# Cyclic AMP metabolism in adipose tissue of exercise-trained rats[1,2,3]

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**Abstract** Cyclic AMP metabolism in epididymal adipose tissue of exercise-trained rats was examined to determine if training induced changes in cyclic AMP production or inactivation. Beginning at 7 weeks of age, male rats were physically trained by 12 weeks of treadmill running. Pair-fed control rats remained sedentary in their cages for the duration of the experiment. Tissue levels of cyclic AMP were measured in epididymal adipose tissue slices incubated with norepinephrine. Adenyl cyclase was assayed in adipocyte ghost cell preparations and low-$K_m$ phosphodiesterase was assayed in homogenates of adipose tissue. In response to norepinephrine stimulation, tissue cyclic AMP levels were reduced in trained compared to untrained rats. Training increased the ratio of activity of phosphodiesterase relative to adenyl cyclase. The results of this study indicate that cyclic AMP production in response to norepinephrine stimulation is not increased by training and may even be reduced, implying that adipose tissue cyclic AMP levels may be under a greater degree of control in trained rats. Modulation of adipose tissue cyclic AMP levels may function to regulate more closely the duration of lipolysis in exercise-trained rats.

**Supplementary key words** adenyl cyclase · phosphodiesterase

Bouts of exercise are accompanied by an increase in circulating catecholamine levels (1–3). Chronic exposure of target organs to elevated levels of catecholamines results in an increased sensitivity of these tissues to the metabolic effects of epinephrine or norepinephrine (4). Prolonged physical training increases the sensitivity of isolated fat cells to the lipolytic action of epinephrine (5, 6), agreeing with the suggestion of Östman and Sjöstrand (7) that chronic exercise increases the sensitivity of tissues to catecholamines. Such an adaptation may be important in the mobilization and utilization of energy substrates by exercising animals (7, 8).

The lipolytic response of adipose tissue to catecholamines is believed to be the end result of the initial activation of adenyl cyclase at the adipocyte membrane with a subsequent increase in the intracellular levels of cyclic 3′,5′-adenosine monophosphate (cAMP) (9). Since the formation of cAMP is the initial step in the lipolytic scheme, this appeared to be a feasible control point for an exercise-induced adaptation of lipolysis to be manifested.

The present investigation was initiated to determine if the control of lipolysis in epididymal adipose tissue of exercise-trained rats differs from that of sedentary rats with respect to cAMP metabolism. Since the levels of cAMP are controlled by both its production and its destruction, we have investigated the effect of exercise training on adipose tissue cAMP levels as well as the activities of two enzymes regulating cAMP levels, adenyl cyclase and phosphodiesterase.

## METHODS

### Animals and experimental design

Male Carworth CFN rats (Carworth, Portage, MI) 7 weeks of age, weighing 130–140 g, were housed in individual cages in a room regulated to 22°C with 12 hr of light and 12 hr of dark. Rats were randomly allotted to groups designated as trained or untrained. Untrained rats remained sedentary in their cages for the duration of the experiment and were pair-fed a stock diet (Ralston Purina Co., St. Louis, MO) according to the food consumption of the trained rats

Abbreviations: cAMP, cyclic 3′,5′-adenosine monophosphate; BSA, bovine serum albumin; FFA, free fatty acids; TCA, trichloroacetic acid.

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

[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] A previous report of a portion of this material has been published: Askew, E. W., A. L. Hecker, V. G. Coppes, and F. B. Stifel. Cyclic AMP metabolism in adipose tissue of exercise trained rats. *Federation Proc.* **36:** 1157, 1977.

who consumed the diet ad libitum. Trained rats were physically conditioned by a standardized 12-week progressive treadmill training program as described previously (6). At the end of 12 weeks of training, rats were running for 120 min per day at 29.5 m per min with 30-sec sprints at 42.9 m per min interspersed at 10-min intervals throughout the run. The treadmill (Quinton Instruments, Seattle, WA) running surface was set at an 8° incline throughout the experiment.

