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Diets high in monounsaturated and polyunsaturated fatty acids decrease fatty acid synthase protein levels in adipose tissue but do not alter other markers of adipose function and inflammation in diet-induced obese rats

Jennifer E. Enns [a,c], Danielle Hanke [b,c], Angela Park [c], Peter Zahradka [a,b,c], Carla G. Taylor [a,b,c,*]

[a] Department of Physiology, University of Manitoba, Canada
[b] Department of Human Nutritional Sciences, University of Manitoba, Canada
[c] Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Research Centre, R2019 – 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6

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ABSTRACT

This study investigates the effects of monounsaturated and polyunsaturated fatty acids from different fat sources (High Oleic Canola, Canola, Canola–Flaxseed (3:1 blend), Safflower, or Soybean Oil, or a Lard-based diet) on adipose tissue function and markers of inflammation in Obese Prone rats fed high-fat (55% energy) diets for 12 weeks. Adipose tissue fatty acid composition reflected the dietary fatty acid profiles. Protein levels of fatty acid synthase, but not mRNA levels, were lower in adipose tissue of all groups compared to the Lard group. Adiponectin and fatty acid receptors GPR41 and GPR43 protein levels were also altered, but other metabolic and inflammatory mediators in adipose tissue and serum were unchanged among groups. Overall, rats fed vegetable oil- or lard-based high-fat diets appear to be largely resistant to major phenotypic changes when the dietary fat composition is altered, providing little support for the importance of specific fatty acid profiles in the context of a high-fat diet.

1. Introduction

Obesity has become a worldwide epidemic, affecting millions of adults and children in developed and developing nations alike. In Canada, more than one in four adults are obese and the prevalence of obesity has roughly doubled over the past two decades [1]. In large epidemiological studies, body mass index and waist circumference (the most widely used measures of obesity) show strong associations with mortality and cardiovascular disease [2,3].

Adipose tissue is central to the development of obesity. Once thought to serve merely as an energy storage depot, adipose tissue is now known to be a dynamic endocrine organ, secreting bioactive molecules termed "adipokines" that contribute to the regulation of metabolic homeostasis. In obesity, excessive adiposity and the dysregulated production of adipokines, such as adiponectin, leptin, and several classes of pro-inflammatory molecules, contribute to the chronic low-grade inflammation that drives the pathogenesis of related conditions, including insulin resistance and cardiovascular disease [4,5].

Although the etiology of obesity is complex, diet is one of the most important determinants [1]. While high fat intake has been shown to play a role in promoting adipose tissue inflammation [6–8], the findings from carefully controlled weight-gain and weight-loss trials that compare low-, moderate- and high-fat diets have suggested that the amount of fat in the diet is less important than the type of fat consumed [9–11]. In this context, researchers have begun to assess whether diets containing certain proportions of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) might favourably affect obesity parameters. Many of these studies have focused on the very long-chain omega-3 ($n-3$) PUFA eicosapentaenoic acid (EPA; C20:5$n-3$) and docosahexaenoic acid (DHA; C22:6$n-3$), which are obtained primarily from fatty fish or fish oil supplements. However, less is known about the essential $n-3$ fatty acid alpha-linolenic acid (ALA; C18:3$n-3$), found in plant-based products such as canola and flaxseed oils. ALA can be elongated and

* Corresponding author at: Canadian Centre for Agri-Food Research in Health and Medicine, St. Boniface Research Centre, R2019 – 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6. Tel.: +1 204 258 1361; fax: +1 204 237 4018.
E-mail address: ctaylor@sbrc.ca (C.G. Taylor).

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desaturated in the body to form EPA and DHA, but its conversion occurs with very low efficiency [12]. At the same time, the effects of ALA in obesity have not been well studied, despite reports that ALA blood levels and cardiovascular endpoints are inversely associated [13–16]. Other promising findings include the inflammation-reducing effects of ALA supplementation in obese subjects [17] and in the Δ6-desaturase null mouse [18], as well as our previous study in the obese *fa/fa* Zucker rat that indicated a role for ALA-rich flaxseed oil in improving adipose tissue function [19].

While $n-3$ PUFA are widely believed to be beneficial to health, a recent meta-analysis has indicated that high intakes of omega-6 ($n-6$) PUFA without a concurrent increase in $n-3$ PUFA have deleterious effects on cardiovascular events and death [20,21]. Although some $n-6$ PUFA are precursors to eicosanoids, which modulate pro-thrombotic, pro-constrictive and pro-inflammatory processes [22], emerging data on fatty acid derivatives involved in the resolution of inflammation indicate that those derived from $n-6$ PUFA can have both pro- and anti-inflammatory properties [23].

Diets rich in MUFA, which have been studied mainly in relation to the oleic acid (C18:1$n-9$)-rich Mediterranean Diet, may also be beneficial in reducing the risk of co-morbidities associated with obesity [24]. The National Institute of Medicine does not propose a specific dietary requirement for MUFA; instead, MUFA intake makes up the balance of dietary fats after the requirements for saturated fatty acids (SFA) and PUFA are met, and MUFA intake may therefore vary widely [25]. Although there is some evidence that MUFA favourably alter serum lipids [26] and improve insulin sensitivity [27], their contribution to adiposity is uncertain [25] and they have no confirmed independent role in the prevention of chronic disease [28].

