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Duration of coffee- and exercise-induced changes in the fatty acid profile of human serum

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Mougios, Vassilis, Susanne Ring, Anatoli Petridou, and Michalis G. Nikolaidis. Duration of coffee- and exercise-induced changes in the fatty acid profile of human serum. *J Appl Physiol* 94: 476–484, 2003. First published September 20, 2002; 10.1152/japplphysiol.00624.2002.—Prolonged moderate exercise increases the concentration of nonesterified fatty acids (NEFA) and the ratio of unsaturated to saturated (U/S) NEFA in human plasma. The present study examined the duration of these effects and compared them with the effects of coffee ingestion. On separate days and in random order, seven men and six women *1*) cycled for 1 h, *2*) ingested coffee containing 5 mg caffeine/kg body mass, *3*) ingested coffee followed by exercise 1 h later, and *4*) did nothing. Blood samples were drawn at 0, 1, 2, 4, 8, 12, and 24 h. Serum was analyzed for lactate, glucose, glycerol, individual NEFA, triacylglycerols, total cholesterol, and HDL cholesterol. Exercise elevated the U/S NEFA and the percentage of oleate, while decreasing the percentages of palmitate and stearate, at the end of exercise but not subsequently. Consumption of coffee triggered a lower lipolytic response with no alterations in U/S or percentages of individual NEFA. These findings may prove useful in discovering mechanisms mediating the effects of exercise training on the fatty acid profile of human tissues.

glycerol; lactate; nonesterified fatty acids; saturated fatty acids; unsaturated fatty acids

FATTY ACIDS ARE INVOLVED IN a multitude of diverse physiological functions, including energy production, lipid biosynthesis, protein modification, regulation of transcription, and intracellular signaling (22). Furthermore, they have been implicated in pathological conditions, such as insulin resistance (29), atherosclerosis (29), and obesity (23). Although they are usually treated as one entity, it is becoming increasingly apparent in recent years that different fatty acids exhibit distinct functions. For example, they have divergent effects on liver lipoprotein metabolism (24) and on glucose transport into skeletal muscle (34). Given this, one may assume that changes in the fatty acid profile of plasma may affect the metabolism of several tissues by modifying the composition of the mixture of fatty acids delivered to them.

Utilization of blood-borne nonesterified fatty acids (NEFA) in working muscles is important for aerobic ATP resynthesis during prolonged exercise of moderate intensity. Although an increase in the total concentration of plasma NEFA (as a result of augmented lipolysis in adipose tissue) during such efforts is well documented, little is known about the effect of exercise on their percent distribution. Studies from our laboratory (25, 26) and by others (7, 19, 38) have found that exercise changes the percentage of individual plasma NEFA, although there is no consensus on this issue. The most striking finding of our studies was an increase in the ratio of unsaturated to saturated (U/S) NEFA in the plasma of athletes (25) and untrained individuals (26). This change may add to the health benefits of exercise, given the protective role of dietary unsaturated fatty acids against cardiovascular disease and the development of insulin resistance (29). It is reasonable to think that the magnitude of the effect(s) of this change will depend on its duration; that is, the longer the U/S remains elevated, the higher its impact on human metabolism will probably be. Because our findings and the findings of the relevant studies cited above were based on blood samples taken solely at the end of exercise, we deemed it worthwhile to investigate how far into the recovery period the changes in individual NEFA are extended.

Numerous studies have investigated the influence of coffee and caffeine on metabolism, with emphasis on their probable glycogen-sparing effect as the explanation for the increase in endurance performance caused by their intake (reviewed in Ref. 14). On the basis of measurements of glycerol and NEFA release from adipose tissue, the majority of the relevant studies have shown caffeine to stimulate lipolysis (e.g., Refs. 4, 11). The vast majority of these studies have measured total NEFA; to our knowledge, there is only one report on the behavior of individual plasma NEFA after caffeine

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Journal of Applied Physiology

ingestion (27), an issue that deserves attention for the reasons presented above.

The aim of the present study was to examine the effects of exercise and coffee ingestion, separately and in combination, on selected variables of human serum related to carbohydrate and lipid metabolism, particularly the fatty acid composition of NEFA and triacylglycerols (TG). Changes in these variables were monitored for 24 h.

MATERIALS AND METHODS

Subjects

Eight male and seven female young healthy individuals volunteered to participate in the study initially. They were nonsmokers and nonobese (body mass index <30 kg/m^2) and trained up to two times per week. They consumed less than one alcoholic drink and less than three caffeine-containing beverages per day. Female participants were eumenorrheic and did not use oral contraceptives. The study was designed and carried out according to the guidelines of the University of Thessaloniki Ethics Committee.

Design

Subjects visited the laboratory on six occasions placed at least 3 days apart. At their first visit, they were informed, orally and in writing, of the design and possible risks of the study and consented to participate. Subsequently, a health history questionnaire was filled out, and body mass as well as height were measured to let us decide whether they were eligible for the study. Finally, they consumed a coffee drink identical to the one they would have to consume for the study to familiarize themselves with it and detect any adverse effects. No such effects were reported. Participants were then asked not to modify their dietary or physical activity habits during the study.

At their second visit, participants underwent body composition and maximal aerobic power assessment. Body composition was estimated through bioelectrical impedance analysis by using a Bodystat 1500 unit (Douglas). Maximal aerobic power was assessed by a graded test on a Monark bicycle ergometer (Vansbro). Subjects started at 60 W (men) or 45 W (women) for 5 min, and power was increased by 45 W every 3 min thereafter. When subjects were within 5 beats/min of their theoretical maximal heart rate (220 − age), or when it became subjectively obvious that work production was becoming so difficult that a normal increment would cause termination of exercise, increments were limited to 15 W. Subjects were asked to maintain a pedaling frequency of 60 rpm, and the test was terminated when their pedaling frequency fell <55 rpm. During this test and subsequent exercise trials, heart rate was monitored continuously by a Polar Accurex monitor (Kempele).