### Tissue collection

Rats were killed by cervical dislocation after a 12-hr overnight fast. Trained rats were not exercised for 24 hr prior to killing. The left epididymal fat pad was removed, rinsed in room temperature 0.15 M KCl, and used to prepare adipocyte ghosts for the adenyl cyclase assay. The right epididymal fat pad was similarly removed and sectioned longitudinally. One half was used for the preparation of crude homogenate for the assay of phosphodiesterase, while the second half was utilized in the assay of tissue levels of cAMP.

### Adipocyte ghost adenyl cyclase

The left epididymal fat pad was minced into 2–4 mm pieces and incubated with collagenase (5 mg/ml, collagenase R, Calbiochem, San Diego, CA) in Krebs-Ringer bicarbonate buffer (6 ml/g, buffer/tissue) containing 4% bovine serum albumin (BSA, fraction V, Sigma Chemical Co., St. Louis, MO) and one-half the usual concentration of $Ca^{+2}$ (0.055 M), pH 7.4. Isolated fat cells were prepared by a modification (6) of the method of Rodbell (10). The adipose tissue was incubated with the buffer containing collagenase (37°C, gentle shaking) until disaggregation was complete (115 min). Cells were washed three times with fresh buffer. The infranatant containing cellular debris was removed by aspiration following centrifugation at 300 *g* for 3 min at room temperature. After the final wash, the infranatant was discarded, leaving the washed fat cells which were used in the preparation of fat cell ghosts according to the method of Harwood, Löw, and Rodbell (11). The isolated fat cells from two rats receiving the same treatment were combined to increase the yield of ghost cell protein. The fat cells were swollen by washing two times with 5-ml aliquots of cold pH 7.4 lysing medium ($MgCl_2$, 2.5 mM; $CaCl_2$, 0.1 mM; $KHCO_3$, 1.0 mM; mercaptoethanol, 1.0 mM; Tris-HCl, 2.0 mM). The swollen cells were floated to the top of the capped conical polyethylene centrifuge tube by a 15-sec centrifugation at 300 *g*. The infranatant was aspirated off and discarded. After the two washes, the swollen cells were lysed by suspending them in 5 ml of the lysing medium and inverting the capped tube slowly for 1 min (20 inversions per min). The turbid suspension was then centrifuged for 1 min at 900 *g*. The infranatant and pellet were removed and collected in a chilled plastic tube. The remaining fat layer was resuspended in 5 ml of the lysing medium and the above procedure was repeated. After five lysing cycles the combined lysates were then centrifuged at 900 *g* at 0°C for 15 min. The 900 *g* pellet was resuspended in 1 mM mercaptoethanol, 1 mM $KHCO_3$ buffer to yield 1–2 mg of protein/ml.

Adenyl cyclase (EC 4.6.1.1) was assayed by a modification of the procedures of Harwood et al. (11) and Birnbaumer, Pohl, and Rodbell (12). The assay medium contained $MgCl_2$, 5 mM; cAMP, 2.5 mM; caffeine, 20 mM; [$\alpha$-$^{32}$P]ATP (New England Nuclear, Boston, M), 1.0 mM, 50 dpm/pmol; L-epinephrine, 20 $\mu$g/ml; Tris-HCl, 25 mM; and an ATP regenerating system consisting of 25 mM creatine phosphate and 1 mg/ml creatine kinase. The final volume of the incubation mixture was 0.05 ml, pH 7.4. The reaction was initiated by the addition of 0.025 ml (30–60 $\mu$g of protein) of the adipocyte ghosts. The reaction was incubated for 10 min at 30°C in a shaking water bath. The reaction was terminated by the addition of 0.1 ml of stopping solution (lauryl sulfate, 70 mM; ATP, 40 mM; cAMP, 1.4 mM containing 30,000 dpm [8-$^3$H]3′-5′,cAMP (Amersham/Searle, Des Plaines, IL, 27 Ci/mmol). The tubes were heated at 100°C for 3 min after which they were placed in an ice bath. The contents of the tubes were diluted with 0.8 ml of water prior to nucleotide separation.

