Effect of exercise on the proportion of unsaturated fatty acids in serum of untrained middle aged individuals

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Abstract

Objectives—To examine whether prolonged moderate exercising by untrained middle aged individuals of both sexes affects the plasma ratio of unsaturated to saturated (U/S) fatty acids in the non-esterified fatty acid (NEFA) and triacylglycerol (TG) fractions.

Methods—Twenty two healthy untrained volunteers (eleven men and eleven premenopausal women), aged 35–55, exercised on bicycles at 50–55% maximal heart rate reserve (estimated 61% maximal oxygen uptake) for one hour. Pre- and post-exercise blood samples were analysed for lactate, glucose, glycerol, individual NEFAs and TG acyl groups, cholesterol, high density lipoprotein cholesterol, urea, cortisol, and testosterone. Biopsy specimens of adipose tissue were analysed for TG acyl group composition.

Results—Serum total NEFAs increased significantly whereas total TG decreased significantly in both sexes. Changes in individual fatty acids, in both the NEFA and TG fractions, generally paralleled changes in the total pool but were not proportionate. As a result, U/S NEFAs increased in both sexes, although significantly only in women. The shift was in the direction of the composition of the major source of blood NEFAs—that is, adipose tissue TG. U/S acyl groups of TG also increased in both sexes, although significantly only in men.

Conclusions—Prolonged moderate exercise increases the U/S ratio of serum NEFAs and TG. This may add to the beneficial effects of exercise given the protective role of unsaturated fatty acids against coronary heart disease.

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Keywords: exercise; middle age; untrained individuals; unsaturated fatty acids; triacylglycerols

We have recently reported that, in adolescent male athletes, prolonged exercise of variable intensity caused significant acute increases in the plasma ratio of unsaturated to saturated (U/S) fatty acids in both the non-esterified fatty acid (NEFA) and triacylglycerol (TG) fractions.¹ This finding may have interesting implications for health since unsaturated fatty acids are known to protect against coronary heart disease.^{2,3}

In the present study we examine whether similar changes accompany prolonged moderate exercising by individuals of both sexes who are older and physically less active and therefore at a higher risk for coronary heart disease than young athletes. In addition to the U/S ratio, other biochemical parameters were measured for a more complete evaluation of the exercise protocol employed. Our results indicate that exercise can indeed increase the U/S ratio in both the serum NEFA and TG fractions of untrained middle aged men and women.

Subjects and methods

Subjects were eleven men and eleven women who volunteered to participate in a project studying the effect of exercise on lipid metabolism. Inclusion criteria were: (a) age 35–55 years and (b) a low level of physical activity (no more than 2 h of exercise or 5000 kJ per week). Exclusion criteria were: (a) menopause, (b) apparent acute or chronic illness, (c) body mass above 140% of desirable mass (according to the 1985 NIH Consensus Panel Weight Table⁴), (d) smoking, (e) alcohol consumption above two drinks per day, and (f) use of medication that might affect lipid metabolism, including oral contraceptives. The study was designed and carried out according to the guidelines of the university ethics committee.

At their preliminary visit to the laboratory, volunteers gave informed consent and filled in a health history questionnaire. Their body mass and height were measured and their percentage body fat was estimated from skinfold thickness measurements.⁵ The subjects then underwent physical examination to exclude cardiovascular disease and completed a graded maximal treadmill test⁶ during which oxygen uptake relative to body mass ($\dot{V}O_2$) was monitored by a Cosmed K2 portable telemetry system (Cosmed, Rome, Italy). Finally, they were asked to record their dietary intake for three days including one weekend day. The dietary records were analysed for total energy, carbohydrate, fat, protein, and alcohol intake by using the Foodtabs (Sanders, London, United Kingdom) computer software.

Subjects came to the laboratory for the main test on a subsequent day, after an overnight fast, and gave a blood sample from an antecubital vein while in a sitting position. Subsequently they were asked to exercise on a cycle ergometer at gradually increasing velocity, reaching 50–55% of their maximal heart rate (HR) reserve (defined as the difference

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Table 1 Characteristics of participants (mean (SEM))

