Exercise-Induced Changes in the Concentration of Individual Fatty Acids and Triacylglycerols of Human Plasma

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Nineteen male handball players played for two 30-minute periods with a 10-minute interval. Blood samples were drawn at rest, at halftime, and at the end of the game. A biopsy of subcutaneous fat was also taken from 14 participants. Mean plasma lactate concentration was not greater than 4 mmol/L at the end of either half. The concentration of nonesterified fatty acids (NEFA) in plasma increased gradually but not uniformly throughout the game. In effect, the percentages of the major NEFA were significantly different at the three time points of sampling: palmitate (16:0) and stearate (18:0) decreased and oleate (18:1) and linoleate (18:2) increased, resulting in an increase of the ratio of unsaturated to saturated fatty acids (U/S) from 1.1 at rest to 1.6 at the end. The concentration of plasma triacylglycerols (TG) declined during the game, but nine of 19 subjects showed increases during one or both halves, implying a stimulation of TG release from the liver during exercise, which can, at times, overcome the increased hydrolysis of TG in muscle capillaries. Changes in the acyl-group distribution of plasma TG were minor but also in favor of unsaturated fatty acids. Changes in NEFA composition tended toward the composition of adipose tissue, in which TG had a U/S ratio of 3.2. Linear regression between changes in the total concentration of plasma NEFA during each half of the game and corresponding changes in the concentration of individual NEFA showed that the contributions (slopes) of myristate (14:0), palmitoleate (16:1), 18:0, and 18:2 were not significantly different from their fractions in adipose tissue TG. In contrast, the contributions of 16:0 and 18:1 were, respectively, higher and lower than their fractions in adipose tissue TG, suggesting that the rates of release were, respectively, higher and lower than the rates of uptake. The observed shift toward unsaturated NEFA and TG may contribute to the favorable modifications of the plasma lipoprotein profile associated with aerobic exercise and with a diet rich in unsaturated fatty acids.

BLOOD-BORNE LIPIDS, primarily nonesterified fatty acids (NEFA) and triacylglycerols (TG), serve as major sources of energy for skeletal muscle, the largest tissue in the body of a vertebrate. The role of these lipids is accentuated during physical activity, which multiplies the caloric demands of working muscles. Prolonged exertion is known to increase the concentration of NEFA in plasma, due to the stimulation of lipolysis by norepinephrine and epinephrine in adipose tissue (reviewed in Buelow[1] and Holloszy[2]). Most of the relevant literature has treated plasma NEFA as one entity, and reports on the behavior of individual members of this family during exercise are scarce and conflicting.[3,4] In view of the particular roles of individual fatty acids and their association with cardiovascular disease through epidemiologic studies, we considered it pertinent to explore how exercise affects the profile of plasma NEFA.

Opinions are also divided regarding the acute effects of prolonged exercise on the concentration of plasma TG. Although most researchers favor a decrease (reviewed in Buelow,[1] Haskell,[5] and Berg et al[6]), no change,[7-9] increases,[10-17] or even opposite responses under identical conditions[18] have been reported. Clarification of this issue is of importance, since acute changes apparently mediate for the well-established beneficial long-term effects of aerobic exercise.[5,6]

We investigated these problems by determining the concentration of individual NEFA and TG acyl groups in plasma taken from athletes before and after exercise of moderate average intensity. We also determined the composition of adipose tissue TG, which are the prime source of plasma NEFA. Our results indicate an increase in the proportion of unsaturated to saturated NEFA with exertion. This change is in the direction of adipose tissue TG composition. As for plasma TG, it appears that physical activity can stimulate their release from the liver.

SUBJECTS AND METHODS

Our research was conducted among selected handball players at the level of the Greek national junior team. The study group consisted of 19 healthy male volunteers aged 15 to 17 who had been training for the previous 4 to 6 years. They averaged 1.84 m in height and 78 kg in mass. Each subject participated in one of four handball matches played between 5 and 10 PM and had eaten a light meal 3 to 4 hours before the start of his game. The rules were modified to ensure continuous 30-minute play in each half, ie, no time-out, no substitution of players, and no suspension from playing was allowed.

Three blood samples were taken from the antecubital veins of each player: one at rest (15 to 20 minutes before playing), one during the 10-minute halftime (2 to 6 minutes after the end of the first half), and one 2 to 6 minutes after the end of the game. Then a biopsy of subcutaneous adipose tissue was taken by needle aspiration from the right buttock of 14 participants as previously described.[19] EDTA plasma was prepared from each blood sample and stored at −20°C along with the biopsies. Analyses were performed within 1 month.

