Detraining of exercise-trained rats: effects on energetic efficiency and brown adipose tissue thermogenesis

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1. Complete energy balance measurements were made in exercise-trained (treadmill running) rats subjected to 27 d of exercise detraining.
2. The 20% difference in body-weight that existed at the end of the training period between sedentary and trained rats was negated by detraining. Detrained rats had twice the body-weight gain of their untrained controls.
3. An elevation (12%) in metabolizable energy (ME) intake (relative to body-weight) was observed in detrained rats while their gross energetic efficiency was augmented by 60%.
4. Energy expenditure, excluding the estimated costs of fat and protein storage, was similar for detrained and untrained rats. Complementing the latter was the finding that thermogenesis in brown adipose tissue, a known energy buffering process, was also similar.
5. Elevated ME intake (relative to body-weight) largely contributed to the increased energetic efficiency of detrained rats.

Accelerated body-weight gain, arising through increased rates of both fat and protein deposition, is one of the commonly reported modifications that occurs in exercise-trained rats that are subjected to a prolonged period of exercise detraining (Booth *et al.* 1974; Dohm *et al.* 1977). Existing reports (Dohm *et al.* 1977) also indicate that rats undergoing detraining do not consume a greater quantity of food than their untrained controls. These observations, combined with the assumption that nutrient absorption from the gastrointestinal tract is unchanged, suggest an increase in energetic efficiency in rats during the period following cessation of regular exercise training.

With the absence of a difference in energy intake it would appear that the next step toward understanding the augmented energetic efficiency of detrained rats would be to examine their energy expenditure. Assuming that extraneous factors are equal, a lower energy expenditure in detrained rats would in effect indicate the presence of an energy-sparing mechanism. This spared energy would then obviously result in improved energetic efficiency.

Energy sparing may occur within many different physiological processes but one that has obtained a substantial amount of attention in recent years is regulatory thermogenesis. Defective thermoregulatory thermogenesis has been labelled as the instigating factor behind the development of obesity in *ob/ob* mice (Trayhurn, 1984). In addition, suppression of thermogenesis, as a strategy to promote energy conservation, has been reported to occur in lactating (Trayhurn & Richard, 1985), food-restricted (Rothwell *et al.* 1982) and fasted (Hayashi & Nagasaka, 1983) rodents.

Little information is available regarding the energetics of detraining and to our knowledge there have been no previous studies reporting full energy balance measurements for detrained animals. The present energy balance study was thus conducted to provide insight into the mechanism by which exercise-trained rats potentially increase their energetic efficiency during a period of detraining. As brown adipose tissue (BAT) is acknowledged as being one of the main sites of regulatory thermogenesis (both cold- (Foster & Frydman,

1978) and diet- (Rothwell & Stock, 1979) induced) in the rat, thermogenic properties of this tissue were evaluated.

MATERIALS AND METHODS

Forty-four male Wistar rats (Canadian Breeding Laboratories, St Constant, Quebec), weighing approximately 205 g at the start of the experiment (approximately 8 weeks old), were placed into three groups. A baseline (initial) group of eight rats was killed immediately and frozen ($-20°$) pending carcass analysis to provide information about initial body energy and composition. The remaining rats were randomly allocated to two preliminary groups of eighteen each and were housed individually in wire-mesh cages suspended above absorbent paper. All animals were maintained on a 12 h light–12 h dark cycle (lights on 06.30–18.30 hours) at a room temperature of $24 \pm 1°$. Rats were provided with water and a commercial laboratory chow (Ralston Purina Co., Indiana, USA) *ad lib.* throughout the entire experimental period. Food intake, excreta and body-weight of all rats were measured every 2 d. Digestible energy intake and metabolizable energy (ME) intake were calculated as previously reported (Richard *et al.* 1986).