Nucleotide separation was accomplished by a two-column procedure as described by Salomon, Londos, and Rodbell (13). The contents of the tubes were transferred to 0.4 × 15 cm glass columns containing 1 ml of resin (Dowex AG 50W-X4, BioRad Laboratories, Richmond, CA). The initial eluate and two successive 1-ml water washes were discarded. Three ml of water was added to the column and the resulting eluate was collected. The eluate was mixed with 0.2 ml of 1.5 M imidazole-HCl, pH 7.2. The mixture was then transferred to a 0.4 × 15 cm glass column containing 0.6 g of neutral alumina that had previously been washed with 8 ml of 0.1 M imidazole-HCl, pH 7.5 buffer. The column was allowed to drain into a scintillation vial. One additional ml of buffer was added and the combined eluates containing [$^{32}$P]- and [$^3$H]-cAMP were counted following the addition of 15 ml of scintillation fluid (Aquasol, New England Nuclear). Column recoveries were monitored by determining [$^3$H]cAMP recovery. Samples were counted in a liquid scintillation spectrometer with automatic external standardization (Packard Instrument Co., Downers

Grove, IL). Adenyl cyclase activity was linear over the protein range assayed and is expressed as pmol cAMP formed/min per mg protein.

### Phosphodiesterase

The low-$K_m$ phosphodiesterase (3′:5′-cyclic-AMP phosphodiesterase; EC 3.1.4.17) was assayed in homogenates of adipose tissue as described by Loten and Sneyd (14), utilizing the one-step incubation procedure of Thompson, Brooker, and Appleman (15). Prior to homogenization, a 0.5-g longitudinal slice of epididymal adipose tissue was incubated for 30 min in 2.0 ml of pH 7.4 Krebs-Ringer bicarbonate buffer containing 4% BSA (Sigma Chemical Co., fatty acid free) and one-half the normal concentration of $Ca^{+2}$ (0.055 M) in the presence of 0.1 mM norepinephrine (L-arterenol bitartrate, Sigma Chemical Co.) as described by Macho and Kolena (16). After the termination of the 30-min, 37°C incubation period, the tissue was removed and rinsed in 0.15 M ice-cold KCl. The tissue was homogenized in 4.0 ml of 0.25 M mannitol buffer, pH 7.4, in a glass–Teflon Potter-Elvehjem homogenizer. The homogenate was centrifuged for 3 min at 300 *g* in a refrigerated centrifuge. The infranatant was filtered through glass wool and assayed for phosphodiesterase. The reaction medium contained Tris-HCl, 14.3 mM, pH 8.0; $MgCl_2$, 34.3 mM; cyclic AMP, 0.001 mM containing 0.5 $\mu$Ci of [8-$^3$H]3′, 5′-cAMP (27 Ci/mmol) in a 0.35-ml final incubation volume. The reaction was started by the addition of 5 $\mu$g of snake venom (*Ophiophagus hannah*, Sigma Chemical Co.) and 0.05 ml of crude homogenate (approximately 0.05 mg protein). The tubes were incubated for 10 min at 30°C and the reaction was terminated by pipetting 35 $\mu$l of the reaction mixture into 0.8 ml of resin (Bio-Rad AG 1X2, 200–400 mesh, Bio-Rad Laboratories) as described by Thompson et al. (15). Radioactivity was assayed in 10 ml of aqueous counting scintillant (ACS, Amersham/Searle) in a liquid scintillation spectrometer with automatic external standardization (Packard Instrument Co.). The reaction was conducted in triplicate and was linear with respect to crude homogenate protein over the range assayed. Results are expressed as pmol [$^3$H]-3′,5′-cAMP converted to [$^3$H]adenosine per minute per mg protein.

### Tissue levels of cAMP

Cyclic 3′,5′-adenosine monophosphate was assayed in the presence and absence of norepinephrine in 0.2-g longitudinal slices of epididymal adipose tissue as described by Macho and Kolena (16). The 0.2-g portions of adipose tissue were incubated in 2.0 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing one-half the usual concentration of $Ca^{+2}$ (0.055 M) and 4% fatty acid-free BSA. The buffer was gassed with 95% $O_2$, 5% $CO_2$ prior to use, and contained 8.0 mM aminophyllin and 0.1 mM L-arterenol bitartrate (Sigma Chemical Co.). The level of norepinephrine, 0.1 mM, was chosen to give maximum stimulation of lipolysis and cAMP formation according to the results of Macho and Kolena (16). The polyethylene flasks were gassed with 95% $O_2$, 5% $CO_2$, capped, and incubated at 37°C for either 5, 10, 20, or 30 min (six rats were represented at each time period.) After the incubation, the tissue was removed and frozen immediately in liquid nitrogen. The samples were stored at −70°C and subsequently were homogenized in 10% TCA while still frozen. The TCA was removed by cold water–ether extraction, and the samples were lyophylized and assayed for cAMP according to the competitive protein-binding method of Gilman (17). The results are expressed as pmol cAMP present per g of tissue or per two fat pads.