	Men (n=11)	Women (n=11)
Age (years)	41.9 (1.6)	44.5 (1.1)
Body mass (kg)	81.4 (2.9)	72.8 (4.4)
Height (m)	1.78 (0.02)	1.62 (0.02)*
Percent desirable body mass	112 (3)	121 (7)
Body mass index (kg/m ²)	25.7 (0.7)	27.7 (1.7)
Percent body fat	21.1 (1.1)	33.1 (1.1)*
Resting HR (beats/min)	66 (3)	73 (2)
Maximal HR (beats/min)	183 (2)	176 (2)
$\dot{V}O_{2\text{MAX}}$ (ml/kg/min)	40.2 (1.3)	31.9 (2.0)**
Energy intake (kJ/day)	7568 (901)	5673 (450)
Percent carbohydrate	39.3 (3.6)	42.8 (2.8)
Percent fat	40.2 (2.3)	38.5 (1.8)
Percent protein	18.3 (1.8)	16.7 (1.5)
Percent alcohol	2.1 (0.9)	2.0 (0.7)

* $p < 0.001$, ** $p < 0.01$: significantly different from the value for men.

Table 2 Characteristics of the exercise protocol (mean (SEM))

	Men	Women
Duration (min)	60	60
Average HR (beats/min) ^a	128 (2)	129 (1)
Percent maximal HR reserve	53 (0)	54 (1)
Percent maximal HR	70 (1)	73 (1)*
Estimated $\dot{V}O_2$ (ml/kg/min)	24 (1)	19 (1)**
Percent $\dot{V}O_{2\text{MAX}}$	61 (1)	61 (3)
Net energy cost (kJ/kg)	23 (2)	18 (1)*
Net energy cost (MJ)	1.88 (0.11)	1.34 (0.12)**

^aThe initial time during which subjects reached the target heart rate (HR) is not included in the averaging of HR but is included in the estimation of energy cost.

* $p < 0.05$, ** $p < 0.01$: significantly different from the value for men.

between the maximal HR exhibited at the treadmill test and the resting HR) above resting HR within five minutes and to remain within that range for 55 minutes more. HR was monitored continuously by a Polar Accurex monitor (Polar Electro, Kempele, Finland). Blood was withdrawn as above within two minutes of termination of the test.

An aliquot of each pre- and post-exercise blood sample was mixed with heparin solution to measure packed cell volume and haemoglobin, while another aliquot was precipitated with perchloric acid (0.3 mol/l) for lactate determination in the supernatant. The remaining blood was used to prepare serum for all other assays. Finally, a biopsy sample of subcutaneous adipose tissue was taken from the right buttock of each participant by needle aspiration.⁷

Packed cell volume and haemoglobin were measured in an electronic counter to estimate changes in plasma volume. Whole blood lactate and serum glucose, glycerol, total cholesterol,

high density lipoprotein (HDL) cholesterol, and urea were assayed by enzymic spectrophotometric methods using reagent kits from Boehringer (Mannheim, Germany). Individual serum NEFAs and TG acyl groups, as well as the acyl group composition of adipose tissue TG, were determined by a combination of thin layer chromatography and capillary gas chromatography as described.¹ Total serum NEFAs were calculated as the sum of individual NEFAs, and total serum TG as the sum of individual TG acyl groups divided by three. All post-exercise serum cholesterol and TG values were corrected for changes in plasma volume as described.⁸ Cortisol and testosterone were measured by enzyme immunoassays with kits from CloneSystems (Bologna, Italy).

Values are expressed as the mean (SEM). Statistical comparisons were performed by applying appropriate Student's *t* tests. Correlation between variables was examined by using Pearson's correlation coefficient. The level of significance (*p*) was set at 0.05.

Results

Table 1 gives the characteristics of the participants. Most characteristics, including age, body mass, percentage of desirable body mass, body mass index, resting HR, maximal HR, energy intake, and its distribution among the energy-providing nutrients were not significantly different between the sexes. On the other hand, height, percentage body fat, and maximal oxygen uptake relative to body mass ($\dot{V}O_{2\text{MAX}}$) were significantly different between the sexes.

Men and women maintained similar average HR and percentages of maximal HR reserve during exercise (table 2). The cumulative mean values of the two parameters were 128 beats/minute and 53% respectively. Based on the average HR during exercise and the relationship between HR and $\dot{V}O_2$ established during the maximal treadmill test, we estimated that exercise was performed at 61% $\dot{V}O_{2\text{MAX}}$. We also calculated the net energy cost of exercise by subtracting the resting $\dot{V}O_2$ from the exercise $\dot{V}O_2$ and taking the energy equivalent of oxygen to be about 20 kJ/litre. Both $\dot{V}O_2$ and net energy cost were significantly higher in men than in women.

