Mechanism of enhanced lipolysis in adipose tissue of exercise-trained rats

L. BUKOWIECKI, J. LUPIEN, N. FOLLEA, A. PARADIS,
D. RICHARD, AND J. LEBLANC
Laboratory of Endocrinology, Department of Physiology, Faculty of Medicine, Laval University, Québec, Province of Québec, G1K 7P4, Canada

BUKOWIECKI, L., J. LUPIEN, N. FOLLEA, A. PARADIS, D. RICHARD, AND J. LEBLANC. *Mechanism of enhanced lipolysis in adipose tissue of exercise-trained rats.* Am. J. Physiol. 239 (Endocrinol. Metab. 2): E422-E429, 1980.—The effects of exercise training and food restriction on the regulation of lipolysis were studied comparatively in adipocytes isolated from male and female rats. Exercise training inhibited cell proliferation in parametrial, but not in epididymal adipose tissue, whereas it significantly reduced adipocyte size in both fat depots. Adipocyte capacity for responding lipolytically to epinephrine (10 μm) or to ACTH (1 μm) was markedly increased by exercise training. Enhanced lipolysis was also observed when cells isolated from exercise-trained animals were stimulated by bypassing with dibutyryl cyclic AMP (5 mM) or theophylline (5 mM) the early metabolic steps associated with hormonal activation of the adenylate cyclase complex. Significantly, binding of (−)-[^{3}H]dihydroalprenolol to cellular receptor sites was not affected by exercise training. It is therefore concluded that exercise training increases adipocyte responsiveness to lipolytic hormones at a metabolic step distal to stimulus recognition by adrenoreceptors, possibly at the level of protein kinases or lipases. Food restriction markedly reduced adipocyte size and partially mimicked the effects of exercise training on adipocyte proliferation and lipolysis.

exercise training; starvation; cellularity; hyperplasia; adipose tissue; parametrial fat; epididymal fat; epinephrine; dibutyryl cyclic AMP; theophylline; ACTH; insulin; lipase; protein kinase

---

THE RATE OF FATTY ACID MOBILIZATION from white adipose tissue triglyceride stores is generally considered to represent an important regulatory step controlling oxidative metabolism in working muscles, particularly during long-term exercise (18). Recently, several groups reported that exercise training increases the lipolytic effects of catecholamines and postulated that adipose tissue might play a fundamental role in adaptation to exercise (1-3, 21, 22, 24). However, the biochemical mechanisms of the adaptative phenomena responsible for enhancing adipose tissue lipolytic capacity are still not well understood. In particular, the nature of the rate-limiting metabolic step(s) controlling adipocyte lipolysis during exercise is not known.

The principal goal of this investigation was to study the effects of exercise training on the regulation of adipocyte lipolysis. To differentiate the effects of exercise training from those indirectly resulting from a possible modification in caloric balance, we comparatively evaluated the lipolytic capacity of adipocytes isolated from sedentary rats, exercise-trained rats, and sedentary pair-weighed rats (animals submitted to a food-restriction regime aimed at matching their body weight with that of the exercising group). Moreover, to assess which metabolic step(s) controlling lipolysis might have been affected by exercise training or food restriction, we evaluated the lipolytic effects of epinephrine, ACTH, insulin, dibutyryl cyclic AMP, and theophylline. The rationale for specifically selecting these five agents was based on the fact that they all modulate lipolysis, albeit via different mechanisms. These studies were completed by direct binding measurements of a potent β-adrenergic antagonist, (−)-[^{3}H]dihydroalprenolol, to adipocyte receptors. Finally, comparative experiments were carried out with male and female rats in order to assess whether exercise training or food restriction would similarly affect adipocyte proliferation and lipolysis in animals of both sexes.

EXPERIMENTAL PROCEDURES

Animals. Experiments were performed on adult (male and female) Wistar rats weighing 200–250 g when purchased from Charles River Breeding Laboratory. The rats were housed in individual cages in a constant-temperature room (25°C) and were exposed to 12-h intervals of light and dark. The animals were divided into three groups: sedentary controls (C), exercise-trained (E), and food-restricted or pair-weighed animals (R). The animals were fed Purina laboratory chow available in a cup distributor outside the cage. Water was available at all times for all groups. Sedentary controls and exercise-trained animals were fed ad libitum, whereas pair-weighed animals were submitted to a food-restriction program aimed at matching their body weight to that of the exercising animals.

The exercise-trained animals were subjected to an endurance program that consisted of 2–3 h of daily swimming over a period of 7–11 wk. The swimming rats were loaded with weights corresponding exactly to 2% of their body weight. The temperature of the water was maintained at 35–36°C. The trained rats were killed 2–3 days after the last exercise.

Adipocyte isolation. Adipocytes were isolated from epididymal or parametrial adipose tissue by a modification (6) of Rodbell's original procedure (23). Rats were killed by decapitation, and the fat pads were removed

0193-1849/80/0000-0000$01.25

and washed several times in Krebs-Ringer bicarbonate buffer containing glucose (50 mg/100 ml) and fatty acid-free albumin (1%). The fat pads were cleaned, blotted on a filter paper, and weighed. The tissue was minced and introduced into a 20-ml polyethylene vial containing fresh buffer and collagenase (5 mg/g adipose tissue). The vial was incubated in a shaking-water bath at 37°C for 15–20 min. The shaking frequency was 160 cycles/min at an amplitude of 28 mm. During all the digestion period, the vial was removed from the water bath at 90-s intervals and gently shaken by hand for 15 s to ensure prompt liberation of cells from tissue pieces. At the end of the incubation, the content of the vial was filtered through a tissue sieve using a 10-mesh screen. The filtrate was collected in a centrifuge tube, diluted with 40 ml of buffer, and centrifuged at room temperature at 80 *g* for 2 min. The infranatant was removed by aspiration with a plastic syringe to which a polyethylene cannula was fixed. The floating adipocytes were then washed 4 times with 40 ml of buffer as described above. A sample of the final cellular suspension was counted in Neubauer's hemacytometer after being diluted with trypan blue stain (0.4% in normal saline). The percentage of cells resistant to staining by trypan blue was usually greater than 98%.