### Other procedures

Protein in adipose tissue homogenates and ghost cells was assayed by a semi-automated procedure (18).

Differences between treatments were evaluated by a one-way analysis of variance (19). The level of statistical significance chosen was $P < 0.05$. Values are expressed as the mean ± the standard error of the mean (SEM).

### Method of expression of data

Because of the number of assays performed in this experiment, sufficient tissue was not available for epididymal adipose tissue cell number determinations. It has been previously demonstrated that exercise training in adult rats significantly reduces adipocyte size (5, 6, 20–22) but not cell number (5, 6, 21, 22). Adenyl cyclase and phosphodiesterase activities in this experiment are presented in two forms: pmol/min per two fat pads, which should be a reflection of the activity per cell, and pmol/min per mg protein, which indicates the efficiency of the enzyme activity relative to the protein content of the tissue.

## RESULTS

### Effect of training on body and fat pad weights

Training decreased body weight between the untrained and trained pair-fed groups 16% (339 ± 3 vs. 286 ± 6 g) while the mass of the epididymal adipose depot was decreased more than 50% (2.46 ± 0.13 vs. 1.01 ± 0.04 g/fat pad). The decrease in adipose tissue mass in trained rats is primarily the result of re-

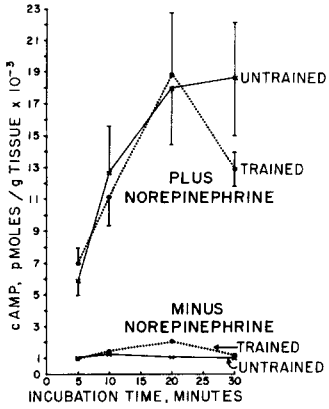


**Fig. 1.** Effect of physical training on basal and norepinephrine-stimulated cAMP release in rat epididymal adipose tissue slices. Mean values ± SEM are shown for four different incubation time periods with six animals represented for each time period for each treatment. "Minus norepinephrine" refers to basal cAMP release in fat pad slices incubated in the absence of norepinephrine. "Plus norepinephrine" refers to cAMP release in fat pad slices incubated in the presence of 0.1 mM norepinephrine. All rats were killed at rest (24 hr since last bout of exercise). The mean values for "plus epinephrine" cAMP levels were not significantly different, $P > 0.05$, between treatments at any of the time periods measured.

duced triglyceride per cell, since adipose tissue cell number is not significantly reduced by exercise training in adult rats (5, 6, 21, 22).

## Adipose tissue cAMP levels

The effect of 0.1 mM norepinephrine on tissue cAMP levels is shown in **Fig. 1.** Norepinephrine maximally stimulated cAMP levels approximately 10-fold compared to basal levels over the time period tested. Trained rats did not significantly differ from untrained rats in cAMP release on a per g of tissue basis. However, due to the approximately two-fold difference in epididymal adipose tissue mass between the two groups, untrained rats possessed significantly greater total norepinephrine-stimulated cAMP release on a per total depot basis (**Fig. 2**). The norepinephrine-stimulated cAMP release was also calculated per mg protein content of the tissue (data not shown). No significant differences existed ($P > 0.05$) between trained and untrained rats when cAMP levels were expressed in this manner.

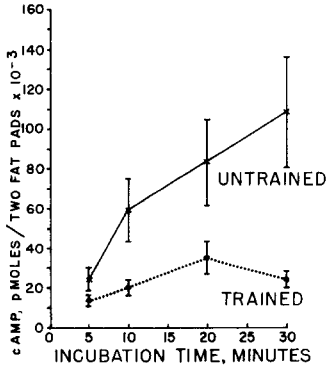


**Fig. 2.** Effect of physical training on total cAMP release per two epididymal fat pads. Values shown represent the mean ± SEM for six rats per group at each of four different incubation time periods. These values are 0.1 mM norepinephrine-stimulated cAMP levels minus basal cAMP levels. All rats were killed at rest (24 hr since last bout of exercise). The amount of cAMP released was significantly greater, $P < 0.05$, for untrained rats at 10, 20, and 30 min incubation times.