One of the previously mentioned groups of rats served as a sedentary control whereas the other performed an exercise-training programme. Exercise training, similar to that described by Richard *et al.* (1986), consisted of treadmill running at 25 m/min, 2 h/d conducted 6 d/week, for a total of 35 d. Following the 35-d training programme, eight rats from each of the sedentary and trained groups were killed (08.00–09.30 hours) by decapitation and then frozen; these slaughtered rats were the sedentary (S) and trained (T) groups respectively. The remaining sedentary (referred to as sedentary–sedentary; SS) and trained (trained–sedentary or detrained; TS) rats were then left inactive in their cages for the following 27 d detraining period. As mentioned previously, body-weight, food intake and excreta were measured every 2 d. The ten SS and ten TS rats were also killed by decapitation following the detraining period.

As previously described (Richard *et al.* 1986), all carcasses, including those that had been previously frozen, had the gastrointestinal contents removed and were then autoclaved for 20 min. Thereafter they were homogenized and freeze-dried before measuring total energy content. The energy content of carcasses, as with faeces, was determined by bomb calorimetry (Parr Instrument Co., Illinois, USA). Body protein was calculated after determining carcass nitrogen by a micro-Kjeldahl procedure. The fat values presented may be slightly overestimated due to the method of calculation used; the energy derived from protein was subtracted from total body energy to provide the energy as nonprotein matter (taken to represent fat). It was thus assumed that carbohydrate represents a negligible part of total body energy.

Initial energy, protein and fat contents of S and T rats were estimated from their weight by reference to the baseline group of rats killed at the beginning of the study. Likewise, initial energy, protein and fat contents of SS and TS rats were estimated from their body-weight by referring to the final energy levels and final body compositions measured for the S and T groups of rats respectively.

S, T, SS and TS rats were killed and interscapular BAT was excised and cleaned of adhering white fat and muscle before being blotted dry and tared. The isolated tissue was homogenized in a sucrose buffer solution and mitochondria were then isolated as detailed by Cannon & Lindberg (1979). Samples of homogenates and mitochondria were taken for protein estimation by a modified Lowry method (Schacterle & Pollack, 1973) with defatted bovine serum albumin used as standard. GDP-binding was measured at room temperature, as previously detailed (Arnold *et al.* 1986), by incubating the BAT mitochondria with 10 μM-[^{3}H]GDP for 7 min in a medium at pH 7·1.
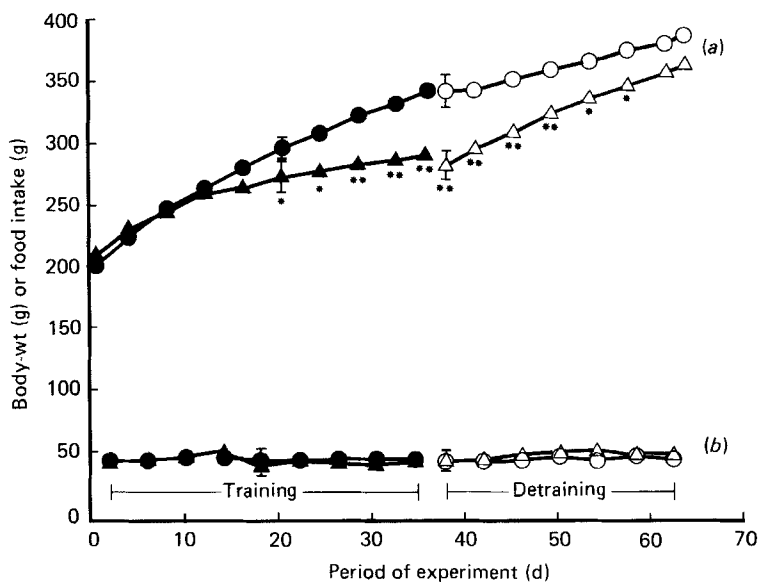
Fig. 1. (*a*) Body-weight and (*b*) food intake (g/2 d period) of sedentary (●, *n* 8) and trained (▲, *n* 8) rats during the 35 d exercise-training period, and sedentary–sedentary (○, *n* 7) and trained–sedentary (△, *n* 10) rats during the 27 d exercise-detraining period. Points are means with their standard errors represented by vertical bars. Body-weight values for sedentary and sedentary–sedentary rats were significantly different from those for trained and trained–sedentary rats respectively: *$P < 0.05$ **$P < 0.01$.

