. Importantly, we recently demonstrated that when exposed to a HF diet, *fat-1* transgenic mice are protected from obesity-linked insulin resistance, glucose intolerance and adipose inflammation despite equivalent weight gain and visceral adiposity as their WT counterparts [16]. The *fat-1* transgenic mouse therefore represents the ideal model to study the effects of ω-3 PUFA on AT in an environment that is not confronted by dietary issues.

To better characterize the actions of ω-3 PUFA specifically in AT we examined AT morphology and performed pathway analysis on data obtained from an Affymetrix microarray of AT from HF-fed *fat-1* transgenic and WT mice. We then corroborated novel observations by qPCR and expanded upon our earlier findings in this model by providing primary evidence that both protectins D1 and DX may contribute to ω-3 PUFA dependent actions in adipose tissue by modulating PPARγ transcriptional activity.

2. Materials and methods

2.1. Animals

Six-week-old male hemizygous *fat-1*(+/–) mice and their WT littermates bred at the Laval University hospital research center were fed high fat diets (diet-93075, 55% kcal from fat, Harlan Teklad) and sacrificed after 8 weeks. At sacrifice epididymal AT excised for the microarray study was rapidly homogenized in QIAZOL (QIAGEN, Toronto, ONT) and snap frozen in liquid nitrogen. Histology was performed in sections of epididymal AT that were placed in 4% paraformaldehyde at sacrifice whereas the samples used for qPCR and lipidomics came from tissues that were rapidly excised and snap-frozen in liquid nitrogen. Animal procedures were approved by the Laval University Committee for the Protection of Animals and carried out in accordance with the guidelines provided by the Canadian Council on Animal Care.

2.2. RNA extraction, qPCR and microarray hybridization

RNA was extracted for qPCR and microarray studies using the RNeasy lipid tissue mini kit (QIAGEN) by following the manufacturer's instructions. For microarray studies the RNA quality testing, quantification and hybridization to the Mouse 430 2.0 Affymetrix gene chip were performed at the McGill University and Génome Québec Innovation Center in Montréal, Québec. For qPCR, RNA was first reverse transcribed to cDNA using the high-capacity cDNA reverse transcription kit from Applied Biosystems. qPCR for *Pparg* (Mm01184322_m1), *Cnr1*(Mm01212171_ s1), *Cnr2* (Mm00438286_m1), *Faah* (Mm00515684_m1) and *Gapdh* (Mm99999915_g1) was then performed using Taqman hydrolysis probes and primers from Applied Biosystems in a CFX96 real-time system from BIO-RAD. The relative expression of genes of interest was then determined by normalization to the reference gene *Gapdh* using the comparative C_T method for relative gene expression [29].