Yields of adipocyte recovery. In each individual experiment, the yield of adipocyte recovery was calculated by estimating triglycerides in samples of cleaned adipose tissue and in samples of the final cellular suspension containing a known amount of cells. Yields of adipocyte recovery after all washings usually varied between 80 and 85%.

Adipocyte incubation and lipolysis. Lipolysis was estimated by measuring the release of extracellular glycerol. The free adipocytes were first preincubated with gentle shaking at 37°C for 15 min in Krebs-Ringer bicarbonate buffer containing glucose (50 mg/100 ml) and fatty acid-free albumin (1%) under an atmosphere of 95% O_2/5% CO_2. After preincubation, the cells were washed twice at 37°C with a modified Krebs-Ringer bicarbonate buffer in which albumin concentration was raised to 4%. This was done to minimize extracellular accumulation of free fatty acids during the final incubation (8). Aliquots (0.5-ml) containing approximately 5×10^5 cells were incubated for 15 min in 20-ml polyethylene vials containing 1 ml of Krebs-Ringer bicarbonate buffer (glucose 50 mg/100 ml; albumin 4%) at 37°C under an atmosphere of 95% O_2/5% CO_2. At the end of the incubation period, the reaction was stopped by placing vials in melting ice. After 5 min, the floating adipocytes were discarded and aliquots of the infranatant were sampled in duplicate for glycerol determination.

Triglyceride and glycerol determinations. Glycerol was estimated fluorometrically by enzymatic cycling methods (14). Triglyceride content of white adipocytes was estimated by measuring glycerol after lipid hydrolysis. In each experiment, aliquots of suspended adipocytes were extracted with chloroform-methanol (2:1) by the method of Folch et al. (9). After extraction, 1 ml of the lower phase was evaporated to dryness and the lipid residue hydrolyzed at 60°C for 90 min with 2.5 ml of freshly prepared ethanolic KOH (KOH 0.5 N; ethanol 95%). The sample was cooled, neutralized with 5 ml of 150 mM $MgSO_4$, and centrifuged. The supernatant was stored at −80°C for glycerol determination. The triglyceride content was calculated taking an average molecular weight for brown adipose tissue triglycerides of 807.

Adipocyte size determinations. A 25-μl aliquot of fat cell suspension was added to 200 μl of trypan blue stain (0.4% in normal saline) in a plastic tube. A 6-μl sample was placed in a Neubauer's hemacytometer and photographed with a Zeiss photomicroscope. Four samples of each cell preparation were photographed. A photograph of a caliper scale was taken at the same magnification as the cells. Amplitude of magnification and negative enlargements were the same for all cell preparations. Adipocyte diameters were directly measured on the photographs using a quadridial caliper (Chemical Rubber). For each cell preparation, mean adipocyte diameter was calculated from 200–300 individual measurements of cell diameter.

Receptor binding studies. (−)-[^{3}H]dihydroalprenolol binding assays were performed in Krebs-Ringer bicarbonate buffer containing 2.7 mM glucose, 1% albumin, and 10 mM HEPES (pH 7.4). Adipocytes were incubated for 10 min at 37°C in a total volume of 250 μl at a concentration of 5.10^4 cells/ml. Incubations were terminated by rapid vacuum filtration of the mixture through Whatman GF/C fiber filters, followed by three washes with 5 ml ice-cold saline containing 10 mM Tris-HCl, 10 mM $MgCl_2$ (pH 7.4). Other experimental conditions were exactly as previously described (5). Specific binding was determined from the difference between total binding and binding in the presence of 10 μM (±) propranolol. Nonspecific binding usually ranged from 30 to 50% of total binding.

Materials. (−)-[^{3}H]dihydroalprenolol (48.6 Ci/mmol) was purchased from New England Nuclear. Other drugs were purchased from the following companies: (−)-epinephrine bitartrate, theophylline, dibutyryl cyclic AMP, fatty acid free albumin from Sigma; crystalline porcine insulin, lot. 615-08E-199 from Eli Lilly; ACTH (Cortrosyn) from Organon, Canada. All other reagents were of the highest purity commercially available.

Statistical evaluation. In all tables and figures, data were evaluated statistically using the unpaired *t* test (26); *n* = the number of individual experiments performed on separate occasions.

RESULTS

A first series of experiments was carried out with male rats weighing 225–250 g at the beginning of exercise training. It can be seen in Table 1 that at the end of the training program (7–11 wk later) body weights of exercised and pair-weighed groups were reduced by 20% in comparison to controls. Thus, sedentary control animals gained weight more rapidly than exercising animals. In both experimental groups, the reduction in body weight was accompanied by a 50–55% decrease in the total mass of epididymal fat. Total triglyceride content in both fat pads and triglyceride content per adipocyte (an index of adipocyte size) were markedly reduced by exercise training (Table 1). However, the total number of adipocytes present in the two epididymal fat pads was not signifi-

TABLE 1. *Effects of exercise training and food restriction on adipose tissue composition in male rats*

	Controls	Exercise-Trained	Food-Restricted (Pair-Weighed)
Body wt, g	413.0 ± 10.1 (7)	330.0 ± 8.4* (7) 79.9†	321.0 ± 4.0* (7) 77.7†
Epididymal fat pad wt, g	4.97 ± 0.21 (7)	2.43 ± 0.11* (7) 48.9	2.78 ± 0.12* (7) 55.9
Total triglyceride content, g	4.16 ± 0.14 (4)	1.80 ± 0.12* (4) 43.2	2.17 ± 0.28* (3) 52.1
Total number of adipocytes, $\times 10^6$	24.25 ± 2.37 (4)	21.76 ± 2.28 (4) 88.9	24.23 ± 4.88 (3) 99.9
Triglyceride content per adipocyte, μg	0.17 ± 0.02 (4)	0.08 ± 0.01* (4) 48.8	0.10 ± 0.02* (3) 55.7

Values are means ± SE of the total number of individual experiments indicated between parentheses. Body weight at the beginning of the training program was 231 ± 1.6 g (animals were 8- to 9-wk-old). Epididymal fat pad weight is the total wet weight of the left and right epididymal fat pads. * Values significantly different ($P < 0.01$ or less) from control values. † Values in these columns are percents of control values.

cantly affected either by exercise training or by food restriction.

