Nutrition 32 (2016) 1295–1298

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Brief report

Higher concentrations of branched-chain amino acids in breast milk of obese mothers

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ARTICLE INFO

Article history:
Received 3 February 2016
Accepted 26 May 2016

Keywords:
Metabolic syndrome
Protein metabolism
Breast-feeding
Free amino acids
Breast milk
Human

ABSTRACT

Objective: Nutrition during fetal life and early childhood is thought to play a crucial role in the risk for developing metabolic syndrome and cardiovascular diseases in the future adult and branched-chain amino acids (BCAA) intake may play a role in the development of obesity. The aim of this study was to compare the breast milk amino acid profiles of obese and normal weight (control) breast-feeding mothers.

Methods: Fifty obese and 50 control breast-feeding mothers were enrolled. Age and parity were similar in both groups. Breast milk samples were collected at the end of the first month of lactation. Free amino acid (FAA) concentrations in breast milk were determined by ultra-performance liquid chromatography tandem mass spectrometry. Comparisons between groups were performed using a two-tailed paired *t* test.

Results: We analyzed 45 breast milk samples from each group. Body mass index was 34.3 ± 3.9 kg/m^2 in the obese group and 21.6 ± 1.4 kg/m^2 in the control group ($P < 10^{-4}$). BCAA concentrations were higher in breast milk of obese mothers (95.5 ± 38.2 μM versus 79.8 ± 30.9 μM; $P = 0.037$), as was tyrosine concentration (13.8 ± 7.1 μM versus 10.6 ± 5.2 μM; $P = 0.016$).

Conclusion: The mature breast milk of obese mothers contained 20% more BCAA and 30% more tyrosine than breast milk of control mothers. Whether altered breast milk FAA profile affects metabolic risk in the breast-fed child remains to be explored.

Introduction

The development of obesity is multifactorial. Interactions between genetic background and exposure to an obesogenic environment trigger the risk for obesity. Obesity, in turn, increases the risk for cardiovascular disease (CVD) and metabolic syndrome (MetS) [1]. The programming effect of nutrition during fetal life and infancy (i.e., nutritional programming) is now established as a major determinant of adult health, late-onset diseases, such as MetS and CVD, or both [2].

Recent studies found that a high intake of branched-chain amino acids (BCAA), when associated with high-fat intake, results in insulin resistance in rats [3]. Activation of the mammalian target of rapamycin pathway by leucine may be the underlying mechanism [3]. The plasma concentrations of BCAA and of two by-products of their catabolism (C3- and C5-acylcarnitines) were reported to be elevated in obese women, and correlated with visceral fat and subcutaneous fat mass in metabolomics studies [3,4]. This could be explained by

The present study was supported by the regional hospital program for clinical research of Western France (PHRC interregional Grand-Ouest; B91049-20). The funder had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. ADL, RH, DD, and CYB conceived and designed the study. VFR, MCAG, and CYB generated, collected, and assembled the data. ADL, MCAG, VFR, and CYB analyzed and interpreted data. ADL wrote the first draft of the manuscript. All authors revised and approved the final version of the manuscript. The authors have no conflicts of interest to declare.

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http://dx.doi.org/10.1016/j.nut.2016.05.013
0899-9007/

alterations of BCAA-catabolizing enzymes in adipose tissue and skeletal muscle [3,5].

Increased plasma BCAA concentrations were found in obese children, with a positive correlation with body mass index (BMI) *Z* score [6,7]. Several authors reported a positive correlation between increased plasma concentrations of BCAA and aromatic amino acids (AAAs), and the development of insulin resistance [3,8]. In metabolomics studies, elevated BCAA allowed the identification of children at risk for insulin resistance [7]. In an adult cohort, an increase in BCAA concentrations was detectable ≤12 y before the onset of type 2 diabetes [8]. Thus, BCAA concentrations may be predictive of the long-term risk for developing metabolic alterations.

Breast-feeding is believed to confer some protection against the development of obesity [9]. The lower protein content of breast milk may help prevent the excess insulin secretion and avoid accelerated early growth observed in formula-fed infants [10]. The composition of breast milk and its participation as a channel of transmission of nonhereditary perinatal nutritional programming is not completely understood. Relatively few studies have focused on the free amino acid (FAA) composition of breast milk [11–14]. In addition to their role as building blocks of protein, AAs are thought to be involved in signaling [15], and AAs could play a role in metabolic programming. The aim of this pilot study was to determine whether the pattern of breast milk FAA differed between obese and normal-weight breast-feeding mothers.

