Acute Reversal of the Enhanced Insulin Action in Trained Athletes

Association with Insulin Receptor Changes

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SUMMARY

We studied the effect of aerobic training and detraining on insulin-stimulated glucose disposal and on erythrocyte insulin receptor binding. Seven endurance-trained athletes were studied at 12 h, 60 h, and 7 days after cessation of training and compared with three untrained, age- and weight-matched controls.

The metabolic clearance rate of glucose as measured by the euglycemic clamp technique was 15.6 ± 1.8 ml/kg/min (mean ± SEM) in the trained subjects 12 h after the last bout of exercise compared with 7.8 ± 1.2 ml/kg/min in the untrained control group. When the trained subjects refrained from physical training, the metabolic clearance rate decreased to 10.1 ± 1.0 ml/kg/min at 60 h and further to 8.5 ± 0.5 ml/kg/min after 7 days of detraining.

The percentage of specific insulin binding to young erythrocytes (density 1.089–1.092), isolated by density gradient centrifugation, decreased from 10.4 ± 0.9 at 12 h after the last exercise to 8.1 ± 0.7%/3 × 10^9 cells after 60 h of detraining ($P < 0.001$). The decrease in insulin binding to erythrocytes was almost entirely accounted for by a decrease in the number of insulin receptors.

We conclude that the increase in peripheral insulin action seen in trained athletes is rapidly reversed, possibly by a mechanism separate from other phenomena associated with chronic training. The parallel findings of decreased in vivo insulin action and decreased insulin binding in young erythrocytes suggest that modulation of in vivo insulin response by detraining may be at least partially mediated by changes in insulin receptor number. Isolated young erythrocytes are better indicators of acute insulin receptor modulation than are whole cell preparations. DIABETES 1985; 34:756–60.

Physical training appears to enhance the action of insulin on the peripheral tissues. Thus, trained individuals require less endogenous insulin secretion to achieve normal glucose levels after an intravenous (i.v.) glucose load,[1] and insulin-stimulated glucose disposal as measured in vivo using the euglycemic clamp technique positively correlates with the degree of physical training.[2] Insulin binding to its receptor on circulating monocytes is also increased, both after acute exercise[3] and in the physically trained state.[1] In animal models, the adipose tissue and muscle seem to be affected by the changes,[4–6] while the role of liver is uncertain.[6] The receptor changes have been confirmed in the adipose tissue, but intracellular alterations in glucose utilization have also been suggested.[4,5] At present, the exact mechanism of these phenomena and its relation to the other well-known physiologic changes associated with physical training remain incompletely understood. Previous studies have examined individuals in a steady state of physical conditioning, an approach that does not allow the study of the temporal interrelationships of the above phenomena.

The purpose of the present study was to examine such a temporal relationship of the changes in glucose metabolism with the insulin receptor changes, as well as with the known time course of other physiologic adaptations induced by physical training. To this effect, we used the approach of detraining highly trained individuals for a period of 7 days. We report here the effects of detraining on the insulin-stimulated glucose disposal in vivo and on the insulin binding to circulating erythrocytes, as well as on an isolated fraction of young erythrocytes, which appears to display short-term modulation better than whole cell populations. We have used erythrocytes because they are the only cell type that can be

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TABLE 1
Physical characteristics of subjects

	Trained group (N = 7)	Untrained group (N = 3)
Age (yr)	30.4 ± 3.9*	24.7 ± 0.7
Weight (kg)	63.3 ± 4.0	60.8 ± 1.9
Height (cm)	174.1 ± 4.1	173.7 ± 4.4
Body surface area (m^2)	1.76 ± 0.07	1.72 ± 0.05
Body density (g/cc)	1.0563 ± 0.0087	1.0418 ± 0.0019
VO_2 max (ml/kg/min)	57.2 ± 3.5	43.4 ± 1.3†

*Mean ± SEM.
†Significant difference ($P < 0.002$) between groups.

