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Neither linoleic acid nor arachidonic acid promote white adipose tissue inflammation in *Fads2*-/- mice fed low fat diets

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ABSTRACT

Dietary n-6 polyunsaturated fatty acids (PUFA) are widely perceived to promote inflammation and contribute to the development of chronic diseases. This dogma has been recently questioned due to evidence that n-6 PUFA, specifically linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6), do not appear to activate inflammatory signalling pathways when consumed in moderate amounts. However, delineating the independent roles of different dietary n-6 PUFA *in vivo* is challenging because LA is continuously converted into AA in a pathway regulated by the fatty acid desaturase 2 (*Fads2*) gene. The objective of this study was to investigate the independent roles of LA and AA on white adipose tissue (WAT) inflammatory signalling pathways using *Fads2*-/- mice. We hypothesized that dietary LA would not induce WAT inflammation, unless it was endogenously converted into AA. Male C57BL/6 wild-type (WT) and *Fads2*-/- mice were fed low-fat isocaloric diets containing either 7% corn oil w/w (CD, containing ~42% LA) or 7% ARASCO oil w/w (AD, containing ~27% AA) for 9 weeks. WAT inflammatory gene expression, protein levels, as well as phospholipid (PL) and triacylglycerol (TAG) fatty acid composition, were analyzed by RT-qPCR, western blots, and gas chromatography, respectively. *Fads2*-/- mice fed CD had high LA, but little-to-no GLA (18:3n-6), DGLA (20:3n-6), and AA in PLs and TAGs compared to their WT counterparts. In comparison, *Fads2*-/- and WT mice fed AD showed minimal differences in n-6 PUFA content in serum and WAT, despite having significantly more AA than CD-fed mice. No differences in gene expression for common inflammatory adipokines (e.g. *Mcp-1, Ccl5, Tnfα*) or key regulators of eicosanoid production (e.g. *Cox-2, Alox-12, Alox-15*) were detected in WAT between any of the diet and genotype groups. Furthermore, no differences in MCP-1, and total or phosphorylated STAT3 and p38 inflammatory proteins, were observed. Collectively, these results demonstrate that neither LA nor AA promote WAT inflammation when consumed as part of a low-fat diet. Therefore, the existing dogma surrounding n-6 PUFA and inflammation needs to be reconsidered.

1. Introduction

Polyunsaturated fatty acids (PUFA) are critical for maintaining cellular function and phospholipid membrane fluidity, as well as regulating cell signalling and immune function [1]. Over the past hundred years, changes in the Western diet have resulted in significant increases in n-6 PUFA content compared to n-3 PUFA. This higher n-6 to n-3 PUFA ratio is hypothesized to contribute to the increased prevalence of chronic diseases, such as obesity and metabolic syndrome [2–4]. Consequently, the current dogma intimates that increased consumption of n-3 PUFA can reduce the risk of chronic diseases, while increased consumption of n-6 PUFA does the opposite.

High levels of n-6 PUFA in the diet are postulated to increase the risk of chronic diseases by enhancing pro-inflammatory mediator production (i.e., eicosanoids) and inflammatory gene expression, while simultaneously reducing levels of pro-resolving n-3 PUFA derived metabolites such as resolvins and protectins [5,6]. Specifically, increased consumption of foods rich in arachidonic acid (AA, 20:4n-6), such as

