Adipose Tissue Cellularity and Lipolysis

RESPONSE TO EXERCISE AND CORTISOL TREATMENT

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ABSTRACT Male rats at 5 wk of age were subjected to 13 wk of intensive treadmill running to study the effect of exercise on adipose tissue cellularity and lipolysis. Untrained controls of the same age remained sedentary in their cages for the duration of the experiment. Adipocyte numbers were similar in epididymal fat pads from trained and untrained rats ($12.7\pm1.3\times10^6$ vs. $15.3\pm1.3\times10^6$ cells/pad), however trained rats had smaller fat pads containing smaller cells (0.09 ± 0.01 vs. 0.20 ± 0.04 μg triglyceride/cell). Adipocytes from trained rats possessed greater epinephrine-sensitive lipase activity than sedentary rats on a per cell, per milligram protein, per gram adipose tissue, or per fat pad basis. Although the smaller cells of the trained rats had greater epinephrine-sensitive lipase activity than the larger cells of the untrained rats, lipolysis was positively correlated with cell size within both treatment groups. Cortisol treatment of intact animals did not significantly affect in vitro adipose tissue lipolysis. The results of this study indicate that exercise training increased the potential of adipose tissue cells to release free fatty acids in response to epinephrine stimulation. Exercise training initiated at 5 wk of age had only a small effect on adipose tissue cell numbers but significantly decreased cell size.

INTRODUCTION[1]

Free fatty acids are mobilized from adipose depots in response to a negative energy balance (1), such as that created by exercise. The rate at which FFA are mobilized may determine their availability to the working muscle (1, 2) and represent an important determinant of the overall rate of oxidative metabolism (3, 4).

Situations that produce a sudden demand for energy stimulate fatty acid mobilization primarily through the release of norepinephrine by the postganglionic sympathetic nerve fibers (1) and the subsequent activation of a hormone-sensitive lipase in adipose tissue. FFA release by adipose tissue of physically trained rats has been shown to be especially sensitive to in vitro epinephrine stimulation (5-8). The exact mechanism for this apparent increase in fatty acid mobilization potential after long-term exercise training is not known. Due to the hyperplastic nature of adipose tissue, two mechanisms merit special consideration as possible explanations of the training-induced increase in lipolytic potential of adipose depots: (*a*) Exercise training may be associated with an increased number of cells with lipolytic activity constant per cell or (*b*) Exercise training may be associated with no change in cell number, but an increased lipolytic activity per cell. Previous investigations of the effect of exercise training on lipolysis have not been accompanied by simultaneous adipose tissue cellularity studies thus precluding an evaluation of the influence of cell size and cell number on lipolysis.

The possibility also exists that exercise training influences adipose tissue lipolytic response to epinephrine by chronic exposure to hormones that are released during the daily exercise periods. Several adipokinetic hormones increase in plasma after exercise (9, 10). Notable among these hormones is cortisol, which has been suggested to act in concert with other hormones to stimulate the formation of enzymes involved in cyclic AMP formation (11, 12) and/or potentiate the sensitivity of adipose tissue lipolysis to cyclic AMP (13, 14).

The present study was designed to investigate the influence of a long-term exercise training program on the cellular response of rat adipose tissue FFA release to epinephrine stimulation and to test the effect of

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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

exogenous cortisol administration on this response. In addition, the effect of a long-term exercise training program on rat adipose tissue adenyl cyclase activity is reported.

METHODS[2]

Three experiments are reported in this study. Experiment I tested the effect of exercise training and cortisol treatment on epinephrine-sensitive lipase and adipose tissue cellularity. Experiment II tested the effect of dietary intake on adipose tissue cellularity and lipolysis. Experiment III tested the effect of exercise training on adipose tissue adenyl cyclase.

Experiment I

Exercise program. Male Carworth CFN rats 5 wk of age, weighing 85–105 g, were initially divided into two groups designated trained (T)[3] and untrained (UT). Trained rats were exercised 5 days/wk on a motor-driven treadmill (Quinton Instruments, Seattle, Wash.) for 13 wk before sacrifice. Both duration and intensity of the exercise were progressively increased throughout the experimental period until the rats were running 120 min/day at 29.5 m/min, 8% grade, with a 30-s sprint every 10 min at 53.6 m/min. After this work load was attained it was held constant for 2 wk before sacrifice. UT rats remained sedentary in their cages for the duration of the experiment with the exception of a brief period of familiarization running on the treadmill during the week before sacrifice. Food and water consumption were ad lib. throughout the experiment. Commercial laboratory chow was fed during the first 8 wk of the experiment and a nutritionally adequate semipurified diet[4] was fed during the final 5 wk. Throughout the training period lighting was regulated to provide 12 h of light and 12 h of darkness. Room temperature was maintained at 20°C.

