

Acute changes in triacylglycerol lipase activity of human adipose tissue during exercise

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Abstract Although physical exercise is known to increase adipose tissue lipolysis, its effect on the activity of triacylglycerol (TG) lipase, the enzyme regulating TG breakdown, is not known. The aim of the present study was to monitor the acute changes in TG lipase activity of adipose tissue induced during moderate exercise. For this purpose a new assay, sensitive to the phosphorylation state of the enzyme, was developed. Ten young sedentary men cycled for 30 min at a heart rate of 120–130 beats min⁻¹. Needle adipose tissue biopsy was performed from the buttock area at rest, at 5, 15, and 30 min of exercise, as well as at 15 min of passive recovery. Five other men served as controls by being biopsied as above without exercising. TG lipase activity was determined by measuring the decrease of endogenous TG concentration during incubation of the homogenized tissue. TG lipase activity increased 6.4-fold above baseline at 5 min of exercise ($P < 0.001$) and fell gradually afterwards, whereas it did not change significantly in the control group. **Conclusion** In conclusion, our data show that TG lipase activity in human adipose tissue peaks early during exercise and subsequently decreases despite the maintenance of the physical stimulus.—Petridou, A., and V. Mougios. Acute changes in triacylglycerol lipase activity of human adipose tissue during exercise. *J. Lipid Res.* 2002. 43: 1331–1334.

Supplementary key words lipolysis • hormone-sensitive lipase • phosphorylation • assay • biopsy

TG lipase (EC 3.1.1.3) catalyzes the hydrolysis of stored TG in adipose tissue (1), an important process for the supply of energy sources in the postabsorptive state and for the control of body weight. TG lipase is under acute neuronal and hormonal regulation (hence referred to as hormone-sensitive lipase, HSL) through the cAMP cascade ending in reversible phosphorylation and activation of the enzyme (2–4). Physical exercise stimulates lipolysis in adipose tissue, as has been shown by the use of microdialysis, arteriovenous difference, and isotope tracer techniques (5–8). This is assumed to be mainly due to increased cate-

cholamine secretion resulting in β -adrenergic stimulation and HSL activation (9), but direct evidence is lacking. Given the multitude of factors intervening between the hydrolysis of TG inside the adipocytes and the appearance of the products in the extracellular space and plasma (e.g., transport across membranes and blood flow), it would be interesting to study the effect of exercise on the lipolytic rate at the intracellular level and on the main determinant of lipolytic rate, i.e., TG lipase activity.

TG lipase activity of adipose tissue is usually measured against a synthetic substrate, 1(3)-[³H]oleoyl-2-*O*-oleylglycerol, because its rate of hydrolysis is 10 times that of TG and independent of the phosphorylation state of the enzyme (10). The latter feature, however, precludes the use of the synthetic substrate to determine the actual activity of the enzyme and to monitor acute changes in response to endogenous or exogenous stimuli. Therefore, to study the effect of exercise on TG lipase activity, an assay sensitive to the phosphorylation state of the enzyme is needed.

The aim of the present study was to investigate the effect of moderate exercise on TG lipase activity in human adipose tissue. To achieve this, we have developed a novel assay of the actual activity of the enzyme against endogenous TG.

METHODS

Subjects

Fifteen healthy sedentary men, aged 21–26, who responded to a public invitation, participated in the study. They were informed orally and in writing of the design and probable risks of the research and consented to participate. The study was designed and carried out according to the guidelines of the University of Thessaloniki Ethics Committee.

Abbreviations: HSL, hormone-sensitive lipase.

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Experimental protocol

Each subject was transported to the laboratory by car on a separate day at 9 AM, after an overnight fast and rest. Following 15 min of rest, an adipose tissue sample was obtained by needle biopsy from the buttock area without anesthesia, as described (11). Subsequently, 10 of the subjects exercised on a Kettler KX1 bicycle ergometer (Ense-Parsit, Germany) for 30 min at a power output corresponding to a heart rate of 120–130 beats min^{-1} , being monitored continuously by a Polar Accurex monitor (Kempele, Finland). The power output had been determined during a preliminary test performed at least 1 week earlier. Adipose tissue biopsy was performed at 5, 15, and 30 min of exercise, as well as after 15 min of passive recovery. All samples were taken with the subjects seated on the bicycle and with a 5- to 10-s interruption of cycling at 5 and 15 min of exercise. The other five subjects were sequentially biopsied as above, the only difference being that they rested throughout the sampling period.