easily and ethically sampled repeatedly in the human subject. Although they are not among the metabolically important target tissues of insulin, receptor changes on them have been shown to correlate with known alterations of glucose metabolism in a number of physiologic and pathologic states.[7–9]

MATERIALS AND METHODS

Subjects. Insulin-stimulated glucose disposal was measured in seven athletes (4 female and 3 male subjects) at (1) 12 h, (2) 60 h, and (3) 7 days after their last exercise bout. Erythrocyte insulin receptor studies were performed at 12 and 60 h. Three subjects, matched for age and weight, but who were not athletically active, served as the controls and were studied on the same time schedule as the athletes. The subjects were asked to keep a quantitative food record for 24 h before the start of the study until its completion. They were asked to maintain their normal eating patterns. Analysis of the records indicated no significant change in food intake during the study.

Our subjects were highly trained endurance athletes; 6 were distance runners who regularly ran 60–100 mi./wk and the seventh was a national level swimmer who trained for 12–15 h/wk, activity at least equivalent to that of the runners. Other relevant subject characteristics are provided in Table 1.

The subjects had completed their last work-out, equivalent to one-sixth of their weekly exercise, 12 h before the first in vivo insulin sensitivity test. All subjects volunteered freely for the study and were informed of the purpose of the study and potential risks associated with it. The study was reviewed and approved by the Ethics Committee of McMaster University.

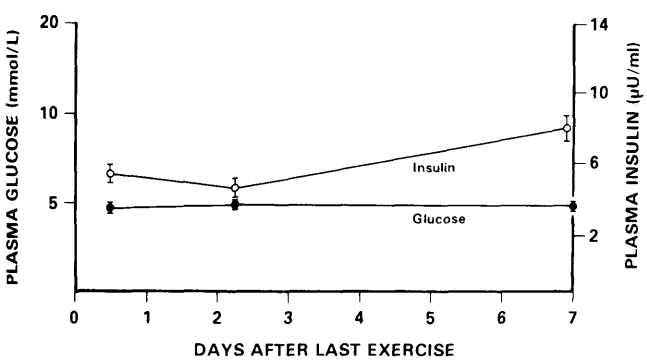

FIGURE 1. Plasma glucose and insulin levels with detraining. Fasting blood samples were obtained from seven athletes at 12 h, 60 h, and 7 days after the last exercise bout. Results are expressed as mean ± SEM.

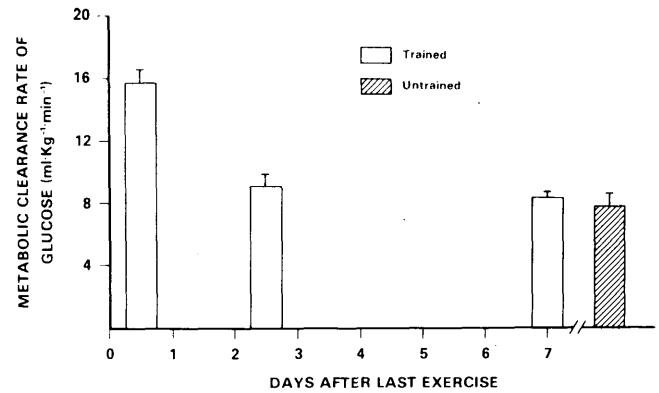

FIGURE 2. Effect of detraining on in vivo insulin resistance. Seven aerobically trained athletes (open bars) were studied at 12 h, 60 h, and 7 days after the last exercise period. Three sedentary controls are shown by hatched bars. Results are expressed as mean ± SEM. The plasma insulin concentration range was 3.5–7.6 μU/ml before the start of the test and 55.8–61.1 μU/ml during the insulin infusion. One of the trained subjects was excluded from the second part of the study because the plasma insulin concentration failed to rise to the level it reached on his initial test.

