Long chain nonesterified fatty acid patterns in plasma of healthy children and young adults in relation to age and sex

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Abstract In a large group of healthy Belgian children and young adults, the absolute and relative concentrations of nonesterified fatty acid patterns were determined by an appropriate gas–liquid chromatographic technique. The statistical analysis of the results showed that concentrations were dependent on age and sex. The absolute concentration of each fatty acid and the total nonesterified fatty acid concentration decreased exponentially with age and were significantly higher for girls than for boys. In addition, it was found that variation of relative concentrations with age was rather small. Furthermore, the concentration of monounsaturated fatty acids was significantly higher and that of saturated acid significantly lower for girls than for boys.—**Rogiers, V.** Long chain nonesterified fatty acid patterns in plasma of healthy children and young adults in relation to age and sex. *J. Lipid Res.* 1981. **22:** 1–6.

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Long chain nonesterified fatty acids (NEFA) or free fatty acids in human plasma are derived from adipose tissue stores and represent a major fuel for tissues, especially in the fasted and exercised state (1).

The total NEFA concentration (2–4) and the NEFA pattern (5–7) of plasma and serum of adults have been determined and studied extensively both in healthy individuals and in patients with various diseases (7–11). For children however, few (7, 11–17) and mainly incomplete data are available as only small groups have been studied (7, 13, 15–17).

As we intended to study the metabolic exchange between the plasma NEFA fraction and the phospholipids of the erythrocyte membrane of children under different pathological conditions, it was first necessary to establish the plasma NEFA pattern (composition and concentration) for a valid control group of healthy schoolchildren and young adults. An appropriate gas–liquid chromatographic (GLC) technique had been worked out previously (18) and storage conditions of plasma and blood samples had been determined (19).

MATERIALS AND METHODS

The materials used were described previously (18).

Subjects

Apparently healthy Belgian schoolchildren and young adults (university students) were studied in different age groups for both sexes: 8–10 yr (group I, 103 boys and 94 girls), 15–17 yr (group II, 134 boys and 61 girls), and 20–25 yr (group III, 45 boys and 42 girls). A single blood sample of 5 ml was taken in the morning at 9 AM after an overnight fast of 12–13 hr by rapid venipuncture with minimum stasis. The samples were collected in ice-cooled heparinized tubes (100 I.U./ml blood). No lipolysis due to the use of heparin occurred as checked by comparison of the total NEFA concentration obtained using heparin with the corresponding value obtained using EDTA as anticoagulant (n = 12). The subjects rested for 30 min before blood samples were taken. Only water consumption was permitted, in view of the changes occurring after eating (20) and coffee drinking (21). For young adults, cigarette smoking was not allowed (22). All subjects were examined as a part of a study concerning the nutritional and cardiological state of the young Belgian population. Apart from the determination of the plasma NEFA pattern, determinations of cholesterol, triglycerides, total lipid and protein were carried out. Blood pressure and ECG were taken; information concerning family history was gathered and the diet was followed by a dietician.

From the nutritional survey of these healthy children and young adults it appeared that the food for the three different groups was very rich in fat. Forty-five percent of the total calories was derived from fat. The mean proportion of saturated fat was 38%, of

Abbreviations: NEFA, nonesterified fatty acids; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

TABLE 1. Absolute concentration of individual plasma NEFA and total NEFA

Fatty Acid	Group	Female	Male
		μM ± S.D.	
$C_{14:0}$	I[a]	16 ± 12	14 ± 12
	II[b]	12 ± 9[d]	9 ± 7
	III[c]	7 ± 6	5 ± 4
$C_{16:0}$	I	119 ± 68	109 ± 55
	II	102 ± 41[f]	74 ± 37
	III	77 ± 32[e]	62 ± 23
$C_{16:1}$	I	26 ± 20	22 ± 17
	II	23 ± 14[e]	16 ± 13
	III	15 ± 12[d]	11 ± 7
$C_{18:0}$	I	49 ± 24	48 ± 21
	II	42 ± 18[e]	33 ± 18
	III	31 ± 13[d]	26 ± 11
$C_{18:1}$	I	191 ± 112	168 ± 93
	II	159 ± 72[f]	112 ± 57
	III	111 ± 62[d]	88 ± 42
$C_{18:2}$	I	77 ± 49	73 ± 44
	II	71 ± 34[f]	53 ± 30
	III	50 ± 26	42 ± 22
$C_{18:3}$	I	8 ± 6[d]	6 ± 5
	II	6 ± 3[d]	5 ± 4
	III	4 ± 3[d]	3 ± 2
Total NEFA	I	499 ± 282	442 ± 249
	II	424 ± 197[f]	308 ± 149
	III	293 ± 151	251 ± 114

[a] Group I: 8–10 yr old; 103 males and 94 females.
[b] Group II: 15–17 yr old; 134 males and 61 females.
[c] Group III: 20–25 yr old; 45 males and 42 females.
[d] $P < 0.1$.
[e] $P < 0.05$.
[f] $P < 0.01$.

