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TABLE OF CONTENTS

What We Know, Think We Know, or Are Starting to Know 03

The Study 04

Results 04

The Critical Breakdown 05

Key Characteristic 05

Interesting Finding 06

Relevance 07

Application to Practice 08

References 09-10

Martín-Grau C, Deulofeu R, Serrat Orus N, Arija V, on behalf of the ECLIPSES Study Group. Trimester-Specific Reference Ranges for Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Serum of Pregnant Women: A Cohort Study from the ECLIPSES Group. Nutrients. 2021; 13(11):4037.

What We Know, Think We Know, or Are Starting to Know

Dietary fat, and lipid stores in the body, are known to play a critical role in relation to pregnancy outcomes [1–3]. Due to their unique role in the development of the brain and central nervous system, much of the research attention has focused on polyunsaturated fats, in particular docosahexaenoic acid [DHA] and arachidonic acid [AA], but also eicosapentaenoic acid [EPA] [2].

The focus on DHA and AA over EPA reflects the fact that from around 20-24 weeks gestation into the third trimester, and through the first 2-years of infancy, the ratio of DHA to AA is crucial in supporting a developmental period known as the "infant brain growth spurt" [4,5]. Benefits to cognitive development appear to require around a 2:1 to 1:1 ration of DHA to AA [2].

There is also a separate, but related, aspect of diet and pregnancy which is deterministic of healthy pregnancy outcomes: gestational term. The gestational age at delivery is one of the most important variables in determining overall health status and cognitive development in children [6]. The related aspect to lipids is that omega-3 fatty acid levels, and supplementation, are associated with significant increases in gestational age at delivery [1,3].

Little, however, is known about the role of saturated fatty acids and monounsaturated fatty acids during the peri-natal period. However, as reviewed in a recent Deepdive, an RCT of a monounsaturated fat enriched diet led to a 25% lower risk of developing gestational diabetes [GDM] [7] Conversely, high dietary saturated fat intake has been associated with GDM risk [8], which may relate to the increased insulin resistance from dietary saturated fat [9].

Yet in this data, only advice exists for omega-3 fatty acid supplementation and pregnancy [2], which likely reflects the overall lack of data in relation to other fatty acids. The present study examined maternal fatty acid status during pregnancy, including all fat subtypes.

The Study

The ECLIPSES study [I couldn' t find the full title of the study acronym, probably for the best by the looks of it] is a randomised controlled trial on the effects of iron supplementation on pregnancy outcomes in 793 otherwise healthy Spanish women. For the present study, data was analysed from a subgroup of women with blood samples taken during the first trimester [T1, ~12 weeks] and the third trimester [T2, ~37 weeks].

Diet was assessed using the Spanish Diet Quality Index, which derived a composite numeric score from nine food groups [protein, dairy, cereals, fruits, vegetables, oils, legumes, tubers, sweets]. The score ranged from 0 to 18; 0-10 was classified as low-medium diet quality, and 11-18 as a high diet quality.

For the analysis of fatty acids, 36 individual fatty acids were analysed. The sum of fatty acids were also analysed for saturated fat [SFA], monounsaturated fatty acids [MUFA], and polyunsaturated fatty acids [PUFA]. The study also calculated the omega-6:omega-3 ratio, and the essential fatty acid [EFA] index, which is the sum of essential omega-3 + omega-6 fats against the sum of non-essential fatty acids [like omega-9 oleic acid, found in olive oil].

Results: Blood samples were taken from 476 women at T1, and 464 at T3. The average age of women in the study was 30yr. Of 36 fatty acids analysed, only 11 were detected in the serum of women analysed.