Plasma lactate, glucose, and glycerol were determined by enzymatic spectrophotometric methods using reagent kits from Boehringer (Mannheim, Germany). Analysis of plasma NEFA and TG was initiated by the addition of appropriate amounts of heptadecanoic acid and triheptadecanoin (Sigma, St Louis, MO) as internal standards. Lipids were extracted as previously described[20] and separated by thin-layer chromatography on silica gel plates. The developer was petroleum ether:diethyl ether:acetic acid 87:13:1 (vol/vol/vol). Lipid spots were located under UV light after spraying with dichlorofluorescein in solution, and spots corresponding to NEFA and TG were excised and incubated in methanol:

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sulfuric acid 96:4 (vol/vol) at 60°C overnight. The methyl esters produced were separated by gas chromatography in a Hewlett-Packard 5890 Series II chromatograph (Waldbronn, Germany) equipped with a 30-m-long Carbowax capillary column from Alltech (Deerfield, IL). The column temperature was programed from 180° to 215°C at 4°C/min and from 215° to 260°C at 10°C/min. The carrier gas was helium at a flow rate of 1.5 mL/min. Methyl esters of the samples were quantified by comparing the area under their peaks in the chromatogram with that of methyl heptadecanoate. Similarly, adipose tissue lipids were extracted and separated by thin-layer chromatography, and the percent molar distribution of the TG acyl groups was determined by gas chromatography.

Values are expressed as the mean ± SEM. Statistical comparisons of means at the three time points were performed using ANOVA. Comparisons involving two-sample means or two estimates of regression coefficients were made by applying Student's *t* test. The level of significance (*P*) was set at .05.

RESULTS

Table 1 lists plasma concentrations of lactate, glucose, and glycerol before, after the first half, and after the end of a handball game. Lactate increased from a mean resting value of 1.78 to 3.75 mmol/L at halftime and declined to 2.54 mmol/L at the end. Likewise, glucose exhibited the lowest concentration at rest (4.20 mmol/L) and the highest at 30 minutes of play (5.92 mmol/L). Glycerol, on the other hand, had a progressive increase from 0.060 at rest to 0.148 mmol/L at the end (2.5-fold). Mean values for each of these parameters at the three time points were significantly different. Moreover, the change in lactate concentration during the first half was significantly different from the change during the second half. This was also the case for changes in glucose concentration. In contrast, changes in glycerol concentration during each half were not significantly different.

The effect of exercise on plasma NEFA levels is shown in Table 2. Being significantly different at the three time points of sampling, the concentrations of individual NEFA underwent successive increases during the game, resulting in an increase of the mean total concentration from 0.189 at rest to 0.623 mmol/L at 60 minutes (3.3-fold). The change during the first half (0.261 ± 0.043 mmol/L) was not significantly different from the change during the second half (0.173 ± 0.030).

The relative change in the mean concentration of individual NEFA throughout the game was not uniform. At one extreme was linoleate (18:2) with a 4.5-fold increase, and at the other, stearate (18:0) with a mere twofold increase. As a result, molar percentages of individual NEFA changed during the game (Table 2). These changes were significant for the four major plasma NEFA. Percentages of the saturated fatty acids palmitate (16:0) and 18:0 decreased gradually, whereas those of the unsaturated fatty acids oleate (18:1) and 18:2 increased. As a consequence, the mean ratios of unsaturated to saturated fatty acids (U/S) were significantly different at the three time points, increasing from 1.08 at rest to 1.61 at the end of the game.

Table 3 lists mean concentrations of the acyl groups of plasma TG. All concentrations decreased progressively, resulting in a decrease of the mean total TG concentration from 0.907 at rest to 0.708 mmol/L at 60 minutes. However, differences at the three time points did not reach statistical significance for any of the individual acyl groups or for the total TG. This lack of significance reflects the lack of uniform changes during the game. More precisely, although most (10) subjects exhibited the pattern imprinted in the mean values (ie, decrease during the first half and further decrease during the second half), all other possible patterns were also observed: decrease and then increase (five subjects), increase and then decrease (two subjects), and even sequential increases (two subjects). It should be noted that, with the exception of arachidonate (20:4), mean levels of each TG acyl group at rest and at the end of the game were significantly different. This was also the case for total TG concentrations.

In accordance with the absolute concentrations, the relative amounts of each TG acyl group were not significantly different at the three time points of sampling (Table 3). However, molar percentages of certain acyl groups underwent changes in the same direction during the two halves of the game, which resulted in significant differences between the resting and 60-minute values. These were, on one side, myristate (14:0) and 16:0, the percentages of which decreased, and, on the other side, palmitoleate (16:1), 18:1, and 20:4, the percentages of which increased. (The percentages of 18:2 showed sequential increases, too, but the mean preexercise and postexercise values were not significantly different.) Likewise, the mean U/S ratio increased slightly with exercise, and the ratio at 60 minutes was significantly different from that at rest.

