Enriching the Starter Diet in n–3 Polyunsaturated Fatty Acids Reduces Adipocyte Size in Broiler Chicks

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Abstract
Epidemiologic studies associate perinatal intake of eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA, 22:6n–3) with reduced adiposity in children, suggesting that these fatty acids may alter adipocyte tissue development. The objective of this study was to determine whether enriching the perinatal diet in EPA and DHA reduces fat deposition in young chicks. Cobb 500 broiler chicks were fed isocaloric diets containing fat (8% wt:wt) from fish oil (FO), lard, canola oil, or flaxseed oil from 7 to 30 d of age. Adiposity (abdominal fat pad weight/body weight) at 30 d was not significantly affected by diet, but FO significantly reduced adipocyte size, increasing the abundance of small adipocytes. Plasma nonesterified fatty acid concentrations suggest that reduced adipocyte size was due, in part, to enhanced mobilization of fatty acids from adipose tissue. Our work indicates that dietary EPA and DHA effectively reduce the size of developing adipocytes in juveniles, which may limit adipose deposition and provide metabolic benefits. Curr Dev Nutr 2017;1:e001644.

Introduction
Approximately 27% of children in the United States are classified as overweight or obese by age 5 y (1). Obese children are more likely to be obese adults, and both childhood and adult obesity increase the risk of cardiovascular disease, diabetes, and other comorbidities (2). Limiting excess fat accumulation in the first few years of life is therefore therapeutically important for children and for the prevention of adult obesity. A plethora of studies have identified factors that influence adiposity in mature animals and humans, in which changes in adipocyte size are the primary basis for differences in fatness. Much less is known about control of adipose mass in juveniles, when both adipocyte hypertrophy and hyperplasia actively contribute to fat deposition.

PUFAs of the n–3 and n–6 series differentially regulate preadipocyte proliferation, adipogenesis, and TG storage, all of which contribute to deposition of adipose tissue before adolescence. n–6 PUFAs tend to be pro-adipogenic, whereas long-chain n–3 PUFAs (particularly EPA and DHA) attenuate lipid accumulation and promote an oxidative adipocyte phenotype [reviewed in (3)]. Large-scale studies in mother-child pairs have associated n–6 PUFA intake with increased adiposity in children, whereas an inverse relation has been shown with dietary n–3 PUFAs (4, 5). These associations suggest that the types of FAs consumed early in life may influence the course of adipose development and subsequently affect the predisposition to obesity.

Avian models are useful for testing the effects of diet on early adipose development because chicks eat independently at hatch, allowing direct manipulation of the diet very early in life. Broiler chickens in particular are a valuable polygenic model of susceptibility to obesity due to inadvertent consequences of selection for rapid growth (6). Broiler chicks begin to deposit excess abdominal fat compared with other breeds within 2 wk of hatch. In contrast, most rodent models of obesity are monogenic or are induced by feeding a diet very rich in fat (7). Therefore, broiler chicks are particularly valuable for testing the effects of dietary n–3 PUFAs on adipose development before the critical period for adipose expansion.

PUFAs of the n–3 series (EPA, DHA) are common in marine species but rare in terrestrial species. n–3 PUFAs can reduce fat accumulation in mammalian adipose tissue (8), and evidence from human research indicates an inverse relation of dietary n–3 PUFAs with adiposity in children (9–11). n–6 series PUFAs tend to be pro-adipogenic, whereas long-chain n–3 PUFAs (EPA, DHA) attenuate lipid accumulation and promote an oxidative adipocyte phenotype in mature animals and humans (4, 12). These associations suggest that the types of FAs consumed early in life may influence the course of adipose development and subsequently affect the predisposition to obesity.

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Supplemental Figure 1, Supplemental Methods, and Supplemental Tables 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://cdn.nutrition.org.

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Abbreviations used: ACOX1, acyl-CoA oxidase 1; CA, canola oil; CPT1, carnitine palmitoyl acyltransferase 1; EGR1, early growth response transcription factor 1; FL, flaxseed oil; FO, fish oil; LA, lard; PNPLA8, patatin-like phospholipase domain containing protein 8.
high in fat. We used Cobb 500 broiler chicks, one of the most widely used commercial broiler lines, to determine whether providing EPA and DHA in the diet early in life attenuates adipose tissue deposition in juveniles. Diets enriched in fish oil (FO; as a source of EPA and DHA) were compared with diets containing equal amounts of lard (LA), canola oil (CA), or flaxseed oil (FL) to evaluate the effects relative to other types of FAs. Experimental diets were provided beginning at 7 d of age to coincide with initial deposition of the abdominal depot and to focus on a developmental window in which hypertrophy and hyperplasia contribute comparably to adipose growth.