Abbreviations: **AA**, arachidonic acid; **AD**, ARASCO diet; **Alox-12**, arachidonate 12-lipoxygenase; **Alox-15**, arachidonate 15-lipoxygenase; **Ccl5**, chemokine ligand 5; **CD**, CORN diet; **COX**, cyclooxygenase; **Cox-2**, cyclooxygenase-2; **D5D**, delta-5-desaturase; **D6D**, delta-6-desaturase; **DGLA**, dihomo-γ-linolenic acid; **Elovl**, elongase; **eWAT**, epididymal white adipose tissue; **FA**, fatty acid; **Fads**, fatty acid desaturase; **Fads2-/-**, fatty acid desaturase 2 knock out; **GLA**, γ-linolenic acid; **iWAT**, inguinal white adipose tissue; **LA**, linoleic acid; **LOX**, lipoxygenase; **MAPK**, mitogen-activated protein kinase; **Mcp-1**, monocyte chemoattractant protein-1; **PL**, phospholipid; **PUFA**, polyunsaturated fat; **STAT**, signal transducer and activator of transcription; **TAG**, triacylglycerol; **Tnf**, tumour necrosis factor; **WT**, wild-type

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meat and eggs, leads to an enrichment of this n-6 PUFA in phospholipids, thereby increasing its availability for the production of pro-inflammatory eicosanoids via cyclooxygenase (COX)- and lipoxygenase (LOX)-mediated pathways [7]. Linoleic acid (18:2n-6), which represents the most common n-6 PUFA in the diet, has been largely assumed to promote inflammation due to its endogenous conversion into AA, which is then subsequently used for pro-inflammatory eicosanoid production [8]. Despite the widespread belief of a positive association between increased dietary n-6 PUFA and inflammation, several lines of evidence suggest that this relationship remains equivocal. First, a systematic review showed that increasing dietary LA by up to 6-fold had no discernible impact on AA levels in serum [9]. Second, Vaughan and colleagues demonstrated that an LA-enriched diet did not induce inflammatory gene expression in mouse liver or adipose tissue [10]. Third, an evidence-based review by Johnson and Fritsche found no indication of a relationship between increased consumption of LA and inflammation in humans [11]. Finally, results from several carefully controlled clinical trials showed that an increased intake of dietary LA [12] or AA [13,14] did not induce inflammation when consumed at moderate levels. Collectively, these findings reinforce the need for further investigations regarding the individual relationships between LA and AA, and inflammation.

However, a major challenge in delineating the independent roles of individual n-6 PUFA is that LA is continuously and endogenously converted into AA, albeit to a limited extent, via the fatty acid desaturase (FADS) pathway [15]. Briefly, the initial rate-limiting desaturation of LA into γ-linolenic acid (GLA, 18:3n-6) is catalysed by the delta-6-desaturase (D6D) enzyme, which is coded by the fatty acid desaturase 2 (*Fads2*) gene. Subsequent elongation and desaturation steps mediated by elongase 5 (*Elovl5*) and the delta-5-desaturase (*Fads1*), respectively, convert GLA to dihomo-γ-linolenic acid (DGLA, 20:3n-6) and then AA. Of relevance to the current study, a whole-body deficiency in *Fads2* leads to significant alterations in the fatty acid profiles of key metabolic tissues, such as liver, muscle, and adipose [16,17]. Thus, the *Fads2-/-* mouse provides a unique nutritional model with which to uncouple the endogenous conversion of LA into AA in order to clarify their independent roles on inflammation.

The present study investigated the independent effects of LA and AA on inflammatory signalling pathways in visceral and subcutaneous white adipose tissue (WAT) in *Fads2-/-* mice. We chose to study inflammatory signalling in two WAT depots for several reasons. First, WAT represents the primary tissue for the storage of dietary fat. Second, WAT secretes a wide array of adipokines that regulate inflammation both locally and systemically. Third, visceral and subcutaneous WAT depots are known to differ in regards to their secretion profiles and their association with inflammation [18]. Based on the current state of knowledge, we hypothesized that a low-fat diet enriched with LA would have little-to-no effect on WAT inflammatory signalling pathways when it is unable to be converted into AA. We also surmised that any pro-inflammatory effects of AA would be greater in visceral compared to subcutaneous WAT.