Experimental treatments. 3 days before the end of the experiment the T and UT animals were each subdivided into two groups. One-half of the T and one-half of the UT rats were injected subcutaneously with 1.25 mg of cortisol (hydrocortisone, Sigma Chemical Co., St. Louis, Mo.) per 100 g body weight. The cortisol was suspended in corn oil (10 mg/ml). Rats received daily cortisol injections 3 days before and on the day of sacrifice. Control rats from each group received injections of corn oil without cortisol. One-half the rats of each group (T, cortisol; T, control; UT, cortisol; UT, control) were sacrificed immediately after running to exhaustion on the treadmill and one-half were sacrificed at the same time in the rested state (48 h since last bout of exercise). T rats were exhausted at 43.9 m/min and UT rats at 20.1 m/min. Rats were exhausted at different work loads because T rats would not exhaust readily at 20.1 m/min and UT rats would not run at 43.9 m/min.

Tissue preparation. Rats were sacrificed by decapitation. Epididymal adipose tissue was immediately removed and rinsed in room temperature 0.15 M KCl. One fat pad was blotted, weighed, and subsequently utilized for adipose tissue cellularity and metabolic studies. The remaining fat pad was homogenized in 4 vol 0.15 M KCl with a Potter-Elvehjem homogenizer. The homogenate was centrifuged 600 *g* for 10 min in a refrigerated (4°C) centrifuge. The infranate was filtered through two layers of cheesecloth and analyzed for protein by an automated Lowry procedure (15).

Fat cell isolation. Fat cells were isolated by a modification (16) of Rodbell's procedure. Siliconized glassware and polyethylene labware was used throughout the isolation. After incubation with collagenase (Sigma Chemical Co., crude collagenase, type I from *Clostridium histolyticum*) fat cells were floated to the surface by centrifugcation 300 *g* for 2 min. The sediment and infranate were removed by gentle aspiration and the cells were washed three times with Krebs-Ringer phosphate buffer, pH 7.4, prepared without calcium or glucose and containing 4% bovine serum albumin (fatty acid poor). All washes were conducted with room temperature buffer. After the final wash, cells from one fat pad were diluted to 40 ml with buffer. Cells were uniformly dispersed in buffer before aliquoting for DNA and epinephrine-sensitive lipase assay by stirring in a polyethylene beaker with a Teflon stirring bar over a magnetic stirrer. Microscopic examination of isolated fat cells stained with methylene blue revealed freely dispersed spheres, each containing a nucleus. The washed cell suspension was free of stromal-vascular cells.

Fat cell DNA and lipid analysis. DNA content of the isolated washed fat cells was determined by a modification of the glass fiber filter method of Novak and Monkus (17). 5-ml aliquots of the stirred adipocyte suspension were filtered, washed, defatted, and dried before reacting the sample with twice the volume of reagents recommended by Novak and Monkus. DNA standards (calf thymus DNA, Sigma Chemical Co.) were spotted on disks previously extracted identically to the samples after the filtering of 5 ml of buffer containing no adipocytes. Samples were read at 610 nm against blanks containing disks through which buffer without cells had been filtered and extracted. The calculation of cell numbers was based on the assumption of 7×10^{-6} μg DNA/cell (18).

Triglyceride content of isolated fat cells was determined by a semiautomated fluorometric procedure (19). Results are expressed as milligrams of triglyceride (triolein) per cell and represent a measure of cell size (20).

Microscopic determination of fat cell diameter. Cell size was also estimated by direct microscopic determination of fat cell diameter (21) in four T and four UT rested rats. A minimum of 100 cells was measured from each rat.

Plasma corticosterone. Plasma corticosterone was assayed by a competitive protein-binding method (22).

Epinephrine-sensitive lipase (ESL). The isolated fat cells were assayed for ESL by a modification of the method of Hubbard and Matthew (23). 1 ml of fat cell suspension was added to 3 ml of Krebs-Ringer phosphate buffer containing no calcium or glucose and 4% bovine

[2] 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.

[3] *Abbreviations used in this paper:* ESL, epinephrine-sensitive lipase; T, trained; UT, untrained.

