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Fatty acid composition of skeletal muscle and adipose tissue in Spanish infants and children

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There is a relationship between the fatty acid profile in skeletal muscle phospholipids and peripheral resistance to insulin in adults, but similar data have not been reported in infancy and childhood. The objective of this study was to investigate the fatty acid composition of skeletal muscle and adipose tissue across the paediatric age range. The fatty acid profile of skeletal muscle phospholipids and adipose tissue triacylglycerols was analysed in ninety-three healthy Spanish infants and children distributed into four groups: group 1 (0 to <2 years, n 10); group 2 (2 to <5 years, n 41); group 3 (5 to <10 years, n 24); group 4 (10 to 15 years, n 18). In skeletal muscle phospholipids, oleic acid (18: 1n-9*cis*) content decreased significantly whereas that of linoleic (18: 2n-6) acid increased significantly with age (P for trend $<0{\cdot}01$). In adipose tissue, the contents of triacylglycerol and linoleic acid increased significantly across the paediatric age range (P for trend $<0{\cdot}01$), whereas dihomo-γ-linolenic (20: 3n-6) and arachidonic (20: 4n-6) showed significant differences between groups. The variations in fatty acid composition observed with age indicated an imbalance in dietary n-3/n-6 long-chain PUFA.

Adipose tissue: Fatty acid composition: Insulin resistance: Skeletal muscle: Children

Long-chain PUFA (LCPUFA) are major components of cellular phospholipids and are involved in the structure and function of cell membranes. Changes in lipid composition of the diet modify both the biochemical and functional characteristics of cell membranes, including permeability, physical properties, transport of molecules and enzyme activity (Bourre *et al.* 1989, 1993).

Maternal diet has an important influence on the fatty acid (FA) composition of breast milk (Guestnet *et al.* 1991) and consequently on the FA content of the plasma and red blood cells of breast-fed infants (Yeh *et al.* 1998). However, the few existing studies on FA composition across the paediatric age range have only been carried out on plasma or red-cell phospholipids, and have been limited to the breast-feeding period. In this area, we have previously published reports on plasma FA composition during pregnancy (Sanjurjo *et al.* 1993) and during labour in both mothers and neonates (Sanjurjo *et al.* 1994). More recently, the plasma phospholipid composition of mother–infant pairs has been studied longitudinally (Rump & Hornstra, 2002).

The probable relationship reported in adults between the LCPUFA composition of skeletal muscle phospholipids and peripheral insulin sensitivity/resistance (Borkman *et al.* 1993; Pan *et al.* 1995) highlights the importance of determining LCPUFA reference values in skeletal muscle phospholipids at all ages, in order to design early preventive strategies for type 2 diabetes. The single study reported in the literature was restricted to early infancy and showed a negative correlation between the content of DHA (22: 6n-3) in skeletal muscle and plasma glucose levels (Baur *et al.* 2000).

The aim of this investigation was to determine the FA profile of skeletal muscle phospholipids and adipose tissue triacylglycerols during infancy and childhood in order to establish reference values in this field.

Subjects and methods

Subjects

Ninety-three children with an age range between 2 months and 15 years who were undergoing minor elective surgery in our hospital were recruited for the study. Surgery was being performed to correct an inguinal hernia or undescended testes, or for minor urological problems. Muscle samples were obtained from the abdominal external oblique muscle, and adipose tissue was obtained from the surrounding area. All the children were well nourished and had no history of chronic or systemic disease; none was subject to dietary restriction. Owing to the type of surgery employed, 90 % of the subjects were boys and only 10 % were girls, the number in each group depending on the availability of the samples.

Abbreviations: FA, fatty acid; LCPUFA, long-chain PUFA.

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The subjects were distributed between four groups: group 1 (0 to <2 years, n 10); group 2 (2 to <5 years, n 41); group 3 (5 to <10 years, n 24); and group 4 (10 to 15 years, n 18). The mean weights in each group were 8·52 (SD 2·92), 15·08 (SD 3·29), 24·2 (SD 6·74) and 40·98 (SD 12·66) kg, respectively. These groups were selected for two main reasons. First, this was the separation used in the dietetic enquiry published for this region (the Basque Country, Spain; see Table 1; Serra-Majem & Aranceta-Bartrina, 2004). Second, this division is associated with the different stages of insulin resistance across the paediatric period. Table 1 shows the energy data for each nutrient, by age and sex. Of the children under 2 years of age in our study, three were exclusively breast-fed for up to 5 months, two for up to 3 months and four for up to 1 month, one infant being formula fed from birth.

