Effect of training on the GH/IGF-I axis during exercise in middle-aged men: relationship to glucose homeostasis

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Manetta, Jérôme, Jean Frederic Brun, Laurent Maimoun, Albert Callis, Christian Préfaut, and Jacques Mercier. Effect of training on the GH/IGF-I axis during exercise in middle-aged men: relationship to glucose homeostasis. *Am J Physiol Endocrinol Metab* 283: E929–E936, 2002. First published June 25, 2002; 10.1152/ajpendo.00539. 2001.—The aim of this study was to compare circulating levels of growth hormone (GH), IGF-I, and IGF-binding protein (IGFBP)-1 and IGFBP-3 in response to a long-duration endurance exercise in trained vs. sedentary middle-aged males and to determine whether a relationship with glucose homeostasis exists. Seven trained men (Tr) were compared with seven age-matched sedentary men (Sed) during two trials of 60 min of cycling exercise performed below ($-$VT) and above ($+$VT) the ventilatory threshold. Insulin sensitivity (S_I) was higher in Tr than in Sed ($P < 0.001$). Basal GH, IGF-I, and IGFBP-1 and -3 were higher in Tr ($P < 0.05$). During $+$VT, Tr had a threefold higher GH response, whereas their blood glucose level was better maintained ($P < 0.05$). Basal IGFBP-1 was correlated with S_I ($P < 0.01$). These data indicate that endurance training in middle-aged men increased the activity of the GH/IGF-I system and improved glucoregulation both at rest and during high-intensity endurance exercise.

endurance training; insulin sensitivity; insulin-like growth factor I; insulin-like growth factor-binding protein-1 and -3; middle age

HIGH LEVELS OF ACTIVITY AND FITNESS have been shown to increase both the secretion of the growth hormone/insulin-like growth factor I (GH/IGF-I) axis (10, 14) and glucose disposal (17, 26, 27). Furthermore, the decline in GH secretion (22, 29, 32), plasma IGF-I (22, 32, 33, 34), and insulin sensitivity (S_I) (11, 12) that occur with aging may be related in part to a decline in physical fitness. This process is particularly relevant for middle-aged individuals, because the insulin resistance (11, 12) and progressive reduction in the GH secretion rate (16, 39) of aging are initiated as early as the third decade of life. It has recently been shown that low plasma IGF-I concentrations are predictive of a decline in whole body glucose uptake in older people (9). Although it is well documented that circulating levels of IGF-I are regulated by insulin and GH, evidence is accumulating that exercise is potentially another important regulator of IGF-I levels (21). Thus these age-related declines in anabolic hormone status (9, 34) and glucose disposal (17, 26) may both be restored by training. However, most IGF-I circulates in blood bound to a family of IGF-binding proteins (designated IGFBP-1 to -6), which have been shown to modulate IGF-I availability for action on tissues (36). IGFBP-1 and IGFBP-3 are considered to be the best characterized of the six circulating IGFBPs (31), but the evidence is conflicting regarding the effects of regularly performed exercise on IGFBP levels. One study found no effect of exercise on IGFBP-1 and -3 in the elderly (34), whereas another observed increased IGFBP-1 levels in middle-aged men (18). An involvement of IGF-I and its binding protein IGFBP-1 in glucose homeostasis has been proposed, because alterations in IGF-I availability may modulate its insulin-like effects (19, 23, 37). In addition, constitutive overexpression of IGFBP-1 results in impaired glucose tolerance with normal S_I (35). There is a paucity of data on the interaction of prolonged exercise, especially at different intensities, and both carbohydrate homeostasis and the GH/IGF-I axis responses. All of the studies considered, however, were performed with elderly subjects (between 60 and 70 yr of age), although, as mentioned above, the critical period for a progressive reduction in both the GH secretion rate and S_I is during early middle age (11, 12, 16, 39); almost nothing seems to be known in the middle-aged population. Our working hypothesis in this study was, therefore, that training would induce important alterations of both glucoregulation and GH/IGF-I axis in middle-aged subjects and that these two mechanisms are related to each other. Therefore, this study was undertaken to investigate in middle-aged subjects *1*) to what extent and at which intensity level (below or above VT or both)

training modifies plasma GH, IGF-I, and IGFBP-1 and -3 and *2*) whether a relationship to glucose homeostasis exists, especially regarding glucoregulation adaptation to a long-duration exercise.

METHODS

Subjects. Seven middle-aged endurance-trained cyclists (Tr) and seven middle-aged sedentary men (Sed) participated in this study. None had a family history of diabetes or hypertension. Smokers or those currently using medication for the control of blood arterial pressure and lipid or carbohydrate metabolism were excluded. No subject exhibited electrocardiogram abnormalities at rest or during a maximal cycle ergometer test. The physical characteristics of the subjects are shown in Table 1. Body composition was assessed with a four-terminal impedance plethysmograph, the Dietosystem Human IM-Scan (25). The training program for the cyclists was a collective activity, as all were members of the same cycling club. They cycled almost 10 h/wk (300 ± 15 km) and had been doing so for 10 ± 1.5 yr.