[4] Diet composition (components expressed as percent of diet). All components except for hydrogenated vegetable oil (Crisco, Procter & Gamble Co., Cincinnati, Ohio) purchased from Nutritional Biochemicals Corp., Cleveland, Ohio; vitamin-free casein, 15.8; L-cystine, 0.3; hydrogenated vegetable oil, 18.0; linoleic acid, 2.0; cerelose, 54.0; alphacel, 4.0; vitamin diet fortification mixture, 2.2; salt mixture, Rogers-Harper, 4.0.

serum albumin (fatty acid poor), pH 7.4. Incubations were conducted in polyethylene vials placed in a shaking water bath for 30 min at 37°C. Epinephrine-stimulated incubations contained 20 μg L-epinephrine (E-4250, Sigma Chemical Co.), control vials contained no epinephrine. The reaction was terminated by the addition of 5 ml heptane : isopropanol : 1 N H_2SO_4 (10 : 40 : 1, vol/vol/vol) and the FFA extracted and titrated by the method of Dole and Meinertz (24). ESL activity was calculated by subtracting the FFA liberated by the control cells from the FFA released by the cells incubated in the presence of epinephrine.

A set of samples identical to the ones described above were simultaneously incubated and frozen by immersing the vials in dry ice-ethanol. The frozen samples were subsequently thawed, sonicated, (8 A, 30 s Branson Sonifier LS-75, Branson Instrument Co., Stamford, Conn.) and analyzed for glycerol by a commercial method (glycerol Stat-Pack, Calbiochem, San Diego, Calif.). The calculation of ESL activity based on glycerol release was analogous to that detailed for FFA. FFA reesterification was calculated by the balance technique described by Vaughan (25).

Experiment II

Experiment II was designed to permit a more rigorous interpretation of the data from experiment I by testing the effect of dietary intake on ESL activity. A second group of Carworth CFN rats, starting at 7 wk of age were trained identically to those of experiment I. Before the start of the experiment, the animals were randomly divided into four groups of 10 rats each, designed T (ad lib.), UT (ad lib.), UT (pair fed), and UT (pair weighed). The two ad lib. groups were fed a commercial lab chow (Purina lab chow, Ralston Purina Co., St. Louis, Mo.) ad lib. The UT (pair fed) group was fed identical quantities of feed as the T (ad lib.) group, and the UT (pair weighed) group was fed a quantity of diet designed to keep their body weight as close as possible to that of the T (ad lib.) group.

All procedures and assays for experiment II were as described for experiment I. Rats were sacrificed in the rested state. Due to the laborious nature of microscopic cell diameter measurements, cell size measurements were done on five randomly chosen rats per treatment group. Values reported in Table II for glycerol analysis represent five to eight rats per group due to the ruining of several samples by forming an opaque emulsion caused by a too intense sonication of adipocytes before analysis.

Experiment III

Adenyl cyclase. Adenyl cyclase was assayed in a second group of Carworth CFN rats. Rats were trained identically to those of experiment I except they were held at the maximum work load for 4 wk instead of 2 wk (15 wk of training). Before the start of the experiment, rats were randomly assigned to two groups designated T or UT. UT rats remained sedentary in their cages for the duration of the experiment and were pair fed according to the food consumption of the T group. Rats were fed the same semipurified diet as those of experiment I. Food was removed from all rats 12 h before sacrifice. Rats were sacrificed in the rested (24 h since the last exposure to exercise) condition by decapitation. One epididymal fat pad was removed, rinsed in ±4°C 0.15 M KCl, and homogenized in 3 vol of 0.05 M Tris-HCl buffer, pH 7.6, with a small TenBroeck homogenizer. The 2,000-*g* protein pellet was prepared and assayed for adenyl cyclase as described by Greene, Herman, and Zakim (26). After the addition of 0.1–0.15 mg of protein, the reaction was incubated for 10 min at 30°C with gentle shaking. Each reaction vessel contained 1.2 μCi of [α-^{32}P]adenosine 5′-triphosphate (47 Ci/mM, ICN, Irvine, Calif.). Reactions were terminated and nucleotides separated on alumina columns as described by White and Zenser (27). 2 ml of the appropriate column fraction was collected, suspended in jelled Aquasol (New England Nuclear, Boston, Mass.), and counted for ^{32}P and ^{3}H in a liquid scintillation counter. Results are expressed as disintegrations per minute of 3′,5′-cyclic [^{32}P]AMP produced per minute per epididymal fat pad.

Statistical analysis. Treatment means were compared by the Neuman-Keul's procedure (28). Where no significant ($P > 0.05$) treatment effects were evident for cortisol, exhaustion, or dietary intake, rats were pooled into two groups, T and UT. The effect of physical training was then estimated by a nonpaired *t* test (28). All values are shown as mean±SEM.