TABLE 1. Adenyl cyclase activity in adipocyte ghosts[a]

| Treatment | Ghost protein mg/two fat pads | Adenyl cyclase activity pmol per min | |
|---|---|---|---|
| | | per two fat pads | per mg protein |
| Untrained | 0.68 ± 0.04 | 48.04 ± 6.13 | 69.60 ± 6.63 |
| Trained | 0.62 ± 0.04 | 44.36 ± 3.78 | 71.78 ± 5.66 |

[a] Values shown represent the mean ± SEM for 12 samples per group. Each sample represents the pooled fat cell ghosts from one epididymal fat pad from each of two rats (two fat pads/sample). Methods of assay were as described under Methods. No significant differences, $P > 0.05$, existed for adenyl cyclase activity between trained and untrained rats.

## Adenyl cyclase and phosphodiesterase

The effect of exercise training on adipocyte ghost adenyl cyclase activity is shown in **Table 1.** The yield of ghost cell membrane protein relative to fat pad weight was not affected by training, but composed a greater percentage of total crude homogenate protein (**Table 2**) in trained than in untrained rats. Adenyl cyclase between training groups was not significantly different when the results were expressed on a mg protein or total depot weight basis.

Total fat pad crude homogenate protein was significantly decreased in trained rats (Table 2). Training resulted in an approximately two-fold increase in phosphodiesterase activity on a per mg protein basis as shown in Table 2. The activity of this enzyme was approximately 15% greater when expressed per total depot, although this difference was not statistically

TABLE 2. Phosphodiesterase activity in adipose tissue homogenates[a]

| Treatment | Crude homogenate protein mg/fat pad | Phosphodiesterase activity pmol per min | |
|---|---|---|---|
| | | per two fat pads | per mg protein |
| Untrained | 21.82 ± 1.26 | 1993 ± 209 | 44.3 ± 4.1 |
| Trained | 13.82 ± 0.66[b] | 2302 ± 138 | 81.4 ± 3.3[b] |

[a] Values given represent the mean ± SEM for 23 rats per group. Low-$K_m$ phosphodiesterase activity was analyzed in the crude homogenate fraction of the epididymal fat pad as described under Methods.

[b] Means are significantly different, $P < 0.05$.

significant ($P > 0.05$). If the total phosphodiesterase activity per depot was related to metabolic body size (pmol per min per fat pad per body weight$^{.734}$), then the activity in trained rats was significantly greater ($P < 0.05$, data not shown).

On a per g of tissue basis, phosphodiesterase and adenyl cyclase activities were, respectively, 402 ± 43 and 9.62 ± 1.16 for untrained rats and 1122 ± 65 and 20.77 ± 1.69 for trained rats. The ratio of mean phosphodiesterase activity to mean adenyl cyclase activity per gram of tissue was 41.7 for untrained rats and 54.0 for trained rats, representing approximately a 30% increase in the ratio of these two enzymes between training groups.

## DISCUSSION

Exercise training similar in intensity and duration to that utilized in the present study results in an increased capacity of the skeletal musculature to extract (23) and oxidize (24–27) fatty acids. Depending upon the intensity and duration of the exercise period, plasma FFA can supply 30–90% of the total energy expenditure (28–30). The major source of fatty acids oxidized during exercise is adipose tissue, the turnover rate of which is increased by physical training (31), implying that this organ is centrally involved in adaptations of lipid metabolism to exercise training (5). An increased lipolytic potential of adipose tissue from trained rats (32, 33) has been suggested as being a complementary response to oxidative adaptations (33–35), although some investigators have suggested that the lipolytic capacity of adipose tissue from sedentary untrained individuals may be adequate to provide sufficient FFA to meet the oxidative requirements for this metabolic fuel during exercise (22).