In vivo studies. The in vivo insulin action studies were done using the method of Greenfield et al.[10] The studies were started between 0800 and 0900 h at least 12 h after the last meal. Intravenous lines were established in the antecubital veins bilaterally using a #21 Angiocath. One line was used for blood sampling while the contralateral i.v. line was used for insulin and glucose infusion. The insulin (soluble, Connaught Laboratories, Toronto, Ontario) was infused with a Harvard syringe-driven pump. Blood glucose levels were measured every 5 min. The rate of glucose infusion was adjusted every 5 min to maintain the basal blood glucose level. The steady-state glucose requirements between 30 and 120 min were used to calculate the rate of body glucose metabolism as the metabolic clearance rate for blood glucose (ml/kg/min). The use of venous blood may give calculated values of clearance that are slightly higher than if "arterialized" blood had been used, but the effect would be proportional for all samples compared. In the nonexercising arm, the evaluation of steady state for blood glucose is not affected by the site of sampling.

Maximal oxygen consumption (VO_2 max) was measured during a progressive treadmill (Quinton, model 2472) running test at a constant speed and stepwise grade increments of 2% every 2 min until exhaustion. Heart rate was continuously monitored as was VO_2 by means of an open circuit gas analysis system. Inspired air volume (Hewlett Packard 4000 VR Pneumotachograph) and mixed expired CO_2 and O_2 (Godard Capnograph and Rapox O_2 analyzer) were measured and recorded on a four-channel physiograph (Narco-Physiograph, E and M Instruments Company, Houston, Texas) for selection of the highest VO_2 achieved as VO_2 max.

Body density was estimated by hydrostatic underwater

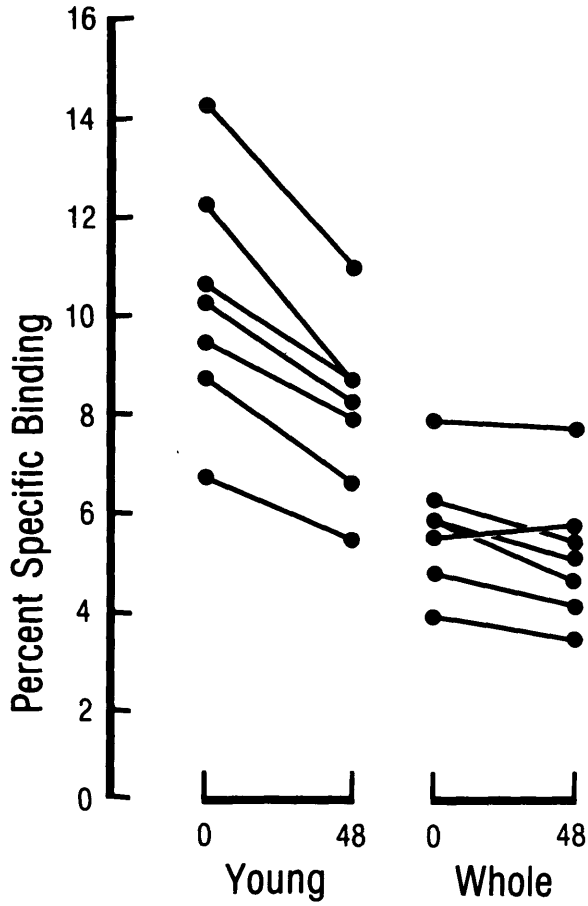

FIGURE 3.^{125}I-insulin binding to erythrocytes from athletes with detraining. ^{125}I-insulin binding studies were performed on young erythrocytes prepared by density centrifugation and in whole cell preparations. The insulin binding was expressed per 3 × 10^9 cells as percent of total added radioactivity. The individual results are presented for 12 h (left) and 60 h after the last exercise bout (right).

weighing at full inspiration. Vital capacity was measured by a dry gas flow meter (American Meter Company 802) and residual volume was evaluated by the helium dilution technique, using a respirometer (Collins 9 liter) and a helium meter (Warren E. Collins Instruments, Braintree, Massachusetts). Body density was calculated according to the formula described by Bruzek et al.[11]

Glucose and insulin measurements. Blood glucose was measured by a Beckman glucose-oxidase analyzer (Beckman Instruments, Toronto, Ontario) and a Reflomat Reflectometer. Serum insulin was measured every 0.5 h during the euglycemic clamp study by standard radioimmunoassay (RIA kit from Amersham Corp., Canada). The assays done at McMaster University were kindly performed by the laboratory of Dr. W. H. C. Walker.

