Response of Rat Tissue Lipases to Physical Training and Exercise[1] (36730)

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(Introduced by H. E. Sauberlich)

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Numerous studies have demonstrated increased blood free fatty acids (FFA) following prolonged exercise (1–5). These FFA are presumably mobilized from adipose depot stores in response to a negative energy balance (6) and may be an important determinant of the rate of oxidative metabolism (7–8). The lipase responsible for fatty acid mobilization is a hormone-sensitive lipase which responds to a variety of hormones, among which are epinephrine, norepinephrine, ACTH, glucagon, and TSH (9). Although the factors responsible for increased lipid mobilization during exercise are not well defined, the release of norepinephrine at sympathetic nerve endings within adipose tissue probably plays a major role (10). The release of FFA in response to exercise in previous studies has been estimated by measuring FFA increase in adipose tissue or blood (11). This measurement probably reflects the net result of mobilization and utilization in response to exercise and is not a satisfactory method of estimating the effect of a long-term physical conditioning program on the capacity of adipose tissue to mobilize FFA in response to adrenergic stimulation.

In addition to FFA mobilized from adipose tissue, esterified fatty acids in the form of triglycerides of chylomycron and low density lipoproteins are also utilized by extrahepatic tissues for energy production (12). These esterified fatty acids are liberated by the enzyme lipoprotein lipase prior to uptake by the tissue of utilization (13). The effect of exercise on rat heart, muscle, and adipose tissue lipoprotein lipase has been reported (14–16), but the effect of physical training[2] on this enzyme is lacking.

This study was initiated to investigate the effects of physical training, exercise, and exhaustion on adipose tissue epinephrine-sensitive lipase (ESL) and heart, skeletal muscle, and adipose tissue lipoprotein lipase (LPL) in rats.

Materials and Methods. The results reported in this paper are from two similar experiments designated I and II. Male rats of the Carworth CFN strain 5 weeks of age weighing 110 g were housed in individual cages and fed Wayne Lab Blox[3] (Expt. I), or a normal synthetic diet[4] (Expt. II) *ad libitum*. Prior to the start of experiments, rats were randomly divided into two groups designated trained and untrained. Untrained rats remained sedentary in their cages for the duration of the experiment, while the trained group was subjected to a physical conditioning program of treadmill[5] running. Trained rats were maintained on the training schedule described by Holloszy (17) for the duration of Expt. I (12 wk). The training schedule was condensed in Expt. II to 7 weeks. At the end of Expt. I, rats were running 5 days/wk

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

[2] "Physical training" will be used to mean a chronic daily exercise program of long duration, whereas "exercise" will indicate a single occurrence of work performance.

[3] Allied Mills, Chicago, IL.

[4] Diet composition (g/kg): lard (106), starch (250), cerelose (250), vitamin free casein (197), cystine (3), linoleic acid (20), vitamin diet fortification mixture (22), salt mixture R. H. (40), cellulose (112). All dietary components purchased from Nutritional Biochemical, Cleveland, OH.

[5] Quinton Instrument Co., Seattle, WA.

at 1.1 mph for 120 min with a 30-sec sprint at 1.8 mph every 15 min. At the end of Expt. II, rats were running 5 days/wk at 1.1 mph for 120 min with no sprints. All treadmill running was at an 8% grade. Throughout the training period, lighting was regulated to provide 12 hr of light and 12 hr of darkness. Room temperature was maintained at 20°.

Experiment I. Rats were sacrificed at weeks 4, 9, and 12 following rest, exercise, or exhaustion. Prior to sacrifice, untrained rats were run to exhaustion (untrained exhausted) and trained rats pair run for the same length of time at 0.5 mph, 8% grade. In addition, trained rats were run to exhaustion (trained exhausted) at 1.25 mph, 8% grade. Exercised animals were decapitated immediately after they were taken from the treadmill while control groups were sacrificed at rest.