All values presented for S and T rats are for the period covering the 35 d training programme. Estimation of the energy cost of the exercise-training programme for T rats was based on calorimetrically determined values (averages) from a previous study (Arnold & Richard, 1987). Briefly, total O_2 (litres) consumed during exercise was calculated as total running time (min) × average body-weight (kg) × exercise O_2 consumption (above resting metabolic rate) (ml/kg per min). The latter values (ml/kg per min) (Arnold & Richard, 1986) were obtained from air samples taken while rats ran for 120 min within a treadmill-adapted metabolism chamber at 25 m/min. Total O_2 (litres) consumed during the exercise programme was converted to units of work using a kilojoule equivalent of 20·9 kJ/l O_2. Values shown for SS and TS rats are only those which were collected for the 27 d detraining period. Values expressed relative to body-weight were calculated using an average of the body-weights from each 2 d measurement period. Results were statistically evaluated by Student's *t* test (non-paired) (Hays, 1981). Values are shown as means with their standard errors.

RESULTS

As seen in Fig. 1(*b*) during the training period, food intake was similar for T and S rats; both groups consumed on average 46 g chow during every 2 d period. Likewise, food intake was also similar for both groups of rats during the detraining period. As anticipated, body-weight (Fig. 1(*a*)) became significantly lower in T animals following 20 d of exercise and continued as such until completion of the training programme. Thus TS (detrained) rats commenced the detraining period with a body-weight 22% lower than that of their

Table 1. *Effects of exercise training and detraining on protein and fat gains*

(Mean values with the standard errors; no. of rats in parentheses)

	Training†				Detraining‡			
	Sedentary (8)		Trained (8)		Sedentary–sedentary (7)		Trained–sedentary (10)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body protein (g)	34·9	0·5	36·3	0·6	63·4	2·1	53·4**	0·9
Final body protein (g)	63·4	2·1	55·0**	0·9	75·0	2·1	70·0*	1·0
Protein gain (g)	28·5	1·8	18·7**	1·1	11·6	0·8	16·6**	0·7
Initial body fat (g)	14·5	0·3	15·4	0·4	36·3	1·9	18·3**	0·2
Final body fat (g)	36·3	2·5	18·0**	0·9	46·4	1·4	34·4**	2·3
Fat gain (g)	21·9	2·4	2·6**	0·9	10·0	0·6	16·1**	0·7

Value were significantly different from corresponding control group (Student's t test): * $P < 0·05$, ** $P < 0·01$.

† Values from the 35 d training period.

‡ Values from the 27 d detraining period.

Table 2. *Energy balance in sedentary and exercise-trained rats for the 35 d training period*

(Mean values with their standard errors for eight rats per group)

	Training			
	Sedentary		Trained	
	Mean	SE	Mean	SE
Metabolizable energy intake:				
kJ	10500	235	9802	176
kJ/kg $W^{0·75}$ per d	771	7	758	10
kJ/kg $W^{0·67}$ per d	697	6	681	9
Body energy gain†:				
kJ	1496	128	522**	44
kJ/kg $W^{0·75}$ per d	109	8	40**	4
kJ/kg $W^{0·67}$ per d	99	7	36**	3
Gross efficiency‡	0·14	0.01	0.05**	0.01
Net energy cost of exercise training§ (kJ)	—		745	11
Energy expenditure excluding the net cost of exercise‖:				
kJ	9005	173	8534	191
kJ/kg $W^{0·75}$ per d	662	8	660	12
kJ/kg $W^{0·67}$ per d	598	7	593	11

W, body-weight.