The study protocol was approved by the Ethics Committee of our hospital and fulfilled the requirements of the Declaration of Helsinki.

Methods

Total lipids were extracted from both kinds of sample by liquid–liquid extraction (Folch *et al.* 1957), using tridecanoine as an internal standard for adipose tissue, and phosphatidyl diheptadecanoine for skeletal muscle. For muscle, the phospholipid fraction was isolated from total lipids by TLC using heptane–isopropylether–acetic acid (70:30:5, v/v) as the resolving system.

After spraying the plate with an ethanol solution of 2′,7′-dichlorofluorescein, lipid fractions were made visible under UV light. The phospholipid band was scraped off, and the fatty acids were transmethylated (Lepage & Roy, 1986). The methyl esters were separated and quantified with a gas chromatograph (HP 5890) by means of a 30m SP 2330 fused silica capillary column, internal diameter 0·25 mm, 0·20 mm film thickness (Supelco Inc. Bellefonte, PA, USA). The instrument was equipped with a flame ionization detector.

The initial oven temperature of 80°C was maintained for 1 min after injection; this was then raised by 50°C/min to 140°C, 5°C/min to 190°C, maintained for 5 min and finally raised by 5°C/min to 215°C, at which it was isothermally maintained for 15 min. Injector and detector temperatures were 250°C. He gas under a pressure of 0·5 bar was used as a carrier.

Identification and quantification were performed by comparison with commercial standards containing known percentages in weight of FA methyl esters (Supelco Inc; Larodan Fine Chemicals, Malmö, Sweden). Results were expressed as percentage of each FA by weight.

Statistics

The FA in some of the groups did not conform to a normal distribution. The descriptive statistics are expressed as median and interquartile range. Comparisons of group means were performed using one-way ANOVA. Distribution and equality of variances between groups were evaluated (Levene's test and Shapiro–Wilk's test), and $\log_{10}$ transformations were performed for some of variables to improve normality and homogeneity of variance. Once the difference among means had been established, the Bonferroni test was applied as a correction for multiple comparisons.

In addition, P values for linear trends between categories (polynomial analysis) were also calculated in the ANOVA. Retransformed mean values were stated as geometric means. A two-tailed significance level of 0·05 was chosen for all comparisons. Statistical analysis was performed with SPSS statistical software (release 10.0, SPSS Inc, Chicago, IL, USA).

Results

The content of the main FA in skeletal muscle phospholipids in the four age groups studied is shown in Table 2, as are some ratios between FA. FA 22: 5n-6/22: 4n-6 represents the DHA deficiency index, whereas 22: 6n-3/22: 5n-6 is the DHA sufficiency index (Holman, 1986; Neuringer *et al.* 1986) and the 22: 6n-3/20: 4n-6 index shows the ratio between the most important members of the n-3 and n-6 families.

As can seen, the levels of palmitic acid (16 : 0) decreased progressively with age (geometric means: 20·19, 17·74, 17·00 and 18·01, respectively for the four groups), but the Bonferroni test was significant only between groups 1 and 3 ($P<0·009$). Oleic acid (18 : $1n-9cis$) showed a negative linear tendency (P for trend $<0·01$; means 9·40, 9·21, 8·13 and 7·70, respectively). The Bonferroni test was significant between groups 2 and 4 ($P<0·05$). On the other hand, linoleic acid (18: $2n-6$) showed a difference between the groups (means 18·38, 23·52, 26·04 and 24·93, respectively). The Bonferroni test was significant between group 1 and the other groups ($P<0·05$, $P<0·001$ and $P=0·001$, respectively).