2. Materials and methods

2.1. Animal husbandry and sample collection

Breeding pairs of *Fads2* heterozygous mice that had been backcrossed for 10 generations to a C57BL/6J background (Jackson laboratory, Bar Harbor, Michigan, USA) were transferred from the University of Illinois at Urbana-Champaign to the University of Guelph to establish a breeding colony. Male wild-type (WT) and *Fads2-/-* mice were obtained by breeding *Fads2* heterozygous C57/BL6 mice. Harems were maintained on a modified AIN-93G diet with corn oil as the principal dietary fat source (D03090904P, Research Diets, New Brunswick, NJ, USA). *Fads2-/-* and WT mice (n = 12–15 mice per genotype per dietary group) were weaned onto one of two modified AIN93G experimental diets at 21 days of age. Mice were fed *ad libitum* for 9 weeks low-fat (16% kcal from fat) experimental diets (Table 1) modified to contain either 7% corn oil w/w (CD, containing ~42% LA) or 7% ARASCO oil w/w (AD, containing ~27% AA, DSM Nutritional Products Canada Inc., Ayr, ON, Canada). Both CD and AD diets were supplemented with a minimal amount (0.2% w/w) of DHA from docosahexaenoic acid single cell oil (DHASCO; DSM Nutritional Products Canada Inc., Ayr, ON, Canada) to prevent long-chain n-3 PUFA deficiency in *Fads2-/-* mice. Mice were housed in ventilated cages (n = 1–4 mice per cage) at 22 °C in a humidity-controlled environment at 12 h (light): 12 h (dark cycle) for study duration. At 12 weeks of age, mice were weighed and euthanized with CO_2. Immediately following euthanization, blood was collected via cardiac puncture, allowed to coagulate at room temperature for 30 min in an Eppendorf tube, and then centrifuged (15 min, 1500 × *g*) at 4 °C to separate serum from solid blood components. Serum, as well as collected inguinal WAT (iWAT) and epididymal WAT (eWAT), were flash frozen in liquid nitrogen and stored at −80 °C. This study was approved by the University of Guelph Animal Care Committee in accordance to the requirements of the Canadian Council of Animal Care (AUP #1524).

Table 1
Macronutrient and fatty acid composition of the Corn (CD) and ARASCO (AD) diets.

	CD[a] kcal (%)	AD[a] kcal (%)
Protein	20	20
Carbohydrate	64	64
Fat	16	16
kcal/gm	4	4
Ingredient	**gm**	**gm**
Corn oil	66.25	0.0
ARASCO oil	0.0	66.25
DHASCO oil	3.75	3.75
Fatty Acid[b]		
12:0	0.1	0.1
14:0	0.6	1.0
16:0	7.8	9.7
18:0	1.3	5.8
20:0	–	–
22:0	–	0.7
24:0	–	–
Total SFA	**9.8**	**17.3**
16:1n7	–	–
18:1n9	17.0	8.4
20:1n7	–	0.2
22:1n9	–	1.0
24:1n9	–	–
Total MUFA	**17.0**	**9.7**
18:2n6	39.9	5.0
18:3n6	–	–
18:3n3	0.9	2.2
18:4n3	–	–
20:3n6	–	–
20:4n6	–	26.5
20:5n3	–	0.1
22:4n6	–	–
22:5n3	–	–
22:6n3	1.5	1.5
Total PUFA	**42.3**	**35.3**

[a] Composition of AIN-93 G modified diets as provided by manufacturer, Research Diets. Diet product numbers are D12041402 (CD) and D12041406 (AD).
[b] Fatty acids expressed as gram (gm)/4000 kcal.

2.2. RNA and protein extraction

Total RNA and protein from WAT were extracted using the Qiagen RNeasy Mini Kit (Qiagen, Mississauga, ON, Canada) and an adapted guanidine hydrochloride protocol from Simões et al. [19]. The quantity and quality of extracted RNA was assessed using a Nanodrop 2.0 spectrophotometer (Fisher Scientific, Waltham, MA, USA) and Agilent