RESULTS

Effect of exercise on body weights and food consumption. After 13 wk of intensive physical training, T rats weighed significantly less (294±7 vs. 404±6 g; $P < 0.001$) than UT rats of the same age (18 wk). T rats also consumed significantly less food per week (101±3 vs. 133±4 g/rat per wk; $P < 0.001$) than their sedentary counterparts, although both groups consumed similar amounts of diet/100 g body weight.

Effect of exhaustion on fat pad composition and lipolysis. Exhaustive exercise performed immediately before sacrifice had no significant effect on fat pad composition or in vitro lipolysis. Therefore data collected from rested and exhausted animals were pooled according to training and cortisol treatment before statistical analysis for cortisol treatment effects.

Effect of cortisol administration on fat pad composition and lipolysis. Subcutaneous injection of cortisol increased resting plasma corticosterone titers in both T and UT rats but had no significant effect on any of the other parameters measured except fat pad weight of UT rats. Cortisol treatment increased plasma corticosterone from 28.2±5.1 μg/100 ml to 98.2±19.1 g/100 ml in UT rats and from 24.9±4.6 μg/100 ml to 84.9±16.7 g/100 ml in T rats. Cortisol treatment did not affect fat pad weight in T rats but was associated with decreased fat pad size in UT rats (4.1±0.3 vs. 3.5±0.2 g/pad). Since cortisol treatment did not significantly affect fat pad cell number, cell size, protein, or ESL, data for subsequent comparisons were pooled according to training.

Effect of physical training on epididymal fat pad composition. Fig. 1 illustrates the effect of 13 wk of treadmill running on epididymal fat pad composition. Since fat pad weight was decreased by cortisol treatment in the UT group, comparison of pooled data for fat pad weights shown in Fig. 1 is not justified on a statistical basis. Analysis of unpooled data for fat pad weights revealed that training significantly decreased fat pad

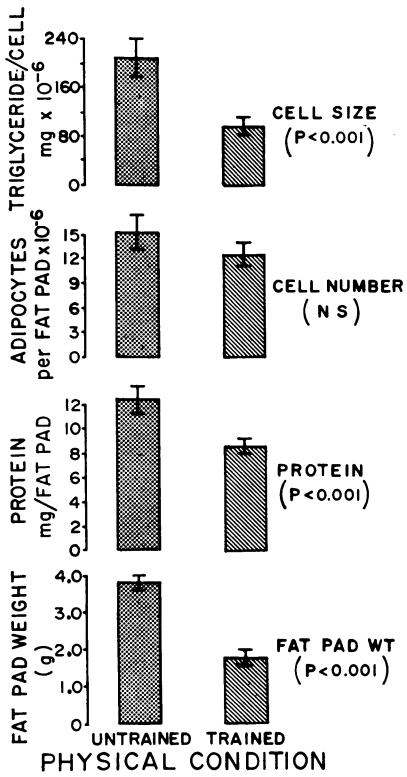

FIGURE 1 Effect of exercise training on epididymal fat pad composition. Values are $\bar{x}\pm$SEM for 31 animals per group. Levels of statistical significance are shown in the right-hand margin.

weight in both control and cortisol-injected rats ($P < 0.05$). Fat pad weights are presented in Fig. 1 for the purposes of uniformity in relation to the other three parameters shown in the figure. Physical training was associated with a decrease in total fat pad weight and protein content ($P < 0.001$). Fat pad cell numbers from T rats were not significantly different from those of UT rats. The adipocyte triglyceride content was significantly decreased by training ($P < 0.001$) indicating that T rats had smaller cells.

Cell size was also estimated by microscopic determination of the diameter of fat cells from four T and four UT rats. These results are shown in Fig. 2. The mean fat cell diameter of the UT rats was significantly greater than that of the T rats (86.3±3.6 vs. 67.8±3.3 μm; $P < 0.01$).

Effect of physical training on ESL and FFA esterification. Neither cortisol administration nor exhaustion had any significant effect ($P > 0.05$) on lipolysis or FFA esterification. Thus, the data were pooled according to physical condition (T or UT) before evaluation of the effects of training.

Fig. 3 shows the effect of physical training on epinephrine-stimulated lipolysis. To aid in interpretation of these data, FFA release was calculated by four commonly used methods of expressing adipose tissue ESL activity. Training significantly increased ESL activity ($P < 0.001$) regardless of the method of expressing the data.