During each of the subsequent four occasions, subjects followed at random one of the protocols described below. Subjects reported to the laboratory in the morning after an overnight fast. They had been asked to abstain from caffeine-containing beverages during the previous 2 days and to record their dietary intake during the previous 3 days. They were unaware of the specific protocol that they were going to follow (except, of course, for the last occasion). Each subject provided 10 ml of blood from an antecubital vein into an evacuated test tube while seated at ~9 AM (taken as *zero time*) and then embarked on one of the following protocols (Fig. 1).
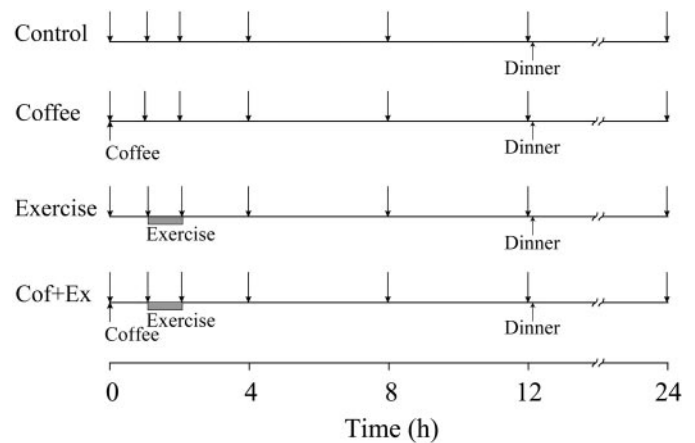

Fig. 1. Design of the 4 experimental protocols. Downward arrows denote blood sampling. Cof+Ex, coffee plus exercise protocol.

Control. Subject remained in a confined area for 12 h with lying, sitting, or walking allowed and without taking any food except water ad libitum and up to one pack of artificially flavored candy or chewing gum to combat the feeling of hunger. Subject provided blood samples as described above at 1, 2, 4, 8, and 12 h. Then the subject had a standardized dinner providing 70 kJ/kg body mass in the laboratory (up to 1 h after blood sampling), recorded food intake, left the laboratory, slept, and fasted until the next morning, when a final blood sample was drawn (at 24 h).

Coffee. Subject ingested 200 ml of warm instant coffee containing 5 mg caffeine/kg body mass (based on a content of 32 mg caffeine/g coffee, determined through high-performance liquid chromatography by the manufacturer, Nestlé) and tablets of an artificial sweetener (Nutrasweet) added to taste. Then the subject rested; provided blood samples at 1, 2, 4, 8, and 12 h; had dinner; and provided a final blood sample at 24 h, as described under *Control*.

Exercise. Subject rested for 1 h, provided a second blood sample, cycled for 1 h at 50–55% of his or her maximal aerobic power, and provided a blood sample immediately postexercise (at 2 h). Then the subject rested; provided blood samples at 4, 8, and 12 h; had dinner; and provided a final blood sample at 24 h, as described under *Control*.

Cof+Ex. Subject ingested coffee as described under *Coffee*, provided a second blood sample at 1 h, cycled for 1 h at the same power output as under *Exercise*, and provided a blood sample immediately postexercise (at 2 h). Then the subject rested; provided blood samples at 4, 8, and 12 h; had dinner; and provided a final blood sample at 24 h, as described under *Control*.

During the last visit to the laboratory (to provide the 24-h blood sample of the last protocol performed), each subject had his or her body mass measured and provided a biopsy of subcutaneous adipose tissue from the right buttock by needle aspiration, as described (5).

Biochemical Analyses

A 0.5-ml aliquot of each blood sample was mixed with EDTA solution to prevent clotting, to measure packed cell volume and hemoglobin (on the same day). Another 0.1-ml aliquot of the 0-, 1-, and 2-h samples of each protocol was precipitated with 0.3 mol/l HClO$_4$ to determine lactate in the supernatant (also on the same day). The remaining blood was allowed to clot, and serum was prepared and stored at −20°C for all other assays.

Table 1. *Characteristics of participants*

	Men ($n = 7$)	Women ($n = 6$)
Age, yr	22.6 ± 0.9	21.4 ± 0.5
Body mass, kg	78.4 ± 4.5	56.6 ± 2.3
Height, m	1.76 ± 0.01	1.65 ± 0.02
Body mass index, kg/m^2	25.3 ± 1.4	20.8 ± 0.7
Body fat, %	13.6 ± 1.7	20.4 ± 0.6
Maximal aerobic power, W	259 ± 12	150 ± 7

Values are means $\pm$ SE; *n*, no. of subjects.

Packed cell volume was measured by microcentrifugation and by correcting for 4% plasma trapped among the packed cells (10). Hemoglobin, lactate, glucose, glycerol, total cholesterol (TC), and HDL cholesterol (HDLC) were assayed by enzymatic photometric methods through the use of reagent kits from Sigma Chemical (St. Louis, MO) for glycerol and Böhringer (Mannheim, Germany) for all other parameters. Individual serum NEFA 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 (25). Total serum NEFA concentration was calculated as the sum of individual NEFA concentrations, and total serum TG concentration was calculated as the sum of individual TG acyl group concentrations divided by 3. All serum TG, TC, and HDLC values at 1–24 h were corrected for changes in plasma volume relative to 0 h, calculated from packed cell volume and hemoglobin, as described (10).

Dietary Analysis

Dietary records were analyzed in Microsoft Access by the use of a food database created in our laboratory on the basis of published data (18).

Statistical Analysis

Data were analyzed through the SPSS software. Values are expressed as means $\pm$ SE. Body mass at the onset and at the end of the study were compared through paired Student's *t*-test. Energy and nutrient intakes were compared through repeated-measures ANOVA. Heart rate, plasma volume, and biochemical parameters were compared initially through three-way (gender $\times$ protocol $\times$ time) ANOVA with repeated measures on protocol and time. Because no significant interaction and no significant main effect of gender on any of the dependent variables was found, data from both genders were combined to increase the power of analysis, and two-way (protocol $\times$ time) ANOVA was performed. Significant differences between protocols or time points were detected by performing simple contrasts. Linear correlation analysis was done by Pearson's product-moment correlation. The level of statistical significance was set at $\alpha = 0.05$.

RESULTS

One male and one female volunteer withdrew from the study before completing all four protocols because they could not respond to the demands of the experimental design; thus they were excluded. The characteristics, at admittance, of the 13 participants who completed the study are presented in Table 1. By the end of the study, their body mass had decreased by 0.8 ± 0.6 kg (not significant).