Erythrocyte insulin binding studies. Heparinized whole blood was shipped on ice by air to Montreal, generally arriving at the laboratory within 4 h after the taking of blood samples. All samples were processed within 24 h by our method described elsewhere.[12] After aspiration of the plasma, the leukocytes and platelets were removed by passage through a cellulose column[13] and a well-defined fraction of young erythrocytes was obtained by density gradient centrifugation through isotonic solutions of dextran of density

TABLE 2
^{125}I-insulin binding in whole cells and isolated young erythrocytes

	Young cells		Whole cells	
Time after exercise (h)	12	60	12	60
Percent binding	10.4 ± 0.9	8.1 ± 0.7	5.8 ± 0.5	5.3 ± 0.5
P (by paired *t*-test)	<0.001		<0.02	
Percent decrease	21.4 ±1.8		10.5 ± 2.8	

The data summarize Figures 3 and 4.

1.089 and 1.092 g/ml. Cells that remained in this density bracket comprised the youngest 5–8% of all erythrocytes and contained practically all the reticulocytes. As we have previously described,[12] very few young cells were lost by imposing the lower limit of 1.089, an approach that has the advantage of defining the fraction by both upper and lower limits of density.

The cells were then washed and incubated at 4°C for 24 h with ^{125}I-labeled insulin in the presence of various concentrations of unlabeled hormone and 0.2 mg/ml of bacitracin. Porcine zinc insulin (Connaught Laboratories) was iodinated by the chloramine-T method to a calculated specific activity of 150–200 Ci/g. Incubations were performed in duplicate or triplicate to a total volume of 50 μl and final cell concentration of between 2.5 and 3.5 × 10^9 cells. All results were corrected to 3 × 10^9 cells on the basis of an automated cell count. Reticulocytes were counted in a standard fashion.[14]

RESULTS

The baseline glucose and insulin levels at 12 h, 60 h, and 7 days after the last bout of exercise are shown in Figure 1. There was no significant change in glucose concentration throughout the study period. However, the plasma insulin concentrations increased between 2 and 7 days of detraining. The metabolic clearance rate of glucose was 15.6 ± 1.8 ml/kg/min (mean ± SEM) for the athletes at 12 h after exercise (Figure 2), compared with 7.8 ± 1.2 ml/kg/min obtained for the sedentary controls ($P < 0.01$). After 60 h of no exercise, the metabolic clearance rate for glucose in the

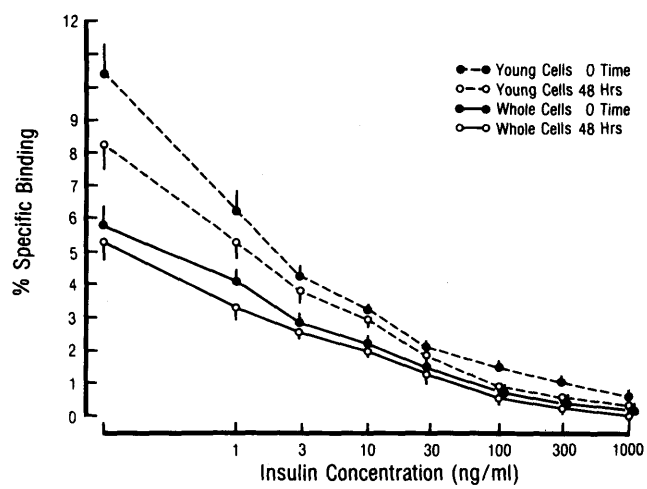

FIGURE 4. Dose-displacement curves for insulin binding. The blood samples were obtained at 12 and 60 h after the last exercise bout from the seven athletes. The results are expressed as the mean ± SEM.

athletes had decreased to 10.1 ± 1.0 ml/kg/min ($P < 0.05$) and remained suppressed after 7 days of detraining (8.5 ± 0.5 ml/kg/min). At 60 h and 7 days of detraining the metabolic clearance rate of glucose exhibited by trained athletes did not differ significantly from sedentary controls.