In an attempt to correlate changes in the profile of plasma NEFA with the profile of their major source, ie, TG of adipose tissue, we obtained biopsies from 14 participants and determined the acyl-group composition of their TG. These data are listed in Table 4 along with the plasma NEFA distribution at rest, 30 minutes, and 60 minutes of exercise for these individuals. Approximately half the acyl groups in adipose tissue TG belonged to 18:1, with 18:2 and 16:0 competing for second place. Two observations are striking, considering the relationship between the composition of plasma NEFA and adipose tissue TG: First, at all three time points, mean percentages of each of the four

Table 1. Plasma Lactate, Glucose, and Glycerol Concentrations (mmol/L) at Rest, After 30 Minutes, and After 60 Minutes of Handball Playing, and Changes During Each Half (mean ± SEM, N = 19)

	Rest	30 Minutes	60 Minutes	ANOVA	30 Minutes − Rest	60 Minutes − 30 Minutes	*t* Test
Lactate	1.78 ± 0.11	3.75 ± 0.38	2.54 ± 0.28	$P < .001$	1.97 ± 0.40	−1.20 ± 0.39	$P < .001$
Glucose	4.20 ± 0.13	5.92 ± 0.33	5.27 ± 0.48	$P < .01$	1.72 ± 0.33	−0.65 ± 0.46	$P < .01$
Glycerol	0.060 ± 0.010	0.127 ± 0.016	0.148 ± 0.016	$P < .001$	0.067 ± 0.021	0.022 ± 0.021	NS

Table 2. Concentration and Percent Molar Distribution of Individual Plasma NEFA at Rest, After 30 Minutes, and After 60 Minutes of Handball Playing (mean ± SEM, N = 19)

Fatty Acid	Concentration (μmol/L)				Percent Molar Distribution			
	Rest	30 Minutes	60 Minutes	ANOVA	Rest	30 Minutes	60 Minutes	ANOVA
14:0	3.0 ± 0.6	8.0 ± 1.1	9.2 ± 1.4	$P < .001$	1.5 ± 0.3	1.8 ± 0.2	1.4 ± 0.2	NS
16:0	59.0 ± 4.3	126.8 ± 11.6	166.8 ± 15.6	$P < .001$	31.6 ± 0.8	28.9 ± 0.6	27.0 ± 0.5	$P < .001$
16:1	6.6 ± 1.0	15.2 ± 2.1	20.4 ± 2.1	$P < .001$	3.7 ± 0.5	3.4 ± 0.3	3.3 ± 0.1	NS
18:0	28.3 ± 2.2	48.1 ± 3.6	57.7 ± 3.5	$P < .001$	15.7 ± 1.0	11.7 ± 0.7	10.4 ± 0.8	$P < .001$
18:1	67.6 ± 6.6	181.5 ± 21.6	260.8 ± 26.3	$P < .001$	35.1 ± 1.1	39.3 ± 1.1	41.6 ± 0.8	$P < .001$
18:2	23.1 ± 3.1	66.9 ± 9.5	102.9 ± 14.2	$P < .001$	11.5 ± 0.8	14.2 ± 0.8	15.4 ± 1.0	$P < .01$
18:3	0.4 ± 0.3	0.6 ± 0.3	1.7 ± 0.5	$P < .05$	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	NS
20:4	1.0 ± 0.3	2.5 ± 0.4	3.4 ± 0.5	$P < .001$	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	NS
Total	189.2 ± 14.8	449.7 ± 46.0	623.0 ± 59.5	$P < .001$	100.0	100.0	100.0	
U/S	1.08 ± 0.06	1.40 ± 0.07	1.61 ± 0.07	$P < .001$				

plasma NEFA that underwent significant changes during exercise (16:0, 18:0, 18:1, and 18:2) are significantly different from the percentage of the same fatty acid in adipose tissue TG. The same holds true for the mean U/S ratios. Second, changes in the percentages of these NEFA in plasma are in the direction of the adipose tissue percentages, ie, decrease where the percentage in plasma at rest was higher than the percentage in adipose tissue (16:0 and 18:0), and increase where the percentage in plasma at rest was less than the percentage in adipose tissue (18:1 and 18:2). Accordingly, U/S ratios of plasma NEFA moved toward the U/S ratio of adipose tissue TG during the game. These trends are depicted schematically in Fig 1. The mean percent convergence of the plasma-NEFA to adipose-tissue percentage for 16:0, 18:0, 18:1, and 18:2 after 60 minutes of exercise was, respectively, 39, 54, 37, and 62. Likewise, the convergence in U/S ratios was 29%.

If the composition of adipose tissue TG determines the changes in the distribution of plasma NEFA during exercise (as the data of Table 4 imply), then the contribution of each fatty acid to the increase in the concentration of plasma NEFA should be proportional to its fraction in adipose tissue TG. To test this hypothesis, we examined the correlation between changes in the total concentration of plasma NEFA during the first or second half of the game and changes in the concentration of each NEFA during the same period. Significant correlations were found for the six major fatty acids (14:0, 16:0, 16:1, 18:0, 18:1, and 18:2), with correlation coefficients ranging from .62 ($P < .05$) to an impressive .98 ($P < .001$).