Since net FFA release is dependent upon both lipolysis and reesterification, glycerol release was also measured. The results are shown in Table I. Several samples were lost due to vial breakage, thus these values represent the mean of 25 rats for T group and 23 rats for the UT group. The effect of training on ESL was apparent from both the increased glycerol release ($P < 0.001$) and FFA release ($P < 0.001$). However, appreciable FFA reesterification was occurring in both

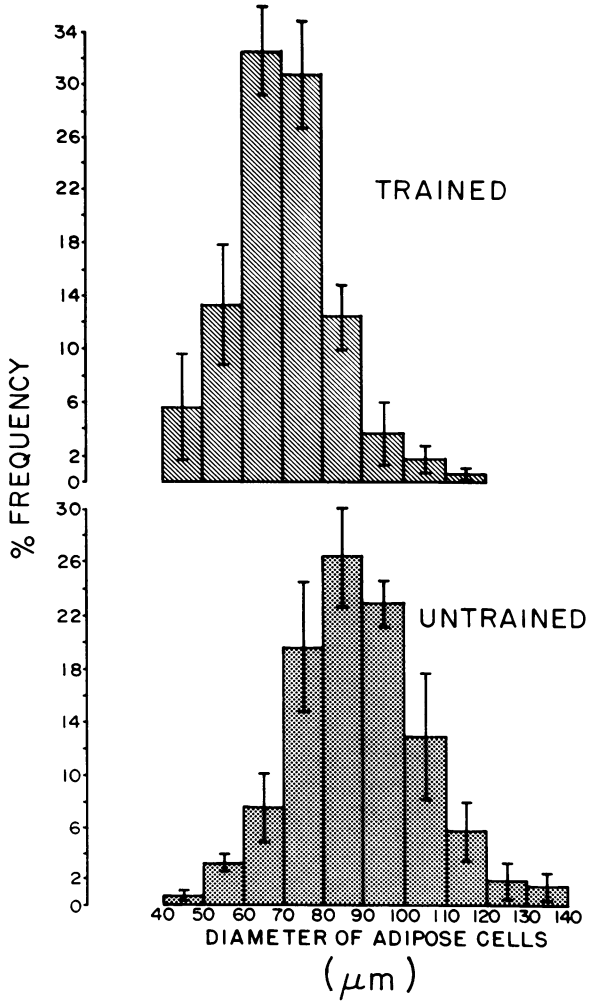

FIGURE 2 Effect of exercise training on size distribution of adipocytes isolated from epididymal adipose tissue. Each value represents the $\bar{x}\pm$SEM of percent frequency of occurrence for four rats. The mean cell diameter for the T rats was 67.8±3.3 compared to 86.3±3.6 μm for the UT rats.

groups with significantly greater ($P < 0.025$) reesterification occurring in the T rats.

Relationship between cell size and lipolysis and reesterification. Although considerable variation existed between individual rats, mean cell size (milligrams triglyceride/10^6 cells) was positively correlated ($P < 0.01$) with lipolysis (microequivalents FFA/h per 10^6 cells) for both the T ($r = 0.57$) and the UT ($r = 0.74$) groups. This correlation was apparently not due to greater reesterification of FFA by smaller cells since FFA reesterification (micromoles FFA/h per 10^6 cells) was not significantly correlated ($r = 0.04$ T, 0.07 UT) with cell size.

In an attempt to separate the effect of cell size from the effect of training on ESL, 9 T and 15 UT rats possessing similar mean cell sizes (88-138 mg triglyceride/10^6 cells) were selected and compared. T rats (102±6 mg triglyceride/10^6 cells) had significantly greater ($P < 0.001$) ESL (3.28±0.68 vs 1.11±0.16 μeq FFA/h per 10^6 cells) than UT rats (104±4 μg triglyceride/10^6 cells).