There were no significant differences among the four protocols in mean daily energy, carbohydrate, fat, or protein intake during the 3 days preceding each protocol (not shown). Energy intake was 9.51 ± 0.84 MJ and was derived from carbohydrate by 44%, fat by 41%, and protein by 15%. Similarly, there were no significant differences in the above parameters at the dinner on the day of each protocol (not shown). Energy intake from the dinner was 4.69 ± 0.64 MJ and was derived from carbohydrate by 43%, fat by 41%, and protein by 16%.

Subjects obtained 2 ± 1 kJ through the artificial sweetener in their coffee during Coffee and Cof+Ex. From 9 AM to 9 PM during each of the four protocols, they obtained 60 ± 12 kJ through the artificially flavored candy or chewing gum, with no significant differences among protocols.

Regarding heart rate during exercise, the interaction of protocol and time was significant ($P < 0.05$), as was the main effect of time ($P < 0.001$). Heart rate tended to be higher during Exercise (averaging 146 beats/min) than during Cof+Ex (averaging 142 beats/min), although the two protocols did differ significantly only at 5 min of exercise ($P < 0.05$).

Regarding plasma volume (Fig. 2), the main effects of protocol and time were significant ($P = 0.05$ and $P < 0.001$, respectively), whereas their interaction was not. Plasma volume decreased after Coffee, reaching 94% of the initial volume at 2 h, and was lower than Control at 1, 2, 4, and 12 h ($P < 0.05$). There were no significant differences between Control and Exercise or Cof+Ex.

The main effects of protocol and time, as well as their interaction, were significant with regard to blood lactate concentration ($P < 0.01$; Fig. 3). Lactate concentration was higher 1 h after coffee ingestion (Coffee or Cof+Ex) compared with no coffee ingestion (Control or Exercise, $P < 0.01$). Exercise alone increased lactate, but the increase did not reach statistical significance ($P = 0.082$ compared with Control at 2 h). The effect of coffee and exercise was additive; the concentration under Cof+Ex at 2 h (2.28 ± 0.32 mmol/l) was significantly higher than that under Exercise ($P < 0.05$) and marginally nonsignificantly higher than that under Coffee ($P = 0.093$).
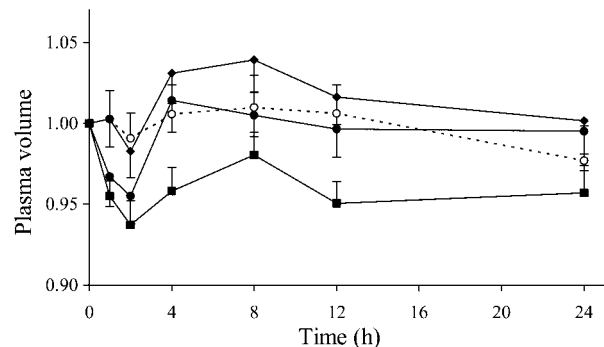

Fig. 2. Mean plasma volume relative to 0 h during Control (○), Coffee (■), Exercise (◆), and Cof+Ex (●) protocols. Values are means $\pm$ SE. See RESULTS for significant differences among protocols.

Fig. 3. Mean blood lactate (*A*), serum glucose (*B*), and serum glycerol (*C*) concentration during Control (○), Coffee (■), Exercise (◆), and Cof+Ex (●) protocols. Values are means ± SE. See RESULTS for significant differences among protocols.
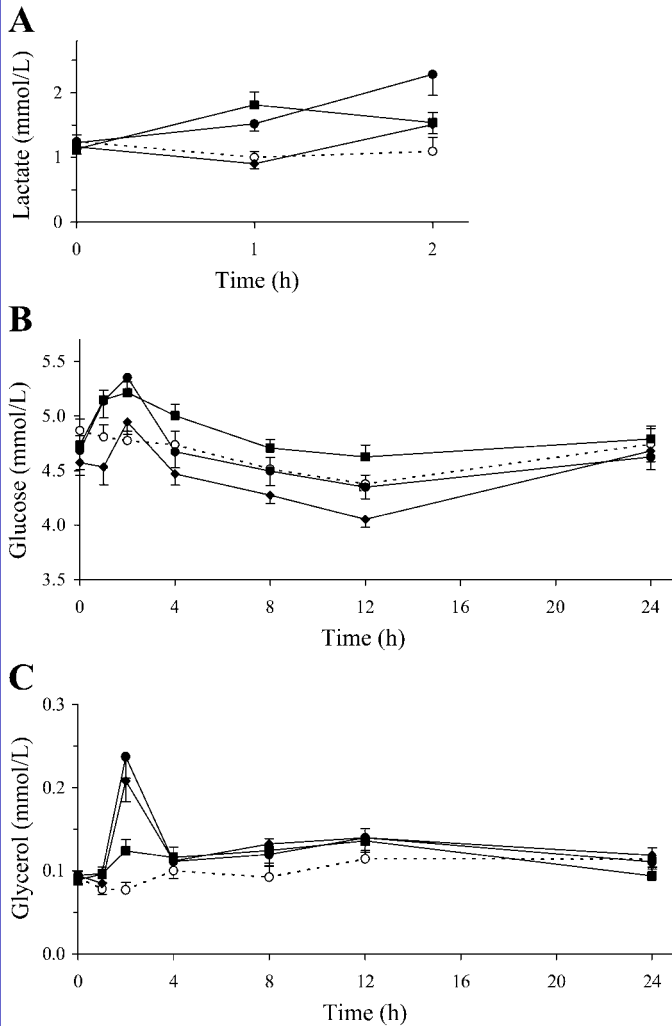
0.085 ± 0.013 and 0.096 ± 0.011 mmol/l at 1 h to 0.208 ± 0.025 and 0.237 ± 0.026 mmol/l at 2 h under Exercise and Cof+Ex, respectively. The values at 2 h under Exercise and Cof+Ex were higher compared with Control and Coffee ($P < 0.001$). Coffee consumption alone caused a lower but significant increase compared with Control at 2 h ($P < 0.01$). It is worth mentioning that the increases at 2 h were the only significant changes in glycerol concentration during the 24-h observation period.