- ***Fatty Acid Status*:** During T1, only total SFA, the SFA C16:0 palmitic acid, and EPA, were moderately correlated with this stage of pregnancy. During T3, the SFA C12:0 lauric acid and C16:0, total SFA, and EPA, had low to moderate correlations with this stage of pregnancy. The strongest correlation at both T1 and T3 was for EPA.
- ***Relationship Between Maternal Factors and Fatty Acid Status*:** Women with a BMI of >30 exhibited significantly higher levels of total SFA, MUFA, and AA in both T1 and T3. Both education status and ethnicity were associated with higher total omega-3 PUFA and DHA in T1, and lower SFA and AA in T3. In T1, low physical activity was associated with lower DHA values. In T3, both total omega-3 and DHA were associated with older age and better diet quality.

The Critical Breakdown

Pros: The analysis in the present study is ambitious and goes beyond the zeroing-in on PUFA in relation to pregnancy, which is warranted due to their particular effects, but perhaps not the full picture. Taking repeated measures at T1 and T3 also allowed for potential differences during this very dynamic life stage to be captured, and repeated measures were available for 97% of women in the study. By quantifying both absolute concentrations of fatty acids, and as a percentage of total fatty acids, the results could be compared to wider research on circulating fatty acid levels during pregnancy. Fatty acid levels were analysed using mass spectrometry, which is the best available method.

Cons: The paper broadly refers to the term serum measurements only, which in generally as biomarkers reflect perhaps the previous 3-4 days of dietary intake [(10)]. It also calculated various fatty acid ratios, but didn' t calculate the ratio of DHA to AA. The quantification of diet, from the perspective of a biomarker study like this, is also inadequate as the outcome here is specific [fatty acids], yet the quantification of diet was general [a composite diet quality score]. Thus, "high diet quality" tells us nothing about what foods may associate with fatty acid status. And even still, circulating levels of saturated fatty acids are a poor reflection of dietary intake of fat, due to the ability to synthesise SFA in the body [(10)].

Key Characteristic

It is important to bear in mind that this study should not be taken as a reflection of dietary fat intake. This could be construed in this way, but what was analysed was circulating serum levels of fatty acids, which may not necessarily reflect dietary intake. Recall from our Research Lecture on linoleic acid as biomarker, that valid biomarkers are ones where the measured *internal* level actually reflects the nutrient of interest. Now, in the present the most prominent circulating saturated fatty acid was C16:0 palmitic acid, which constituted 20-38% of total fatty acids during T1 and 28-57% during T3. However, C16:0 is also synthesised from dietary carbohydrate intake, and may in fact be more reflective of carbohydrate in the diet than saturated fats [(11)]. This is something to bear in mind, because this study could easily be taken to reflect some ideal reference range for dietary intakes, when this is not what is being shown.

Interesting Finding

In this analysis, both AA and DHA declined between T1 and T3. The timing of this would not be considered ideal, given that the third trimester is when the magic starts to happen, so to speak, in relation to the rapid incorporation of these fatty acids into brain central nervous system tissues [12]. Now, the question then becomes, what do these levels represent? Is the decline an issue, or a reflection of increased foetal uptake, or indeed uptake into breastmilk? Previous research has shown that the body will maintain constant AA and DHA levels in breastmilk through internal metabolism, although this has been investigated during lactation, not pregnancy [13].

A Norwegian cohort study which measured red blood cell [RBC] fatty acid levels at the start of the third trimester showed AA and DHA levels of 11% and 6%, respectively, and DHA correlated strongly with cognitive testing at 1yr of age [14]. But RBC is not the same as serum, so these percentages of fatty acids are not really comparable in meaning. So, what does this decline in AA and DHA in the present mean? We don' t know. There is no meaningful dietary analysis to relate this to [i.e., lack of DHA supplementation, change in dietary intake, etc.], and it may not even have any practical, physiological relevance. Because serum only reflects 2-3 days of previous dietary intake, it could also just reflect diet immediately prior to either T1 or T3 measurement.

