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Original Research

Elevated dairy fat intake in lactating women alters milk lipid and fatty acids without detectible changes in expression of genes related to lipid uptake or synthesis☆

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ARTICLE INFO

Article history:
Received 18 September 2014
Revised 2 January 2015
Accepted 13 January 2015

Keywords:
Human milk
Dairy fat
Gene expression
Mammary gland

ABSTRACT

Previous work has demonstrated that elevated maternal lipid intake (particularly from dairy products) is associated with increased lipids and altered fatty acid profile in milk produced by healthy lactating women. To investigate our primary hypothesis that a maternal diet rich in full-fat dairy products would simultaneously increase milk lipid percent and expression of genes related to the uptake and/or de novo biosynthesis of milk lipids, we provided 15 lactating women with diets enriched in full-fat or nonfat dairy products for 14 days each in a randomized, crossover study with a 2-week washout period. Milk fat (%) was lower when women consumed the low-fat compared with the full-fat dairy diet (2.41% ± 0.31% vs 3.35% ± 0.28%, respectively; $P < .05$); concentrations of more than 20 fatty acids also differed. However, neither conservatively evaluated microarray data nor quantitative real-time polymerase chain reaction analysis uncovered any treatment effects on expression of genes related to lipid synthesis or uptake. These data suggest that alteration in gene expression in the lactating human mammary gland is likely not the primary mechanism by which consumption of a high-fat diet affects milk fat percent in healthy, lactating women.

1. Introduction

Of the energy-yielding nutrients in human milk, lipids are likely the most affected by maternal diet. One of the first studies to show this was conducted by Insull and colleagues [1], who reported rapid, reversible shifts in milk fat output in response to dietary macronutrient manipulation in a single, hospitalized breast-feeding woman. Specifically, daily milk fat output was 25% higher when she consumed a high-fat compared with low-fat diet. Karmarkar et al [2] also observed

Abbreviations: BH, Benjamini and Hochberg; BMI, body mass index; *c*9,*t*11-CLA, *c*9,*t*11-conjugated linoleic acid; FASN, fatty acid synthase; MFG, milk fat globule; rtPCR, quantitative real-time polymerase chain reaction; RIN, RNA integrity number.

☆ K.M. Yahvah, S.L. Brooker, J.E. Williams, M. Settles, M.A. McGuire, and M.K. McGuire have no conflicts of interest related to this research.

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http://dx.doi.org/10.1016/j.nutres.2015.01.004
0271-5317/

a 25% increase in milk fat content with dietary lipid supplementation. Similarly, in previous work from our laboratory [3], milk fat (%) was 18% higher when subjects consumed a high-fat diet. Mohammad et al [4] also reported 12% greater milk fat (%) in lactating women consuming a high-lipid diet compared with an isocaloric, low-fat diet. These intervention studies provide considerable evidence that the macronutrient composition of a woman's diet can impact total milk lipids.

Understanding how maternal lipid intake influences lipid composition of human milk is important, as sufficient levels are critical for neonatal and infant health. Indeed, milk lipids represent the largest source of energy for the nursing neonate. Delineating the mechanisms by which a woman's diet can influence cellular functions that direct the uptake, synthesis, and packaging of milk lipids is an important step in elucidating factors impacting this important component of human milk. Although considerable excellent research has been devoted to studying regulation of milk production in the lactating rodent (eg, Refs. [5–7]), previous efforts to study gene expression in the lactating human mammary gland have been hindered by difficulty with obtaining mammary biopsy samples from healthy, lactating women. However, a promising noninvasive technique using RNA extracted from the milk fat globule (MFG) has recently been developed (eg, Refs. [8–10]). This technique is possible because during milk synthesis, small amounts of RNA-containing cytoplasm from secretory mammary epithelial cells are captured within the MFG and released into the ductiles with other milk constituents [11]. This secretory epithelial cell–derived RNA can then be isolated in sufficient quantity and quality from only 10 mL of milk to perform gene expression analysis. Importantly, gene expression data derived from MFG RNA agree well with those generated from RNA obtained directly from mammary biopsy samples [12].