Table 1. Energy and nutrient intake in the pediatric population of the Basque Country (from Serra-Majem & Aranceta-Bartrina, 2004)

	2–5				5–10				10–15			
	Male		Female		Male		Female		Male		Female	
Groups (years) Sex	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (kcal)	1627	173	1543	126	1975	169	1832	238	2259	248	1932	181
Proteins (% energy)	16·6	1·4	17·1	1·9	16·2	1·4	16·9	1·9	17	1·8	16·6	1·3
Carbohydrate (% energy)	43·4	3·5	44·8	2·3	43·6	3·8	41	3·5	42·2	4·9	42·6	2·2
Lipids (% energy)	39·8	4	38·5	2·9	39·5	3·1	40·9	3·5	40·2	2·3	39·9	2·1
Saturated FA (% energy)	14·4	1·5	14·1	2·2	13·7	1·5	14·1	2·1	13·9	0·8	13·3	1
MUFA (% energy)	16·3	3·1	14·8	1·8	15·8	1·5	16·3	1·7	15·9	1·9	15·8	1·2
PUFA (% energy)	4·6	1	4·5	0·8	4·9	0·7	5·2	0·9	5·2	0·7	5·5	1
Cholesterol (mg/1000 kcal)	193	79	164	55	178	51	189	61	181	41	184	52

Table 2. Fatty acid composition of skeletal muscle phospholipids in the four age groups (g/100 g)

	Group 1 (0 to <2 years) *n* 10	Interquartile range	Group 2 (2 to <5 years) *n* 41	Interquartile range	Group 3 (5 to <10 years) *n* 24	Interquartile range	Group 4 (10 to 15 years) *n* 18	Interquartile range	*P (between groups)*	*P* for trend
Lauric (12 : 0)	0·27	0·10–0·46	0·18	0·09–0·44	0·16	0·0–0·26	0· 15	0·02–0·44		
Miristic (14 : 0)	0·98	0·72–1·18	0·73	0·60–0·87	0·64	0·57–0·84	0·56	0·43–0·82		
Palmitic (16 : 0)	19·4	17·6–22·1	17·6	15·9–19·5	17·3	15·5–18·9	17·9	15·8–20·4	(1 *v.* 3)**	
Palmitoleic (16 : 1*n*-7)	0·35	0·28–0·50	0·38	0·3–0·46	0·34	0·28–0·37	0·34	0·30–043		
Stearic (18 : 0)	18·5	17·3–21·7	18·5	17·3–19·9	18·4	17·3–19·3	18·1	17·7–21·9		
Oleic (18 : 1*n*-9)	9·0	8·2–10·9	9·1	7·9–9·9	7·9	7·1–8·9	8·2	6·7–9·0	(2 *v.* 4)*	$p < 0·01$
Elaidic (18 : 1*n*-9trans)	0·24	0·17–0·40	0·32	0·10–0·40	0·38	0·32–0·43	0·30	0·20–041		
Linoleic (18 : 2*n*-6)	19·2	14·4–22·8	23·5	21·3–26·1	25·9	22·8–29·4	25·2	22·6–29·6	(1 *7v.* 2)*, (1 *v.* 3)***, (1 *v.* 4)***	$p < 0·01$
α-Linolenic (18 : 3*n*-3)	0·18	0·05–0·24	0·16	0·10–0·24	0·14	0·08–0·20	0·17	0·12–0·20		
Dihomo-γ-linolenic (20 : 3*n*-6)	1·9	1·3–3·1	1·6	1·4–1·9	1·6	1·4–1·8	1·5	1·3–1·7		
Arachidonic (20 : 4*n*-6)	12·8	9·6–15·6	13·8	12·6–14·7	14·6	13·5–15·3	14·3	12·4–15·6		
Eicosapentaenoic (20 : 5*n*-6)	0·46	0·11–0·81	0·54	0·36–0·72	0·53	0·36–0·70	0·46	0·35–0·59		
Docosapentaenoic (22 : 5*n*-6)	0·55	0·34–0·86	0·46	0·40–0·59	0·48	0·32–0·53	0·38	0·36–0·45		
Docosahexaenoic (22 : 6*n*-3)	3·0	2·0–3·9	3·3	2·7–4·1	3·2	2·6–3·5	2·8	2·3–3·2		
Docosatetraenoic (22 : 4*n*-6)	0·96	0·83–1·66	0·83	0·72–1·01	0·71	0·62–0·90	0·74	0·65–0·88		
n-3/*n*-6	0·13	0·09–0·17	0·13	0·11–0·15	0·11	0·10–014	0·11	0·09–0·11		
22 : 6*n*-3/20 : 4*n*-6	0·22	0·17–0·27	0·25	0·20–0·28	0·21	0·19–0·27	0·20	0·18–0·22		
22 : 5*n*-6/22 : 4*n*-*6*	0·53	0·37–0·69	0·56	0·46–0·65	0·63	0·47–0·71	0·47	0·44–0·61		
22 : 6*n*-3/22 : 5*n*-3	2·6	2·3–3·3	3·0	2·4–3·3	2·9	2·7–3·5	2·8	2·7–3·1		

Bonferroni test * < 0·05, ** < 0·01, *** < 0·001.

