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Corresponding author:
Yixiang Su

suyx@mail.sysu.edu.cn

Author Affiliation:
Sun Yat-sen University

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None declared.

Human milk lipid profiles around the world: a pooled data analysis

Zhang, Z¹; Wang, Y²; Yang, X³; Chen, Y⁴; Zhang, H⁵; Xu, X⁶; Zhou, J⁷; Chen, M⁸; Su, M⁹; Yang, Y¹⁰; Su, Y¹¹.

Review question / Objective: This review study compiled the published concentrations of total fat, phospholipids, cholesterol and individual fatty acids in human milk, and investigated their variation with respect to geographical area, lactation stage and publication year.

Eligibility criteria: Studies were eligible for inclusion if they reported the total fat, phospholipid, cholesterol, and/or FA composition of human milk. Studies were included in this meta-analysis if they 1) analyzed 24 h milk samples or single samples from healthy mothers, with data reported as g/100 g or g/100 mL for total fat, phospholipids and cholesterol, and % of total FAs for fatty acids; 2) contained data presented as means or medians, with the standard error of the mean (SEM), standard deviation (SD), range, 95% confidence interval (CI) and/or interquartile range; 3) measured FA concentrations by high-performance liquid chromatography, gas chromatography or gas-liquid chromatography; and 4) were written in English or Chinese. Studies were excluded if they 1) used donor human milk samples that underwent additional processing; 2) had samples with an unidentified type or lactation stage; 3) pooled samples from multiple mothers or lactation stages; 4) were maternal dietary restriction studies or studies of mothers with diseases, such as gestational diabetes, preeclampsia or HIV; or 5) were reviews or reported data measured using a human milk analyzer. References from the retrieved articles were manually screened for additional eligible studies.

INPLASY registration number: This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 14 April 2022 and was last updated on 14 April 2022 (registration number INPLASY202240079).

INTRODUCTION

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concentrations of total fat, phospholipids, cholesterol and individual fatty acids in human milk, and investigated their

variation with respect to geographical area, lactation stage and publication year.

Condition being studied: Lipids are present in human milk in the form of fat globules, which mainly consist of triglycerides surrounded by a structural membrane composed of phospholipids, cholesterol, proteins and glycoproteins. Fat from human milk provides approximately 50%–60% of the energy intake of young infants, as well as providing essential fatty acids (FAs) and fat-soluble vitamins. Triacylglycerols make up 98%–99% of the total fat content of human milk and infant formulae. Their properties depend on the length and degree of unsaturation of the FAs esterified to the glycerol backbone. The most widely studied FAs in human milk are the long-chain polyunsaturated FAs (PUFAs). Epidemiological studies have found that children exposed to higher PUFA concentrations in breast milk have significantly higher rates of mental illness, increased risks of developing allergies and other negative outcomes. To counter these risks, information on lipid profiles can provide guidance for defining adequate nutrient intakes for infants and can serve as the basis for the development of infant formulae. Many individual studies worldwide have investigated the FA, total fat, phospholipid and cholesterol contents of human milk. There have also been some pooled data analyses of lipids in human milk, but these have only focused on specific FAs, like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), total fat or phospholipids. The composition of human milk changes dynamically with feeding, time of day and lactation period. It also varies between individual mothers and between women of different ethnicities, and is modulated by the maternal diet. Fat is one of the most variable nutrients in human milk. However, a consolidated lipid profile, based on multiple studies and reflecting geographical differences and changes through the progression of lactation and over different decades, is not yet available.

METHODS

Search strategy: A systematic literature search, up to March 2021, was performed using the PubMed, Embase, Web of Science and Medline databases for English language papers and Wanfang and China National Knowledge Infrastructure databases for Chinese language papers. The following search terms were used: (fatty acid* OR lipid* OR fat OR phospholipid* OR cholesterol* OR triacylglyceride) AND (human milk OR breastmilk OR breast-milk OR breast milk). Further information was retrieved through a manual search of references from recent reviews (including meta-analyses) and relevant published original studies, as well as through searches of Google Scholar.

Participant or population: The review will include studies conducted on healthy mothers. Moreover, we will exclude mothers who were dietary restriction and underwent additional processing.

Intervention: No intervention.

Comparator: No comparator.

Study designs to be included: Research papers of all designs including interventional studies, cross-sectional, case-control, cohort designs and review articles will be included. Articles with no full text available like conference abstracts will be excluded. For multiple reports from the same study, we included the most recent and/or most complete study.