Eleven serum NEFA were detected in considerable amounts by gas chromatography, namely, laurate (12:0), myristate (14:0), palmitate (16:0), palmitoleate [16:1(n-7)], stearate (18:0), oleate [18:1(n-9)], vaccenate [18:1(n-7)], linoleate [18:2(n-6)], α-linolenate [18:3(n-3)], gondoate [20:1(n-9)], and arachidonate [20:4(n-6)]. The main effects of protocol and time, as well as their interaction, were significant with regard to total NEFA ($P < 0.001$; Fig. 4). Under Control, total NEFA increased during the day (from 0.30 ± 0.05 mmol/l at 0 h to 0.75 ± 0.08 mmol/l at 12 h) and decreased through the next morning. Coffee ingestion caused an increase at 1 h ($P < 0.01$, Coffee or Cof+Ex vs. Control or Exercise). At 2 h, all intervention protocols displayed higher total NEFA concentrations compared with Control ($P < 0.01$). What is more, the concentration under Cof+Ex (0.97 ± 0.07 mmol/l) was higher than the ones under Coffee and Exercise ($P < 0.01$), implying an additive effect. In addition, total NEFA were higher at 2 h under Exercise compared with Coffee ($P < 0.05$). Total NEFA remained higher under Coffee, Exercise, and Cof+Ex compared with Con-
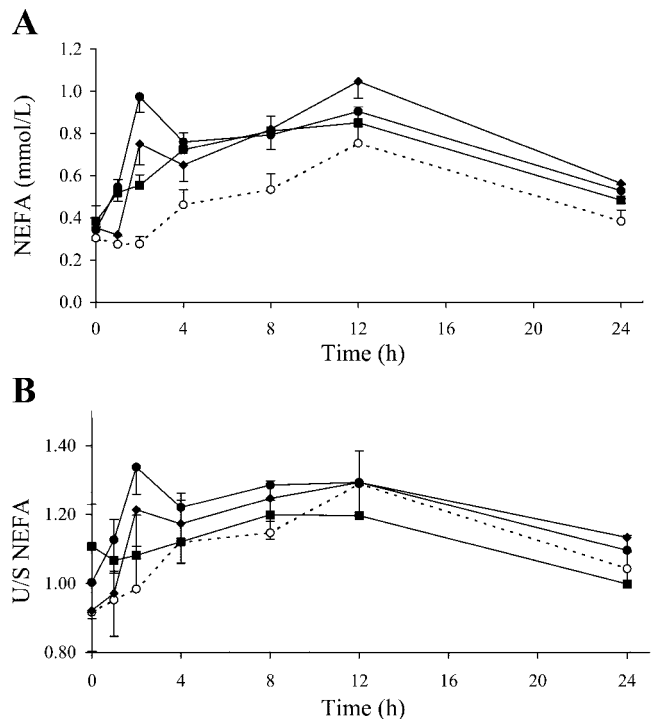
Fig. 4. Mean serum nonesterified fatty acid (NEFA) concentration (*A*) and unsaturated-to-saturated ratio (U/S) (*B*) during Control (○), Coffee (■), Exercise (◆), and Cof+Ex (●) protocols. Values are means ± SE. See RESULTS for significant differences among protocols.

The interaction of protocol and time as well as both main effects were significant with regard to serum glucose ($P < 0.01$; Fig. 3). Glucose declined gradually during the day under Control (from 4.87 ± 0.10 mmol/l at 0 h to 4.38 ± 0.14 mmol/l at 12 h). Coffee ingestion, with or without exercise, caused a transient increase at 1 and 2 h ($P < 0.05$ compared with Control), which was highest after the combination of the two treatments (5.35 ± 0.14 mmol/l at 2 h under Cof+Ex). Exercise alone also increased glucose at 2 h ($P < 0.05$ compared with Control). Glucose decreased 2 h after exercise (i.e., at 4 h) and was not significantly different from Control from that point on. On the contrary, coffee ingestion alone maintained glucose higher than Control up to the 12-h sampling point ($P < 0.05$).

Concerning glycerol, the main effect of protocol and time as well as their interaction were significant ($P = 0.05$, $P < 0.001$, and $P < 0.001$, respectively; Fig. 3). Glycerol concentration under Control fluctuated between 0.077 ± 0.009 mmol/l (at 2 h) and 0.114 ± 0.016 mmol/l (at 12 h). Exercise induced an increase from

Table 2. *Serum concentrations of nonesterified fatty acids during the experimental protocols*