Methods

Animals and diets
All animal procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee. Forty mixed-sex Cobb 500 broiler chicks were fed ad libitum a commercial starter diet from hatch until 7 d, then switched to 1 of 4 experimental diets. Experimental diets were produced by adding fat (8% wt:wt) from LA, CA, FL, or FO to a commercially formulated starter diet base (Supplemental Table 1). Final energy content of each diet was 1981 kcal/kg. Chicks were fed experimental diets from 7 to 30 d. At 30 d, chicks were weighed, then killed by carbon dioxide asphyxiation, and tissues and blood were collected for experimental procedures. Complete experimental details are provided in Supplemental Methods.

Statistical analysis
Data were analyzed for effects of diet by using ANOVA implemented in SAS (version 9.4; SAS Institute, Inc.), with P < 0.05 as the criterion for significance. A significant F-test was followed by post hoc comparisons with the use of Fisher’s least-significant-difference test to identify pairwise differences between diet groups.

Results

Effects on body composition and growth rate
The FA composition of the diet significantly affected final body weight (P = 0.045), with FO birds weighing less than those in the LA group (Table 1; P = 0.02). Neither absolute nor relative (adjusted for body weight) weights of the abdominal adipose depot or of breast muscle differed significantly between diet groups (P > 0.05). Plasma concentrations of NEFAs (P = 0.002), but not glucose (P = 0.130), were affected by diet, with increased NEFAs in FO chicks compared with each of the other diet groups. As expected, the FA profile of the abdominal fat reflected the dietary FA composition (Supplemental Table 2). Feed intake did not differ across diets (data not shown).

Despite similarities in fat pad weight, diet significantly affected abdominal adipocyte volume (P = 0.020). Average adipocyte size was smallest in FO chicks, differing significantly from chicks fed LA or CA diets (Table 1). Adipocyte number varied with diet (Table 1), but differences were not significant (P = 0.093). FO promoted a shift in adipocyte size, favoring the abundance of relatively small adipocytes compared with diets enriched in LA or CA (Figure 1A). The frequency of very small (<2000 μm³) adipocytes was significantly increased in FO compared with either LA or CA (P < 0.05). Conversely, frequencies of cells in each size bin >4000 μm³ were lower (P < 0.05) in FO than in CA chicks and in FO than in LA chicks for most bins. Adipocyte volumes tended to be smaller in chicks fed the FL diet, with frequencies intermediate between those of FO and LA or CA in most size bins.

Effects on relative mRNA expression in visceral white adipose tissue and liver
Dietary fat source significantly influenced expression of PPAR-γ (PPARG), early growth response transcription factor 1 (EGR1), patatin-like phospholipase domain–containing protein 8 (PNPLA8), and pyruvate dehydrogenase kinase 4 (PDK4) in abdominal adipose tissue (Figure 1B). Both PPARG and EGR1 were expressed at significantly lower levels in FO and CA chicks relative to LA. The expression of PNPLA8 was significantly reduced in FO, CA, and FL chicks compared with LA chicks. Genes associated with FA oxidation [acyl-CoA oxidase 1 (ACOX1) and carnitine palmitoyl acyltransferase 1 (CPT1)], lipogenesis [FA synthase (FASN)], lipid storage [lipoprotein lipase (LPL)], gluconeogenesis [phosphoenolpyruvate carboxykinase 1 (PCK1)], and

### TABLE 1  Effects of dietary LA, CA, FL, and FO on body, adipose, and muscle weights and on serum metabolites in broiler chicks