The dose-response relationship for the stimulation of lipolysis by epinephrine in adipocytes isolated from sedentary controls in shown in Fig. 1. Maximal lipolysis was observed between 1 and 10 μM epinephrine.

As shown in Fig. 2, prolonged exercise training markedly increased the sensitivity of isolated adipocytes to the lipolytic action of epinephrine. Epinephrine-stimulated lipolysis was also significantly greater in adipocytes isolated from pair-weighed rats. Exercise training had a greater effect on adipocyte lipolytic capacity than food restriction whether results were expressed on a per cell number basis as presented in Fig. 2 or whether they were calculated per cell triglyceride [triglyceride contents of adipocytes isolated from exercised and pair-weighed animals were identical (Table 1)].

Similar results were observed for all three groups of animals when lipolysis was stimulated by bypassing with dibutyryl cAMP the early metabolic steps associated with hormonal stimulation of the adrenoreceptor-adenylate cyclase complex (Fig. 2). This observation provided a first indication that the major metabolic alterations induced by exercise training occur at a metabolic step distal to stimulus recognition by adrenoreceptors.

To further investigate this problem, we analyzed the characteristics of (−)-[^{3}H]dihydroalprenolol binding to cell surface receptor sites. The saturation curves for receptor site occupancy by (−)-[^{3}H]dihydroalprenolol in adipocytes isolated from control, exercise-trained, and food-restricted animals are shown in Fig. 3. There was no significant difference in (−)-[^{3}H]dihydroalprenolol binding between adipocytes isolated from the three experimental groups of animals whether binding was tested at low (5–10 nM) or high (40–110 nM) radioligand concentrations. This indicates that the proprieties of adipocyte surface adrenoreceptors are not modified by exercise training or food restriction.

To characterize the alterations of adipocyte metabolism induced by exercise training, we initiated a second series of experiments with female rats. The rationale for using female animals was based on the following facts: *a*) adult female rats, in contrast to males, lose little or no weight during intensive exercise training (22, 24, 28); *b*) although it is well established that hyperplasia ceases in epididymal adipose tissue before rats are 2-mo-old (4, 22, 24, 28), it was reported by Lemonnier (12, 13) that total adipocyte number continues to increase in other fat depots (parametrial, perirenal, subcutaneous) for a much

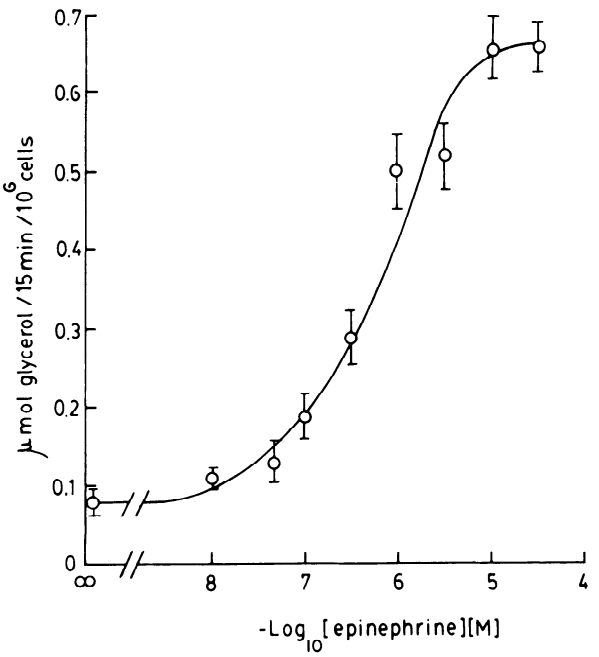

FIG. 1. Dose-response curve for stimulation of lipolysis by epinephrine in adipocytes isolated from epididymal adipose tissue of control sedentary male rats (225–250 g). Incubation conditions were exactly as described in EXPERIMENTAL PROCEDURES. Values are means ± SE of 4 individual experiments.

longer time. Because of these differences, it was decided to determine whether alterations in adipocyte size, number, and/or lipolytic capacity observed with male rats could also be reduplicated with females.

Preliminary experiments were conducted to assess whether it would be possible to detect hyperplasia in adipose tissue of female rats during the period of time (7–11 wk) required to train adult animals to exercise. It can be seen in Fig. 4 that total adipocyte number does in fact markedly increase during the early adult life of female rats, whereas adipocyte size remains unchanged. This hyperplasia results in an increase of parametrial adipose tissue weight and triglyceride content.

On this basis, we have run a similar experiment as described above for male rats with three groups of female animals: sedentary controls (C), exercise-trained (E), and food-restricted (R). It can be seen by comparing data of Table 2 (females) with the data of Table 1 (males) that mean body weight of exercise-trained male animals was 83 g inferior (or −20.1%) to that of sedentary controls, whereas with females the difference of body weights between exercised and control groups was much smaller