The primary objective of this study was to investigate the effects of increased maternal dairy fat intake on lipid-related gene expression in the lactating human mammary gland using RNA obtained from MFG. We hypothesized that a maternal diet rich in full-fat dairy products would increase milk lipid percent while eliciting enhanced expression of genes related to the uptake and/or de novo biosynthesis of milk lipids. A secondary objective was to examine the effects of increased dairy fat intake on selected non–lipid-related cellular functions in the mammary gland. Specifically, our group is interested in the fact that dairy (bovine) fat is the major dietary source of several biologically active fatty acids, and we have a long-standing interest in one of these: *c*9,*t*11 conjugated linoleic acid (*c*9,*t*11-CLA) [13]. Previous studies using cell culture systems and animal models have demonstrated that *c*9,*t*11-CLA has potent anticarcinogenic (especially in the mammary gland) and growth-modulating properties [14–16]. This is of particular interest in the context of human lactation because having breast-fed an infant is associated with a reduced risk for developing some forms of breast cancer [17,18]. Among the metabolic pathways activated during lactation is the synthesis of *c*9,*t*11-CLA from *trans*-vaccenic acid (*t*11-18:1) [19]. Consequently, it is probable that lactating mammary tissue experiences increased exposure to *c*9,*t*11-CLA—especially in breast-feeding women who consume large amounts of dairy fat. This increased mammary exposure to *c*9,*t*11-CLA may contribute, at least in part, to the protective effects of lactation on breast cancer risk. Therefore, a secondary hypothesis of this study was that increased dairy fat (and, consequentially, *c*9,*t*11-CLA) consumption would influence expression of genes related to cancer development (eg, cell cycle and apoptosis) in the lactating human mammary gland.

2. Methods and materials

2.1. *Experimental design and dietary treatments*

All procedures were approved by the University of Idaho Human Assurances Committee and the Washington State University Institutional Review Board. Written informed consent was obtained from each subject. This study was designed as a randomized, crossover, dietary intervention trial, with 2 treatments each lasting 14 days and separated by a 14-day washout period. To be eligible for inclusion, women needed to be successfully lactating, at least 4 months postpartum, and feeding their children "considerable" amounts of formula or supplementary foods. This last criterion was necessary because we anticipated a drop in milk fat during the low-fat dairy period, and we wanted to make sure that infants continued to have access to adequate nutrition throughout the study. Women were advised to continue to breast-feed on demand during each intervention period to make up for any possible caloric deficit in the milk. All women were self-described as healthy and were recruited from the Moscow, ID/Pullman, WA region. No other inclusion or exclusion criteria (eg, parity, body fat, etc) were imposed.

During each treatment period, participants were provided with and asked to consume either 4 servings of full-fat dairy products ("full-fat dairy treatment") or 4 servings of nonfat dairy products ("low-fat dairy treatment") on a daily basis. The provided foods included full-fat and skim milk, full-fat and nonfat yogurt, and full-fat and fat-free cheese. To help control for potential confounding effects, subjects were asked to refrain from consuming all other dairy products, including butter, during the 14-day intervention periods so that most of the dairy fat consumed was that provided by us. Dairy products were delivered weekly to the subjects' homes along with reminders of study guidelines and sampling dates.

2.2. *Dietary analysis*

Subjects were provided with electronic food scales and instructed to keep careful diet records on 2 weekdays and 1 weekend day during the week prior to the first dietary intervention (baseline) and again during each of the intervention periods. Food records were analyzed as previously described [3] using the ESHA Genesis nutrient analysis program (ESHA, version 7.7.0, Salem, OR, USA).

2.3. *Sample collection*

To obtain representative samples for milk fat analysis, milk samples were collected by complete breast expression 1 day before the treatment scheme was initiated (baseline) and on days