Fatty Acid	0 h	1 h	2 h	4 h	8 h	12 h	24 h
			Control				
12:0	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000‡	0.001 ± 0.000‡	0.002 ± 0.000†	0.002 ± 0.001	0.002 ± 0.001
14:0	0.009 ± 0.001	0.008 ± 0.002	0.008 ± 0.001‡§	0.012 ± 0.002†	0.012 ± 0.002†‡	0.017 ± 0.002‡	0.011 ± 0.002
16:0	0.107 ± 0.017	0.098 ± 0.015†§	0.096 ± 0.010†‡§	0.144 ± 0.021†‡§	0.173 ± 0.025†‡§	0.220 ± 0.017‡§	0.129 ± 0.015
16:1(n-7)	0.006 ± 0.002	0.007 ± 0.002	0.007 ± 0.001‡§	0.014 ± 0.003	0.014 ± 0.003‡§	0.020 ± 0.002	0.009 ± 0.002
18:0	0.035 ± 0.006	0.030 ± 0.004†§	0.032 ± 0.003†‡§	0.043 ± 0.005†‡§	0.055 ± 0.006†‡	0.066 ± 0.006‡	0.039 ± 0.004
18:1(n-9)	0.090 ± 0.019	0.082 ± 0.015†§	0.086 ± 0.014†‡§	0.164 ± 0.032†§	0.189 ± 0.032§	0.287 ± 0.037	0.123 ± 0.021
18:1(n-7)	0.004 ± 0.001	0.004 ± 0.001†§	0.004 ± 0.001‡§	0.008 ± 0.002†§	0.009 ± 0.002	0.013 ± 0.002	0.006 ± 0.001
18:2(n-6)	0.042 ± 0.009	0.038 ± 0.006†§	0.037 ± 0.006†‡§	0.064 ± 0.011§	0.068 ± 0.013†‡§	0.113 ± 0.014	0.057 ± 0.012
18:3(n-3)	0.002 ± 0.001	0.001 ± 0.000†	0.001 ± 0.000‡§	0.002 ± 0.000	0.002 ± 0.000†‡	0.003 ± 0.000	0.002 ± 0.000
20:1(n-9)	0.001 ± 0.001	0.001 ± 0.000§	0.001 ± 0.001‡§	0.003 ± 0.002	0.002 ± 0.001	0.005 ± 0.003	0.002 ± 0.001
20:4(n-6)	0.004 ± 0.001	0.003 ± 0.001†§	0.004 ± 0.001§	0.007 ± 0.002	0.006 ± 0.002	0.009 ± 0.003	0.003 ± 0.001‡
Sum	0.303 ± 0.053	0.273 ± 0.041†§	0.277 ± 0.033†‡§	0.462 ± 0.072†‡§	0.533 ± 0.076†‡§	0.754 ± 0.077‡	0.384 ± 0.054
			Coffee				
12:0	0.001 ± 0.000	0.002 ± 0.001‡	0.002 ± 0.001	0.003 ± 0.001	0.004 ± 0.001*	0.004 ± 0.001	0.003 ± 0.002
14:0	0.011 ± 0.002	0.014 ± 0.001‡	0.014 ± 0.002‡§	0.019 ± 0.002*	0.023 ± 0.003*	0.024 ± 0.002	0.018 ± 0.007
16:0	0.128 ± 0.020	0.172 ± 0.016*‡	0.185 ± 0.013*‡§	0.235 ± 0.021*	0.266 ± 0.021*	0.280 ± 0.019‡	0.167 ± 0.022
16:1(n-7)	0.009 ± 0.002	0.011 ± 0.002	0.011 ± 0.002‡§	0.017 ± 0.003	0.021 ± 0.004	0.022 ± 0.003‡	0.010 ± 0.002
18:0	0.035 ± 0.005	0.059 ± 0.005*‡	0.063 ± 0.004*	0.073 ± 0.005*	0.075 ± 0.005*	0.075 ± 0.006‡	0.055 ± 0.011
18:1(n-9)	0.133 ± 0.031	0.175 ± 0.028*‡	0.189 ± 0.023*‡§	0.251 ± 0.035*	0.286 ± 0.032	0.297 ± 0.036‡	0.152 ± 0.026
18:1(n-7)	0.006 ± 0.002	0.008 ± 0.002*	0.009 ± 0.001*‡§	0.012 ± 0.002*	0.014 ± 0.002	0.015 ± 0.002‡	0.007 ± 0.001
18:2(n-6)	0.054 ± 0.012	0.070 ± 0.012*‡	0.073 ± 0.012*‡§	0.102 ± 0.017	0.112 ± 0.015*	0.123 ± 0.015	0.062 ± 0.010‡
18:3(n-3)	0.002 ± 0.000	0.002 ± 0.001*	0.002 ± 0.000§	0.002 ± 0.001	0.003 ± 0.001*	0.003 ± 0.001	0.002 ± 0.001
20:1(n-9)	0.002 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.002 ± 0.000	0.003 ± 0.001	0.002 ± 0.000	0.001 ± 0.000
20:4(n-6)	0.003 ± 0.001	0.004 ± 0.001*	0.003 ± 0.001§	0.004 ± 0.001	0.005 ± 0.001‡	0.006 ± 0.001	0.004 ± 0.001*
Sum	0.384 ± 0.073	0.519 ± 0.062*‡	0.553 ± 0.050*‡§	0.722 ± 0.080*	0.811 ± 0.071*	0.850 ± 0.077‡	0.483 ± 0.074
			Exercise				
12:0	0.001 ± 0.000	0.001 ± 0.000†	0.003 ± 0.001*	0.002 ± 0.000*	0.002 ± 0.001	0.004 ± 0.001	0.003 ± 0.001
14:0	0.010 ± 0.001	0.008 ± 0.001†	0.021 ± 0.003*†	0.016 ± 0.002	0.021 ± 0.003*	0.027 ± 0.002*	0.017 ± 0.002
16:0	0.127 ± 0.018	0.115 ± 0.014†§	0.251 ± 0.029*†§	0.214 ± 0.022*	0.265 ± 0.025*	0.332 ± 0.021*†	0.189 ± 0.018
16:1(n-7)	0.007 ± 0.002	0.007 ± 0.002	0.021 ± 0.004*†§	0.015 ± 0.003	0.025 ± 0.00*	0.030 ± 0.004†	0.015 ± 0.003
18:0	0.038 ± 0.005	0.034 ± 0.004†§	0.061 ± 0.006*§	0.065 ± 0.007*	0.070 ± 0.005*	0.095 ± 0.006*†	0.049 ± 0.005
18:1(n-9)	0.112 ± 0.024	0.100 ± 0.018†§	0.268 ± 0.043*†§	0.227 ± 0.037	0.296 ± 0.047	0.386 ± 0.039†	0.187 ± 0.031
18:1(n-7)	0.005 ± 0.001	0.006 ± 0.001§	0.012 ± 0.002*†§	0.011 ± 0.002	0.014 ± 0.002	0.018 ± 0.002†	0.009 ± 0.002
18:2(n-6)	0.046 ± 0.009	0.043 ± 0.009†§	0.105 ± 0.018*†§	0.090 ± 0.014	0.114 ± 0.019*	0.142 ± 0.020	0.084 ± 0.017†
18:3(n-3)	0.002 ± 0.001	0.001 ± 0.000	0.003 ± 0.001*	0.003 ± 0.001	0.004 ± 0.001*	0.004 ± 0.001	0.003 ± 0.001
20:1(n-9)	0.001 ± 0.000	0.001 ± 0.000§	0.002 ± 0.000*	0.002 ± 0.000	0.002 ± 0.000	0.003 ± 0.000	0.002 ± 0.000
20:4(n-6)	0.003 ± 0.001	0.003 ± 0.001	0.004 ± 0.001	0.005 ± 0.001	0.006 ± 0.001†	0.006 ± 0.001	0.005 ± 0.001
Sum	0.352 ± 0.058	0.319 ± 0.043†§	0.750 ± 0.100*†§	0.650 ± 0.078*	0.820 ± 0.098*	1.047 ± 0.079*†	0.562 ± 0.073
			Cof+Ex				
12:0	0.003 ± 0.002	0.002 ± 0.001	0.004 ± 0.001	0.002 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.003 ± 0.001
14:0	0.012 ± 0.004	0.015 ± 0.003	0.029 ± 0.004*†	0.019 ± 0.002	0.021 ± 0.002	0.022 ± 0.002	0.014 ± 0.002
16:0	0.118 ± 0.018	0.178 ± 0.023*‡	0.313 ± 0.031*†‡	0.242 ± 0.012*	0.256 ± 0.022*	0.292 ± 0.016*	0.180 ± 0.018
16:1(n-7)	0.006 ± 0.001	0.013 ± 0.003	0.030 ± 0.004*†‡	0.018 ± 0.001	0.022 ± 0.002*	0.024 ± 0.002	0.014 ± 0.003
18:0	0.040 ± 0.008	0.061 ± 0.005*‡	0.080 ± 0.006*‡	0.082 ± 0.004*	0.070 ± 0.006	0.078 ± 0.005	0.053 ± 0.004
18:1(n-9)	0.106 ± 0.017	0.178 ± 0.026*‡	0.350 ± 0.030*†‡	0.267 ± 0.022*	0.282 ± 0.030*	0.327 ± 0.033	0.178 ± 0.027
18:1(n-7)	0.005 ± 0.001	0.008 ± 0.001*‡	0.016 ± 0.002*†‡	0.012 ± 0.001*	0.013 ± 0.001	0.015 ± 0.002	0.009 ± 0.002
18:2(n-6)	0.047 ± 0.007	0.081 ± 0.015*‡	0.141 ± 0.012*†‡	0.107 ± 0.011*	0.114 ± 0.015*	0.130 ± 0.013	0.070 ± 0.011
18:3(n-3)	0.002 ± 0.000	0.003 ± 0.001	0.004 ± 0.001*†	0.003 ± 0.000	0.003 ± 0.001	0.004 ± 0.001	0.002 ± 0.000
20:1(n-9)	0.001 ± 0.000	0.001 ± 0.000*‡	0.002 ± 0.000*	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.001 ± 0.000
20:4(n-6)	0.003 ± 0.001	0.004 ± 0.001*	0.006 ± 0.001*†	0.005 ± 0.001	0.006 ± 0.001	0.007 ± 0.001	0.004 ± 0.001
Sum	0.343 ± 0.052	0.546 ± 0.067*‡	0.975 ± 0.074*†‡	0.759 ± 0.038*	0.793 ± 0.068*	0.904 ± 0.062	0.529 ± 0.060