Epinephrine-sensitive lipase activity was assayed by a modification of the method of Ho, Ho and Meng (18). Epididymal adipose fat pads were removed, rinsed in room temperature 0.15 *M* KCl and incubated with gentle shaking for 1 hr, 37°, pH 7.3, in Krebs–Ringer–bicarbonate buffer containing 5% bovine serum albumin.[6] The buffer was gassed with 95% O_2, 5% CO_2, prior to assay. Tissue-to-buffer ratio was 1:5 (w/v). L-Epinephrine[6] was present at 20 μg/ml buffer for one fat pad while the contralateral pad served as a nonepinephrine control. Following incubation, a 2.0 ml aliquot of the buffer surrounding the pad was removed, and FFA were extracted and titrated according to the method of Dole and Meinertz (19). Epinephrine-sensitive lipase (ESL) activity is expressed as: μEq FFA released/hr/g adipose tissue (epinephrine-stimulated pad minus the control fat pad).

Heart and skeletal muscle (quadriceps muscle group) were removed, rinsed in 0.15 *M* KCl and homogenized in 9 vol of Chappell–Perry buffer (20), pH 7.4. Heart was minced with scissors and muscle disrupted with an Omni-mixer[7] for 5 sec at 16,000 rpm, prior to homogenizing in a glass–Teflon Potter–Elvehjem homogenizer. Homogenates were centrifuged at 600*g* for 10 min in a refrigerated centrifuge, and 0.2–0.5 ml of the 600*g* supernatant was used for assay of lipoprotein lipase. Lipoprotein lipase was assayed by the method of Korn (21) using serum-activated Ediol[8] as substrate. Reactions were linear with respect to enzyme and incubation period. Following a 60-min incubation, the reaction was terminated and FFA were extracted and titrated as described above. Lipoprotein lipase (LPL) activity is expressed as: μEq FFA released/hr/g tissue or mg protein.

Experiment II. In Expt. II, rats were sacrificed at rest after 7 wk of training. At that time trained rats were running 120 min/day at 1.1 mph, 8% grade, no sprints. ESL was measured as described in Expt. I, except only one pad was used. The pad was split logitudinally to provide an epinephrine and control segment. The remaining fat pad, heart, and skeletal muscle were homogenized in Chappel–Perry buffer and 600*g* supernatants were prepared and assayed for LPL as described in Expt. I. Due to a shortage of rat serum, rabbit serum was used to activate Ediol for adipose LPL assays, and rat serum was used for heart and muscle LPL. Statistical comparisons (nonpaired *t* test) were performed according to Steel and Torrie (22). Protein was determined by an automated Lowry procedure [cited in (23)].

Results and Discussion. The effect of training period on ESL is shown in Table I. No training effect was evident at 4 wk, but by 9 wk and at 12 wk adipose tissue from trained rested rats had a significantly greater ($p < .01$) capacity to release FFA in response to epinephrine stimulation. It was noted that trained rats weighed less and had smaller fat pads than sedentary controls following 12 wk of training. To aid in interpretation of data, ESL activity was expressed in several ways and the results are summarized in Table II. The FFA content of fat pads following a 60-min incubation in the presence of epinephrine was significantly greater for trained animals ($p < .01$) indicating that FFA released into the medium was represen-

[6] Sigma Chemical Co., St. Louis, MO, fraction V BSA, USP purity epinephrine.

[7] Ivan Sorvall, Inc., Norwalk, CT.

[8] Lipostrate-CB, Calbiochem, Los Angeles, CA.

TABLE I. Effect of Training Period on Rat Adipose Tissue Epinephrine-Stimulated Lipolysis (Expt. I).[a]

Training period[b] (wk)	Physical condition		Significance
	Trained	Untrained	
4	9.81 ± 0.95 (6)	8.11 ± 0.95 (6)	NS
9	3.62 ± 0.54 (6)	1.36 ± 0.42 (4)	$p < .01$
12	4.10 ± 0.52 (5)	1.94 ± 0.20 (6)	$p < .01$

[a] Values expressed as μEq FFA/hr/g tissue, $\bar{X}$ ± SEM, number of animals in parentheses. Method of assay as described under materials and methods. Rats were sacrificed in rested state.