Linear regression showed that for each of these fatty acids, the regression coefficient (slope) related to the changes during the first half was not significantly different from the one related to the changes during the second half. This suggested, in statistical terms, that the two coefficients were estimates of a common slope or, in physiologic terms, that the dependence of the individual changes on the total changes was uniform during the two halves. This, in turn, allowed us to simplify the picture by pooling the data from the two halves for each fatty acid. Figure 2 presents the cumulative points and lines produced by linear regression, and Table 5 lists the characteristics of these correlations and lines. As with the data from the individual halves, the combined data showed significant correlations between changes in the total concentration of plasma NEFA and changes in the concentration of each of the six major NEFA, with correlation coefficients now ranging from .67 to .97.

None of the Y intercepts of the lines describing these correlations were significantly different from 0. However,

Table 3. Concentration and Percent Molar Distribution of Plasma TG Acyl Groups at Rest, After 30 Minutes and After 60 Minutes of Handball Playing (mean ± SEM, N = 19)

Fatty Acid	Concentration (μmol/L)				Percent Molar Distribution			
	Rest	30 Minutes	60 Minutes	ANOVA	Rest	30 Minutes	60 Minutes	ANOVA
14:0	86 ± 23	67 ± 15	53 ± 13*	NS	2.8 ± 0.4	2.5 ± 0.3	2.2 ± 0.3†	NS
16:0	882 ± 117	734 ± 84	660 ± 66†	NS	31.9 ± 0.6	31.3 ± 0.7	30.6 ± 0.7‡	NS
16:1	71 ± 11	62 ± 8	57 ± 7*	NS	2.5 ± 0.1	2.6 ± 0.1	2.7 ± 0.1*	NS
18:0	173 ± 26	151 ± 21	133 ± 18*	NS	6.1 ± 0.4	6.2 ± 0.4	6.1 ± 0.5	NS
18:1	1,035 ± 116	882 ± 77	835 ± 63*	NS	38.6 ± 0.8	38.7 ± 0.7	39.5 ± 0.8*	NS
18:2	433 ± 39	371 ± 25	351 ± 21*	NS	16.6 ± 0.9	17.1 ± 1.1	17.2 ± 1.1	NS
18:3	16 ± 3	13 ± 2	11 ± 2*	NS	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	NS
20:4	23 ± 3	22 ± 3	22 ± 2	NS	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1†	NS
Total	2,720 ± 318	2,302 ± 215	2,124 ± 169†	NS	100.0	100.0	100.0	
Total TG	907 ± 106	767 ± 72	708 ± 56†	NS				
U/S	1.48 ± 0.06	1.54 ± 0.07	1.60 ± 0.07‡	NS				

*$P < .05$, †$P < .01$, ‡$P < .001$: Significantly different from the value at rest.

Table 4. Percent Molar Distribution of Acyl Groups in Adipose Tissue TG of 14 Handball Players and Distribution of NEFA in Their Plasma at Rest, After 30 Minutes, and After 60 Minutes of Playing (mean ± SEM)

Fatty Acid	Adipose Tissue TG	Plasma NEFA		
		Rest	30 Minutes	60 Minutes
14:0	1.8 ± 0.4	1.4 ± 0.2	1.9 ± 0.3	1.5 ± 0.2
16:0	18.8 ± 1.5	32.4 ± 0.7*	29.2 ± 0.6*	27.4 ± 0.6*
16:1	3.7 ± 0.4	3.5 ± 0.4	3.6 ± 0.4	3.4 ± 0.1
18:0	4.9 ± 0.3	14.3 ± 0.9*	11.1 ± 0.8*	9.3 ± 0.7*
18:1	50.9 ± 1.9	35.7 ± 1.1*	39.3 ± 1.3*	41.2 ± 0.9*
18:2	19.3 ± 0.9	12.1 ± 0.9*	14.3 ± 0.9*	16.4 ± 1.1*
18:3	0.6 ± 0.1	0.2 ± 0.2	0.1 ± 0.1*	0.3 ± 0.1*
20:4	0	0.4 ± 0.2†	0.5 ± 0.1‡	0.5 ± 0.1*
U/S	3.18 ± 0.31	1.11 ± 0.06*	1.40 ± 0.08*	1.65 ± 0.08*

*$P < .001$, †$P < .05$, ‡$P < .01$: Significantly different from the adipose tissue value.

the most interesting information lay in the slopes: Comparison to the fractions of the same fatty acids in adipose tissue TG showed that most differences were not significant, with two remarkable exceptions, 16:0 and 18:1. The slope for 16:0 was higher than its fraction in adipose tissue TG, whereas the slope for 18:1 was lower than its fraction in adipose tissue TG. It is noteworthy that these two fatty acids had the highest correlation coefficients. The sum of the slopes for the six fatty acids was 1.000, very close to the sum of their fractions in adipose tissue TG (0.994).

DISCUSSION

Utilization of blood-borne NEFA as a substrate for aerobic catabolism in working muscles plays an important role during prolonged exercise of moderate intensity. Although an increase in the concentration of NEFA in blood during prolonged efforts (as a result of augmented lipolysis in adipose tissue) is well documented, little is known about the effect of exercise on the percent distribution of plasma NEFA. Plasma TG, on the other hand, are considered to contribute little to energy provision during physical activity.[1] It is probably because of this that acute changes in TG concentration have received less attention than the long-term effects of exercise.