Values are means ± SE in mmol/l. Cof+Ex, coffee plus exercise protocol. Significantly different from the respective value under *Control, †Coffee, ‡Exercise, and §Cof+Ex, respectively ($P < 0.05$).

trol up to the 8-h sampling point ($P < 0.05$). Additionally, the value at 12 h of Exercise (1.05 ± 0.08 mmol/l, the highest observed) was higher than the corresponding values under Control and Coffee ($P < 0.05$).

The concentrations of the individual serum NEFA (Table 2) generally paralleled the changes described above for their sum, although fewer differences were found to be significant. The curves of 16:0 and 18:1(n-9) during the four protocols bore the most striking resemblance to the corresponding curves of total NEFA. Those were the most abundant serum NEFA, each accounting for approximately one-third of total.

With regard to U/S NEFA, the interaction of protocol and time as well as the main effect of time were

significant ($P < 0.05$ and $P < 0.001$, respectively; Fig. 4), but the main effect of protocol was not. The cumulative U/S NEFA of the four protocols at each time point increased gradually during the day, being higher than 0 h from 2 to 12 h ($P \leq 0.001$). Coffee ingestion did not elicit significant changes compared with Control. U/S NEFA at the end of exercise (i.e., at 2 h of Exercise and Cof+Ex) was higher than for Control and Coffee ($P < 0.05$). U/S NEFA correlated positively with total NEFA concentration ($r = 0.58$, $P < 0.001$) and glycerol concentration ($r = 0.38$, $P < 0.001$).

Concerning the effect of each treatment on each serum NEFA as a percentage of total, the only significant interactions of protocol and time were found in 16:0, 18:0, and 18:1(n-9) ($P < 0.01$, $P < 0.001$, and $P < 0.01$, respectively). In particular, after exercise (with or without prior coffee ingestion), 16:0 decreased compared with Control, but significance was reached only between Exercise and Control ($P < 0.05$ at 2 h). In contrast, after coffee ingestion, the percentage of 16:0 fluctuated only slightly up to 24 h. The percentage of 18:0 was markedly reduced at the end of Exercise compared with Control and Coffee ($P < 0.01$). It is worth mentioning that the decrease of 18:0 under Exercise and Cof+Ex reached 21 and 29%, respectively, compared with the preexercise levels. Finally, the percentage of 18:1(n-9) at 2 h under Exercise and Cof+Ex was higher compared with Control ($P < 0.05$), whereas there were no differences between Coffee and Control.

Table 3 presents the fatty acid composition of adipose tissue TG, which contained predominantly 18:1(n-9). Its percentage and the U/S (1.55 ± 0.09) were higher than any corresponding value of serum NEFA. On the contrary, the percentages of 16:0 and 18:0 in adipose tissue TG were lower than any corresponding value of serum NEFA.

Serum TG concentration (Fig. 5) decreased during all intervention protocols until dinnertime and then increased on the next morning. The interaction of protocol and time as well as the main effect of protocol were not significant, but the main effect of time was ($P < 0.01$). On the other hand, neither the interaction nor any main effect was significant with respect to the U/S TG acyl groups (not shown).
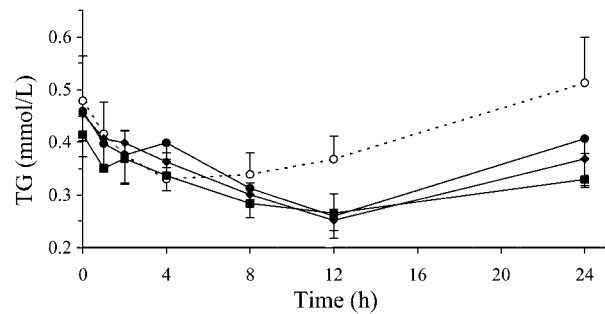

Fig. 5. Mean serum triacylglycerol (TG) concentration during Control (○), Coffee (■), Exercise (◆), and Cof+Ex (●) protocols. Values are means ± SE. See RESULTS for significant differences among protocols.