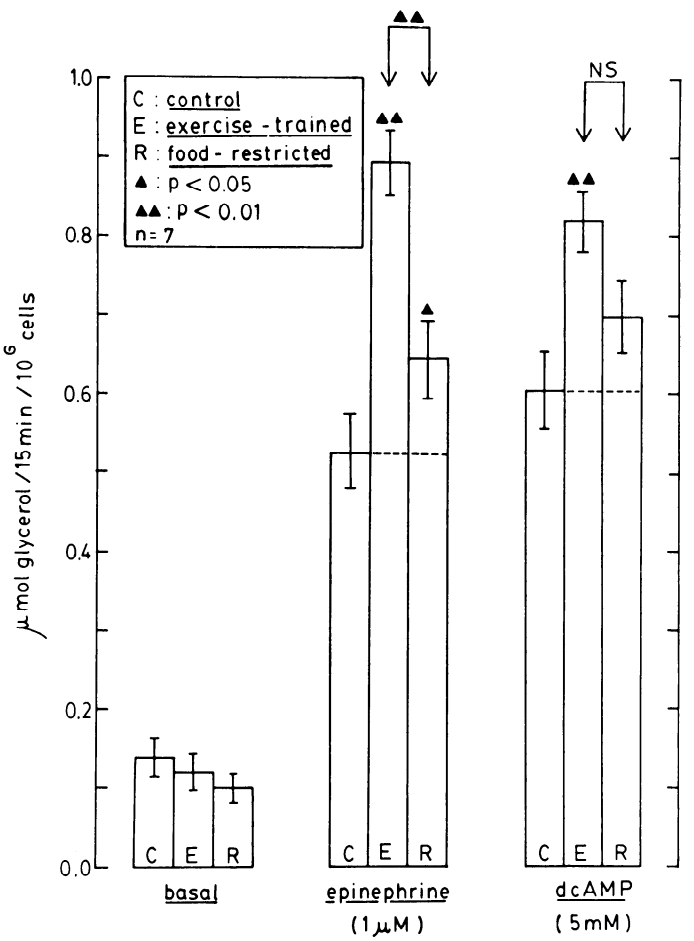

FIG. 2. Effects of epinephrine and dibutyryl cAMP on lipolysis in adipocytes isolated from epididymal adipose tissue of control, exercise-trained, and food-restricted rats. Body weight and adipose tissue composition of animals is given in Table 1. Values are means ± SE; n = number of individual experiments.

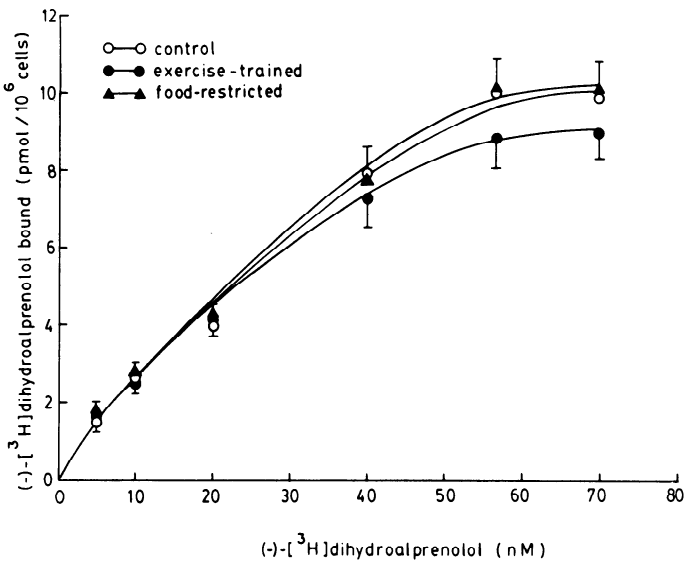

FIG. 3. Specific binding of (−)-[^{3}H]dihydroalprenolol to epididymal adipocytes isolated from control, exercise-trained, and food-restricted rats as a function of increasing concentration of ligand. Specifications of animals are given in Table 1. Binding was measured as described in EXPERIMENTAL PROCEDURES.

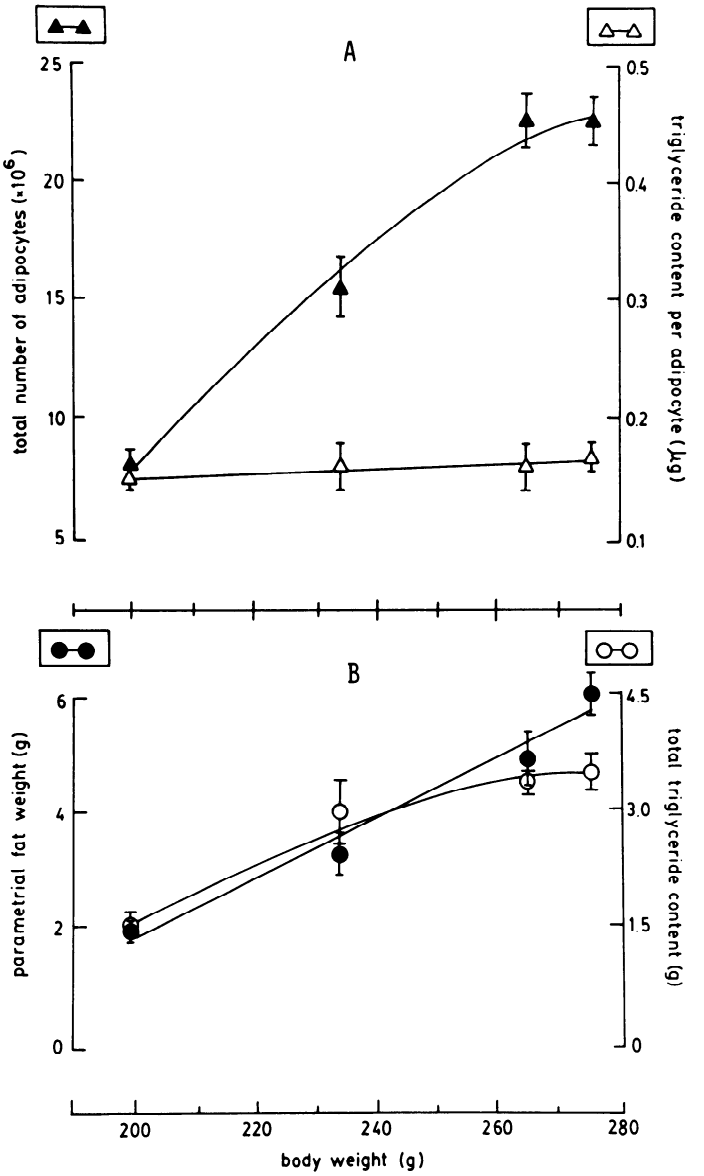

FIG. 4. Growth of parametrial adipose tissue in young adult female Wistar rats. At beginning of experiment rats were 8- to 9-wk-old. *A*: total number of adipocytes present in both parametrial fat depots and triglyceride content per adipocyte are plotted versus body weight. *B*: wet weight of parametrial fat and its triglyceride content are given. Each point represents mean ± SE of 7–12 individual experiments.