TG lipase assay

The determination of TG lipase activity was performed immediately after biopsy, without storage of the adipose tissue sample. The sample was transferred into a glass homogenizer (Kontes, Vineland, NJ) containing 300 μl of a potassium phosphate buffer (0.1 mol/l, pH 7.0), to which the following substances (all from Sigma, St. Louis, MO) had been added in order to prevent protein dephosphorylation, phosphorylation, and degradation: EDTA (1 mmol/l), "phosphatase inhibitor cocktail I" (containing microcystin LR, cantharidin, and (-)-*p*-bromotetramisole in DMSO) in 100-fold dilution, inhibitor of the cAMP-dependent protein kinase (rabbit sequence, 2.5 $\mu\text{mol/l}$), antipain (20 mg/l), leupeptin (20 mg/l), and pepstatin (10 mg/l). The sample was weighed and homogenized with approximately 20 manual strokes, while buffer was being added to a final volume of 20 times the weight of the tissue. The total time from sampling to complete homogenization was approximately 30 s. The homogenate was then transferred to a test tube and was incubated at 37°C under continuous stirring. At 0 and 30 min, 10 μl aliquots were removed in triplicate from the homogenate and were transferred into 1 ml of 2-propanol-heptane-1 N H_2SO_4 , 40:10:1 (v/v/v), to denature TG lipase. This solution also contained 70 μg of triheptadecanoyl glycerol (Sigma) as the internal standard for the subsequent chromatographic analysis.

Lipids were extracted from the above solution as described (12) and were separated by TLC on silica gel plates with petroleum ether-diethyl ether-acetic acid, 80:20:1 (v/v/v), as a developer. Lipid spots were located under ultraviolet light after spraying with a solution of dichlorofluorescein in ethanol and the spot corresponding to TG was excised and incubated in 0.5 ml of methanolic sodium methoxide (Sigma) at 50°C for 10 min (13). The fatty acid methyl esters thus produced were extracted with hexane and were separated by gas chromatography as described (14). Methyl esters were quantified by comparing the area under their peaks in the chromatogram to that of methyl heptadecanoate (produced by transmethylation of the internal standard). The μmol of TG in each aliquot of the homogenate were calculated as the sum of the μmol of the methyl esters divided by 3. TG lipase activity was then calculated as the rate of TG decrease over the incubation period. The coefficient of variation of the assay was 12%.

Statistical analysis

Results are reported as the mean \pm SE. Significant changes in the percentage of each TG acyl group from 0 to 30 min of incubation were detected by performing paired Student's *t*-tests. Comparisons of TG lipase activity between exercise and rest, as

well as among the five time points of adipose tissue sampling were performed by two-way (condition \times time) ANOVA with repeated measures on time. Post-hoc pairwise comparisons were performed through simple main effects analysis. The level of statistical significance was set at $\alpha = 0.05$. The SPSS (version 10.0) was used for all analyses.

RESULTS

There were no significant differences in age, weight, and height between the exercise and the control group. The age of the 15 subjects was 24.1 ± 0.5 years, their weight 81.2 ± 1.9 kg, and their height 1.81 ± 0.02 m. During the 30 min of cycling, the average power output of the exercise group was 106 ± 5 W and the average heart rate was 127 ± 1 beats min^{-1} . The weight of the 75 adipose tissue samples taken (five from each volunteer) was 27.0 ± 1.0 mg.

Seven fatty acid methyl esters derived from the TG of adipose tissue were detected in considerable amounts by gas chromatography, namely myristate (14:0), palmitate (16:0), palmitoleate (16:1 ω 7), stearate (18:0), oleate (18:1 ω 9), vacenate (18:1 ω 7), and linoleate (18:2 ω 6). Their molar percentage distribution at the onset of incubation is presented in **Table 1**. The distribution after 30 min of incubation (not shown) was very similar to that at the onset.

The time course of TG lipase activity of the two groups is presented in **Fig. 1**. ANOVA showed that the interaction of condition and time was significant ($P = 0.024$). In the exercise group, activity increased significantly 5 min after the onset of cycling ($P < 0.001$), reaching 6.4 times the baseline value (0.00992 ± 0.00198 vs. 0.00155 ± 0.00086 $\mu\text{mol TG min}^{-1} \text{mg}^{-1}$), and decreased gradually and significantly ($P < 0.05$) thereafter, reaching the lowest value during recovery. Enzyme activity did not change significantly with time in the control group and averaged 0.00203 $\mu\text{mol TG min}^{-1} \text{mg}^{-1}$. The only significant difference between the two groups was at 5 min ($P = 0.018$). TG decrease during the 30 min incubation period ranged from 6% in the baseline samples to 31% in the samples taken at 5 min of exercise.