Significant differences between boys and girls of the same age group are indicated, as estimated by a two-tailed z test for a normal distribution. In the abbreviation used for the fatty acids, the first two digits indicate the number of carbon atoms and the third digit indicates the number of double bonds. Values are mean ± S.D.

monounsaturated fat 42%, and of polyunsaturated fat 20% of the total dietary fat. Thirteen percent of the total caloric intake was protein and 42% carbohydrate. The total caloric intake was 97–100% of the energy requirement.

The social-economic status of group I and II was lower middle class. These groups were generally composed of children from workers and employees. The status of the university students was higher (university level). However, no significant differences could be detected in their average dietary intake.

Informed consent was obtained from the parents of the children and from the young adults.

Methods

The composition and concentration of NEFA were determined by the GLC technique that has been previously described in detail (18). In short, after blood samples were collected, the plasma was separated from the red blood cells within a limited time (19) and the analysis was performed immediately. A known concentration of heptadecanoic acid was added as internal standard and the total lipids were extracted twice using isopropanol–n-heptane–1 N sulfuric acid 39:8:1 (v/v).

The various lipid fractions were separated by one-dimensional thin-layer chromatography (TLC) with n-heptane–diethylether–acetic acid 40:10:1 (v/v). The NEFA spots were scraped off and extracted with purified diethylether. After methylation with boron trifluoride in methanol (14%, w/v), the methyl esters were extracted with n-heptane and separated by GLC on a 5% free fatty acid phase column. A digital integrator (Hewlett-Packard 3380 A) was used for the calculation of the results. Reference and blank determinations were carried out simultaneously.

RESULTS

Absolute concentration of plasma NEFA

The absolute concentration of each individual fatty acid and the total NEFA concentration (sum of the absolute fatty acid concentrations in μM) were not symmetrically distributed but were skewed to the right. The assumption that these data fall on a normal distribution is not completely justified, but the errors introduced are small and may be neglected (23).

The concentrations (mean ± S.D.) of the individual plasma NEFA and the total NEFA concentration of healthy boys and girls in the three groups are shown in **Table 1.** The results for arachidic ($C_{20:0}$) and arachidonic ($C_{20:4}$) acid are not shown, because these fatty acids occur only in trace amounts (0.2–0.5%). In the plasma samples with very low total NEFA concentration, especially in those of group III, both fatty acids were absent or not present in sufficient concentration to be detected and automatically integrated.

Concerning a possible relation to age, it was concluded that for both sexes, the absolute concentration of each fatty acid and the total NEFA decreased exponentially with age (from 8 to 25 years). **Fig. 1a** shows the exponential decrease of the total NEFA for girls and boys ($y = 711\ e^{-0.04x}$, $r = 0.91$; and $y = 673\ e^{-0.06x}$, $r = 0.84$, respectively). Since both weight and surface area change over the age range examined, the total NEFA concentration was plotted versus these two parameters. Identical results were obtained as shown in Fig. 1b and 1c. Comparing the mean concentration of each fatty acid and the total NEFA of group I with the corresponding value of group II and of group II with III, significant differ-