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>CA</th>
<th>FL</th>
<th>FO</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>1752 ± 46.7a</td>
<td>1650 ± 54.6ab</td>
<td>1694 ± 45.1ab</td>
<td>1562 ± 45.0a</td>
<td>0.045</td>
</tr>
<tr>
<td>Breast weight, g</td>
<td>337.9 ± 13.8</td>
<td>360.2 ± 16.5</td>
<td>354.6 ± 13.6</td>
<td>346.1 ± 7.9</td>
<td>0.573</td>
</tr>
<tr>
<td>Relative breast weight, %</td>
<td>19.4 ± 1.0</td>
<td>21.9 ± 0.6</td>
<td>21.0 ± 1.0</td>
<td>22.1 ± 1.7</td>
<td>0.400</td>
</tr>
<tr>
<td>Adipose tissue weight, g</td>
<td>26.0 ± 1.9</td>
<td>24.5 ± 1.2</td>
<td>22.3 ± 1.2</td>
<td>24.3 ± 1.8</td>
<td>0.455</td>
</tr>
<tr>
<td>Relative adipose weight, %</td>
<td>1.48 ± 0.09</td>
<td>1.51 ± 0.07</td>
<td>1.33 ± 0.07</td>
<td>1.56 ± 0.12</td>
<td>0.499</td>
</tr>
<tr>
<td>NEFAs, mM</td>
<td>6.25 ± 1.98ab</td>
<td>6.49 ± 0.72ab</td>
<td>6.55 ± 1.41ab</td>
<td>10.04 ± 0.96a</td>
<td>0.002</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>175.7 ± 12.8</td>
<td>184.5 ± 10.8</td>
<td>189.4 ± 7.0</td>
<td>168.1 ± 5.7</td>
<td>0.130</td>
</tr>
<tr>
<td>Adipocyte volume, μm³</td>
<td>3651 ± 420.7a</td>
<td>3706 ± 184.1a</td>
<td>3098 ± 95.2ab</td>
<td>2546 ± 153.1b</td>
<td>0.020</td>
</tr>
<tr>
<td>Adipocyte number</td>
<td>75.4 ± 13.3</td>
<td>70.8 ± 9.5</td>
<td>93.7 ± 9.8</td>
<td>90.3 ± 8.1</td>
<td>0.093</td>
</tr>
</tbody>
</table>

¹Values are means ± SEMs for all chicks in each diet group, n = 10/group. Means with shared superscript letters do not differ significantly, P < 0.05. CA, canola oil; FL, flaxseed oil; FO, fish oil; LA, lard.
²Derived by using single-factor ANOVA for effect of diet.
³Breast weight (g)/body weight (g) × 100.
⁴[Abdominal adipose depot weight (g)/body weight (g)] × 100.
⁵Calculated from adipocyte volume and adipose depot weight.
inflammation [chemokine C-C ligand 20 (CCL20), colony stimulating factor 1 receptor (CSF1R)] were not significantly affected by dietary fat type. In liver, expression of ACOX1, but not CPT1 or FASN, was significantly affected by diet (Figure 1C). Expression of ACOX1 was higher in FO than in all other diet groups.
FO and FL chicks on the basis of expression of CPT1 and ACOX1. It is also possible that FO, and to some extent FL, increased the abundance of smaller adipocytes by suppressing their progression through differentiation, rather than altering the balance between lipid storage and mobilization. This possibility is supported by a study in which dietary perilla oil (−52% α-linolenic acid, 18:3n−3) downregulated the later stages of adipocyte differentiation in rats (17). Additional characterization with stage-specific markers of adipocyte differentiation is necessary to investigate this possibility in our study.

None of the genes that we profiled were specifically affected by the diets (FO and FL) that reduced adipocyte size. However, all 3 diets enriched in unsaturated FAs reduced expression of PPARG, EGR1, and PNPLA8 compared with LA. PPARG is a well-characterized transcriptional regulator of both adipocyte differentiation and maintenance of the mature adipocyte phenotype (18). Increased PPARG expression with dietary SFA-compared with PUFA-enriched diets is consistent with comparable studies in mature broilers (19). EGR1 is a pleiotropic transcription factor that has been linked to multiple aspects of adipocyte function (20). Elevated EGR1 expression in adipose tissue is associated with obesity in humans and mice, whereas loss of EGR1 enhances adipocyte metabolism and confers protection from obesity (21). Decreased expression of this gene in the FL, FO, and CA groups relative to LA may therefore reflect beneficial effects of dietary fat quality on adipocyte metabolism. Calcium-independent phospholipase A2γ (iPLA2γ, encoded by PNPLA8), is a phospholipase that catalyzes the release of FA side chains from mitochondrial phospholipids to generate production of eicosanoids and other lipid second messengers that regulate cellular energetics. The specific roles of iPLA2γ in adipose tissue are not known, but PNPLA8−/− mice are resistant to diet-induced obesity, with reduced adipocyte size relative to wild-type controls (22). Although expression was not consistently associated with adipocyte size in our study, reduced levels across all PUFA-enriched diets suggest that dietary FAs may regulate mitochondrial lipid mediators and subsequently adipocyte metabolism through PNPLA8.

In conclusion, we showed that dietary FO attenuates adipocyte hypertrophy in juvenile chicks that are prone to rapid fat accumulation. The mechanisms underlying this effect remain to be determined but may include increased mobilization of stored FAs for oxidation by other tissues or disruption of adipocyte maturation. Although the effect of diet on adipocyte size did not manifest as significantly less adipocyte mass during the relatively brief period of feeding used herein, it may nonetheless be sufficient to elicit favorable metabolic effects in children who are prone to obesity.

Acknowledgments

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References