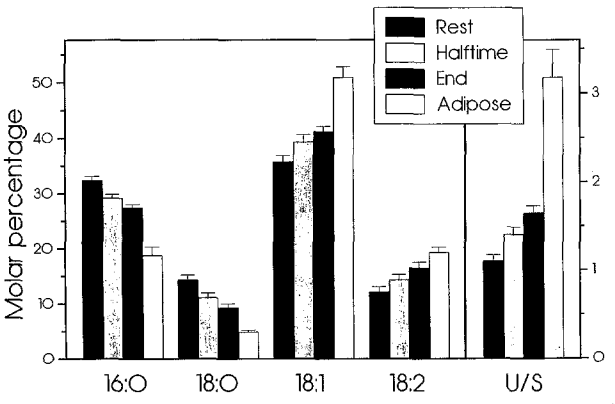

Fig 1. Mean molar percentages of the four major fatty acids (16:0, 18:0, 18:1, and 18:2) and the U/S ratio in plasma NEFA of 14 handball players at rest, at halftime, and at the end of a game, as well as in the acyl groups of their adipose tissue TG. Error bars represent the SEM. All rest, halftime, and end values are significantly different from adipose tissue values ($P < .001$).

We have examined a number of biochemical changes, with an emphasis on aspects of lipid metabolism, during exercise of variable intensity (handball playing). The two indices of carbohydrate utilization examined, ie, plasma lactate and glucose concentrations, showed similar changes during the game: increase at 30 minutes of exercise and decline to values above resting levels at 60 minutes (Table 1). Lactate values were less than 4 mmol/L, which is considered to mark the anaerobic threshold,[21] pointing to a predominance of aerobic metabolism in muscle. The lower lactate concentration after 60 minutes of play as compared with the 30-minute value indicates reduced utilization of carbohydrates during the second half. Taking this into account, the glucose values suggest that output from the liver was reduced during the second half.

Plasma glycerol and all NEFA, on the other hand, exhibited a different pattern, ie, sequential increases at the two halves of the game (Tables 1 and 2). This is typical of prolonged efforts[1,2] and indicates a continuous stimulation of lipolysis throughout the game.

Physical activity caused significant changes in the relative amounts of plasma NEFA (Table 2). Our data afford a clear distinction between the major saturated fatty acids 16:0 and 18:0, whose percentages decreased, and the major unsaturated fatty acids 18:1 and 18:2, whose percentages increased. We have been able to trace only two relevant reports in the literature. Horstman et al[3] found a decrease in the percentage of 18:1 and an increase in the percentage of 18:2 with heavy exercise, which caused the total plasma NEFA concentration to decrease, and the opposite effect with moderate exercise, which caused the NEFA concentration to increase. Vihko et al[4] reported decreased percentages of 16:0, 18:0, and 18:2 and increased percentages of 16:1 and 18:1 after intermittent exercise of variable intensity, which elevated the total plasma NEFA concentration. The consensus of these results and our own is that, with the exception of 18:2, the relative amounts of saturated plasma NEFA decrease and those of unsaturated NEFA increase when exercise conditions favor an increase in the total plasma NEFA concentration. A possible explanation for the discrepancy regarding 18:2 will be presented later.

The response of plasma TG concentration to exercise was not as clear as that of NEFA (Table 3). Although the predominant change was a decrease, interestingly enough, nine of 19 athletes exhibited increases in total TG concentration during one or both halves of the game. Several other studies have found an exercise-induced increase in blood TG concentration,[10-18] although the methodology used in a number of them casts doubt on the validity of the relevant data. As already pointed out,[14] the usual enzymatic assays for TG measure the level of glycerol produced after total TG hydrolysis. Since prolonged exercise elevates blood glycerol concentration, TG values will be falsely elevated unless the glycerol naturally present in a sample is determined, as well. Correction for free glycerol has indeed been