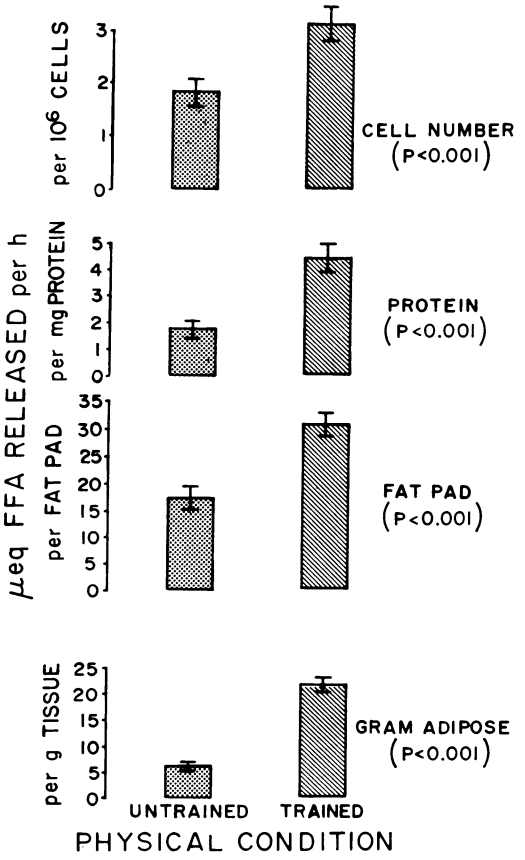

FIGURE 3 Effect of exercise training on isolated fat cell ESL activity, expressed several ways. Activity was measured as described under Methods. Notations in right-hand margin represent basis of expression of lipolytic activity and level of statistical significance. Values are $\bar{x}$±SEM for 31 animals per group.

TABLE I
*Effect of Physical Training on Lipolysis and Reesterification**

Treatment	Lipolysis: Glycerol release‡	Lipolysis: Theoretical FFA release§	Lipolysis: Actual FFA release‖	FFA reesterification¶
Untrained	0.7±0.1	2.2±0.3	1.2±0.2	1.0±0.1
Trained	1.6±0.2	4.8±0.6	2.8±0.3	2.0±0.5
Significance level	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.025$

* Values are $\bar{x}$±SEM for 23 rats per untrained group, 25 rats per trained group, assays conducted as described under Methods.
‡ μmol glycerol/10^6 cells per h.
§ μmol glycerol × 3 (assuming no reesterification).
‖ μmol FFA/10^6 cells per h net result of lipolysis and reesterification.
¶ μmol FFA/10^6 cells per h, Theoretical-Actual, applying the net balance technique of Vaughn (25).

Effect of dietary intake on lipolysis. Since the rats in experiment I were fed ad lib. and UT rats consumed more diet than T rats, it was not possible to state with certainty that the increase in ESL observed in trained rats was the result of training. The results of experiment II are shown in Table II and illustrate that dietary intake was not a significant factor in the increase in ESL occurring in T rats. T and UT (pair-fed) rats consumed an average of 82% as much diet as the UT (ad lib.) group, and the UT (pair-weighed) group consumed 72% as much diet as the UT (ad lib.) rats. There was no significant difference in body weights between the T and UT pair-weighed rats. Pair-fed UT rats were intermediate in body weight between T and UT (ad lib.) rats. Dietary intake did not have any statistically significant effect on fad pad weight, cell numbers, cell size, or ESL in UT rats (ad lib. vs. pair fed vs. pair weighed). Confirming the results of experiment I, T rats possessed significantly smaller ($P < 0.05$) fad pads, smaller adipose tissue cells, and greater ESL activity. As in experiment I, adipocytes per fat pad tended to be less, but not significantly so.

Effect of exercise training on adenyl cyclase. The effect of exercise on epididymal adipose tissue adenyl cyclase is shown in Fig. 4. Although the UT rats were pair fed with the T rats in this experiment, they had significantly greater ($P < 0.001$) body weights (354±7 vs. 289±6 g) and fat pad weights (4.0±0.3 vs. 1.4±0.1 g). Since food intake was identical, these differences were probably due to an increased energy demand for the work of exercise. Although the mass of the epididymal fat pads of T rats was less than that of the UT rats, the adipose organ from T rats contained significantly ($P < 0.01$) greater adenyl cyclase activity

TABLE II

*Effect of Dietary Intake on Adipose Tissue Cellularity and Lipolysis**

Treatment	Body wt	Fat pad wt	Adipocytes per fat pad	Adipocyte cell size‡	ESL§	
	g	*g*	*×10⁶*	*μm*	*μeq FFA/ h/fat pad*	*μmol glycerol/ h/fat pad*
Untrained (ad lib.)	437±13[a] (9	2.6±0.2 (9)	16.4±2.0 (8)	71.2±2.6 (5)	6.2±3.0 (9)	4.9±1.5 (8)
Untrained (pair fed)	367±10[b] (10)	2.8±0.3 (10)	16.3±2.7 (8)	76.4±1.7 (5)	7.1±3.5 (10)	2.4±0.8 (7)
Untrained (pair weighed)	310±4[c] (10)	2.2±0.2 (10)	12.5±1.6 (8)	71.8±1.4 (5)	9.0±3.3 (10)	4.4±1.5 (8)
Trained (ad lib.)	300±8[c] (10)	1.2±0.1[a] (10)	10.8±2.3 (10)	53.8±2.2[a] (5)	16.4±4.3 (10)	13.0±4.0[a] (5)

* Values shown represent $\bar{x}$±SEM, number of animals in parenthesis. Means not sharing a common letter-superscript are significantly different by Newman-Keul's analysis, $P < 0.05$.