Increased adipose tissue adenyl cyclase activity has been suggested as a possible mechanism for the increased lipolytic responsiveness of adipocytes from trained rats (5). The results of the present study do not indicate that exercise training increases adipocyte adenyl cyclase activity. Although total epididymal fat pad cell number was not measured in this study, previous work from this laboratory has shown that the number of adipocytes per epididymal fat pad is not significantly changed by training (5, 6); thus, the expression of adenyl cyclase activity per epididymal fat depot can probably be extrapolated to adenyl cyclase activity per cell. In addition, total fat cell ghost membrane protein was unaffected by training, providing a reliable common denominator for the expression of adenyl cyclase activity. The results of the adenyl cyclase portion of this study differ from a previous report of increased adenyl cyclase activity in homogenates of adipose tissue from trained rats (5). The difference in results between these two studies may be in part due to the tissue preparation used and the choice of adenyl cyclase stimulator. The previous assay utilized NaF as the in vitro stimulator of adenyl cyclase while epinephrine was used in the present study. Fluoride is believed to stimulate adenyl cyclase activity by a more direct catalytic effect on the enzyme independent of membrane receptors, whereas epinephrine is believed to act via membrane receptors (11, 36).

Cellular cAMP levels in adipose tissue from trained rats appeared to be decreased following norepinephrine stimulation depending upon the method chosen to express the data. Conservatively, it can be said that, under the conditions of this study, norepinephrine-stimulated cAMP levels were not increased in adipose tissue of exercise-trained rats. A lack of correspondence was noted between norepinephrine-stimulated cAMP levels and the activity of adenyl cyclase per epididymal adipose depot. Total cAMP levels per fat pad appeared to be reduced in adipose tissue slices from trained rats, whereas adenyl cyclase activity in adipocyte ghosts was unaffected by training. Although it is possible that the differences in cAMP concentrations between trained and untrained rats could be a result of unequal inhibition of phosphodiesterase activity by aminophyllin, it is also possible that cAMP production in trained rats could have been partially inhibited by an elevated level of tissue FFA released during lipolysis but not transported out of the tissue and into the medium. Previous results from our laboratory have indicated that fat pads from trained rats incubated with epinephrine have a higher tissue level of FFA than corresponding fat pads from untrained rats (33). FFA have been reported to inhibit elevations in cAMP in fat cells stimulated by epinephrine (37), suggesting that differences in FFA concentration in proximity to adenyl cyclase may reduce its ability to respond to epinephrine.

Phosphodiesterase activity appeared to be increased in adipose tissue of trained rats, although the magnitude of the increase depended in part upon the method chosen to present the data. As discussed previously for adenyl cyclase, total phosphodiesterase activity per depot can probably be extrapolated to activity per cell. In these terms, training resulted in only a 15% increase in low-$K_m$ phosphodiesterase activity, a difference that was not statistically significant ($P > 0.05$). On the other hand, if phosphodiesterase activity was expressed per mg of protein, its specific activity was almost doubled by training.

In terms of adipose tissue crude homogenate pro-

tein, training resulted in a reduced adipose tissue mass with an elevated tissue protein content per unit mass but a reduced total amount of protein per depot. The type of exercise program utilized in this experiment has consistently resulted in reduced total fat pad protein in this and other studies (5). The finding that fat cell membrane protein was not reduced by training while crude homogenate protein was reduced may reflect decreased stromal-vascular cell components in the network required to contain the smaller volume of adipocytes in fat pads of trained rats. Since it is not known if training affected phosphodiesterase protein the same way it did crude homogenate protein, it is impossible to state that the increased phosphodiesterase activity per mg of crude homogenate protein represents an increase in actual enzyme specific activity. Comparisons of the ratio of phosphodiesterase and adenyl cyclase activity within treatment groups can be made to assess if training altered the ratio of the activity of the enzymes responsible for the production and inactivation of cAMP. Since each treatment group serves as its own control in this type of comparison, problems of interpretation relative to changes in tissue protein content are largely circumvented. On a per gram of tissue basis, trained rats exhibited approximately a 30% increase in phosphodiesterase activity relative to adenyl cyclase activity. These results indicate that training altered the activity of the phosphodiesterase relative to adenyl cyclase.