Table 3 lists the pre- and post-exercise serum values of the biochemical parameters determined. Lactate concentration increased slightly

Table 3 Pre- and post-exercise values of biochemical parameters of participants (mean (SEM))

	Men		Women	
	Pre	Post	Pre	Post
Lactate (mmol/l)	1.17 (0.10)	1.83 (0.20)*	1.28 (0.08)	1.69 (0.18)**
Glucose (mmol/l)	5.47 (0.20)	4.73 (0.25)***	5.16 (0.15)	4.97 (0.17)**
Glycerol (mmol/l)	0.099 (0.011)	0.157 (0.033)**	0.097 (0.014)	0.164 (0.023)*
Total NEFA (mmol/l)	0.424 (0.032)	0.530 (0.071)**	0.458 (0.034)	0.670 (0.066)*
Total TG (mmol/l)	1.07 (0.11)	0.97 (0.09)**	0.78 (0.09)	0.67 (0.07)**
Total cholesterol (mmol/l)	5.72 (0.30)	5.47 (0.30)**	5.73 (0.20)	5.66 (0.20)
HDL-cholesterol (mmol/l)	1.09 (0.09)	1.07 (0.09)	1.43 (0.09)	1.52 (0.10)
Total/HDL-cholesterol	5.60 (0.48)	5.50 (0.50)	4.18 (0.32)	3.88 (0.29)
Urea (mmol/l)	6.34 (0.37)	6.30 (0.38)	5.91 (0.39)	5.79 (0.39)
Cortisol (nmol/l)	292 (31)	317 (35)	280 (33)	284 (35)
Testosterone (nmol/l)	22.03 (2.26)	25.62 (2.68)***	2.14 (0.36)	2.36 (0.33)

NEFA, non-esterified fatty acids; TG, triacylglycerol; HDL, high density lipoprotein.

* $p < 0.01$, ** $p < 0.05$, *** $p < 0.001$: significantly different from the pre-exercise value.

Table 4 Pre- and post-exercise serum concentration and percentage molar distribution of non-esterified fatty acids (mean (SEM))

Fatty acid	Men		Women	
	Pre	Post	Pre	Post
Concentration (μmol/l)				
14:0	9.2 (1.2)	10.8 (2.1)	8.2 (1.1)	12.1 (1.2)*
16:0	123.2 (12.4)	151.4 (23.2)	128.8 (8.0)	184.1 (15.3)*
16:1	10.8 (1.6)	14.5 (3.1)	11.0 (1.0)	18.8 (2.1)*
18:0	53.1 (4.0)	54.9 (5.4)	63.7 (4.6)	69.7 (6.4)
18:1	165.1 (11.7)	215.6 (29.1)**	176.9 (14.3)	274.8 (27.5)***
18:2	56.7 (5.1)	75.0 (11.3)	63.4 (10.6)	102.6 (17.7)*
18:3	0.3 (0.2)	1.1 (0.5)	0.6 (0.3)	1.6 (0.8)
20:4	5.3 (0.4)	6.5 (0.6)	5.5 (0.6)	6.6 (0.7)
Total	423.7 (32.3)	529.9 (70.5)**	458.1 (34.4)	670.2 (65.9)***
Percentage molar distribution				
14:0	2.12 (0.12)	1.97 (0.14)	1.84 (0.26)	1.85 (0.12)
16:0	28.70 (0.86)	28.50 (0.97)	28.49 (0.88)	27.97 (0.64)
16:1	2.48 (0.22)	2.58 (0.18)	2.40 (0.10)	2.79 (0.12)*
18:0	12.66 (0.55)	10.96 (0.63)**	14.19 (0.91)	10.66 (0.58)*
18:1	39.27 (1.07)	40.59 (1.46)	38.40 (1.08)	40.76 (1.29)**
18:2	13.38 (0.85)	13.84 (1.06)	13.32 (1.07)	14.77 (1.07)*
18:3	0.07 (0.05)	0.21 (0.10)	0.10 (0.06)	0.21 (0.07)**
20:4	1.33 (0.14)	1.36 (0.15)	1.25 (0.15)	1.00 (0.05)
U/S	1.305 (0.033)	1.436 (0.074)	1.265 (0.064)	1.489 (0.065)***

U/S, unsaturated/saturated.