Mean values were significantly different from those for the sedentary rats: ** $P < 0·01$.

† Values of 22·4 kJ/g and 39·2 kJ/g were taken for the energy content of protein and fat respectively (Davidson *et al.* 1975).

‡ Gross efficiency was calculated as body energy gain/metabolizable energy intake.

§ The net cost of exercise training (energy expenditure above resting metabolic rate) was based on calorimetrically determined values in a previous study (Arnold & Richard, 1987).

‖ Energy expenditure was calculated as metabolizable energy intake − body energy gain.

Table 3. *Energy balance in sedentary and exercise-detrained rats for the 27 d detraining period*

(Mean values with their standard errors; no of animals in parentheses)

	Detraining			
	Sedentary–sedentary (7)		Trained–sedentary (10)	
	Mean	SE	Mean	SE
Metabolizable energy intake:				
kJ	8069	171	8326	136
kJ/kg $W^{0.75}$ per d	639	5	715**	7
kJ/kg $W^{0.67}$ per d	589	5	654**	6
Body energy gain†:				
kJ	650	63	1102**	84
kJ/kg $W^{0.75}$ per d	52	6	94**	6
kJ/kg $W^{0.67}$ per d	48	5	86**	6
Gross efficiency‡	0·08	0·01	0·13*	0·01
Energy expenditure§:				
kJ	7418	224	7224	96
kJ/kg $W^{0.75}$ per d	587	10	621*	9
kJ/kg $W^{0.67}$ per d	541	9	568*	8
Heat loss in storing protein and fat‖ (kJ)	466	31	691**	39
Energy expenditure excluding heat loss in storing protein and fat§:				
kJ	6952	246	6533	89
kJ/kg $W^{0.75}$ per d	550	12	562	9
kJ/kg $W^{0.67}$ per d	507	12	513	8

W, body-weight.

Mean values were significantly different from those for the sedentary–sedentary rats: * $P < 0.05$, ** $P < 0.01$.

† Values of 22·4 kJ/g and 39·2 kJ/g were taken for the energy content of protein and fat respectively (Davidson *et al.* 1975).

‡ Gross efficiency was calculated as body energy gain/metabolizable energy intake.

§ Energy expenditure was calculated as metabolizable energy intake − body energy gain.

‖ Values of 1·25 and 0·36 kJ/kJ were used to estimate heat loss in storing protein and fat respectively (Pullar & Webster, 1977).

untrained (SS) controls. However, after completion of the 27 d of detraining, body-weight was observed to be equal in TS and SS rats; detrained animals displayed a near doubling in body-weight gain compared with that of their controls.

Table 1 shows the effects of exercise training and detraining on body composition. Exercise caused a sharp decline in both protein and fat gains; in comparison with the gains observed in their S controls, exercise-trained (T) rats showed decreases in protein and fat gains of 34 and 88% respectively. On commencing detraining, body protein and fat levels were 16 and 50% lower respectively in TS rats compared with their controls. However, at the time of killing, body protein and fat levels of TS rats were lower by only 7 and 26% respectively, compared with SS control rats.

Table 2 shows energy balance values for the 35 d training period. The 7% lower ME intake (kJ) of T compared with S rats was not statistically significant. Likewise, ME intake relative to body-weight also remained similar between the two groups. Both body energy gain and gross efficiency were decreased with exercise training; the latter variables were almost three times lower in T *v.* S rats. Energy expenditure, corrected for the cost of exercise training, was observed to be similar whether expressed relative to body-weight or in absolute (kJ) terms.

