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Dietary flaxseed oil reduces adipocyte size, adipose monocyte chemoattractant protein-1 levels and T-cell infiltration in obese, insulin-resistant rats

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ABSTRACT

Background: Adipocyte dysfunction is characterized by an increase in adipocyte size and changes to their adipokine profiles. Immune cell infiltration into adipose tissue is thought to contribute to the metabolic complications of obesity, with local and systemic consequences for the inflammatory status of the obese individual. Dietary interventions with omega-3 fatty acids from marine sources have been successful at reducing inflammation. The aim of this study was to determine whether flaxseed oil containing the plant-based omega-3 fatty acid α-linolenic acid (ALA) is an effective modulator of inflammation and adipocyte dysfunction.

Methods: Seventeen-week old male *fa/fa* and lean Zucker rats were fed a control diet (faCTL, lnCTL) and *fa/fa* rats were fed an ALA-rich flaxseed oil supplemented diet (faFLAX) for 8 weeks. Adipose tissue and serum were collected and analyzed for cytokine (IL-6, IL-10, IL-18, IL-2, IFN-γ, TNF-α), haptoglobin, monocyte chemoattractant protein-1 (MCP-1) and adipokine (leptin, adiponectin) levels. Splenocytes were isolated and *ex vivo* mitogen-stimulated cytokine production was measured. Digital images of adipose tissue sections were used to quantify adipocyte area. Macrophage and T-cell infiltration were assessed in adipose tissue by immunohistochemistry.

Results: faFLAX rats had 17% smaller adipocytes and 5-fold lower MCP-1 levels in adipose tissue than faCTL rats. Adipose tissue levels of IL-10 were 72% lower in the faFLAX group compared to baseline, and TNF-α levels decreased 80% (equal to lnCTL levels) in the faFLAX group compared to faCTL. There were no changes in *ex vivo* cytokine production by splenocytes between faFLAX and faCTL. Macrophage infiltration was not different among groups; however, faFLAX rats had less T-cell infiltration than faCTL rats.

Conclusions: Dietary intervention with ALA-rich flaxseed oil in obese Zucker rats reduced adipocyte hypertrophy, protein levels of inflammatory markers MCP-1 and TNF-α, and T-cell infiltration in adipose tissue. Modest improvements to other parameters of obesity were also observed. The results suggest that, due to its ability to improve adipocyte function, ALA-rich flaxseed oil confers health benefits in obesity.

1. Introduction

Obesity is associated with low-grade inflammation resulting from chronic activation of the innate immune system [1]. Inflammation has been linked to the pathogenesis of several other chronic diseases, including metabolic syndrome, cardiovascular disease, type 2 diabetes mellitus (T2DM) and cancer [2,3]. Adipose tissue actively participates in inflammation and immunity through the production and release of pro-inflammatory molecules (e.g. leptin, tumor necrosis factor [TNF]-α, interferon [IFN]-γ, interleukin [IL]-6, IL-18) and anti-inflammatory molecules (e.g. adiponectin, IL-10) from both the adipocytes and infiltrating immune cells [4]. Obesity results in increased adipocyte size (hypertrophy) and adipose tissue dysfunction, creating an imbalance of pro- and anti-inflammatory factors which can alter the immune response and inflammatory status both locally and systemically. For example, increased fat mass and larger adipocytes lead to elevated circulating leptin concentrations and leptin, in turn, exerts pro-inflammatory actions by activating various immune cells [5].

Obesity is also characterized by macrophage infiltration in adipose tissue [6] and macrophages are a major source of

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pro-inflammatory cytokines, including high mobility group box 1 (HMGB1), TNF-α and IL-6 [6,7]. Recent research indicates that T-cells also play a prominent role in adipose tissue inflammation and the development of obesity [8]. In fact, it has been suggested that T-cells are among the first immune cells to enter the adipose tissue and may stimulate pre-adipocytes to recruit macrophages via release of factors such as monocyte chemoattractant protein-1 (MCP-1) [9].

Omega-3 fatty acids are considered to be anti-inflammatory and thus may counteract some of the damaging effects of the obese condition. Higher intakes of dietary omega-3 fatty acids are associated with reduced lymphocyte proliferation [10,11] and Th1 cell development [12], lower circulating levels of leptin [13–15], C-reactive protein [16–20], and other pro-inflammatory cytokines [12,16,17,20], as well as a lower risk of infection [21]. The majority of studies investigating omega-3 fatty acids in relation to immune function and inflammation have used dietary fish oil as the source of omega-3 fatty acids (i.e. eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) [22]. Alternatively, plant-based sources of omega-3 fatty acids contain α-linolenic acid (ALA) which can be elongated and desaturated to EPA and DHA [22]. Whether intervention with dietary ALA is an effective modulator of inflammation or adipose dysfunction associated with obesity is unknown.

The *fa/fa* Zucker rat is a model of obesity with a defect in the leptin receptor, and as such, exhibits leptin resistance, adipose tissue dysfunction and impaired immune function [23]. We hypothesized that dietary intervention with omega-3 fatty acids in the form of ALA-rich flaxseed oil would promote a decrease in pro-inflammatory markers and an increase in anti-inflammatory markers in *fa/fa* Zucker rats, thereby leading to improved immune function and a healthier adipocyte phenotype in an obese state. Thus, the objective of this study was to investigate the effects of dietary ALA-rich flaxseed oil on the adipose tissue by assessing pro- and anti-inflammatory markers (splenocyte *ex vivo* cytokine production, circulating and adipose tissue levels), adipocyte size, and macrophage/T-cell infiltration in adipose tissue of obese *fa/fa* rats.