Since phosphodiesterase acts to decrease cAMP concentrations in adipose tissue, an increase in its activity relative to adenyl cyclase would appear to be inconsistent with a training-induced enhancement of lipolysis. There is, however, increasing evidence to suggest that factors in addition to cAMP concentrations are involved in mediating the effects of hormones on lipolysis (8, 38–40). Robison et al. (9) found intracellular cAMP rate-limiting to lipolysis only below 300 pmol/g, with further increases in cAMP having no effect on glycerol release. An increase in phosphodiesterase activity relative to adenyl cyclase activity may represent an adaptation to chronic exercise indicative of a more regulated control over catecholamine-induced cAMP levels.

Indirect evidence for this postulate may be observed by comparing the elevation of plasma FFA commonly seen in trained and untrained individuals after a period of exercise. The post-exercise amplitude and duration of plasma FFA elevation is less in trained than in untrained subjects (41–43), perhaps indicating a greater degree of regulation of plasma FFA levels. The control of this "overshoot" of FFA mobilization has been attributed in part to an increased oxidative capability (42, 43). The results of the present study (reduced total tissue norepinephrine-stimulated cAMP levels and increased phosphodiesterase activity relative to adenyl cyclase activity in trained rats) are consistent with more closely regulated cAMP levels, perhaps contributing to the modulation of FFA release after the termination of exercise. Insofar as tissue levels of cAMP which are produced by in vitro catecholamine concentrations sufficient to maximally stimulate lipolysis appear to be far in excess of those required for maximum activation of lipolysis (9), a mechanism for controlling excess cAMP production would appear advantageous to the animal. This type of adaptation would not decrease the maximal rate of lipolysis attained but rather would decrease the duration of lipolysis once the adrenergic stimulus was removed. Such control may permit an animal subjected to chronic catecholamine stimulation (such as during daily bouts of exercise) to exert a greater degree of control over the duration of the lipolytic response. Östman and Sjöstrand (7) have suggested that physical training results in an increased sensitivity of tissues to the vascular and metabolic actions of norepinephrine which in turn permits the animal to develop a better tolerance to conditions (such as exercise) requiring an increased sympathetic transmitter secretion in order to maintain homeostasis. The possibility that exercise training may increase the lipolytic sensitivity of adipose tissue to lower stimulating levels of catecholamines by altering either the number or affinity of the binding sites on the cell membrane is an alternative hypothesis consistent with previous reports of increased in vitro lipolysis in response to relatively high levels of epinephrine stimulation (32).

Factors in addition to cAMP formation may be involved in mediating training-related effects on lipolysis. Glucocorticoid hormones released during exercise have been suggested as a possible inducing mechanism for enhanced epinephrine-stimulated lipolysis in trained animals (5). Lamberts et al. (44) found that treating rats with cortisol enhanced epinephrine-stimulated lipolysis of the cells as well as cAMP-dependent protein kinase activity of these fat cells. These investigators suggest that glucocorticoids produce their effect on the lipolytic system of the fat cell by acting on either protein kinase and/or the hormone-sensitive triglyceride lipase.

Other possibilities for lipolytic regulation within the adipose tissue cell involving a feed back regulator have been proposed (45) and could represent an alternative method of lipolytic control. A recent report by Shepherd et al. (46), published while this manuscript was being prepared, also suggests that training increases adipose tissue phosphodiesterase without enhancing adenyl cyclase, leading these investigators

to conclude that the overall effect of training was to blunt the system for cAMP production in rat adipocytes. The results of the present study agree with the major findings of Shepherd et al. (46), e.g., that adipose tissue from trained rats produces less cAMP in response to norepinephrine stimulation than that from untrained rats and that phosphodiesterase is increased in response to training. It should be noted that Shepherd et al. (46) measured high-$K_m$ phosphodiesterase whereas we assayed the insulin-sensitive low-$K_m$ form, perhaps explaining the difference in magnitude of phosphodiesterase response to training between the two studies.

The results of this study indicate that adipose tissue cAMP production in response to pharmacologic levels of catecholamines is not increased by training and may actually be reduced. These results imply that adipose tissue cAMP levels may be under a higher degree of control in trained than in untrained rats. The net effect of such a training-induced modulation of cAMP levels on the magnitude and duration of lipolysis during exercise may result in better coordination between release of fatty acids by adipose tissue and their oxidation by skeletal muscle.

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