After receiving a complete and accurate verbal description of the procedure, risks, and benefits associated with the study, the subjects provided written consent. The experimental protocol was approved by the Committee on Research for the Medical Sciences.

Protocol. The subjects came to the laboratory on four separate days for *1*) the glucose tolerance test (*day 1*), *2*) an incremental maximal exercise test for the determination of the maximal oxygen uptake ($\dot{V}O_{2\ max}$) and ventilatory threshold (VT) (*day 2*), and *3*) two 60-min steady-state exercise tests, one below (−15%) and the other above (+15%) their individual VT. These tests were performed in random order (*days 3* and *4*).

All tests were separated by ≥4 days and never more than 7 days. All subjects were requested to refrain from exercise performance for the 3 days before the glucose tolerance and exercise tests.

Insulin action. An intravenous glucose tolerance test was performed according to the minimal model of Bergman et al. (2) with TISPAG software from the Department of Physiology, University of Montpellier I (7), which uses a nonlinear least squares estimation. Subjects were asked to fast for 12 h before the test, which began at 9 AM. A cannula was inserted in the cephalic vein at the level of the cubital fossa for blood sampling at various times, and a glucose injection was administered via the contralateral cephalic vein. Glucose (0.5 g/kg, solution at 30%) was slowly injected over 3 min. Insulin (0.02 U/kg body wt; i.e., 1–2 U) was immediately injected intravenously at 19 min. Blood samples were drawn twice

before the glucose bolus and at 1, 3, 4, 8, 10, 15, 19, 20, 22, 30, 41, 70, 90, and 180 min after the end of glucose injection.

Measurement of S_I. The minimal model is a mathematical analysis of the frequently sampled intravenous glucose tolerance test (2). This program gave the value of S_I, which is a measure of the dose-response relationship between plasma insulin and glucose disposal. It gave the values of SI from the following equations

$$dG(t)/dt = -[p1 + X(t)]\ G(t) + p1\ Gb$$

$$G(0) = Go$$

$$dX(t)/dt = -p2\ X(t) + p3\ [I(t) - Ib]$$

$$X(0) = 0$$

where G(t) and I(t) are plasma glucose and insulin concentrations, X(t) is the insulin in a compartment remote from plasma ("insulin action"), and p1-p3 are model parameters. G0 is the glucose concentration that one would obtain immediately after injection if there were instantaneous mixing in the extracellular fluid compartment. Gb and Ib are basal values of glucose and insulin. Parameter p1 represents the fractional disappearance rate of glucose independent of any insulin response, and p3 and p2 determine the kinetics of insulin transport into and out of, respectively, the remote insulin compartment where insulin action is expressed. S_I is an index of the influence of plasma insulin to change glucose's own effect on glucose concentration. Thus $S_I = p3/p2$. The validity of our procedure using a reduced number of samplings has been tested and previously published elsewhere (6).

Incremental maximal exercise test. The subject's maximal oxygen consumption ($\dot{V}O_{2\ max}$) was measured during 8–12 min of exercise on an electronically braked cycle ergometer (550 ERG, Bosch, Berlin, Germany). Fractions of oxygen and carbon dioxide in the expired air were measured by a mass spectrometer (Marquette MGA 1100, St. Quentin, France). The calibration of the mass spectrometer was checked before each test with standard calibration gases. A 3-l syringe was used to calibrate the volume turbine by use of flow rates similar to subject ventilation. Heart rates were monitored throughout the exercise test. Exercise testing started with a 3-min warm-up at 40 W. The workload was increased by steps of 20 W for the sedentary group and 30 W for the trained group every minute until maximal exercise was reached. This was evaluated in terms of maximal heart rate, respiratory exchange ratio (RER) values (>1.15), and O_2 consumption ($\dot{V}O_2$) stability. The estimation of the ventilatory threshold (VT) was performed by analysis of the behavior of carbon dioxide production ($\dot{V}CO_2$) vs. $\dot{V}O_2$ following the V-slope according to the method of Beaver et al. (1).

Steady-state exercise tests. Subjects arrived at the laboratory at 8 AM after an overnight fast (i.e., 12 h). A Teflon catheter was inserted in the cephalic vein at the level of the cubital fossa for blood sampling at various times. At 8:30 AM, a resting blood sample was drawn for subsequent analysis, and subjects then exercised either below (−15%) or above (+15%) their VT for 60 min on an electronically braked cycle ergometer (550 ERG). During the 60 min of exercise, the subjects were instructed to maintain a pedaling rate of 75 rpm. Ventilation ($\dot{V}E$), RER, $\dot{V}O_2$, and $\dot{V}CO_2$ were measured continuously, as described in the preceding section. During this period, $\dot{V}O_2$ and $\dot{V}CO_2$ varied by <0.1 l/min, and $\dot{V}E$ varied by <0.5 l/min.