Participants and methods

The analysis conducted in this study was part of the REGULACT project (EudraCTClinical Trials Registry, eudract.ema.europa.eu, 2009-A00912-55), aiming at comparing leptin and macronutrients in the breast milk of obese and normal-weight mothers.

Participants

Two groups of mother–infant dyads were recruited during postpartum hospitalization at the University Maternity Hospital of Poitiers and the Maternity Hospital of Chatellerault, France among mothers who elected to breastfeed their baby. Obese (BMI ≥30 kg/m^2) and normal-weight (BMI 18.5–24.99 kg/m^2) mothers were matched for age (±5 y) and parity. Inclusion criterion was the continuation of breast-feeding at 1 mo postpartum. Exclusion criteria were a preexisting chronic or gestational disease, smoking during pregnancy, twin pregnancy, prematurity, a low birth weight (less than third centile for gestational age according to French references AUDIPOG), or hospitalization in the neonatal period.

This study was conducted according to Helsinki Declaration. Written consent was obtained from all participating mothers. This protocol was approved by the CPP Ouest III ethics committee (September 7, 2009).

Breast milk samples

Breast milk was collected at 1 mo of lactation between 09:00 h and 11:00 h, during breast-feeding, and from the contralateral breast using a breast pump (Symphony, Medela™, Switzerland). Breast milk was aliquoted and frozen at −80°C until analysis.

Sample preparation

Determination of FAA concentrations in breast milk by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) was previously described [13]. Briefly, the protocol comprised the addition of a labeled standard pool, a delipidation step by centrifugation and a de-proteinization step by addition of sulfosalicylic acid and centrifugation. FAA from supernatant were finally derivatized using AccQ•Tag™ Ultra reagent.

Liquid chromatography–mass spectrometry

Liquid chromatographic separation was performed on an Acquity H-Class® UPLC system (Waters Corporation, Milford, CT, USA). UPLC-MS/MS analysis was carried out on a Xevo TQD® (Waters Corporation). Integration and quantitation were performed using the Waters TargetLinks™ software.

Statistical analyses

Data are expressed as mean ± SD and [minimum/maximum]. Between-group comparisons were performed using a parametric two-tailed paired *t* test. P value < 0.05 was considered statistically significant. All statistical analyses were carried out using R 3.2.2 software.

Results

Within the two groups of 50 mother–infant pairs that completed the study, 45 pairs of breast milk samples were available for FAA analysis. The mean age did not differ between control and obese mothers (30.1 ± 4.2 versus 30.2 ± 4.7 y, ns). BMI was 34.3 ± 3.9 kg/m^2 in the obese group, and 21.6 ± 1.4 kg/m^2 in the controls ($P < 10^{-4}$). Parity was 1.9 ± 0.8 versus 2 ± 1.2 in the normal-weight and obese groups, respectively (ns).

Concentrations of valine, isoleucine, and total BCAA were ≈17%, 44%, and 20% significantly higher, respectively, in breast milk from obese mothers than in breast milk from normal-weight mothers. The concentration of the AAA tyrosine was 30% significantly higher, and alanine concentration was 12% significantly higher in breast milk of obese mothers (Table 1). Other AAs did not differ between groups.

Discussion

To the best of our knowledge, the present study is the first to demonstrate that the FAA composition of breast milk differs between normal-weight and obese mothers. Notably, BCAA and tyrosine concentrations were higher in the breast milk of obese mothers than normal-weight mothers.

In the present study, the volume of milk suckled by infants was not directly measured. Yet the volume of breast milk collected by means of a breast pump while the baby suckled the contralateral breast did not reach significance with the 45 pairs selected for the present FAA analysis (48.9 ± 13.8 versus 54.3 ± 17.9 mL; $P = 0.12$ in the obese versus control group). Moreover, none of the macronutrient concentrations differed: 1 ± 0.2 versus 1 ± 0.2 g/100 mL for protein in the obese and control group, $P = 0.86$; 3.8 ± 1.6 g versus 3.3 ± 1.3 g/100 mL for fat, $P = 0.11$; and 6.8 ± 0.3 versus 6.9 ± 0.3 g/100 mL, $P = 0.96$ for carbohydrate. Taken in aggregate, this evidence suggests both the volume of breast milk ingested, and macronutrient and energy intake were similar in both groups, which could account for