Table 3 shows energy balance measurements for the 27 d detraining period. ME intakes
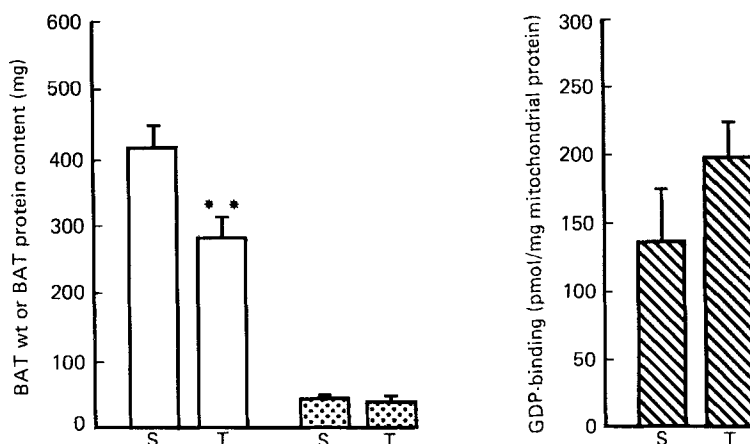
Fig. 2. Brown adipose tissue (BAT) weight (□), total BAT protein content (▦) and GDP-binding (▧) in sedentary (S, n 8) and exercise-trained (T, n 8) rats. Values are means with their standard errors represented by vertical bars. Values for T rats were significantly different from those for S rats: **$P < 0.01$.

were not significantly different for TS and SS rats, when expressed in absolute terms (kJ). However, when expressed relative to body-weights, the ME intake in detrained animals was significantly greater. Body energy gain and gross efficiency were a little more than 1·5-fold higher in TS compared with SS rats. Calculated energy expenditure, relative to body-weight, was seen to be significantly higher in TS rats. However, after subtracting the energy associated with protein and fat storage, energy expenditure expressed in absolute or relative terms was not significantly different between TS and SS groups of rats.

Fig. 2 shows some characteristics of BAT from S and T rats. Physical activity reduced both the weight and total protein content of this tissue. The 20% lower total protein content in BAT of T rats compared with S rats was not significant. GDP-binding by BAT mitochondria was unaltered by the exercise-training programme.

Fig. 3 shows characteristics of BAT from TS and SS rats. Total protein content of BAT was very similar for TS and SS rats despite a 13% greater tissue weight in SS animals. There was no difference in GDP-binding by BAT mitochondria between TS and SS rats.

DISCUSSION

The present results confirm those of past studies that have also shown a reduction of protein and fat gains in exercising male rats (Oscai, 1973; Richard et al. 1986). The reduced body energy gain of exercised rats in the present study appears to have resulted largely from their increased energy expenditure (due to physical activity), that was not compensated by an increase in food intake. Although food intake was not significantly altered in our exercise-trained rats, reduction in food intake has previously been reported to contribute, in addition to the cost of exercise, to decreased body energy gains in trained rats (Crews et al. 1969; Richard et al. 1986).

Energy expenditure (after allowing for the cost of exercise), expressed relative to body-weight or in absolute terms, was similar for exercise-trained and sedentary rats. In addition, BAT thermogenesis was also found to remain unchanged by exercise training, a finding that is consistent with previous observations made in this laboratory (Richard
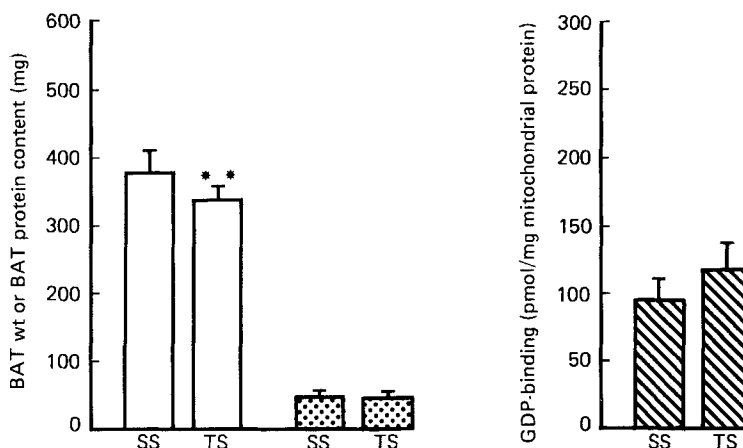
Fig. 3. Brown adipose tissue (BAT) weight (□), total BAT protein content (▦) and GDP-binding (▨) in sedentary–sedentary (SS, *n* 7) and trained-sedentary (TS, *n* 10) (exercise-detrained) rats. Values are means with their standard errors represented by vertical bars. Values for TS rats were significantly different from those for SS rats: **$P < 0.01$.