(15 g or 5.3%). Nevertheless, parametrial fat pad weights, triglyceride content per adipocyte, and adipocyte diameters were all significantly decreased in exercise trained, as well as in food-restricted female animals (Table 2). Remarkably, exercise training inhibited adipocyte proliferation normally occurring in parametrial adipose tissue of sedentary control animals (Table 2 and Fig. 4). This effect of exercise training was partially mimicked by food restriction (Table 2).

It can be seen in Fig. 5 that exercise training enhanced the lipolytic response of adipocytes to all stimuli, whether positive (epinephrine, ACTH, theophylline, dibutyryl cyclic AMP) or negative (insulin). Again, values of stimulated lipolysis in the semistarved group were significantly higher than in controls, but remained lower than in exercise-trained animals.

TABLE 2. *Effect of exercise training and food restriction on adipose tissue composition in female rats*

	Controls	Exercise-Trained	Food-Restricted
Body wt, g	275.9 ± 3.6 (12)	261.3 ± 4.4* (9) 94.7‡	235.6 ± 4.0† (11) 85.4‡
Parametrial fat wt, g	6.13 ± 0.40 (12)	2.44 ± 0.20† (9) 39.8	4.05 ± 0.38† (11) 66.1
Total triglyceride content, g	3.54 ± 0.23 (9)	1.02 ± 0.20† (9) 28.9	2.3 ± 0.20† (9) 65.1
Total number of adipocytes, $\times 10^6$	22.82 ± 1.17 (9)	12.25 ± 0.85† (9) 53.7	19.26 ± 1.18* (9) 84.4
Triglyceride content per adipocyte, μg	0.17 ± 0.01 (9)	0.10 ± 0.01† (9) 63.2	0.13 ± 0.01† (9) 80.6
Diameter of adipocyte, μm	113.67 ± 2.91 (8)	76.58 ± 1.78† (8) 67.4	95.93 ± 1.40† (7) 84.4

Values are means ± SE of the total number of individual experiments indicated between parentheses. Body weight at the beginning of the training program was 195 ± 1.4 g (animals were 8- to 9-wk-old). Parametrial fat weight is the total wet weight of the left and right parametrial fat pads. Significantly different from control values: * $P < 0.05$; † $P < 0.01$. ‡ Values in these columns are percents of control values.

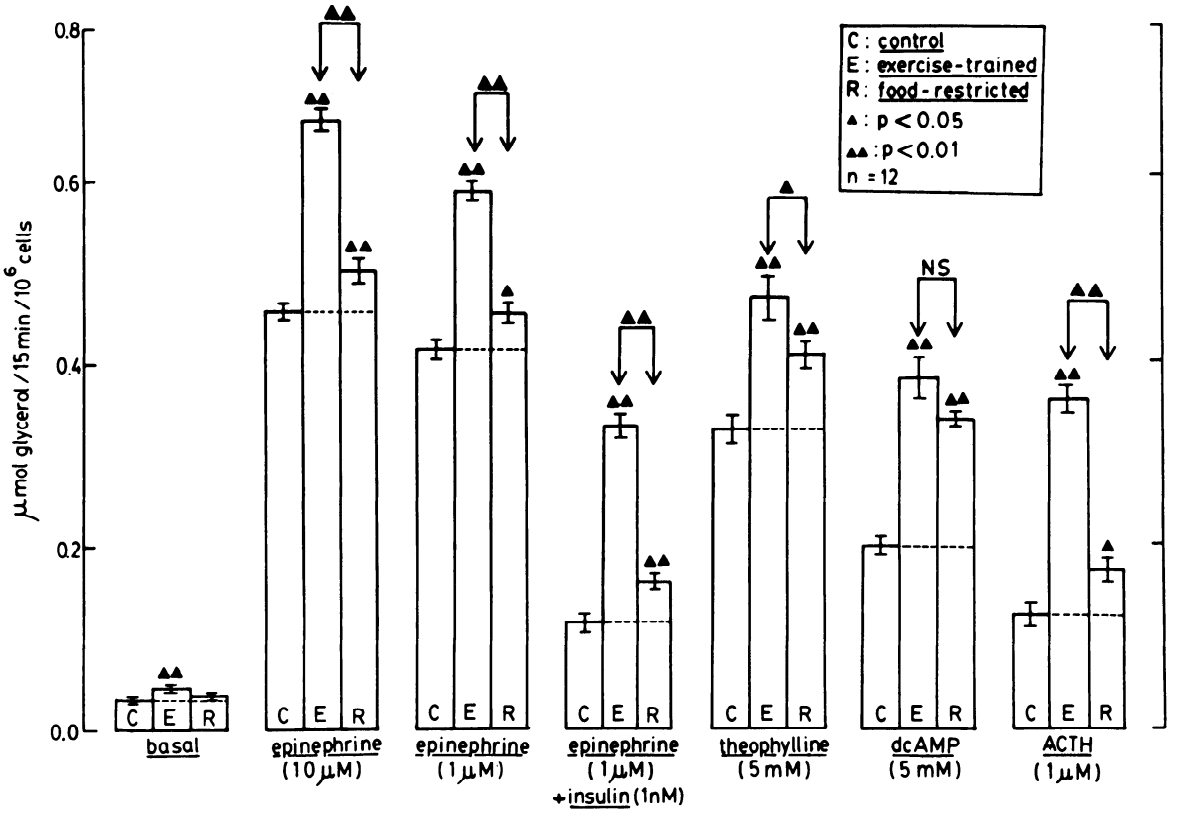

FIG. 5. Effects of epinephrine, dibutyryl cAMP, theophylline, ACTH, and of mixture epinephrine + insulin on lipolysis in adipocytes isolated from parametrial adipose tissue. Body weights and adipose tissue composition of animals are given in Table 2. Values are means ± SE; n = number of individual experiments.

DISCUSSION

Previous evidence indicates that prolonged exercise training inhibits the normal increase of body weight during the early adult life of male rats. This phenomenon is principally mediated by a decrease in the size of adipose tissue fat depots (4, 12, 13, 20, 21, 28). The present data confirm and extend these observations by demonstrating that several parameters (body weight, fat pad weight, total triglyceride content, triglyceride content per adipocyte and cellular diameter) are all decreased after exercise training or food restriction in both male as well as female rats.