Table 3. Fatty acids composition of adipose tissue triacylglycerols in the four age groups (g/100 g)

	Group 1 (0 to < 2 years) *n* 10	Interquartile range	Group 2 (2 to < 5 years) *n* 41	Interquartile range	Group 3 (5 to < 10 years) n 24	Interquartile range	Group 4 (10 to 15 years) *n* 18	Interquartile range	*P* (between groups)	*P* for trend
Lauric (12 : 0)	2·0	1·7–2·4	1·1	0·7–1·4	1·1	0·6–1·5	0·73	0·59–1·10		
Miristic (14 : 0)	3·8	3·7–4·6	3·1	2·7–3·7	3·0	2·6–3·5	2·6	2·0–3·2		
Palmitic (16 : 0)	23·4	20·6–24·4	21·1	20·1–22·0	21·1	20·3–22·2	20·7	18·3–21·9		
Palmitoleic (16 : 1*n*-7)	3·5	3·1–4·5	2·6	2·0–2·9	2·9	2·1–3·6	3·1	2·4–3·8		
Stearic (18 : 0)	3·9	3·5–4·7	7·0	5·8–7·5	6·9	5·9–7·5	5·6	4·3–6·9		
Oleic (18 : 1*n*-9)	41·9	40·1–42·8	41·9	39·5–45·0	41·1	39·1–43·2	42·5	38·1–46·4		
Elaidic (18 : 1*n*-9trans)	0·54	0·38–0·71	0·96	0·76–1·17	1·2	1·1–1·6	1·0	0·8–1·4		
Linoleic (18 : 2*n*-6)	13·1	11·1–13·6	13·8	11·8–16·0	14·6	12·5–15·8	15·8	12·3–18·1		$p < 0{\cdot}01$
α-Linolenic (18 : 3*n*-3)	0·58	0·47–0·70	0·44	0·37–0·52	0·51	0·40–0·53	0·53	0·48–0·61		
Dihomo-γ-linolenic (20 : 3*n*-6)	0·19	0·15–0·30	0·14	0·13–0·18	0·14	0·11–0·18)	0·20	0·14–0·23	(1 *v.* 2)**, (1 *v.* 3)*, (3 *v.* 4)* (2 *v.* 4)*	
Arachidonic (20 : 4*n*-6)	0·23	0·19–0·31	0·20	0·18–0·23	0·23	0·19–0·26	0·30	0·21–0·37		
Docosapentaenoic (22 : 5*n*-6)	0·027	0·012–0·039	0·029	0·015–0·034	0·037	0·022–0·045	0·045	0·017–0·068		
Docosahexaenoic (22 : 6*n*-3)	0·18	0·10–0·27	0·16	0·13–0·23	0·16	0·12–0·28	0·21	0·16–0·24		
Docosatetraenoic (22 : 4*n*-6)	0·089	0·067–0·117	0·081	0·062–0·101	0·089	0·073–0·118	0·13	0·08–0·14		
n-3/*n*-6	0·073	0·053–0·083	0·050	0·043–0·065	0·0·51	0·042–0·072	0·054	0·049–0·064		
22 : 6*n*-3/20 : 4*n*-6	0·52	0·43–0·94	0·78	0·59–1·22	0·77	0·54–1·31	0·69	0·40–0·98		
22 : 5*n*-6/22 : 4*n*-6	0·33	0·13–0·40	0·33	0·18–0·44	0·31	0·26–0·50	0·39	0·18–0·64		
22 : 6*n*-3/22 : 5*n*-3	1·8	1·5–2·5	1·5	1·3–1·9	1·52	1·20–1·89	1·37	1·28–1·52		

Bonferroni test * < 0·05, ** < 0·01.