et al. 1986). Therefore, the present and recently reported (Richard *et al.* 1986) results provide evidence that expenditure components, such as regulatory diet-induced thermogenesis and thermoregulatory thermogenesis, are not altered with exercise training. However, at this point, one must acknowledge a certain limitation with the calculation of energy expenditure; energy expenditure as such represents the sum of all expenditure components and thus each component cannot be reliably quantified.

Little knowledge exists concerning the mechanism that permits trained–detrained rats to gain more body-weight than control rats while consuming equal amounts of food. A previous study measuring food intake in detraining rats failed to express food intake relative to body-weight (Dohm *et al.* 1977). Due to differences in body-weight between the various groups of rats it would seem that a better interpretation of energy intake, and energy expenditure, would be provided by expressing these results relative to the metabolic body size (kg body-weight $(W)^{0.75}$), or perhaps according to a more adequate expression for intraspecific energy metabolism, kg $W^{0.67}$ (Heusner, 1985). Expressed as such, ME intake in the present study was more than 10 % higher in detrained rats.

In the present study the energy content of faeces (kJ/g) was similar for TS and SS rats. Previously, Dohm *et al.* (1977) found no difference in faecal protein, carbohydrate and lipid levels with detraining. Thus it may be surmized that detrained (TS) rats do not absorb more nutrients (energy) per portion of food than control rats.

Energy expenditure, expressed relative to body-weight, was found to be significantly greater in TS rats. However, as energy gain was likewise elevated in these same rats and as all rats were sedentary during the detraining period, it was assumed justifiable to deduct the energy cost of storage from the overall energy expenditure before comparing detrained rats with their untrained controls. After correcting energy expenditure for the differences in storage costs, TS and SS rats were found to have similar energy expenditures.

In detraining rats, the absence of an energy-sparing mechanism, such as reduced regulatory thermogenesis, was also confirmed by our assessment of BAT thermogenesis; BAT mitochondrial proton conductance pathway activity, as evaluated by GDP-binding, was unaltered in detrained rats.

An approximate 40% decrease in gross efficiency was observed in the SS rats during the detraining period when compared with the younger S rats. One possible explanation for this change in efficiency may relate to the age of the rats. As rats age both energy intake and energy deposition decrease. In fact, energy deposition decreases more rapidly than energy intake; energy intake remains higher to presumably compensate for the larger cost of maintenance. Consequently an index based on the ratio of energy gain: total energy intake inevitably decreases with age and would approach zero as energy gain plateaus.

The experiments conducted by Hill *et al.* (1984, 1985) examined the effects of energy restriction on energy utilization in rats and can be compared to the present study of detraining. Both groups examined the energy metabolism of rats with reduced body-weight gains, be it diet- or exercise-induced. In the work of Hill *et al.* (1984, 1985) it was observed that rats with reduced body-weight, due to underfeeding, reduced maintenance energy requirements in proportion to their reduction in body-weight. More specifically, it may be proposed from the latter work that underfed rats, due to their smaller body-weight would also have a proportionally lower basal metabolic rate.

In summary, cessation of exercise training resulted in a near complete reversal of the exercise effects on body composition; body fat and protein levels approached, in a catch-up manner, those levels in untrained controls following 27 d of detraining. During the detraining period no alteration in energy expenditure, excluding storage costs, was observed. Rather, the accelerated body-weight gain and hence increased energetic efficiency of detrained rats was largely due to their increased energy intake (relative to body-weight).

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