Data shown in Table 1 demonstrate that the decrease in total epididymal fat weight of exercise-trained male rats is mainly mediated by a decrease in adipocyte size. Indeed, exercise training had little or no effect on the total number of adipocytes present in epididymal fat. However, in female rats, the decrease in parametrial adipose tissue wet weight can only be explained by a combination of two factors: a reduction in adipocyte size associated with a decrease in the total number of adipocytes (Table 2). It appears therefore that exercise training exerts a dual action on parametrial adipose tissue by reducing adipocyte size and by concurrently inhibiting cellular proliferation (Figs. 4 and 5; Table 2). Oscai et al. (20, 21) have previously reported a similar inhibitory effect of exercise training on cellular proliferation in epididymal adipose tissue of young, growing rats (in these experiments, animals of approximately 5 days of age were subjected to a program of swimming over a period of 14–28 wk). However, at approximately 12–14 wk of age, cell

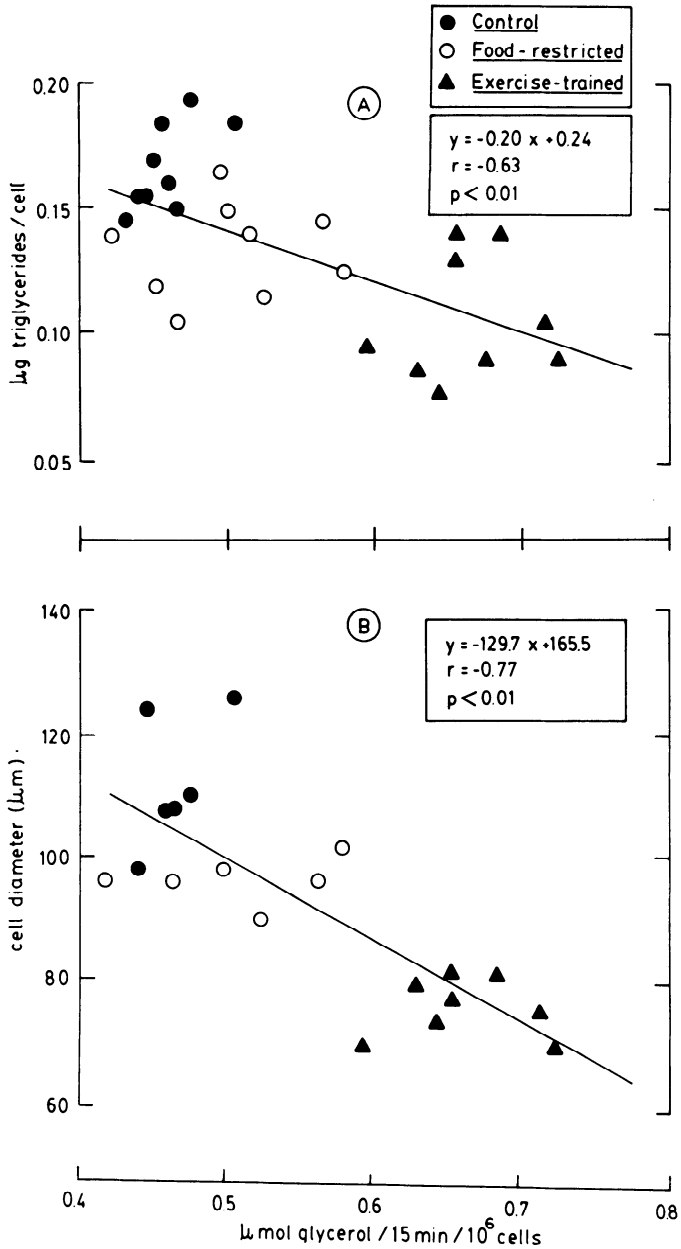

FIG. 6. Correlation between cell size and lipolytic response to norepinephrine (1 μM) in adipocytes isolated from control, exercise-trained, and food-restricted female rats. Each point represents individual measurements of series of experiments performed with female rats described in Table 2 and Fig. 5.

number becomes fixed in epididymal fat and after this age, exercise training affects only cell size and not cell number (4, 12, 13, 20, 21, 28).

The present studies provide a first demonstration that exercise training inhibits adipocyte proliferation in adult animals and indicate that it affects gonadal adipose depots differently in male and female rats. Epididymal adipose tissue is one of the most studied fat depots, but as far as hyperplasia is concerned, it is certainly not representative of other fat depots in which cellular multiplication continues during a much longer period of time in early adult life (12, 13). The present data clearly indicate that the concept that exercise training inhibits cellular proliferation only in very young animals needs to be revised.

Several laboratories have previously postulated that adipocyte metabolism and its hormonal responsiveness might be related to cell size (11, 17, 22, 23). However, factors other than cell size per se (e.g., age, degree of obesity, exercise training) play also an important role in the regulation of adipocyte metabolism (1, 11, 22). In the present studies, we have shown that the decrease in adipocyte size induced by exercise training or food restriction is accompanied by an increase of adipocyte lipolytic response to norepinephrine. This was verified in male as well as female rats (Tables 1 and 2; Fig. 2). A correlation analysis of the data presented in Table 2 and Fig. 5 disclosed that adipocyte size and lipolytic response to norepinephrine were negatively correlated when all three groups of animals were analyzed together (Fig. 7). Although the correlation was highly significant, it does not necessarily imply that there exists a direct cause-effect relationship between decreased adipocyte size and increased norepinephrine responsiveness. Indeed, no correlation could be demonstrated between lipolysis and adipocyte diameter (or triglyceride content) within each of the three treatment groups.

Two other laboratories reached a similar conclusion by using different experimental approaches. Owens et al. (22) reported that moderate exercise training had little effect on cell size, but significantly increased adipocyte responsiveness to epinephrine. On the other hand, Askew and Hecker (1) obtained a similar reduction in cell size in two groups of animals trained at two levels of exercise. They showed that the group receiving the greatest amount of daily exercise had a significantly greater epinephrine-stimulated lipolysis.