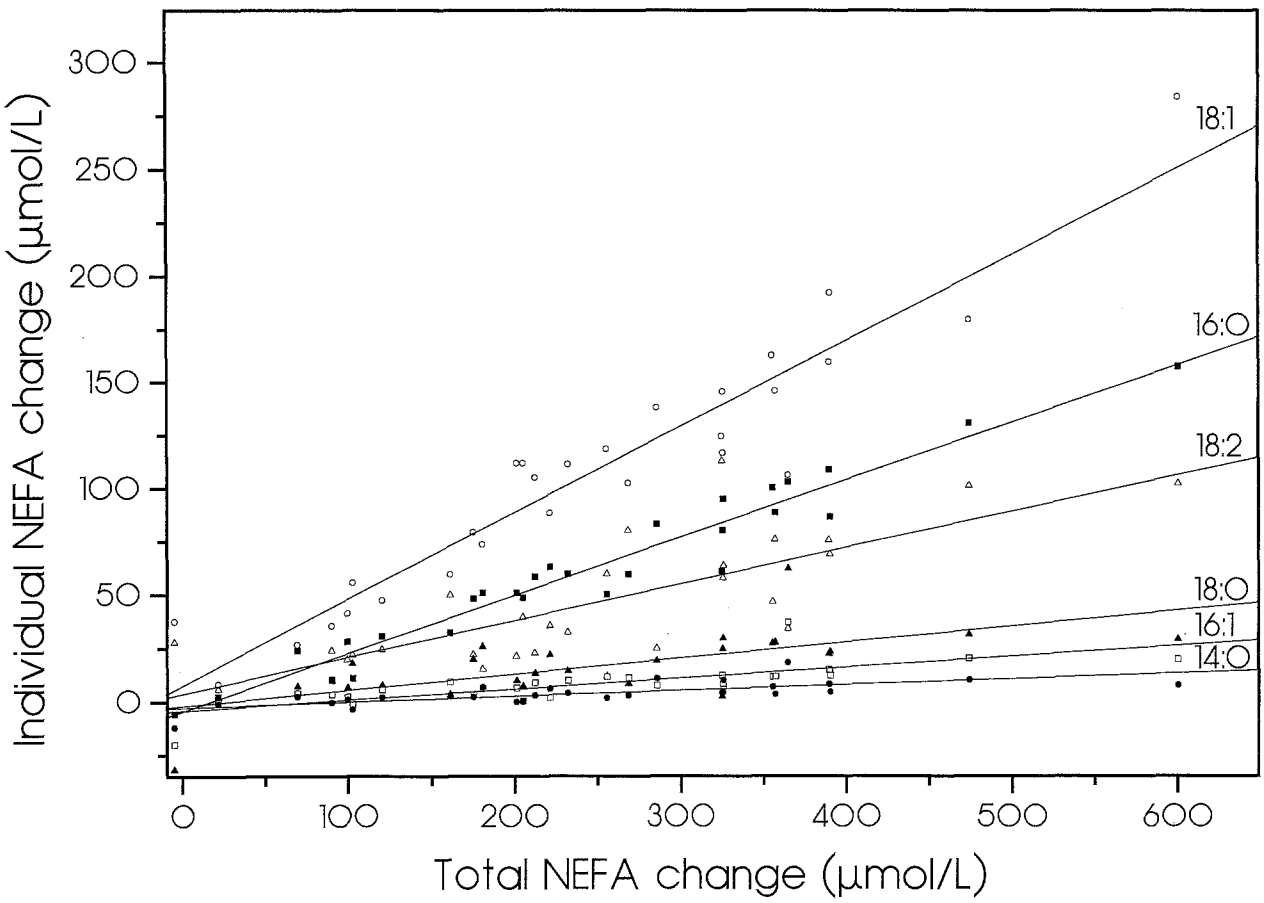

Fig 2. Changes in the concentration of 14:0 (●), 16:0 (■), 16:1 (□), 18:0 (▲), 18:1 (○), and 18:2 (△) in the plasma of 14 handball players during each half of a game (pooled data, N = 28) as a function of corresponding changes in the total concentration of plasma NEFA.

made in a few of the studies cited above,[10,12] whereas the increases in TG concentration seen in some other studies[11,13,14,16] are probably too great to be exclusively due to increased glycerol production.

Researchers who observed an elevation of plasma TG with exercise have offered the explanation of increased synthesis by the liver as a result of increased secretion of NEFA from adipose tissue into the bloodstream,[10,11,17] a hypothesis proposed by others, too.[22] Apart from the fact that evidence for an exercise-induced stimulation of TG synthesis in the liver is lacking, it should be noted that available data show a dramatic reduction in liver uptake of NEFA during exercise as a result of decreased hepatic blood flow and decreased NEFA concentration in arterial blood.[23] Moreover, exercise reduces the potential of the liver to synthesize fatty acids,[22] due to inactivation of key enzymes by the decrease in the ratio of insulin to glucagon. Therefore, it seems improbable that the acute increase in plasma TG concentration found in some exercising individuals in the present study and in the studies mentioned earlier is due to a stimulation of TG synthesis in the liver. Instead, we favor the hypothesis of augmented release of already synthesized TG. This has been already proposed by Pilardeau et al,[16] who found a significant increase after 7 minutes of heavy exercise, a time they considered too short to warrant enhanced synthesis.

We believe we are faced with the challenging possibility of an as-yet-unidentified process in the liver that is stimulated during exercise and supplements the well-established release of glucose with lipid fuel in the form of TG. We are currently investigating the precise conditions of activation of the process and the reason for the considerable interindividual variation observed in this and other studies.[9,18] For the moment, there appears to be a competition between increased output by the liver and increased hydrolysis by lipoprotein lipase, with the latter factor winning in the majority of cases.