[b] At 4 wk rats were running 45 min/day at 1.0 mph; 9 wk, 96 min/day at 1.1 mph; and 12 wk, 120 min/day at 1.1 mph (1.8 mph sprint every 15 min for 30 sec).

tative of what was occurring in the fat pad itself. When ESL activity was expressed on a per fat pad basis, there was no difference between the trained and untrained groups. This was due to larger size of the fat pads from untrained rats. However, when the total fat pad activity was expressed on a kilogram of body weight basis, the effect of training was again evident ($p < .01$). Since adipose tissue protein was not measured in Expt. I, ESL activity could not be expressed on a protein basis. However, adipose tissue protein was measured in Expt. II, and the results of this experiment are shown in Table III. Confirming the results of Expt. I, ESL activity was significantly greater ($p < .05$) in trained animals on a per gram of tissue basis. The same training effect was indicated when the results were expressed on a milligram of protein basis, but the differences were not great enough for statistical significance at $p < .05$. Adipose tissue from trained rested rats was more sensitive to epinephrine stimulation in this study and agrees with the proposal by Lafrance *et al.* (24) that, in the rat, chronic exposure to endogenous or exogenous catecholamines leads to an increased sensitivity to the metabolic effects of noradrenalin and adrenalin. Daily treadmill running should provide a stimulus for chronic endogenous catecholamine production (25–26). It is also possible that the differences in response to epinephrine stimulation observed in this study may be in part due to a decreased sensitivity of fat cells from untrained rested rats. Hubbard and Matthew (27) have pro-

TABLE II. Effect of 12 wk of Training on Several Adipose Tissue Variables (Expt. I).[a]

Variable	Physical condition		Significance
	Trained	Untrained	
Body wt (g)	282 ± 14 (5)	377 ± 8 (5)	$p < .01$
Fat pad wt (g)	1.27 ± 0.12 (5)	2.19 ± 0.12 (6)	$p < .01$
Epinephrine stimulated lipolysis[b]	4.10 ± 0.52 (5)	1.94 ± 0.20 (6)	$p < .01$
Fat pad FFA[c]	4.02 ± 0.24 (5)	2.41 ± 0.17 (6)	$p < .01$
ESL/fat pad[d]	4.99 ± 0.26 (5)	4.37 ± 0.38 (5)	NS
ESL/fat pad/kg[e]	17.89 ± 1.42 (5)	11.61 ± 1.07 (5)	$p < .01$

[a] Values expressed as $\bar{X}$ ± SEM, number of animals in parentheses. Method of assay as described under Materials and Methods. Rats were sacrificed in rested state.

[b] μEq FFA/hr/g tissue released into media in response to epinephrine.

[c] μEq FFA/g content of fat pad following 1 hr incubation in the presence of epinephrine.

[d] Epinephrine stimulated lipolysis/1 fat pad, μEq FFA/hr/1 fat pad.

[e] Same as *d* except μEq/hr/1 fat pad/kg body weight.

TABLE III. Effect of 7 wk Training on Adipose Tissue Epinephrine-Stimulated Lipolysis (Expt. II).[a]

Epinephrine-stimulated lipolysis	Physical condition		Significance
	Trained	Untrained	
μEq FFA/hr/g tissue	4.50 ± 0.82 (8)	2.80 ± 0.39 (8)	$p < .05$
μEq FFA/hr/mg protein	0.81 ± 0.19 (8)	0.55 ± 0.11 (7)	NS

[a] Values expressed as $\bar{X}$ ± SEM, number of animals in parentheses. Method of assay as described in Materials and Methods. Protein was determined on 600g infranatant of homogenized adipose tissue, ESL determinations were on intact adipose tissue. Rats were sacrificed in rested state.

posed that the lipid content per cell or the surface area to volume ratio of the cell can alter noradrenaline-stimulated lipolysis. The effect of physical training on fat cell size and numbers is currently under investigation by our laboratory.

The effect of exercise and exhaustion following 12 wk of training is shown in Fig. 1. Exercise and exhaustion significantly increased ($p < .01$) basal lipolysis (FFA release without epinephrine) in both untrained and trained rats. The stress of running probably elicited adrenalin or other lipolytic hormone secretions prior to sacrifice that carried over with the tissue to the *in vitro* assay.