In this context, we recently found that it is possible to reduce adipocyte size by more than 50% simply by starving warm-acclimated (25°C) rats for 2 days in the cold (5°C). In this situation, the marked decrease in the size of adipocytes was paralleled by a decrease in lipolytic capacity, thus exactly the opposite of what is observed after exercise training (unpublished observations). It would appear therefore that enzymatic changes, possibly at the level of the system of protein kinases-lipases (vide infra), are more important than changes in adipocyte size for the regulation of adipocyte lipolysis.

A schematic representation of the sequence of metabolic events leading to fat mobilization in white adipose tissue is given in Fig. 7. It is indicated that ACTH (4), epinephrine (1), and insulin (4) modulate adenylate cyclase activity and lipolysis via binding to their respective cell surface receptors. It is also shown that the early metabolic events resulting from the interaction of receptors with the adenylate cyclase complex can be bypassed by two drugs: theophylline (2), which presumably increases basal cyclic AMP levels by inhibiting phosphodiesterases, and dibutyryl cyclic AMP (3), which mimics the action of cyclic AMP directly at the level of protein kinases.

Because an enhancement of lipolytic response was invariably observed in adipocytes isolated from exercise-trained or food-restricted animals when cells were stim-

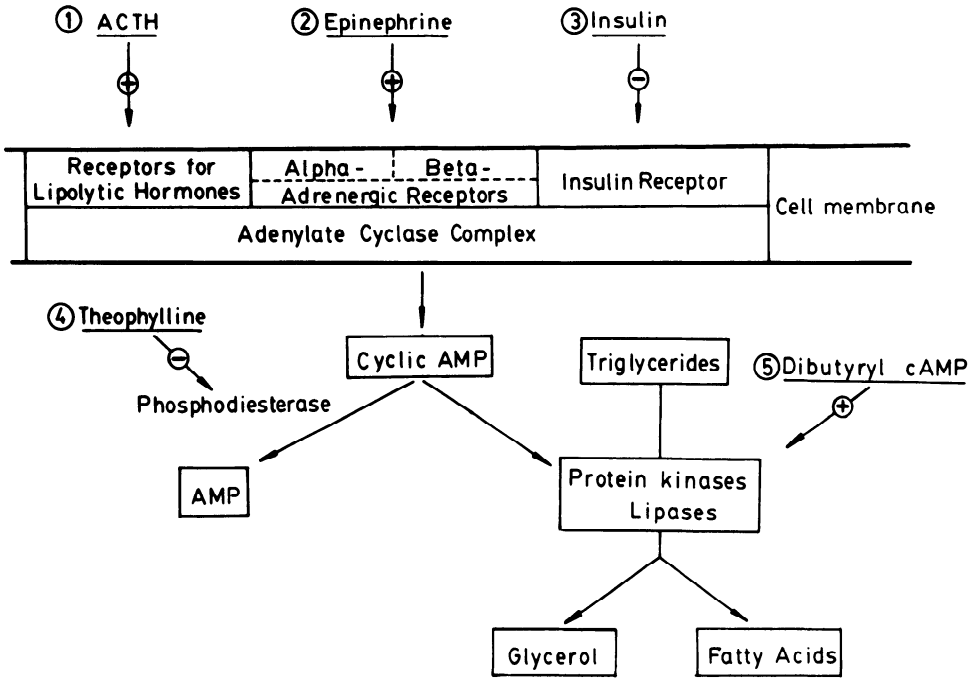

FIG. 7. Schematic representation of principal metabolic events controlling lipolysis in adipocytes. Numbers 1 to 5 refer to the 5 lipolytic agents tested in experiment described in Table 2 and Fig. 5.

ulated, not only by epinephrine, but also by polypeptide hormones (ACTH) or lipolytic drugs (theophylline, dibutyryl cyclic AMP), it can be concluded that *a*) exercise training increases the lipolytic capacity of adipocytes at a metabolic step distal to stimulus recognition by receptors; *b*) this metabolic step probably lies at the level of protein kinases or lipases; and *c*) food restriction partially mimics the effects of exercise training on adipocyte lipolysis, possibly via a similar mechanism. Moreover, the increased capacity of adipocytes for lipolysis cannot be explained by alterations in the number of affinity of adrenoreceptors (Fig. 3), by changes in the activities of adenylate cyclase or phosphodiesterase, or by modifications in tissue levels of cyclic AMP (3, 24, 25). Thus, all the available evidence indicates that exercise-training alters the enzymatic system that responds to cyclic AMP, i.e., either the system of protein kinases-lipases or some metabolic factor controlling the activity of these enzymatic complexes. The data in Fig. 4 suggest at least two separate mechanisms for adaptation. One involves a specific effect of exercise on the response to activators of adenylate cyclase (ACTH, epinephrine) that is not mimicked by food restriction. The second effect is on the response to dibutyryl cyclic AMP that is mimicked by food-restriction. Further work should be carried out to clarify these points.

Finally, it might be pointed out that hyperthyroidism (7, 15) and cold-acclimation (unpublished observations) can also increase the response of fat cells to all lipolytic agents. It would therefore appear that the enhancement of adipocyte lipolytic capacity is an adaptive phenomenon characterizing physiological situations in which energy expenditure exceeds energy gain, such as in exercise training, food restriction, cold acclimation, and hyperthyroidism.

This investigation was supported by grants from the Medical Research Council of Canada, The Juvenile Diabetes Foundation, USA, and the Conseil de la Recherche en Santé du Québec.

L. Bukowiecki is a Scholar of the Conseil de la Recherche en Santé du Québec. J. LeBlanc is an Associate of the Medical Research Council of Canada.

A preliminary report of part of this work was presented at the International Congress of Biochemistry of Exercise, 3rd, Brussels, 1979.

Received 16 April 1980; accepted in final form 28 July 1980.