Given the high prevalence of obesity and obesity-related conditions as well as dietary recommendations to reduce SFA intake, it is important to determine the contributions of MUFA and PUFA to the development of these metabolic imbalances. Our study compared the effects of MUFA- and PUFA-rich diets containing different amounts and ratios of $n-6$ and $n-3$ fatty acids in a diet-induced obesity rodent model, which closely mimics the phenotype and pathogenesis of human obesity. The fatty acid profiles of the vegetable oils making up the fat content of the diets allowed us to evaluate the metabolic consequences of two high $n-6$ PUFA diets containing very low or moderate levels of ALA, and three high MUFA diets with relatively constant amounts of linoleic acid and low, moderate and high levels of ALA. Thus, the objective of our study was to investigate the effects of the different fatty acid profiles of vegetable oils on adipose tissue function and inflammation and relationships with adipose tissue fatty acid composition in diet-induced obese rats.

2. Materials and methods

2.1. Animals and diets

The animal and diet protocol has been described previously [29]. Briefly, 6 week-old-obese-prone CD (Charles River Sprague-Dawley) male rats (Charles River, St-Constant, PQ) were acclimatized for 2 weeks and randomized ($n=10$/group) to one of six dietary treatments for 12 weeks. The experimental diets were formulated with different oils (high-oleic canola oil, conventional canola oil, a 3:1 blend of conventional canola and flaxseed oil, safflower oil or soybean oil), which allowed comparisons of MUFA and PUFA with varying proportions of $n-6$ and $n-3$ fatty acids among diet groups (Table 1). A lard-based diet high in saturated fat that is commonly used to develop diet-induced obesity in obese prone rats served as the control [30,31]. All diets contained 55% of total energy as fat, 30% as carbohydrate and 15% as protein; this formulation was based on similar interventions that induced changes in body weight, glucose, insulin and other metabolic parameters in Wistar rats [30,31]. The diets were prepared in 10 kg batches as needed and stored at 4 °C. Feed intake (corrected for spillage) and weekly body weights were recorded. The experimental protocol was approved by the University of Manitoba Protocol Management and Review Committee and conducted according to the Canadian Council on Animal Care Guidelines. Other indices for these rats related to hepatic steatosis, glucose homeostasis and inflammatory markers have been reported elsewhere [29].

2.2. Blood and tissue collection

Fasting blood samples were obtained via the jugular vein at weeks 0, 4, and 8, and trunk blood was collected at 12 weeks (study end) after rats were euthanized with carbon dioxide. Blood samples were centrifuged at 1500 *g* for 15 min at 4 °C. The serum layer was collected, aliquoted and stored at −80 °C for biochemical analyses. Various organs, including adipose tissue (epididymal fat pads), were dissected, weighed, flash-frozen in liquid N_2, and stored at −80 °C for analysis.

2.3. Serum biochemistry

Serum concentrations of leptin, monocyte chemoattractant protein (MCP)-1 and active plasminogen activator inhibitor (PAI)-1 at weeks 0, 4, 8 and 12 were determined using a Rat Serum Adipokine Milliplex® MAP multiarray kit (EMD Millipore, St. Charles, MO). Serum adiponectin at the end of the study was measured using an ELISA kit (Alpco Diagnostics, Salem, NH).

2.4. Fatty acid composition

The fatty acid composition of epididymal adipose tissue was analysed as previously described [32] using methanolic hydrochloric acid as the methylating agent. The methylated samples were analysed by gas chromatography (GC) using a Varian 450-GC Gas Chromatograph with an FID detector (Varian, Lake Forest, CA) and a GC capillary column (length 100 m, diameter 0.25 mm and film thickness 0.25 μm; Varian, Lake Forest, CA). Fatty acid composition of diet samples was also verified by GC.

2.5. Western immunoblotting

The protein fraction was extracted from frozen epididymal adipose tissue and quantified as previously described [33]. Western immunoblotting was performed by separating proteins (15 μg) by SDS-PAGE, transferring to a polyvinylidene fluoride membrane and probing with primary (1:1000 dilution) and horseradish peroxidise-conjugated secondary antibodies (1:10,000 dilution). Quantification of band intensities was carried out using a FluorChem®Q gel scanning system with a charge-coupled device camera (Proteinsimple, Santa Clara, CA) and AlphaView® Software (Version 1.3.0.6; Alpha Innotech Corporation, Proteinsimple). Data are expressed as arbitrary units relative to the loading control. Western immunoblot analysis was conducted using the following antibodies: fatty acid synthase, p42/44 MAPK, eEF2 and perilipin (Cell Signaling Technology, Inc., Danvers, MA); interleukin-18, GPR41 and GPR43 (Santa Cruz Biotechnology, Inc., Dallas, TX); MCP-1, pigment epithelial-derived factor, fatty acid binding protein 4 and CD36 (Abcam, Cambridge, MA); and adiponectin (EMD Millipore).