*p<0.001, **p<0.05, ***p<0.01: significantly different from the pre-exercise value.

although significantly with exercise (by 0.66 mmol/l in men and 0.41 mmol/l in women), whereas glucose decreased significantly (by 14% in men and 4% in women). The decrease in glucose was significantly greater in men than in women (p<0.001). Glycerol increased significantly (by 59% in men and 69% in women) and so did the total NEFA concentration (by 25% in men and 46% in women). Total TG decreased significantly (by 9% in men and 14% in women). Total cholesterol decreased significantly in men only (by 4.4%) whereas HDL-cholesterol did not change significantly in either sex. The ratio of total to HDL-cholesterol tended to decrease but changes were not significant. Urea did not change significantly in either sex neither did cortisol. Testosterone increased significantly in men only (by 16%).

Table 4 (upper half) contains the pre- and post-exercise concentrations of the eight NEFAs detected in the participants' serum,

i.e. myristate (14:0; carbon atoms:double bonds), palmitate (16:0), palmitoleate (16:1), stearate (18:0), oleate (18:1), linoleate (18:2), linolenate (18:3), and arachidonate (20:4). The only significant change observed in men was an increase in 18:1. Women, on the other hand, exhibited significant increases in five NEFAs (14:0, 16:0, 16:1, 18:1, and 18:2). To facilitate comparisons, changes in these and the following parameters are depicted schematically in fig 1.

From the concentrations of the individual NEFAs, we calculated their percentage molar distribution (Table 4, lower half). In men, the only significant change was a decrease in 18:0. Women exhibited a higher number of significant changes: the percentages of four NEFAs (16:1, 18:1, 18:2, and 18:3) increased, whereas the percentage of 18:0 decreased. As a result of the opposite changes in the relative amounts of unsaturated and saturated NEFAs in women, their ratio increased

Table 5 Pre- and post-exercise serum concentration and percentage molar distribution of triacylglycerol (TG) acyl groups (mean (SEM))

Fatty acid	Men		Women	
	Pre	Post	Pre	Post
Concentration(μmol/l)				
14:0	68 (7)	55 (6)*	51 (9)	36 (7)
16:0	875 (105)	766 (77)**	680 (85)	563 (70)**
16:1	103 (17)	90 (13)**	80 (12)	63 (9)
18:0	118 (15)	104 (9)	102 (10)	86 (8)**
18:1	1377 (157)	1262 (128)	985 (121)	866 (94)**
18:2	610 (68)	582 (66)	397 (64)	353 (62)**
18:3	19 (4)	16 (3)	7 (2)	6 (2)
20:4	33 (4)	33 (4)	26 (4)	27 (4)
Total	3203 (334)	2908 (265)**	2328 (265)	2000 (218)**
Total TG	1068 (111)	969 (88)**	776 (88)	667 (73)**
Percentage molar distribution				
14:0	2.14 (0.12)	1.92 (0.09)**	2.20 (0.30)	1.76 (0.21)
16:0	27.13 (1.35)	26.40 (1.25)	29.28 (1.23)	28.26 (1.35)
16:1	3.15 (0.30)	3.10 (0.28)	3.35 (0.18)	3.10 (0.12)
18:0	3.76 (0.28)	3.65 (0.23)	4.58 (0.38)	4.54 (0.40)
18:1	43.03 (1.67)	43.40 (1.51)	42.29 (1.58)	43.70 (1.67)
18:2	19.17 (1.36)	19.88 (1.37)*	16.89 (1.86)	17.09 (1.85)
18:3	0.57 (0.08)	0.51 (0.08)	0.29 (0.09)	0.25 (0.08)
20:4	1.04 (0.11)	1.11 (0.10)	1.12 (0.12)	1.30 (0.13)
U/S	2.096 (0.150)	2.196 (0.155)**	1.848 (0.159)	1.979 (0.174)

U/S, unsaturated/saturated.

*p<0.01, **p<0.05: significantly different from the pre-exercise value.

Figure 1 Changes in concentration of individual serum non-esterified fatty acids (NEFAs) and triacylglycerol (TG) acyl groups, as well as in the unsaturated/saturated (U/S) ratio in men and women. Asterisks indicate significant changes.

significantly (by 18%, Table 4, lower half). There was also a 10% increase in U/S NEFAs in men which just misses statistical significance ($p = 0.06$).

Table 5 presents the pre- and post-exercise serum concentrations and percentage molar distributions of TG acyl groups. Significant decreases in the concentrations of three or four of the eight fatty acids were found in both sexes. The most consistent change was a decrease in 16:0. As far as the percentage molar distribution of TG acyl groups is concerned, the only significant changes were a decrease in 14:0 and an increase in 18:2 in men. Finally, U/S TG acyl groups increased in both sexes (by 5–7%), but only the change in men reached statistical significance.