Eligibility criteria: Studies were eligible for inclusion if they reported the total fat, phospholipid, cholesterol, and/or FA composition of human milk. Studies were included in this meta-analysis if they 1) analyzed 24 h milk samples or single samples from healthy mothers, with data reported as g/100 g or g/100 mL for total fat, phospholipids and cholesterol, and % of total FAs for fatty acids; 2) contained data presented as means or medians, with the standard error of the mean (SEM), standard deviation (SD), range, 95% confidence interval (CI) and/or interquartile

range; 3) measured FA concentrations by high-performance liquid chromatography, gas chromatography or gas-liquid chromatography; and 4) were written in English or Chinese. Studies were excluded if they 1) used donor human milk samples that underwent additional processing; 2) had samples with an unidentified type or lactation stage; 3) pooled samples from multiple mothers or lactation stages; 4) were maternal dietary restriction studies or studies of mothers with diseases, such as gestational diabetes, preeclampsia or HIV; or 5) were reviews or reported data measured using a human milk analyzer. References from the retrieved articles were manually screened for additional eligible studies.

Information sources: Mentioned above under search strategy.

Main outcome(s): A total of 186 studies were included in the analysis. According to random effects models based on worldwide data, the summarized inverse-variance weighted means (IWMs) (with 95% confidence intervals) as percentages of total fat were 41.9% (40.9%–43.0%) for saturated fatty acids (SFAs), 37.0% (36.1%–38.0%) for monounsaturated fatty acids and 20.9% (19.2%–22.5%) for polyunsaturated fatty acids (PUFAs). However, the study heterogeneity was high for most types of fatty acids. Between geographical regions, the IWMs for the percentages of SFAs and PUFAs in total fat varied from 35.0% to 54.5%, and 13.0% to 26.9% ($P < 0.001$), respectively. The total fat concentration of colostrum (2.40 g/100 mL) was significantly lower than that of transitional (3.25 g/100 mL) and mature milk (3.50 g/100 mL). Significant regional and lactation stage differences were also observed for some individual fatty acids. The concentrations of total fat, SFAs and C16:0 were significantly negatively correlated with publication year, i.e., they were lower in more recent studies ($P < 0.001$ – 0.049). In contrast, a significant positive correlation was observed between the concentrations of PUFAs, total n-6, C18:2 n-6 and C18:3 n-3 and publication year ($P < 0.001$ – 0.002). Our results suggest

that the pooling of data on human milk lipid profiles in different studies should be done with caution due to the high between-study heterogeneity. The concentration of lipids, including total fat, cholesterol and specific fatty acids, fluctuates in human milk according to lactation stage, geographical region and publication time.

Additional outcome(s): The sample size-weighted means (SWMs) values for the total fat, cholesterol, phospholipid and FA concentrations were similar to the pooled IWM values but the SDs for most of the lipid profiles were large, indicating a relative high individual variations.

Quality assessment / Risk of bias analysis: This systematic review and meta-analysis of breast milk lipid content (fats, phospholipids, cholesterol and FAs) is conducted to determine whether lactation period, publication year of the data source and geographical location should be considered when analyzing the lipid content of breast milk. Therefore, this review will not assess the effectiveness of the finding, the risk of bias, or the certainty of the evidence.

Strategy of data synthesis: Data analyses were performed using two methods. First, the inverse-variance weighting method was applied using the “rma.uni” function of the Metafor package of the statistical software RStudio (version 1.1.383, 2009-17), which has been previously validated. For total fat, phospholipid, cholesterol and individual FA concentrations, the inverse-variance weighted means (IWMs) and 95% CIs were calculated. Heterogeneity between studies was assessed using the I^2 parameter. Fixed effects models were used to pool data when heterogeneity was low or moderate ($I^2 < 50\%$) and random effects models were performed when heterogeneity was high ($I^2 \geq 50\%$). Second, the sample size weighting method (shown in the Supplemental File) was used, in which the overall sample size-weighted mean (SWM) and SD values were calculated based on the means and SDs of all eligible studies using the equations of “combining groups” recommended by the

Cochrane Handbook and the study of Zhang et al.

Subgroup analysis: We also performed subgroup analysis based on lactation stages to identify potential sources of heterogeneity.

Sensitivity analysis: Not applicable.

Country(ies) involved: China.

Keywords: human milk, lipids, fat, fatty acids, breast-feeding, infants.

Contributions of each author:

Author 1 - Zheqing Zhang.

Email: zzqaa501@163.com

Author 2 - Yingyao Wang.

Author 3 - Xiaoguang Yang.

Author 4 - Yiyong Chen.

Author 5 - Hong Zhang.

Author 6 - Xuebin Xu.

Author 7 - Jin Zhou.

Author 8 - Hengying Chen.

Author 9 - Mengyang Su.

Author 10 - Yuexin Yang.

Author 11 - Yixiang Su.

Email: suyx@mail.sysu.edu.cn