REFERENCES

1. ASKEW, E. W. AND A. L. HECKER. Adipose tissue cell size and lipolysis in the rat: response to exercise intensity and food restriction. *J. Nutr.* 106: 1351–1360, 1976.
2. ASKEW, E. W., C. G. HUSTON, C. G. PLOPPER, AND A. L. HECKER. Adipose tissue cellularity and lipolysis. Response to exercise and cortisol treatment. *J. Clin. Invest.* 56: 521–529, 1975.
3. ASKEW, E. W., A. L. HECKER, V. G. COPPES, AND F. B. STIFEL. Cyclic AMP metabolism in adipose tissue of exercise-trained rats. *J. Lipid Res.* 19: 729–736, 1978.
4. BOOTH, M. A., M. C. BOOTH, AND A. W. TAYLOR. Rat fat cell size and number with exercise training, detraining and weight loss. *Federation Proc.* 33: 1959–1962, 1976.
5. BUKOWIECKI, L., N. FOLLÉA, J. VALLIÈRES, AND J. LEBLANC. β-Adrenergic receptors in brown adipose tissue. Characterization and alterations during acclimation of rats to cold. *Eur. J. Biochem.* 92: 189–196, 1978.
6. BUKOWIECKI, L., N. FOLLÉA, A. PARADIS, AND C. COLLET. Stereoselective stimulation of brown adipocyte respiration by catecholamines via β_1-adrenoreceptors. *Am. J. Physiol.* 238 (*Endocrinol. Metab.* 1) E552–E563, 1980.
7. CORREZE, C., M. H. LAUDAT, P. LAUDAT, AND J. NUNEZ. Hormone-dependent lipolysis in fat-cells from thyroidectomized rats. *Mol. Cell. Endocrinol.* 1: 309–327, 1974.
8. FAIN, J., R. SHEPHERD, C. MALBON, AND F. MORENO. Hormonal regulation of triglyceride breakdown in adipocytes. In: *Disturbances in Lipid and Lipoprotein Metabolism*, edited by J. M. Dietschy, A. M. Gotto, Jr., and J. A. Ontko. Bethesda, MD: Am. Physiol. Soc., 1978, p. 213–228.
9. FOLCH, J. M. LESS, AND G. A. SLOANE-STANLEY. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497–509, 1956.
10. HARTMAN, A. D., A. I. COHEN, C. J. RICHANE, AND T. HSU.

Lipolytic response and adenyl cyclase activity of rat adipocytes as related to cell size. *J. Lipid Res.* 12: 498–505, 1971.

11. HOLM, M. G., B. JACOBSSON, P. BJÖRNTORP, AND U. SMITH. Effects of age and cell size on rat adipose tissue metabolism. *J. Lipid Res.* 16: 461–464, 1975.
12. LEMONNIER, D. Effect of age, sex and site on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. *J. Clin. Invest.* 51: 2907–2915, 1972.
13. LEMONNIER, D., AND A. ALEXIU. Nutritional genetic and hormonal aspects of adipose tissue cellularity. In: *The Regulation of the Adipose Tissue Mass*, edited by J. Vague and L. Boyer. New York: Elsevier North Holland, 1976.
14. LOWRY, O., AND J. PASSONNEAU. *A Flexible System of Enzymatic Analysis.* New York: Academic, 1972.
15. MALBON, C. C., F. J. MORENO, R. CABELLI, AND J. FAIN. Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. *J. Biol. Chem.* 253: 671–678, 1978.
16. MCGARR, J. A., L. B. OSCAI, AND J. BORENSTAJN. Effect of exercise on hormone-sensitive lipase activity in rat adipocytes. *Am. J. Physiol.* 230: 385–388, 1976.
17. MOLGAARD-HANSEN, F., J. HOIRIIS-NIELSEN, AND J. GLIEMANN. The influence of body weight and cell size on lipogenesis and lipolysis of isolated rat fat cells. *Eur. J. Clin. Invest.* 4: 411–418, 1974.
18. NEWSHOLME, E. A., AND C. START. *Regulation in Metabolism.* London: Wiley, 1973.
19. OSCAI, L. B., P. A. MOLE, L. M. KRUSACK, AND J. O. HOLLOSZY. Detailed body composition analysis on female rats subjected to a program of swimming. *J. Nutr.* 103: 412–418, 1973.
20. OSCAI, L. B., S. P. BABIRAK, J. A. MCGARR, AND C. N. SPIRAKIS. Effect of exercise on adipose tissue cellularity. *Federation Proc.* 33: 1956–1958, 1974.
21. OSCAI, L. B., P. BABIRAK, F. B. DUBACH, A. MCGAAR, AND C. N. SPIRAKIS. Exercise or food restriction: effect on adipose tissue cellularity. *Am. J. Physiol.* 227: 901–904, 1974.
22. OWENS, J. L., O. FULLER, D. O. NUTTER, AND M. DIGIROLAMO. Influence of moderate exercise on adipocyte metabolism and hormonal responsiveness. *J. Appl. Physiol.* 43: 425–430, 1977.
23. RODBELL, M. Metabolism of isolated fat cells. *J. Biol. Chem.* 239: 375–380, 1964.
24. SHEPHERD, R. E., W. L. SEMBROWICH, H. E. GREEN, AND P. D. GOLLNICK. Effect of physical training on control mechanism of lipolysis in rat fat cell ghosts. *J. Appl. Physiol.* 42: 884–880, 1977.
25. SHEPHERD, R. E., E. G. NOBLE, G. A. KLUG, AND P. D. GOLLNICK. Control mechanisms of lipolysis and cyclic-AMP accumulation in adipocytes in response to training (Abstract). *Med. Sci. Sports* 11: 108, 1979.
26. SNEDECOR, G. W., AND W. G. COCHRAN. *Statistical Methods.* New Delhi: Oxford and IBH Publishing, 1967.
27. SUSINI, C., M. LAVAU, AND J. HERZOG. Adrenaline responsiveness of glucose metabolism in insulin-resistant adipose tissue of rats fed a high-fat diet. *Biochem. J.* 180: 431–433, 1979.
28. TAYLOR, A. W., J. GARROD, M. A. BOOTH, D. C. SECORD, AND S. M. CARY. The effects of exercise-training and feeding patterns on rat adipose cell size and number. *Can. J. Appl. Sport Sci.* 1: 93–97, 1976.
29. ZINDER, O., AND B. SHAPIRO. Effect of cell size on epinephrine and ACTH-induced fatty acid release from isolated fat cells. *J. Lipid Res.* 12: 91–95, 1971.