The shift toward unsaturated NEFAs observed in men and women with exercise was in the direction of the composition of the major source of blood NEFAs—that is, adipose tissue TG. The U/S ratio of the latter (table 6) was significantly higher than any U/S ratio in table 4 ($p < 0.001$). Moreover, changes in U/S NEFAs correlated positively with changes in

Table 6 Percentage molar distribution of acyl groups in adipose tissue triacylglycerol (mean (SEM))

Fatty acid	Men	Women
14:0	2.15 (0.17)	2.00 (0.11)
16:0	22.23 (0.57)	20.92 (0.57)
16:1	4.38 (0.33)	5.51 (0.35)*
18:0	4.18 (0.23)	3.28 (0.20)**
18:1	50.94 (0.78)	51.93 (1.01)
18:2	15.34 (0.56)	15.65 (0.82)
18:3	0.38 (0.02)	0.36 (0.03)
20:4	0.40 (0.05)	0.35 (0.05)
U/S	2.531 (0.100)	2.849 (0.118)

U/S, unsaturated/saturated.

* $p < 0.05$, ** $p < 0.01$: significantly different from the value for men.

total NEFA concentration (correlation coefficient 0.65, $p = 0.001$, $n = 22$).

Discussion

We have determined acute changes in a number of biochemical parameters of blood—with emphasis on serum lipids—caused by one hour of cycling at 53% maximal HR reserve or 61% $\dot{V}O_{2\text{MAX}}$ upon untrained middle aged men and women. This exercise protocol stimulated adipose tissue lipolysis as evidenced by the significant increases in both serum glycerol and total NEFA concentrations. However, the increases in individual NEFA concentrations were not proportionate as shown by the significant changes in the percentages of several of them. Remarkably, the percentages of saturated NEFAs decreased, whereas those of unsaturated NEFAs increased (table 4). As a result, the NEFA U/S ratio increased. This change correlated positively with the change in total NEFA concentration, suggesting that the higher the release of fatty acids from adipose tissue TG, the greater the shift toward unsaturated NEFAs. The correlation can be explained by the acyl group composition of TG from the participants' subcutaneous adipose tissue, where the U/S ratio was about twice that of serum NEFAs. This ratio can be taken only as indicative since adipose tissue exhibits regional differences in fatty acid composition^{9 10} and in the lipolytic response to exercise¹¹ as well as gender differences in distribution throughout the body.

Total serum TG concentration decreased significantly in both sexes. This confirms the generally recognised potential of a single bout of exercise to induce transient decreases in TG,¹² although several studies on untrained men who performed bicycle exercise at intensities and duration similar to ours found no significant effect on TG concentration.^{13–16} We could not trace any pertinent studies on women. The decreases in total TG concentration in our study were accompanied by increases in U/S acyl groups. Since changes in plasma TG concentration in the post-absorptive state reflect changes in the balance between output by the liver and hydrolysis by lipoprotein lipase and hepatic TG lipase, an increase in U/S acyl groups in the face of decreasing TG concentration suggests a selectivity of the combined activity of these two lipases toward saturated acyl groups.

Changes in cholesterol and its HDL fraction were not as clear-cut as changes in TG in our study, in agreement with most similar studies

cited above.^{13 14 16} There was, however, a tendency towards a lower ratio of total to HDL-cholesterol.

We have included urea as an index of amino acid metabolism and two hormones (cortisol and testosterone) with opposite metabolic effects on skeletal muscle proteins (catabolic and anabolic respectively) in our evaluation of the exercise protocol. Most changes in these parameters were not significant, therefore we can assume that protein metabolism was not considerably affected.

In our previous study of the effect of exercise on the composition of plasma NEFAs and TG we found an increase in U/S NEFAs of 49%, concomitant with an increase in total NEFA concentration of 229%, and an increase in U/S acyl groups of TG by 8% accompanying a decrease in total TG concentration of 22%.¹ All of those changes were greater than the corresponding changes in the present paper. Mode of exercise, training state, body composition, and age may be responsible for these differences. Nevertheless, changes are in the same direction in both of our studies. We therefore conclude that untrained middle aged men and women, as well as adolescent male athletes, experience an increase in U/S ratio in both the serum NEFAs and TG fractions with prolonged moderate exercise.

The physiological significance of the above findings is not known. We are currently investigating the duration of the observed changes and whether there are chronic effects of exercise training on U/S ratios. Such research should enable the importance of the increase in the proportion of unsaturated serum fatty acids

for lowering the risk of coronary heart disease to be evaluated.

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