‡ Five rats per group were randomly chosen and cell diameters for 100 cells per rat were measured as described under Methods.

§ ESL activity was determined as described under Methods. Activity shown is that portion attributable to epinephrine stimulation. T rats had a significantly greater FFA release ($P < 0.03$) compared to pooled UT rats, 16.4±4.3 (10) vs. 7.4±1.8 (29).

than UT rats. Adipose tissue cell number was not measured in this experiment, however, since the training regime was almost identical to that of experiments I and II, it may be assumed that cell numbers were not influenced by training. Therefore the increase in adenyl cyclase activity per fat pad probably represents an increased activity per cell.

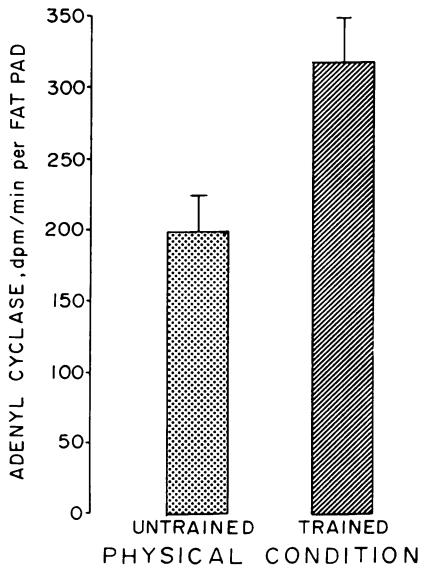

FIGURE 4 Effect of exercise training on epididymal fat pad adenyl cyclase activity. Activity was measured as described under Methods. Values represent $\bar{x}$±SEM for 11 rats in the UT group and 23 rats in the T group. The mean for the T group is significantly greater ($P < 0.01$) than that of the UT.

DISCUSSION

The adipose organ is unique among the other organs of the body for its ability to markedly expand and contract in mass under the influence of age, endocrine status, and energy balance (23, 29, 30). Recent studies have demonstrated the need to accompany investigation of adipose tissue metabolism with simultaneous cellularity studies to aid in interpreting experimental findings. Previous studies from this laboratory have demonstrated that physical training increased in vitro fat pad sensitivity to epinephrine stimulation or a per gram of tissue basis (7). These results, however, did not allow an estimation of individual fat cell lipolytic activity. Oscai, Spirakis, Wolf, and Beck (31) subsequently demonstrated that exercise initiated at an early age can influence fat cell numbers and size. Since cell numbers and cell size both exert strong influences on epinephrine-stimulated lipolysis (16, 23, 32), it became of interest to test the hypothesis that the apparent increase in ESL resulting from long-term exercise training could be attributed to (*a*) an increased lipolytic activity per cell or (*b*) to an altered number of cells per pad.

The results of this study demonstrated that long-term exercise training did not significantly affect adipose tissue cell numbers but did increase ESL per cell. Although fat cell numbers per epididymal fat pad tended to decrease in response to training, the decrease was not statistically significant. The lack of a significant physical training effect on fat cell numbers may be related to the stage of development of the rats when first exposed to exercise. The first 6 wk of life is a time of rapid cell proliferation in the rat (30). It is reasonable to assume that implementation of exercise earlier in this

hyperplastic stage of adipose development might exert a greater effect on cell numbers as shown by Oscai et al. (31) than when exercise was begun later in the developmental stage. The rats used in this study were probably past the primary hyperplastic state of fat depot development. Exercise took place during the stage of simultaneous hyperplasia and hypertrophy (6–15 wk) (30) and thus it could have exerted a smaller effect on cell numbers.

As the adipocyte decreases in cell size, the surface area-volume ratio increases. A training-induced increase in surface area-volume ratio has been suggested as a possible mechanism that may result in a more rapid release of FFA due to the accessibility of the cells to circulating and adrenergic catecholamines (33, 34). Similar to previous investigations on the relationship of cell size to lipolysis (32, 35) the results of this study revealed that within treatment groups, ESL tended to increase as cell size increased. This relationship was true for both the T and UT rats. Although exercise training influenced cell size and lipolysis in opposite directions, it did not alter the positive relationship that existed between these two variables. In addition, a comparison of selected T and UT rats sharing a common distribution of mean cell sizes indicated that T rats possessed greater ESL than UT rats. This suggests that exercise influences the sensitivity of adipocytes to epinephrine in a manner other than changes solely in cell size.