2. Materials and methods

2.1. Animals and diets

After a 5–8 day acclimatization period, 17-week old male *fa/fa* Zucker rats (Harlan, Indianapolis, IN) were randomly assigned to a baseline group (faBASE; $n = 7$), from which tissues were collected and stored immediately, or groups fed a control diet (faCTL; $n = 7$) or an ALA-rich flaxseed oil diet (faFLAX; $n = 7$) for 8 weeks; lean Zucker rats were assigned to the control diet (lnCTL; $n = 7$) for 8 weeks. The diet formulations were based on the AIN-93G diet [24] (Table 1). Although the fatty acid composition of the diets differed, the proportions of polyunsaturated fatty acid, monounsaturated fatty acid and saturated fatty acid were similar (Table 1). Fresh batches of the diet were prepared weekly and stored at −20 °C; rats were given fresh feed three times per week. Feed intake (corrected for spillage) and weekly body weights were recorded. The University of Manitoba Protocol Management and Review Committee approved the protocol for the animal care procedures and the experimental procedures were in accordance with Canadian Council on Animal Care guidelines [25].

2.2. Serum and tissue collection

After 8 weeks, rats were fasted overnight and euthanized by CO_2 asphyxiation and decapitation. Trunk blood was collected, placed on ice and centrifuged to separate serum which was stored at −80 °C. Dissected peri-renal and epididymal adipose tissue was weighed; a small portion was placed in Cryo-Gel embedding medium (Instrumedics Inc., St. Louis, MO) and frozen in a dry ice/ethanol bath, while the other portion was frozen in liquid nitrogen; both were stored at −80 °C. Dissected spleens were weighed and processed immediately.

Table 1
Diet formulation and fatty acid profile.

	FLAX diet	CTL diet
Diet ingredients[a] (g/kg)		
Cornstarch[b]	363	363
Maltodextrin	132	132
Sucrose	100	100
Egg white	212.5	212.5
Cellulose	50	50
AIN-93G-MX mineral mix	35	35
AIN-93-VX vitamin mix	10	10
Choline	2.5	2.5
Biotin mix[c]	10	10
Tert-butylhydroquinone[d]	0.014	0.014
Soy oil	42.5	85
Flaxseed oil[e]	42.5	0
Fatty acid composition[f]		
Saturated fatty acids	12.2	15.8
C16:0	7.5	10.5
C18:0	3.9	4.2
Monounsaturated fatty acids	19.1	22.5
C18:1	18.8	22.1
Polyunsaturated fatty acids	67.3	59.9
C18:2 (n6)	33.4	51.6
C18:3 (n3)	33.7	8.0
Polyunsaturated:saturated	5.5	3.8
Omega-6/omega-3	1.0	6.4

[a] Ingredients from Harlan Teklad (Madison, WI) unless otherwise indicated.
[b] Cornstarch from Castco Inc. (Etobicoke, ON).
[c] 200 mg biotin/kg cornstarch because egg white was the protein source.
[d] Tert-butylhydroquinone from Sigma–Aldrich (St. Louis, MO).
[e] Flaxseed oil from Omega Nutrition Canada Inc. (Vancouver, BC).
[f] g/100 g total fatty acids as analyzed by gas chromatography. Only fatty acids present in amounts >1% are included.

2.3. Splenocyte isolation and stimulation

Single-cell suspensions of spleen were prepared by pressing tissues through nylon screens into sterile Krebs Ringer Bicarbonate Buffer (130 mM NaCl, 10 mM HEPES [pH 7.4], 5.2 mM KCl, 1.4 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 1.4 mM $MgSO_4$ and 10 mL antibiotic/antimycotic) containing 0.5% bovine serum albumin (Sigma–Aldrich). Red blood cells in the spleen cell suspensions were lysed by resuspending the splenocytes in 1–2 mL ammonium-chloride–potassium buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM ethylenediaminetetraacetate, pH 7.4). Cell concentration was determined using a haemacytometer. Splenocytes (1.25×10^6 cells/mL) were suspended in cell culture medium (RPMI 1640 [Sigma–Aldrich, St. Louis, MO] pH 7.2 supplemented with 5% heat-inactivated fetal calf serum [Invitrogen Corporation, Burlington, ON], 25 mL HEPES buffer, 10 mL antibiotic/antimycotic [Invitrogen] and 4 μL 2-mecaptoethanol) and stimulated (Stim) with 2.5 mg/L concanavalin A (ConA; Catalog #L2630, Sigma–Aldrich) by incubating cells at 37 °C in a 5% CO_2 and 95% humidified air incubator for 48 h. A set of splenocytes were incubated without mitogen as controls (un-stimulated; Unstim). After incubation, samples were centrifuged and supernatants were stored at −80 °C until analyzed for cytokine production.

2.4. Splenocyte cytokine analysis

TNF-α, IFN-γ, IL-2, IL-6, IL-4, and IL-10 in cell culture supernatant fractions were determined simultaneously using a