With regard to TC and HDLC, the main effect of time was significant ($P < 0.001$), whereas the main effect of protocol and the interaction were not. TC exhibited small and roughly parallel fluctuations under all protocols (not shown); cumulatively for all protocols, it increased by an average of 4% at 8–12 h compared with that at 0 h ($P < 0.01$) and returned to a value that was 2% below the 0-h value at 24 h ($P < 0.05$). HDLC followed a similar pattern (not shown) but exhibited higher increases compared with TC; that is, HDLC at 4–12 h was higher compared with that at 0 h ($P < 0.001$), reaching a 9% increase at 12 h, and was 2% above the 0-h value at 24 h (not significant). As a result, the HDLC/TC ratio (Fig. 6) displayed a significant main effect of time ($P < 0.001$), with the cumulative value of the four protocols at 2–12 h being higher compared with 0 h ($P < 0.05$). Additionally, a significant protocol × time interaction appeared ($P < 0.001$), owing to the fact that the values at 12 and 24 h under the intervention protocols were higher compared with Control (Coffee, by 3%; Exercise and Cof+Ex, by 5%; $P < 0.05$).

DISCUSSION

The present study emphasized the effect of exercise and/or coffee ingestion on the profile of serum NEFA and TG for 24 h. To our knowledge, this is the first attempt to characterize the influence of coffee ingestion on the fatty acid profile of either human or animal serum and the first study that monitored the fatty acid profile of serum for 22 h postexercise. A limitation of

Table 3. *Fatty acid profile of adipose tissue triacylglycerols*

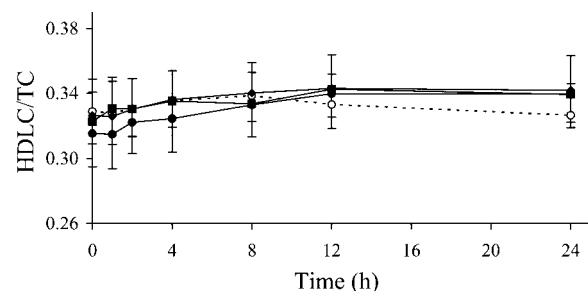| Fatty Acid	Molar Percentage
12:0	0.39 ± 0.05
14:0	3.50 ± 0.22
16:0	30.61 ± 1.05
16:1(n-7)	4.23 ± 0.31
18:0	5.14 ± 0.23
18:1(n-9)	44.45 ± 1.18
18:1(n-7)	0.99 ± 0.03
18:2(n-6)	10.00 ± 1.02
18:3(n-3)	0.15 ± 0.03
20:1(n-9)	0.42 ± 0.03
20:4(n-6)	0.11 ± 0.02

Values are means ± SE.

Fig. 6. Mean ratio of serum HDL cholesterol to total cholesterol (TC) during Control (○), Coffee (■), Exercise (◆), and Cof+Ex (●) protocols. Values are means ± SE. See RESULTS for significant differences among protocols.

our study is the use of serum concentration rather than flux data, which would offer more insight into the mechanisms of the observed effects.

Our data indicate that, compared with the resting state, exercise caused a sustained increase in the serum NEFA concentration that was significant up to the end of the fasting period (10 h postexercise). The increase in NEFA concentration was accompanied by an increase in their U/S, which was significantly higher than Control immediately postexercise [in agreement with our previous studies (25, 26)] but not thereafter (shown for the first time). Changes in U/S NEFA with time under all protocols paralleled the corresponding changes in glycerol and NEFA concentrations, supporting the hypothesis that they are due to the stimulation of lipolysis in adipose tissue, whose TG have a higher U/S than serum NEFA. However, other possibilities, such as differences in NEFA clearance, cannot be excluded.

We decided to compare the effect of exercise on the serum fatty acid profile to that of coffee ingestion, because caffeine is a known lipolytic agent and because exercisers, particularly competitive athletes, often include coffee in their diet to take advantage of the potential ergogenic effects of caffeine (33). We chose coffee as the vehicle of caffeine because we preferred a dietary rather than pharmacological intervention and because the actions of coffee and pure caffeine have been reported to differ (16). Furthermore, even though coffee is probably the most common dietary source of caffeine, its effect on human metabolism is much less studied compared with pure caffeine. A dosage of instant coffee equivalent to 5 mg of caffeine/kg body mass was chosen, because this is most often employed in similar studies (e.g., Ref. 11) and because it results in urinary caffeine concentrations below the 12 mg/l limit set in doping control (33). Concerning the exercise stimulus, its intensity and duration were chosen so as to cause considerable lipid mobilization and to be tolerable by the majority of the population.

Coffee ingestion appeared to stimulate lipolysis, as evidenced by the significantly higher serum glycerol concentration at 2 h and the significantly higher NEFA concentration at 1–8 h compared with Control. Increased serum glycerol and NEFA levels after coffee or caffeine ingestion have been reported by other studies as well, although there are studies that have not found significant changes (reviewed in Ref. 14). The lipolytic effect of coffee was significantly lower than that of exercise, as evidenced by the lower glycerol and NEFA concentrations under Coffee compared with Exercise at 2 h. Apparently as a consequence of this, coffee ingestion did not elicit significant alterations in U/S NEFA, although, as discussed above, other possibilities cannot be excluded. This finding contrasts with the increase in U/S NEFA found after the ingestion of caffeine capsules (27), probably due to the different way of caffeine administration. The combination of coffee ingestion and exercise in our study elicited a higher lipolytic response than exercise alone, as evidenced by the glycerol (though not statistically justified) and NEFA con-

centrations at 2 h. This, along with the fact that glycerol peaked 2 h after coffee ingestion, indicates that it was appropriate to allow 1 h between coffee ingestion and exercise to maximize the lipolytic effect.