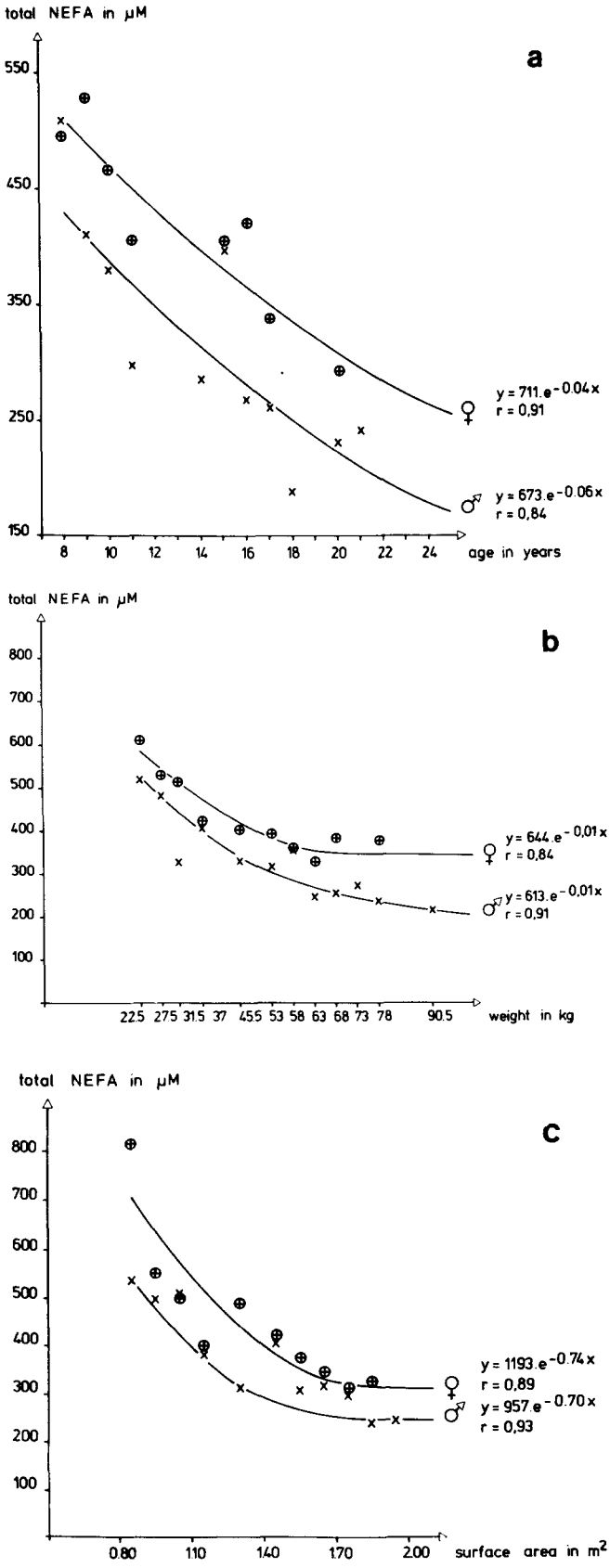

Fig. 1. Relation of plasma total NEFA for healthy males (n = 282) and females (n = 197) to a) age (8–25 yr), to b) weight, and to c) surface area.

TABLE 2. Relative concentration of individual NEFA

Fatty Acid	Group	Female	Male
		mol % ± S.D.	
$C_{14:0}$	I[a]	2.9 ± 1.3[f]	3.0 ± 1.3[f]
	II[b]	2.8 ± 1.8	2.7 ± 1.5
	III[c]	2.2 ± 1.1	2.1 ± 0.9
$C_{16:0}$	I	24.1 ± 2.1[f]	25.0 ± 2.8[d]
	II	25.3 ± 2.5	25.5 ± 2.8
	III	27.8 ± 5.1	26.7 ± 4.4
$C_{16:1}$	I	4.8 ± 1.8	4.6 ± 2.1
	II	5.4 ± 2.3	4.9 ± 2.3
	III	4.1 ± 2.2	4.5 ± 2.8
$C_{18:0}$	I	10.6 ± 2.4	12.2 ± 3.2[e]
	II	10.4 ± 2.3	11.4 ± 3.0
	III	10.7 ± 2.8	11.0 ± 2.3
$C_{18:1}$	I	38.9 ± 3.9[d]	37.7 ± 4.3
	II	37.5 ± 3.5	35.8 ± 3.6
	III	36.9 ± 4.4	37.0 ± 5.7
$C_{18:2}$	I	16.9 ± 4.3	16.3 ± 4.0
	II	17.6 ± 4.3	17.2 ± 5.0
	III	16.7 ± 4.9	17.7 ± 4.7
$C_{18:3}$	I	1.8 ± 1.2	1.4 ± 1.0[d]
	II	1.3 ± 1.2	1.6 ± 1.4
	III	1.4 ± 1.1	1.1 ± 0.8

[a] Group I: 8–10 yr old; 103 males and 94 females.
[b] Group II: 15–17 yr old; 134 males and 61 females.
[c] Group III: 20–25 yr old; 45 males and 42 females.
[d] $P < 0.05$,
[e] $P < 0.01$, and
[f] $P < 0.001$, significant age-dependent differences are indicated when Group I is compared with Group III by a z test.