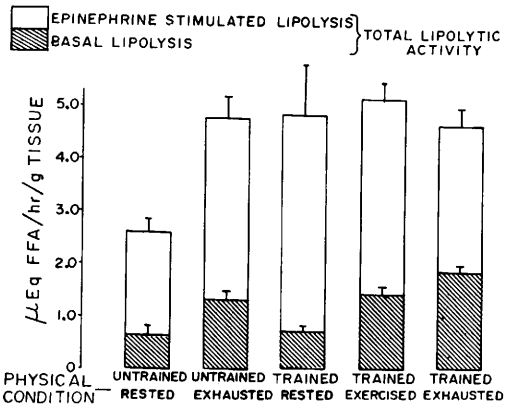

FIG. 1. Effect of training, exercise and exhaustion on rat adipose tissue lipolytic activity. Values expressed as $\bar{X}$ ± SEM. Untrained exhausted rats ran 142 ± 19 min at 0.5 mph, trained exercised rats were pair run with untrained rats and ran 147 ± 23 min at 0.5 mph, trained exhausted rats ran 291 ± 30 min at 1.25 mph. Method of assay as described under Materials and Methods. (Expt I).

Exhaustion of untrained animals was associated with increased ESL ($p < .01$) when compared to untrained rested animals. Trained animals, whether exercised or exhausted, failed to show a similar increase when compared to their rested counterparts. Unlike untrained animals, the potential to respond to epinephrine stimulation appeared to be present in trained animals regardless of prior physical activity. Contrary to the comparisons of ESL activity in rested animals, there were no differences between adipose tissue from trained and untrained rats in response to exogenous epinephrine stimulation following exercise. The increase in ESL activity following exhaustion in untrained rats may be related to hormonal secretion elicted by the stress of exercise. Under similar conditions in another study (28), we found elevated plasma glucocorticoids following exhaustion. Growth hormone has also been reported to be increased during exercise (29–30). Fain, Kovacev and Scow (31) have demonstrated increased lipid mobilization in adipose of fasted rats following a 1-hr incubation with growth hormone and glucocorticoids. Scow and Chernick (32) have reviewed the effects of growth hormone and glucocoricoids on lipolysis and suggest that they may act by stimulating synthesis of protein involved in cyclic AMP formation. It is possible that exhaustion may elicit hormonal secretions that stimulate the activation or formation of an enzyme associated with lipolysis, thus accounting for the greater ESL activity in untrained rats. Conceivably, protein synthesis could have occurred during the time (142 ± 19 min) it took untrained rats

to exhaust. An explanation of the failure of trained rats to exhibit a similar exhaustion response might be that they had previously responded to hormonal stimuli elicited by their daily running regime (as evidenced by their greater ESL activity compared to untrained rested animals) and were not capable of further response upon exhaustion. It should be stressed that the effects of exercise and exhaustion seen in these studies may be due to a multitude of factors such as availability of epinephrine receptor sites, cyclic AMP levels, enzyme levels, metabolite levels, and hormonal influence. The exact nature of ESL increase with exhaustion cannot be determined from our results. Whether the increase in ESL activity in untrained exhausted rats is of a similar nature to that occurring in trained rested animals should merit further investigation. The fact that ESL activity in untrained exhausted rats is similar to that of trained exercising rats should not be interpreted to mean that untrained exercising rats mobilize fatty acids to the same extent as trained exercising rats. This is further emphasized by the findings of Gollnick *et al.* (11) that the adipokinetic response of exercising rats is under both adrenergic and nonadrenergic control.

Lipoprotein lipase. Unlike ESL, LPL activity was not increased by training in rested animals in either muscle, heart, or adipose tissue (Table IV). However, LPL of heart ($p < .025$) and muscle ($p < .01$) was elevated following exhaustion of untrained animals (Fig. 2). Similar results have been reported by Nikkila, Torsti and Penttila (14, 15). The same trend toward increased LPL activity with exercise and exhaustion was seen in the trained animals but was not statistically significant. These results imply that LPL activity in heart and muscle is increased in untrained animals with exercise, probably reflecting increased lipoprotein triglyceride fatty acid uptake as an energy source. The observation that trained animals did not manifest the same magnitude of increase may be due to a heavier reliance on other energy sources such as glucose, FFA, or intracellular lipids.

