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Original article

Increased consumption of salmon during pregnancy partly prevents the decline of some plasma essential amino acid concentrations in pregnant women☆

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SUMMARY

Background & aims: Oily fish is a good source of n-3 long-chain polyunsaturated fatty acids. Since these fatty acids may change efficiency of amino acid (AA) absorption, we determined whether increased salmon consumption influences plasma AA concentrations in pregnant women and their newborns.
Methods: Pregnant women were randomly allocated to remain on their habitual diet ($n = 61$; control group) or to consume two 150 g farmed salmon portions per week from 20 weeks pregnancy until birth ($n = 62$; salmon group). Plasma AA concentrations were determined in women at w20, w34 and w38 of pregnancy and in umbilical cord at delivery.
Results: Concentrations of arginine, valine, leucine and lysine were affected by both time of pregnancy and salmon intake ($p < 0.05$), with a smaller gestation-associated decrease in the salmon group. Total essential AA concentrations were similar in both groups at w20, but at w38 were higher in salmon group ($p < 0.05$). Cord plasma AA concentrations, higher than in maternal plasma ($p < 0.01$), were similar in the two groups ($p > 0.05$).
Conclusions: Two portions/wk of oily fish increased plasma essential AA concentrations during pregnancy and could contribute to a maternal health benefit. Two portions/wk of salmon did not affect plasma AA concentrations in the newborn.
Clinical trials identifier: NCT00801502.

1. Introduction

Amino acid (AA) availability is one of major factors determining fetal growth.[1–3] Besides their obvious role in protein synthesis, both essential and non-essential AA regulate key metabolic pathways involved in growth, development and immunity.[4] Low availability of functional AA such as arginine, cysteine, glutamine or proline impairs fetal growth, which is linked to a higher risk of chronic diseases in adult life.[5] During pregnancy, nitrogen needs of the fetus are ensured by placental transfer from maternal blood.[3–7] The plasma AA profile is constant during the post-absorptive state and is closely related to that of the diet.[8] Meat is the major dietary source of AA in Western diets.[9] However, AA availability from food depends on factors such as the origin of meat consumed (e.g. beef, chicken or fish), the cooking conditions, and the content of fats and

Abbreviations used: n-3 LC-PUFA, n-3 long chain-polyunsaturated fatty acid; AA, amino acid; Tau, taurine; Thr, threonine; Ser, serine; Asn, asparagine; Glu, glutamic acid; Gln, glutamine; Pro, proline; Gly, glycine; Ala, alanine; Cit, citrulline; Val, valine; Cys, cysteine; Met, methionine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Orn, ornithine; Lys, lysine; His, histidine; Arg, arginine; CG, control group; SG, salmon group; w20, 20 week of gestation; w34, 34 week of gestation; w38, 38 week of gestation; TAA, sum of all amino acids; EAA, sum of essential amino acids; BCAA, sum of branched chain amino acids; AAA, sum of aromatic amino acids.

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carbohydrates.[10–12] Fish flesh seems to have a texture that allows better disintegration during digestion leading to higher AA absorption than other meats.[11] Furthermore, the presence of n-3 long chain-polyunsaturated fatty acids (n-3 LC-PUFA) in the meal promotes gastric emptying and so could improve the efficiency of protein digestion and AA absorption.[13]

The Salmon in Pregnancy Study (SiPS) focuses on pregnant women whose offspring are at high risk of developing atopic diseases.[14] To our knowledge it is the first intervention trial with oily fish rich in n-3 LC-PUFA during pregnancy. Since there is potential for oily fish to promote protein digestion and AA availability, we hypothesised that plasma AA profiles will be different between pregnant women who consume oily fish and those who do not and in their offspring at birth.

2. Materials and methods

2.1. Subjects

The SiPS is a single-blind, randomized, controlled intervention with salmon during pregnancy; the full study design and baseline subject characteristics have previously been reported.[14] In brief, women ($n = 123$), within the catchment area of the Princess Anne Hospital (Southampton, United Kingdom), who rarely ate oily fish and were at risk of having a child with atopy were randomly assigned to either remain on their habitual diet (control group [CG]; $n = 61$) or were provided with farmed salmon and requested to consume two 150 g portions of salmon per week from 20 weeks pregnancy until birth (salmon group [SG]; $n = 62$). Full details of the farmed salmon have previously been reported[14]; each 150 g salmon portion contained 30.5 g protein (on average). The AA composition of a salmon portion (g/150 g) was: Asp 3.05; Thr 1.31; Ser 1.21; Glu 4.44; Pro 1.05; Gly 1.43; Ala 1.80; Val 1.53; Cys 0.32; Met 0.88; Ile 1.37; Leu 2.42; Tyr 1.01; Phe 1.16; Lys 2.73; His 0.88; Arg 1.78. The study and all procedures were approved by the Southampton and South West Hampshire Research Ethics Committee (07/Q1704/43). The study was conducted according to the principles of the Declaration of Helsinki, and all women gave written informed consent. The SiPS is registered at www.clinicaltrials.gov (clinical trials identifier NCT00801502).