Significant sex-dependent differences were found for $C_{16:0}$ in Group I ($P < 0.01$), for $C_{18:0}$ in Groups I ($P < 0.001$) and II ($P < 0.05$), and for $C_{18:1}$ in Groups I ($P < 0.05$) and II ($P < 0.01$) (z test). Values are mean ± S.D.

ences were found (z score is calculated for a normal distribution).

In addition, sex-dependent differences were also detected. For every age group, the absolute concentration of each fatty acid and the total NEFA were higher for girls than for boys. The significance, calculated by the z score for a normal distribution, is indicated in Table 1.

Plasma NEFA patterns

A normal distribution was found for the concentration of each fatty acid (expressed in mol % of the total NEFA concentration). This was true for all samples studied, for every age group, and for both sexes. No significant differences existed between the observed distribution of each individual fatty acid and the expected normal distribution with the same mean percentage concentration and the same S.D. as estimated by the X^2 score.

The relative concentrations of NEFA of healthy children and young adults are shown in **Table 2.**

Relation to age was examined by calculating the z

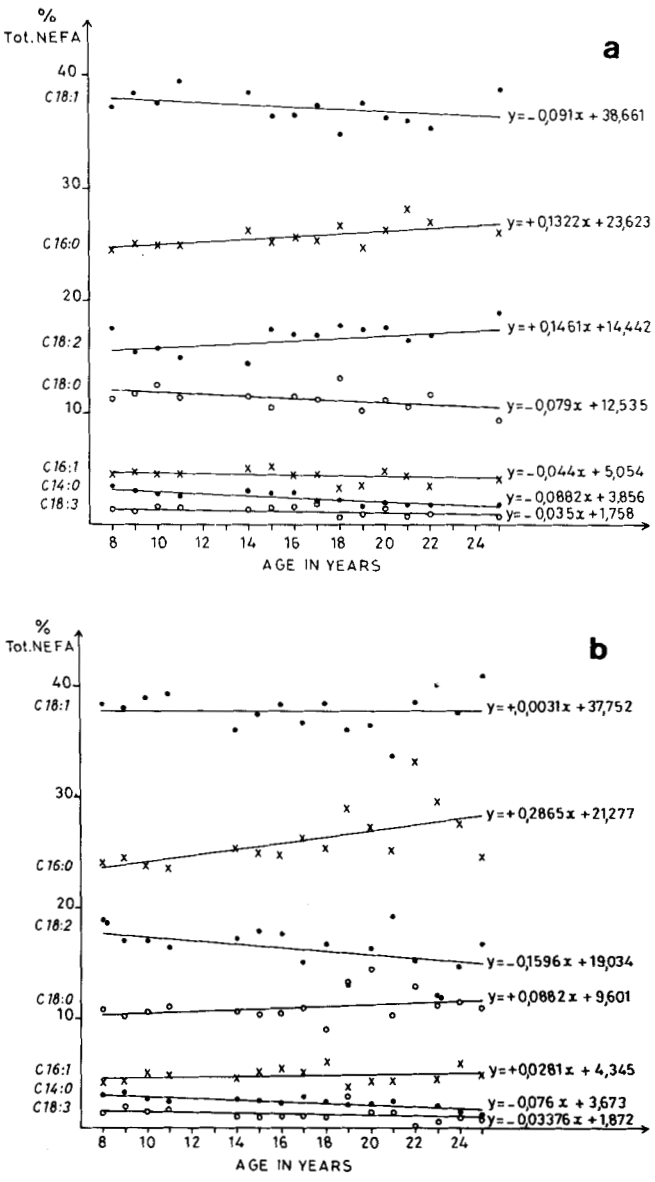

Fig. 2. Relation of plasma percentage NEFA pattern to age. Regression lines are shown for a) healthy males and b) healthy females from 8 to 25 yr old.

score (two-tailed test) and comparing the results of group I with those of group III. The significant differences found are indicated in the footnote of Table 2. The variations with age are small, except for $C_{14:0}$ and $C_{18:3}$. As these two fatty acids occur only in small concentrations in plasma NEFA, greater errors are expected in their GLC determination (18). Regression lines were calculated, but rather poor correlation coefficients were obtained. Results are shown for healthy boys and girls (**Figs. 2a and 2b**).

Sex-dependent differences were very small and amounted to about 10% (footnote of Table 2). Generally, the saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) were higher for boys and the monounsaturated acids higher for girls. The percentages of ($C_{16:0}$ + $C_{16:1}$) and ($C_{18:0}$ + $C_{18:1}$) were the same for both sexes.