Insulin receptor binding to isolated young erythrocytes also fell during the 48-h period of no exercise (12–60 h) in all subjects studied (Figures 3 and 4, Table 2). In the unfractionated red cells, insulin binding at tracer concentration displayed a less consistent and quantitatively smaller decrease than in the isolated young cells (Figure 3, Table 2). The decrease in insulin binding appeared to be due to a decrease in the receptor number rather than the affinity, as is suggested by an identical concentration of unlabeled hormone required to decrease binding by 50% in the young cells (2.8 ± 0.7 versus 3.0 ± 0.4 ng/ml, Figure 4).

DISCUSSION

We have shown that the increase in the insulin-stimulated glucose metabolism induced by physical training can be rapidly reversed. This dissociates it from most other adaptive changes seen in trained individuals. More specifically, the changes in the maximal oxygen capacity and the changes in body composition as regards the proportions of muscle and fat are not as rapidly reversed and, therefore, do not appear to account for the effect seen. The receptor binding, on the other hand, shows a similarly rapid alteration.

On the basis of our in vivo data it is impossible to say whether the changes seen are related to insulin sensitivity (i.e., the insulin level required to achieve a given biologic effect) or to responsiveness (the maximal possible insulin effect).

The erythrocyte receptor changes we observed parallel our findings on the insulin effect. As only a small proportion of the receptors need to be occupied to achieve maximal biologic effect, responsiveness is not affected by ordinary changes in receptor numbers. However, the number of receptors occupied at any given submaximal ambient insulin concentration—and, therefore, the magnitude of its biologic effect—depends on the number and the affinity of the receptors, and modulation of either or both can alter sensitivity to insulin. If, therefore, changes of the erythrocyte insulin receptor can be extrapolated to the homeostatically important target tissues of insulin, they would suggest a sensitivity modulation. At the present time, the result of such an extrapolation can be treated only as circumstantial evidence.

It is possible that factors other than insulin action per se are responsible for the changes in insulin-stimulated glucose utilization. Muscle glycogen stores have been suggested as one.[15] We have previously shown[16] that 12 h after vigorous exercise, muscle glycogen stores have been replenished only at 67% of baseline values whether the diet was enriched in carbohydrate[17] or not.[16] By 24 h postexercise, however, complete replenishment has occurred, even in the absence of carbohydrate enrichment. Differences in the glycogen stores could, then, explain the changes seen in glucose clearance between 12 and 60 h postexercise. If this is the case, these changes are, at least partially, the effect of the last bout of exercise rather than being related to the physically trained state. More detailed time-course studies are needed to examine this alternative hypothesis.

It is interesting that receptor modulation was observed in a cell type that is not known to be otherwise affected by training. Any of a large number of the known homeostatic, hormonal, and neurogenic changes associated with exercise could be the signal for such modulation. A humoral factor has been suggested by in vitro studies.[18]

Finally, our study demonstrates an important methodologic point. We have shown that an isolated fraction of young erythrocytes is capable of a more pronounced acute modulation than are whole cell samples. This is not surprising in view of the fact that insulin has a demonstrable biologic effect only very early in the life of the erythrocyte[19] and its physiologic importance in the mature cell is questionable. Erythrocytes have been found less useful than monocytes in detecting insulin receptor changes in various physiologic and pathologic states.[7] Our technique of isolating young cells appears a promising way of eliminating much of this disadvantage. The coefficient of variation in the baseline values for percent specific binding was identical in isolated young and whole cells. This indicates that the procedure does not introduce "noise" to the data. Actually, as we have shown before,[12] our technique permits better standardization of insulin receptor measurements in situations in which the subjects are not homogenous in terms of their erythropoietic status. We, therefore, believe that it represents a significant improvement on the standard approach to using erythrocytes for the assessment of insulin receptor status.

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