Changes in the distribution of acyl groups in plasma TG (Table 3) were not as large as those in plasma NEFA, reaching statistical significance only after the end of the game. Had the changes in the concentration of TG been more uniform, we might have seen clearer changes in the percentages of acyl groups. At present, it is interesting to note the similarity between NEFA and TG: in both, there was a decrease in the percentages of saturated fatty acids and an increase in the percentages of unsaturated fatty acids.

The changes we observed in the profile of plasma NEFA

during exercise have to be explained by the fact that either NEFA release into the bloodstream or NEFA uptake into the tissues or both were at proportions different from the ones at rest. As for the first possibility, the predominant source of plasma NEFA are the TG of adipose tissue. A further contribution is made by fatty acids, which are not removed immediately from the capillaries once they are produced through hydrolysis of circulating TG by lipoprotein lipase.[24] The latter source is unlikely to have caused the changes in NEFA distribution because, in that case, the changes in acyl-group distribution of the remaining plasma TG could not have been in the same direction, ie, downward for 16:0 and 18:0 and upward for 18:1 and 18:2. However, this is exactly what happened: as shown in Table 3 and as mentioned earlier, the percentage of 16:0 in plasma TG decreased, whereas that of 18:1 increased, with the changes being significant after 60 minutes of exercise.

Our data favor the hypothesis that the major determinant of changes in the distribution of plasma NEFA observed with exercise is the acyl-group composition of adipose tissue TG. This is supported by two lines of evidence: First, the significant changes in the percentages of individual NEFA, ie, 16:0, 18:0, 18:1, and 18:2, are in the direction of the corresponding percentages in adipose tissue TG (Table 4 and Fig 1), confirming an older proposal.[3] It is evident that the composition of plasma NEFA changes during exercise because of the increased mobilization of adipose tissue TG, which have a different acyl-group composition. The difference between the profiles of adipose tissue TG and plasma NEFA at rest is remarkable, and only part of it is bridged after 60 minutes of exercise. This leaves open the possibility of more dramatic changes with efforts of longer duration.

The finding that percentages of the major plasma NEFA move toward the corresponding percentages in adipose tissue TG during exercise may explain the discrepancy between our results and those of the investigators who found a decrease in the percentage of 18:2 with exercise.[3,4] The composition of adipose tissue was not determined in those studies, but it is possible that, due to metabolic and/or dietary particularities of the study groups, the percentage of 18:2 in plasma NEFA at rest was higher than that in adipose tissue TG. It is probably no coincidence that this disagreement arose with regard to an essential fatty acid, the concentrations of which in tissues depend on dietary intake more than the nonessential fatty acids.

The second line of evidence for the dependence of changes in the distribution of plasma NEFA on the composition of adipose tissue TG comes from the direct relationship (established in Fig 2 and Table 5) between changes in the total concentration of plasma NEFA and changes in the concentration of each of the six major NEFA that comprise over 99% of the total. The regression coefficients for 14:0, 16:1, 18:0, and 18:2 were not significantly different from their fractions in adipose tissue TG. The simplest conclusion that can be drawn from this is that the rates of release of these fatty acids are proportional to their percentages in adipose tissue TG. Alternatively, the rates could be different but counterbalanced by rates of uptake from plasma equal to the corresponding rates of release.

While attesting to the prevalence of adipose tissue composition in determining changes in the profile of plasma NEFA during exercise, the results listed in Table 5 raise the question as to what determines the contribution of the two most abundant fatty acids, 16:0 and 18:1, to the increase of plasma NEFA. Since the regression coefficient between changes in the total concentration of plasma NEFA and changes in the concentration of 16:0 (.271) is higher than its fraction in adipose tissue TG (0.188), one has to accept that its rate of release is higher than its rate of uptake, owing to either a preferential release from adipose tissue or a diminished uptake from the other tissues (or both). Conversely, since the regression coefficient between changes in the total concentration of plasma NEFA and changes in the concentration of 18:1 (.405) is lower than its fraction in adipose tissue TG (0.509), one has to assume that its rate of release is lower than its rate of uptake, due to either a diminished release or a preferential uptake (or both). Our data do not allow a distinction between the two possibilities for 16:0 and 18:1, and the available literature is confusing. Evidence has been presented for preferential mobilization of both fatty acids from adipose tissue,[25] preferential mobilization of 18:1 and retention of 16:0,[26] equal or not significantly different fractional uptakes of the two fatty acids by skeletal muscle and liver at rest,[27] as well as by the liver during exercise,[24] and preferential uptake of 18:1 as compared with 16:0 by the exercising muscles.[28] Of all these reports, only the latter accommodates our findings. We therefore tend to favor the differential uptake of 16:0 and 18:1 by the exercising muscles over the differential release from adipose tissue as the reason for the observed differences between their fractions in adipose tissue TG and their contribution to the increase of plasma NEFA.