We found a significant increase in the percentage of the major unsaturated fatty acid, 18:1(n-9), and significant decreases in the percentages of the major saturated fatty acids, 16:0 and 18:0, at the end of exercise (with or without prior coffee ingestion). As with the increase in U/S, these changes were in the direction of the composition of the main source of plasma NEFA, that is, adipose tissue TG. The decrease in 16:0 agrees with some studies (25, 38) but disagrees with other studies that found no change (7, 19, 26). The decrease in 18:0 agrees with all available studies (7, 25, 26, 38) except one, which found no change (19). The increase in 18:1(n-9) agrees with all available studies (7, 19, 25, 26, 38). Regarding the lack of significant changes in the percentages of the other fatty acids after exercise, this generally agrees with most of the relevant studies, with the exception of 18:2(n-6), which has been reported to increase (25), decrease (19, 38), or remain unchanged with exercise (7, 26). Data on the kinetics of 12:0 and 20:1(n-9) after exercise are presented for the first time. The discrepancies noted above are probably due to differences in mode of exercise, training state, age, and (possibly more importantly) fatty acid composition of the adipose tissue of participants. Nevertheless, it is apparent that some consensus emerges from these studies, that is, after exercise, the percentage of 18:0 decreases, that of 18:1(n-9) increases, whereas those of 14:0, 18:3(n-3), and 20:4(n-6) do not change.

The U/S of serum NEFA increased gradually during fasting, approaching the U/S of adipose tissue TG. Fasting has been shown to increase the life span of mice (30). Additionally, caloric restriction (which usually involves repeated fasting periods) is known to delay the onset of diseases associated with ageing and extend the life span of a variety of animals (13). On the other hand, the miscellaneous health benefits of unsaturated fatty acids are well known (29). Whether there is a link between the increase in U/S NEFA during fasting and longevity is an intriguing issue that requires further investigation.

We have determined a number of additional parameters to obtain a more complete picture of the effects of exercise and/or coffee on human physiology and biochemistry. The observed tendency of heart rate during moderate exercise to be lower after caffeine ingestion has also been found in one study (31) and, to a smaller degree, in two more (9, 12), although other studies have failed to detect such an effect (28, 32). The observed decrease in plasma volume after coffee ingestion alone is in accordance with its diuretic action, whereas the higher plasma volume values calculated after the combination of coffee and exercise confirm the ability of the latter to counteract the dehydrating effect of the former (39).

Blood lactate was significantly higher after coffee ingestion, both at rest and postexercise. Indeed, the effect of coffee and exercise was additive. This agrees

with a study (11) that employed the same dose of caffeine and a similar exercise protocol (60 min of walking at 50% of maximal oxygen uptake). Caffeine has also been found to augment the effect of maximal anaerobic exercise on blood lactate (3, 4, 8). Therefore, caffeine appears to increase lactate release (although not from the exercising muscles; Ref. 15) and/or decrease lactate removal from blood across the full spectrum of exercise intensity.

Serum glucose after coffee ingestion remained significantly higher than Control during the entire fasting period. This is in accordance with findings of decreased insulin-stimulated whole body and muscle glucose uptake after caffeine administration (21, 36). The failure of serum glucose to remain significantly higher than Control under Exercise and Cof+Ex, although increased amounts of the same substrates were available as well, is probably due to the fact that exercise deprived the liver and the exercised muscles of much of their glycogen.

Serum TG decreased sharply throughout the fasted state, indicating a marked imbalance between output from the liver and uptake by peripheral tissues. The lack of significant differences among protocols suggests that neither exercise nor coffee affected plasma TG kinetics considerably. The same seems to hold for TC. On the contrary, a significant effect of exercise and coffee, both separately and in combination, on the HDLC/TC ratio was noticed at 12 and 24 h. These findings confirm the ability of a single bout of exercise to elicit favorable changes in plasma lipids (35) but are novel with respect to coffee. In view of the evidence that regular coffee consumption negatively affects the blood lipid profile (i.e., it increases total and LDL cholesterol; Ref. 20), our finding is of interest and deserves further investigation.

The physiological significance of the exercise-induced increase in the U/S serum fatty acids lies in the beneficial role of the former in human health. Apart from the well-established negative correlation between the intake of unsaturated fatty acids and the risk for cardiovascular disease, evidence has been presented in recent years linking fatty acids with insulin sensitivity. In particular, the latter has been positively correlated with the percentage of polyunsaturated fatty acids and negatively correlated with the percentage of 16:0 in the phospholipids of human skeletal muscle (6, 37). The fatty acid composition of human skeletal muscle phospholipids was shown to be affected by exercise training; the most consistently observed changes were increases in the percentages of 18:1(n-9) and n-3 fatty acids, as well as decreases in the percentages of 16:0 and n-6 fatty acids (1, 2, 17). Although the mechanism behind these alterations is not known, they can be partly explained by the findings of the present study, i.e., the increased proportion of unsaturated NEFA [mainly 18:1(n-9)] to saturated NEFA (mainly 16:0) in plasma after exercise, which could be reflected in the relative uptake of these fatty acids by muscle. When these fatty acids or those produced from them by desaturation inside the muscle fibers are incorporated into synthesized phospholipids, the fatty acid profile of the latter may change in the direction reported by the studies mentioned above.

In conclusion, our data show that exercise at 50–55% of maximal aerobic power for 1 h increased the total serum NEFA concentration above control for 10 h postexercise. In addition, it elevated the U/S NEFA and the percentage of 18:1(n-9), while decreasing the percentages of 16:0 and 18:0, at the end of exercise but not subsequently. Consumption of coffee containing 5 mg of caffeine/kg body mass triggered a lower lipolytic response with no alterations in U/S or in the percentages of individual NEFA. Combination of the two stimuli elicited a higher lipolytic response than exercise alone, with similar changes in U/S and in the percentages of individual NEFA. The exercise-induced changes in the fatty acid profile of serum reflected the fatty acid profile of adipose tissue. These findings may prove useful in discovering mechanisms mediating the effects of exercise training on the fatty acid composition of human tissues.

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