DISCUSSION

Absolute concentration of plasma NEFA

Braun (2), using a titrimetric technique, found an asymmetric distribution for the total NEFA concentration. Regouw et al. (4) and Soloni and Sardina (3), working with a colorimetric method, proposed a distribution with a bimodal pattern. With our GLC technique, positive skewed distributions were found for every individual fatty acid and for the total NEFA concentration.

The absolute concentrations for each fatty acid and the total NEFA were quite variable, as shown by the large standard deviations. The plasma NEFA fraction is metabolically labile and is influenced by such factors as stress, exercise, and starvation (24–26).

The mean plasma total NEFA concentrations, reported here for every age group and for both sexes were low, compared with values obtained by colorimetric and titrimetric methods (2–4). These techniques with low specificity give results which are too high due to interference by non-fatty acid compounds. On the other hand, the GLC values of the total NEFA concentrations for healthy adults 25–35 years old, as mentioned by Sampson and Hensley (6), correspond with ours for young adults.

Since most studies concerning the plasma NEFA content in healthy children and adults have been carried out on very small groups, any sex or age influence has been very difficult to establish. We have found that the absolute concentration of each fatty acid and the total NEFA concentration decrease with age (from 8 to 25 yr). In a preliminary study, multiple blood samples were obtained from the same individuals, e.g., children of group I and adults of group III. No significant differences could be detected in the total NEFA concentration (n = 6). Consequently the higher values found for the total NEFA concentration in the younger children were probably not due to greater stress or fear associated with the venipuncture.

Braun (2) reported an increase of the total NEFA concentration with age (from 16 to 50 yr old). This is consistent with our own results[1] for healthy adults 25 to 60 yr old, in whom the plasma total NEFA concentration increased in both men and women. In a

[1] Rogiers, V. Unpublished results.

titrimetric study, Loeb (14) determined that total NEFA concentration was higher for healthy children (4 months to 15 yr old) than for healthy adults (17 to 51 yr old), but in the children there were no differences due to age or sex. Crum, Fass, and Pollack (10) described an increase of the total NEFA content with age (from 3 to 76 yr). Their results are not comparable with ours, because in their study the two groups of subjects differed in the duration of their fasts: the children fasted for 6–12 hr and the adults for 12–18 hr.

Concerning a possible influence of sex on the plasma NEFA pattern, Braun (2) and Soloni and Sardina (3) discovered a lower total NEFA concentration for adult men than for women. Our results for children and young adults support their findings, for every age group, the absolute concentrations of the individual fatty acids and the total NEFA were higher for girls than for boys. Hormonal influences are probably very important. Growth hormone (27, 28) and estrogens (29) are reported to affect the NEFA content in human plasma. It should be noted that the sex differences of the absolute fatty acid concentrations were the most significant in the 15–17 yr old age group (Table 1).

Plasma NEFA patterns

Studies concerning the plasma NEFA pattern of healthy adults and children usually do not mention variations due to sex or age. On the contrary, a possible dependence on sex or age of the adipose tissue fatty acid composition has been reported by several investigators (30–33). As NEFA are mobilized from adipose tissue stores, a certain influence of sex and age on the plasma percentage NEFA pattern may be expected.

Our results show a slight dependence on age; for both sexes the percentage concentration of $C_{16:0}$ increased and $C_{14:0}$ decreased from 8 to 25 yr. The fatty acid pattern of adipose tissue in the newborn is quite different from that in adults and seems to be stabilized by the age of 4 (34) or 5 yr (35). Only Insull and Bartsch (30) described an age dependence from 15 to 50 yr: $C_{16:0}$ and $C_{18:1}$ increased and $C_{14:0}$ and $C_{18:0}$ decreased.

The sex dependence of the plasma NEFA pattern, described here, is in agreement with published results on the fatty acid pattern of adipose tissue. Krut and Bronte-Stewart (31) reported a lower percentage concentration of saturated acids and a higher concentration of monounsaturated acids in women. It is suggested that the most important difference between both sexes may be the ratio of the monounsaturated to the saturated fatty acids. Insull and Bartsch (30) found slightly greater proportions of $C_{18:0}$ in men. Heffernan (32) and Scott et al. (33) reported that the percentage concentrations of $C_{18:0}$, $C_{14:0}$, and $C_{16:0}$ were higher and those of $C_{18:1}$ and $C_{16:1}$ were lower for men than for women, due to diet or sex-related differences in the metabolic activity of adipose tissue.

Our results indicate that both the absolute and relative plasma NEFA concentrations are dependent on age and sex. Consequently, when studying the plasma NEFA pattern of children under pathological conditions, it is essential to compare the results with those of age and sex matched controls.

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