Sixteen women withdrew from the study before giving birth (7 in CG and 9 in SG).[14] There were 107 births in the study and umbilical cord blood was collected from 101 of these births.[14] Not all samples were available for AA analysis: Numbers of samples analyzed in the CG were 59 (maternal w20), 55 (maternal w34), 41 (maternal w38) and 25 (umbilical cord) (Supplemental Fig. 1). Numbers of samples analyzed in the SG were 62 (maternal w20), 55 (maternal w34), 44 (maternal w38) and 30 (umbilical cord) (Supplemental Fig. 1). As reported previously,[14] the two groups did not differ in maternal age, height, or weight, pregnancy duration, mode of delivery or birth weight of offspring. No adverse events or negative health effects were observed.

2.2. Food frequency questionnaire

Women completed an administered 100-item food frequency questionnaire (FFQ) at 20 (w20) and 34 (w34) weeks of gestation that recorded frequency of consumption of the 100 foods over the preceding twelve weeks. This FFQ was based on a validated questionnaire for pregnant women,[15,16] with additional detail collected concerning the quality and quantity of fish consumption.[14] Weekly consumption of protein-rich foods such as “pork”, “chicken”, “lamb”, “beef”, “minced meat”, “sausages”, “ham and luncheon meat”, “white fish”, “fish finger/dishes”, “oily fish” and “shellfish” was examined; the total frequency of consumption of ‘protein-rich foods’ represented the sum of their frequencies of consumption. Total energy and protein intakes were calculated from reported frequency data for all foods listed on the FFQ, using UK food composition data.[15]

2.3. Maternal and cord blood samples collection

At w20 (i.e. before the start of the intervention), w34 and w38 of pregnancy, venous blood samples were collected into heparin and plasma prepared; women were in the fasting state when the samples were collected. Plasma was deproteinized with sulfosalicylic acid (50 mg/ml) and supernatants frozen immediately and stored at −80 °C until analysis. At delivery (w38), umbilical cord blood samples were collected and deproteinized plasma prepared as described above.

2.4. Pregnancy outcomes

Key pregnancy outcomes such as birth weight, infant head circumference at birth, and infant length were measured by the midwife who attended the birth. These parameters are presented for the newborns whose umbilical cord plasma AA were measured here.

2.5. Plasma amino acid analysis

Plasma AA analysis was performed using lithium ion exchange chromatography on a conventional HPLC system (Dionex, Voisins Le Bretonneux, France) coupled with post column derivatization using ninhydrin through a PCX 5200 postcolumn derivatizer (Pickering Laboratories, Mountain View, CA, USA) and detection at both 440 and 570 nm as described previously.[17] Column (cation exchange 5 μm, 4.0 × 100 mm), lithium elution buffer, ninhydrin derivatization medium and amino acid standard solution were purchased from Pickering Laboratories. Integration of chromatograms was accomplished using Chromeleon software Version 6.80 from Dionex.

Before analysis, L-α amino β-guanidinopropionic acid (AGPA), D-glucosaminic acid (GLSA) and nor-leucine (NOR) were added in each sample at 250 μmol/l each as internal standards. AA concentrations were calculated using a standard AA solution established under the same conditions (Method reproducibility = 6%, sensitivity threshold < 5 μmol/l). Pooled human plasma was used as an internal laboratory control and presented a coefficient of variation of 6%. The laboratory is registered in the European quality control program ERNDIM. Results are given as mean ± standard deviation and expressed as μmol/l and related to the packed cell volume. Mean of usual values for healthy European women[17] and for pregnant women[18,19] were used as references.

2.6. Statistical analysis

Comparisons of data for each maternal variable between diet groups (CG, SG) over time (w20, w34, w38) were made using two-factor ANOVA. *A posteriori* Bonferroni testing was performed to evaluate specific differences between groups. Comparisons of data at the same time between diet groups, or between cord blood and maternal blood at w38 were performed by Mann Whitney *U* test. All statistical analyses were performed with the Statistical software Prism 5 (v5.04) for Windows. A p-value <0.05 was considered as statistically significant.

3. Results

3.1. Food frequency questionnaire analysis

Protein and energy intakes did not differ between groups. Mean ± standard deviation daily protein intakes (g) at w20 and