An increased reesterification of FFA by larger fat cells of sedentary rats might create the illusion of an "apparent" increase in FFA release by smaller fat cells of T rats; however, this was not the case in this study. Fat cells from T rats reesterified more FFA than cells from UT rats, agreeing with a previous report demonstrating enhanced glyceride synthesis by adipose tissue of T rats (36). These results would appear to rule out the possibility that the alteration of lipolysis was secondary to an alteration in carbohydrate metabolism (i.e., decreased conversion of glucose to glyceride glycerol in T rats).

As a working hypothesis, we attempted to test cortisol as a lipolytic-potentiating agent in adrenally sufficient rats. Assuming that the stress of repeated treadmill running elicited enhanced cortisol secretion by the adrenals (9, 10, 37), adipose tissue from T rats would have been chronically exposed to a hormone known to function in enzyme induction of lipolysis (11-13). However, under the conditions of this study, no evidence was found to implicate cortisol as the primary effector in the potentiation of adipocytes from T rats to epinephrine stimulation. These results agree with those of Taylor, Murray, and Secord (38) but do not rule out the involvement of cortisol in the adaptation of lipolysis to exercise training. The length of time rats were exposed to exogenous cortisol may not have been sufficient to elicit an effect on lipolysis, although the dose level and period of administration were sufficient to affect carbohydrate metabolism in liver and muscle of these same rats (39). Alternatively, cortisol may have required supplemental levels of another hormone such as growth hormone (11-13) to exert an effect.

Exhaustive exercise performed immediately before sacrifice did not significantly influence subsequent ESL determinations in the isolated adipocytes. The results agree with a previous study of the effect of exhaustion on ESL in T rats, but do not agree with the reported increase in ESL after exhaustion of UT rats (7). The reason for this discrepancy is not apparent, but may relate to the comparatively longer period of time required to prepare isolated adipocytes after exhaustion in this study as opposed to that elapsed between exhaustion and the initiation of intact fat pad incubations.

The results of the present study agree with and extend previous findings of increased sensitivity of adipose tissue FFA release to in vitro epinephrine stimulation after physical training. This adaptation appears to be a result of increased lipolytic activity per cell that is associated with but not established to be the direct result of smaller cell size. The adaptive increase in ESL is not a function of reduced intake caused by the hypophagia of strenuous exercise. Neither does it appear to be attributable solely to exposure of adipose tissue to increased blood cortisol titers. The results of this study do not rule out a cooperative interaction of cortisol working in conjunction with other hormones secreted in response to the stress of chronic exercise.

Numerous reports have demonstrated 3′,5′-cyclic AMP to be involved in a variety of metabolic control mechanisms (40). The release of FFA by adipose tissue in response to catecholamine stimulation is believed to result from the activation of adenyl cyclase at the cell membrane with a subsequent increase in intracellular cyclic AMP which activates a protein kinase which in turn activates a hormone-sensitive lipase (40). When FFA or glycerol release of adipose tissue incubated in the presence of epinephrine is measured in vitro, the entire sequence of events resulting in lipolysis of stored triglycerides is commonly termed "epinephrine-sensitive lipase" activity. The results of the portion of this study on adenyl cyclase suggest that the adaptive increase in adipose tissue lipolytic potential in trained rats may be largely mediated through adenyl cyclase. This contention is further indicated by comparing the magnitude of increase in rat adipocyte epinephrine-stimulated lipolysis in the trained rats of experiment I ($\sim 80\%$) with the ($\sim 60\%$) increase in adenyl cyclase activity observed in experiment III.

The metabolic responses of adipose tissue to exercise training appear to be in concert with oxidative adaptations occurring in skeletal muscle. The apparent increase in the ability of adipose tissue to synthesize triglycerides and mobilize FFA (7, 36) after a rigorous physical training program may be an essential secondary adaptation to an increased capability of skeletal muscle to oxidize fatty acids (41, 42) and ketone bodies (43, 44) during exercise. In a broader sense, the present study demonstrates the adaptability of adipose tissue metabolism to the stress of exercise and ascribes the adipose organ a central role in the adaptation of energy metabolism to physical training.

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