Summary. The results of this study indicate that physical training causes an adaptive increase in epinephrine-stimulated fatty acid mobilization potential in adipose tissue of rested animals. Exhaustion caused an increase in ESL activity of similar magnitude in untrained animals. The exercise effect was not additive with the training effect when trained animals were exercised or exhausted.

Lipoprotein lipase was unaffected by training and increased in heart and muscle of untrained animals following exhaustion. Trained animals exhibited a similar but smaller trend toward increased LPL activity with exercise or exhaustion.

Taken together, the results of this study are compatible with the concept that exercise increases lipolysis in adipose tissue and LPL mediated triglyceride fatty acid uptake by muscular tissues. Physical training appeared to increase the responsiveness of adipose tissue to adrenergic stimulation. An increased adipokinetic response by adipose tissue of trained animals would be compatible with the observation in this (33) and other studies (34–35) that training increases the capacity of muscular tissue for fatty acid oxidation.

TABLE IV. Effect of 7 wk Training on Heart, Muscle, and Adipose Tissue Lipoprotein Lipase (Expt. II).[a]

Tissue	Physical condition		Significance
	Trained	Untrained	
Skeletal muscle	0.42 ± 0.08 (6)	0.36 ± 0.05 (9)	NS
Heart	0.82 ± 0.15 (9)	0.84 ± 0.09 (8)	NS
Adipose tissue	2.86 ± 0.49 (6)	2.76 ± 1.00 (7)	NS

[a] Values expressed as μEq FFA/hr/mg protein, $\bar{X}$ ± SEM, number of animals in parentheses. Method of assay as described in Materials and Methods. Rats were sacrificed in rested state.

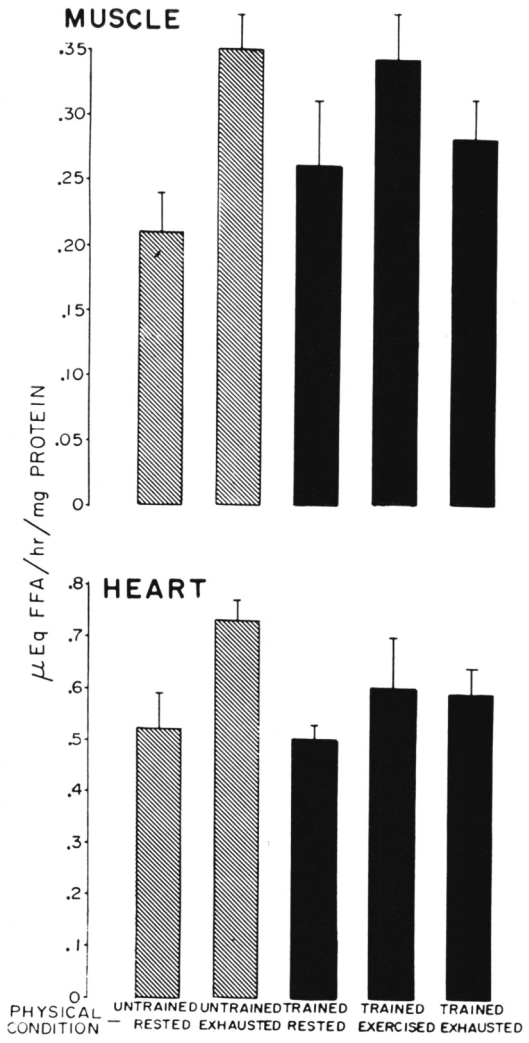

FIG. 2. Effect of training, exercise, and exhaustion on rat heart and skeletal muscle lipoprotein lipase activity. Values expressed as $\bar{X} \pm$ SEM. Exercise and exhaustion conditions same as in Fig. 1. Method assay as described under Materials and Methods (Expt I).

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