DISCUSSION

The purpose of the present study was to monitor, for the first time, TG lipase activity in human adipose tissue

TABLE 1. Molar percentage distribution of acyl groups in adipose tissue TG (mean \pm SE)

Acyl Group	Percentage
14:0	2.5 ± 0.1
16:0	21.5 ± 0.2
16:1 ω 7	4.9 ± 0.1
18:0	3.5 ± 0.1
18:1 ω 9	50.1 ± 0.4
18:1 ω 7	2.0 ± 0.0
18:2 ω 6	15.5 ± 0.3

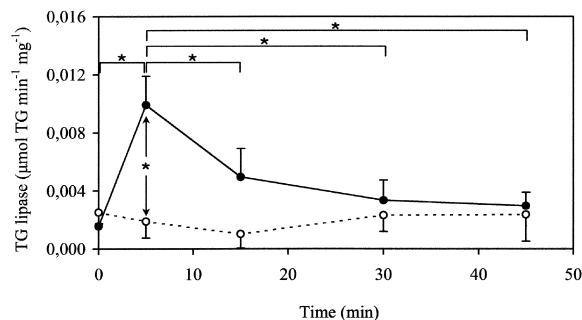


Fig. 1. Triacylglycerol lipase activity (mean and SE) of human adipose tissue during 30 min of moderate cycling and 15 min after passive recovery (closed circle) or during rest (open circle). Asterisks denote significant differences in pairwise comparisons ($P < 0.05$).

during exercise. To that end, a new method was devised measuring the actual activity (according to the phosphorylation state) of TG lipase in its natural place of action and on its natural substrate, i.e., adipose tissue TG. The lack of such a method has been pointed out by other researchers (15, 16).

To preserve, throughout the incubation period, the phosphorylation state of TG lipase at the time of adipose tissue sampling and the integrity of the enzyme, the homogenization buffer contained: *i*) EDTA to chelate Ca^{2+} and Mg^{2+} . Ca^{2+} is a known activator of proteinases, whereas Mg^{2+} is required by enzymes utilizing ATP, such as protein kinases and acyl-coenzyme A synthetases (involved in TG resynthesis), as well as by protein phosphatase-2C (17, 18), which, along with protein phosphatases-1 and 2A, has been shown to dephosphorylate TG lipase (18); *ii*) a phosphatase inhibitor mixture; *iii*) an inhibitor of the cAMP-dependent protein kinase to prevent additional phosphorylation of TG lipase; *iv*) three proteinase inhibitors (antipain, leupeptin, and pepstatin).

An additional advantage of our assay is the use of mild homogenization so as to conserve the morphology of the intracellular lipid droplets. This is important because TG hydrolysis requires the translocation of the lipase from the cytosol to the surface of the droplets (19, 20). Adhesion of the lipase to the droplets is modulated by proteins, such as perilipins. The latter are located at the droplet surface and, in response to lipolytic stimuli, undergo conformational changes facilitating the interaction of the lipase with TG (21, 22). Therefore, it was crucial to preserve all factors affecting TG lipase activity in a state as close to the *in vivo* condition as possible.

To document the reproducibility of our assay and to control a possible carry-over effect of sequential biopsying on TG lipase activity, we have included a control group who rested throughout the sampling period. Our data suggest that the assay was reproducible and that no carry-over effect occurred.

The main finding of the present study was that TG lipase activity increased early during exercise, reaching a peak at 5 min, which was over 6-fold higher than baseline. It is interesting that, although the subjects continued to

exercise, lipase activity gradually decreased and was significantly lower at all time points compared with 5 min. In accordance with this finding, TG lipase activity in rat muscle was found to be significantly higher than baseline after 1 and 5 min, but not after 10 or 60 min of electrical stimulation (23). This decrease in lipase activity despite the maintenance of the physical stimulus may be attributed to the well-known desensitization of β -adrenergic receptors, which would result in decreased activation of the cAMP cascade and, hence, decreased activation of TG lipase. Indeed, it has been reported that β -adrenergic stimulation of lipolysis in human adipose tissue was significantly higher before exercise than after 60 min of moderate exercise (24).

The absence of significant changes in the percentage distribution of TG acyl groups during the assay suggests that there was no preferential hydrolysis of some acyl group(s) by the lipase. This finding contrasts with published data that show a preferential release of polyunsaturated fatty acids from synthetic TG-analogs by crude preparations of rat HSL (25) and from isolated human adipocytes stimulated by lipolytic agents (26). The reasons for the discrepancy between the results of the present study and the aforementioned ones may be the shorter incubation time (0.5 vs. 1.5–24 h) or the different experimental conditions.

In conclusion, the present study demonstrates that TG lipase activity in adipose tissue peaks at 5 min of exercise and decreases afterwards, although the exercise stimulus continues. To measure acute changes in lipase activity, a novel assay, sensitive to the phosphorylation state of the enzyme, was developed. This assay may be further used to study the effect of different exercise conditions on TG lipase activity. Additionally, it would be interesting to examine the effect of exercise on the activity of the enzyme in different population groups, such as the obese, females, and athletes. Finally, our assay permits research on the effect of pharmacological or dietary interventions on TG lipase activity aimed at understanding the mechanism of action of known lipolytic agents and the development of new ones, given the intense interest of modern societies in discovering effective ways to reduce body weight and, in particular, body fat.

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