n-6 PUFA		
LA (C18:2n-6), $n = 446$	T1, $n = 476$	33.30 ± 5.19
	T3, $n = 476$	31.13 ± 6.95
DHGLA (C20:3n-6), $n = 450$	T1, $n = 473$	2.26 ± 0.58
	T3, $n = 474$	1.27 ± 0.40
AA (C20:4n-6), $n = 451$	T1, $n = 475$	8.31 ± 2.05
	T3, $n = 471$	3.83 ± 1.33
Σ Total n-6 PUFA, $n = 444$	T1, $n = 474$	44.35 ± 5.07
	T3, $n = 476$	36.50 ± 7.27
n-3 PUFA		
EPA (C20:5n-3), $n = 446$	T1, $n = 467$	0.36 ± 0.24
	T3, $n = 470$	0.13 ± 0.10
DHA (C22:6n-3), $n = 456$	T1, $n = 474$	2.24 ± 0.66
	T3, $n = 472$	1.24 ± 0.46
Σ Total n-3 PUFA	T1, $n = 470$	2.77 ± 0.83
	T3, $n = 470$	1.36 ± 0.53

***Table** from the paper showing the levels of fatty acids as a percentage of total fatty acids present in serum. Both AA and DHA are highlighted in yellow, and the numbers in green represent the percentage of these fatty acids measured during T1 [8.31% for AA and 2.24% for DHA] and during T3 [3.83% for AA and 1.24% for DHA].*

Relevance

As outlined in the introduction, dietary fat intake and available circulating fat play a critical role in healthy pregnancy, both in relation to cognitive development and gestational age. The present study provides some interesting insight into the dynamic changes in circulating fatty acid levels at both T1 and T3 stages of pregnancy. But, that is really all it provides for now [i.e., my understanding is there are other papers currently in press going into further analysis]. Importantly, however, would be an analysis of the relationships between these data and actual outcomes.

Let's reiterate that this is a study on biomarkers of short-term dietary intake, and as such, the analysis at either time-point may reflect only very recent intake. Let's also reiterate that this is an analysis of *circulating* levels, not dietary intake. The example of serum C16:0 palmitate, the saturated fatty acid, highlighted under ***Key Characteristic*** above is indicative of the importance that internal nutrient biomarkers reflect the *actual intake* of the nutrient of interest.

Further, it is also the case that a particular tissue compartment may not be the best representation of the given nutrient, even if it does have a relationship with intake. For example, in this study only EPA was correlated with both T1 and T3, yet DHA is the prime omega-3 of interest during pregnancy. But again, this study measured *serum*, which is a good reflection of EPA but not ideal for DHA [10,15].

As far as potential changes go, these findings should also be taken with a pinch of potassium-enriched salt, because they may not actually mean much for serum levels to decline. Or they may; we need a more robust analysis relating serum levels to actual outcomes before this could be determined. Certainly, lower serum DHA has been associated with cognitive decline in the elderly [16]. It would be very interesting to see whether these declines in relative AA and DHA concentrations reflect maternal metabolism and preferential uptake of these fatty acids into the developing nervous system and into breastmilk.

The study provides interesting insight into a range of circulating, serum fatty acid levels in otherwise healthy young women at both first and third trimesters. But the relevance and clinical utility of these markers needs to be added to this line of evidence for it to be meaningful.

Application to Practice

This does not change best-practice advice as it relates to lipids and pregnancy, which includes:

- 2-3 servings (280-360g/week) of oily fish low in mercury per week, but additionally supplement with 200-300mg DHA/d through pregnancy and lactation [5];
- Where fish is excluded for individual considerations, supplementation with a direct source of 500-600mg/d DHA is preferable from 20-weeks' gestation [fish oil or algae oil], either through maternal supplementation or infant formulas;
- Infant formulas are recommended to contain at least 0.2% and 0.35% DHA and AA, respectively [or 2:1 AA to DHA] [17].

There may be a call for longer omega-3 supplementation during pregnancy, as many of the studies showing increased gestational age and birth weight have started supplementation before the third trimester [1].

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