The content of the main FA in adipose tissue triacylglycerols at different ages is shown in Table 3. The concentration of linoleic acid, as in skeletal muscle, increased progressively with age (means 12·36, 14·07, 15·06 and 15·22, respectively; P for trend $<0{\cdot}01$). Arachidonic acid (20 : 4*n*-6) varied between the different age groups (geometric means 0·26, 0·21, 0·23 and 0·30, respectively), the Bonferroni test showing statistically significant differences between groups 2 (2 to <5 years) and 4 (10–15 years) ($P<0{\cdot}05$). Dihomo-γ-linolenic acid varied between groups too: the Bonferroni test result was $<0{\cdot}01$ between groups 1 and 2, and $<0{\cdot}05$ between groups 1 and 3, and 3 and 4.

Discussion

We report here the FA composition of skeletal muscle phospholipids and adipose tissue triacylglycerols in ninety-three paired tissue samples obtained across the paediatric age range. Important pitfalls of our study were obviously the lack of dietary data detailing the amount and type of fat ingested in each age period and the lack of data on physical activity.

A strong correlation between FA intake and FA composition of adipose tissue has been shown in adults, especially for essential and *trans* FA (Baylin *et al.* 2002). There is a correlation between the type of infant diet (breast-feeding or formula) and the composition of muscle membrane, especially DHA (Baur *et al.* 2000). Another study equally proved the relationship between diet and skeletal muscle FA composition (Andersson *et al.* 2002). Nevertheless, in Table 1, we offer results from the nutritional enquiry specifically performed on our own population. The most remarkable results related to the FA composition of skeletal muscle phospholipids were a decrease in oleic acid and an increase in linoleic acid content with age. As mentioned earlier, there is only one study on the FA composition of skeletal muscle phospholipids during the breast-feeding period (Baur *et al.* 1998). This study was limited to the early infancy period and studied both breast-fed and formula-fed infants. A comparison of these results with ours seems therefore inappropriate. With respect to the results obtained for adipose tissue, the most important findings were the changes in *n*-6 LCPUFA composition. The linoleic (as in skeletal muscle), di-homo-γ-linolenic and arachidonic acids contents varied across the paediatric age range (between groups 1 and 3, $P=0{\cdot}004$; between groups 1 and 4, $P=0{\cdot}008$).

Our results as a whole, in both skeletal muscle and adipose tissue, suggest the existence of a dietary *n*-3:*n*-6 LCPUFA imbalance, probably as a result of a decline in use of the Mediterranean diet. This ratio between *n*-3 and *n*-6 is habitually employed in the literature and is based on the metabolic and functional competitiveness between both families of FA. Owing to the consumption of fish, this ratio is normally higher in the Mediterranean countries, whereas *n*-6 FA are increased through the consumption of corn oil and sunflower oil. A lower value of this ratio negatively affects endothelial function and insulin resistance.

Two epidemiological studies performed in the Basque Country suggest a progressive loss of and deviation from the Mediterranean diet, both in adults (percentage of energy data collected in the 1960s and 1980s: proteins 12·8 %, 12·7 %; carbohydrates 57·4 %, 45·4 %; saturated FA 8·4 %, 13·4 %; MUFA 13 %, 17·6 %, respectively), and in children (data from 1995 and 2001: saturated FA 16 %, 13·5 %; MUFA 17 %, 17 %; PUFA 5 %, 6·5 %, respectively) (Serra-Majem & Aranceta-Bartrina, 2002). The ideal intake of this kind of diet is saturated FA 7–10 %, MUFA 15–20 %. The significant increase in linoleic acid content and the *n*-3:n LCPUFA imbalance may indicate a greater risk of future cardiovascular disorders (Weyman *et al.* 1975) and may be responsible for the development of pro-inflammatory disorders and peripheral insulin resistance (Abbey *et al.* 1993; Okuyama *et al.* 1996*a*). In fact, in some countries such as Japan, experts have recommended that the intake of linoleic acid should be reduced in children (Okuyama *et al.* 1996*b*).

Preliminary unpublished results derived from this study show a significant association between the arachidonic acid content of the muscular phospholipids and the Homeostatic Model Assessment (HOMA) index. These data agree with those of a recently published study (Savva *et al.* 2003).

In conclusion, we consider the most significant results of our study to be the increase in linoleic acid and decrease in oleic acid across the paediatric age range, both indicating for our children a Westernization of their diet and a loss of the Mediterranean diet in our area. These results should stimulate the study of the possible relationship between the FA composition of the tissues and peripheral insulin resistance, allowing the design of better dietary strategies for preventing type 2 diabetes from the paediatric age.

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