It is noteworthy that the percentage of 16:0 in plasma

Table 5. Linear Regression (y = a + bx) Between Changes in Total Concentration of Plasma NEFA (x) and Changes in Concentration of Individual NEFA (y) During Each Half of a Handball Game for 14 Players Who Provided Adipose Tissue Specimens (N = 28)

Fatty Acid	Correlation Coefficient	Significance (P)	a (μmol/L)	Standard Error	t Test (a = 0)	b	Standard Error	Fraction in Adipose Tissue TG (f)*	t Test (b = f)
14:0	.70	<.001	−2.7	1.5	NS	0.027	0.005	0.018	NS
16:0	.97	<.001	−4.5	3.5	NS	0.271	0.012	0.188	$P < .001$
16:1	.75	<.001	−4.1	2.5	NS	0.051	0.009	0.037	NS
18:0	.67	<.001	−1.9	4.6	NS	0.075	0.016	0.049	NS
18:1	.95	<.001	7.6	7.1	NS	0.405	0.026	0.509	$P < .001$
18:2	.80	<.001	3.8	7.1	NS	0.171	0.025	0.193	NS

*Data from Table 4.

NEFA decreased during exercise (Table 4) despite the fact that its contribution to the increase in the concentration of plasma NEFA (27%, derived from b of Table 5) was higher than its percentage in adipose tissue TG (19%, Table 4). The reason is that its percentage in plasma NEFA at rest was even higher (32%, Table 4). Conversely, the percentage of 18:1 in plasma NEFA increased during exercise, although its contribution to the increase in the concentration of plasma NEFA (40%) was less than its percentage in adipose tissue TG (51%), the reason being again that its percentage in plasma NEFA at rest was even lower (36%). This, then, poses a more fundamental question: Why is there a difference between the profiles of plasma NEFA at rest and adipose tissue TG? Our research was not designed to answer this question, but our findings about the preferential release or diminished uptake of 16:0 and the diminished release or preferential uptake of 18:1 during exercise might give a partial answer. Of course, they will have to be extended to the resting state and to the other fatty acids (18:0 and 18:2) that exhibit significant differences between their percentages in plasma NEFA and adipose tissue TG.

Whereas the change in the profile of plasma NEFA with exercise can be explained by an increased mobilization from adipose tissue, no explanation is readily available for the small but significant change in the profile of plasma TG. It is possible that lipoprotein lipase, which is activated during exercise,[1] shows some selectivity for saturated acyl groups. Alternatively, the process of increased TG output from the liver we proposed earlier might discriminate for TG enriched in unsaturated acyl groups.

The increase in the proportion of unsaturated to saturated NEFA (by almost 50% after 1 hour of exercise, Table 2) and TG (by 8%, Table 3) may have important physiologic repercussions. Aerobic exercise is known to increase high-density lipoprotein cholesterol and decrease low-density lipoprotein cholesterol in plasma,[5,6] with both effects reducing the risk of cardiovascular disease. On the other hand, dietary intake of fat has been linked to the incidence of coronary heart disease through modification of levels of cholesterol and cholesterol-carrying lipoproteins in blood. Low-density lipoprotein production has been directly related to intake of saturated fatty acids, whereas low-density lipoprotein catabolism has been directly related to intake of monounsaturated fatty acids.[29] Polyunsaturated fatty acids have also been implicated, since an increased intake decreased plasma cholesterol.[30,31] It is reasonable to assume that the effect of each of these categories of fatty acids is determined by its proportion relative to the other fatty acids in blood either directly or through uptake by tissues involved in lipoprotein metabolism. In this sense, it is impressive that the shifts in the distribution of plasma NEFA and TG caused by exercise (downward for saturated and upward for monounsaturated and polyunsaturated fatty acids) are totally consistent with a protective role against atherosclerosis. We believe that our results can establish a link between diet and exercise in the prevention of cardiovascular disease. It remains to be seen how long after cessation of exercise these changes last.

In conclusion, the main findings of our research are as follows: (1) Aerobic exercise of variable intensity for a total of 1 hour caused decreases in the percentages of the major saturated plasma NEFA, 16:0 and 18:0, and increases in the percentages of the major unsaturated NEFA, 18:1 and 18:2, such that the U/S ratio increased by almost 50%; (2) Changes in the profile of plasma TG were in the same direction, but not as impressive. The increase in the total concentration of plasma TG observed in many individuals suggests the existence of a pathway for elevated TG output by the liver in response to an exercise-associated stimulus, which competes with increased TG clearance from the bloodstream; (3) The percentages of 16:0, 18:0, 18:1, and 18:2 in plasma NEFA tend toward the percentages in adipose tissue TG as exercise progresses; (4) It appears that the rate of release of 16:0 from adipose tissue is higher than the rate of uptake from plasma, and the reverse is probably true for 18:1; and (5) The increase in the U/S ratio of plasma NEFA and TG may be part of a common mechanism underlying the beneficial effects of aerobic exercise and a diet rich in unsaturated fatty acids on the profile of plasma lipoproteins and the risk of cardiovascular disease.

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