Sample collection and analysis. Blood samples were drawn at rest (*time 0*) and at 5, 15, 30, 45, and 60 min during exercise. The samples were immediately placed in a tube

Table 1. *Characteristics and ergometric parameters of sedentary and endurance-trained subjects*

	Sedentary (n = 7)	Trained (n = 7)
Age, yr	52.33 ± 1.6	51.7 ± 1.4
Height, cm	173.33 ± 1.9	175 ± 1.6
Weight, kg	75.41 ± 2.4	73.4 ± 2.2
BMI, kg/m^2	25.08 ± 0.6	23.96 ± 0.5
Body fat, %	22.08 ± 1.5	20.05 ± 0.8
$\dot{V}O_{2max}$, ml·min^{-1}·kg^{-1}	33.37 ± 1.1	53.2 ± 2.3*
W_{max}, W	204 ± 9.3	331.43 ± 14.9*

Values are means ± SE. BMI, body mass index; $\dot{V}O_{2max}$, maximal O_2 uptake; W_{max}, maximum workload. *Significant difference between trained and sedentary subjects, $P < 0.001$.

containing lithium heparin (glucose, insulin, GH, IGF-I, and IGFBP-1 and -3) or EDTA (lactate). The plasma was immediately separated by centrifugation at 4°C and was stored at −80°C until analysis.

Plasma glucose (Sigma Diagnostics, Lyon, France) and lactate (Boehringer Mannheim, Meylan, France) concentrations were determined by specific enzymatic methods adapted to the spectrophotometer (DU 640; Beckman, Paris, France). Plasma insulin (Insik-5; Sorin Biomedica, Milan, Italy) was measured by radioimmunoassay. The within-assay coefficient of variation (CV) for insulin was 6.6%; the between-assay CV was 6.2%.

Plasma catecholamine concentration was determined using reverse-phase high-performance liquid chromatography procedures (460 Electrochemical Detector; Waters).

Serum somatomedin C/IGF-I was assayed with a kit from Medegenix (Brussels, Belgium). This is a double-antibody disequilibrium assay that includes an ethanol acid extraction procedure from serum samples. After the extraction procedure, RIA is performed employing the addition of sample and rabbit anti-IGF-I, followed by a 2-h incubation at 2–8°C. ^{125}I-labeled IGF-I is then added, followed by a second incubation for 20 h at 2–8°C. The preprecipitated carrier, the second antibody, and polyethylene glycol are added in a single step. The assay is centrifuged after the second 2-h antibody incubation at 2–8°C. The detection limit is 2 nmol/l. This assay does not cross-react (<1%) with IGF-II, hGH, fibroblast growth factor, or platelet-derived growth factor. Within-assay CVs range between 9.1 and 10.1%; between-assay CVs range between 10.3 and 15.2%.

Serum IGFBP-1 was assayed with the DSL ACTIVE IGFBP-1 coated-tube immunoradiometric assay (IRMA) kit (from Diagnostic System Laboratories). This is a two-site IRMA, in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilized on the inside wall of the tubes; the other antibody is radiolabeled for detection. The analyte present in patient samples, standards, and controls is bound by both of the antibodies to form a sandwich complex. Unbound materials are removed by decanting and washing the tubes. The detection limit is 0.01 ng/ml. Within-assay CVs range between 3.4 and 6%; between-assay CVs range between 1 and 3.5%. No cross-reactivity with IGFBP-2, -3, or -4 has been detected.

Serum IGFBP-3 was assayed with the DSL IGFBP-3 radioimmunoassay kit (Diagnostic System Laboratories). This is a classical radioimmunoassay, where there is competition between a radioactive and a nonradioactive antigen for a fixed number of antibody-binding sites. The separation of free and bound antigen is achieved by using a double-antibody system. The detection limit is 0.01 ng/ml. Within-assay CVs range between 5.3 and 6.7%; between-assay CVs range between 4.2 and 8%. No cross-reactivity with IGFBP-2, -3, and -4 has been detected.

Hematocrit was determined at rest and during exercise to ensure that measurements of metabolite and hormone concentrations were not influenced by changes in plasma volume. Water was given ad libitum during exercise tests.

Statistical analysis. A Student's *t*-test was used to compare the physical characteristics and S_I between the two groups. The significance of differences between the Tr and Sed groups during moderate and hard exercise intensity was determined using a two-way analysis of variance (ANOVA). To assess the patterns of response in the groups, plasma substrates and/or hormone concentrations were compared by ANOVA with repeated measures. To evaluate the relationship between S_I and basal hormonal responses, Spearman's rank order analysis was performed. Significance was defined as $P < 0.05$. Data are presented separately as means ± SE.

RESULTS

Physical characteristics, ergometric parameters, and $\dot{V}_{O_2}$ measurement. The Sed and Tr groups did not differ with respect to age, height, weight, body fat, body mass index (BMI), or VT. However, $\dot{V}_{O_2 \, max}$, maximal power (W_{max}), and power at VT (W/VT) were higher in Tr than in Sed ($P < 0.001$). When intensity during the two sessions (−VT and +VT) was expressed as a percentage of $\dot{V}_{O_2 \, max}$, these percentages were quite similar for Tr and Sed: respectively, 50.1 ± 2.2 vs. 51.2 ± 3.1% below VT and 71.2 ± 1.6 vs. 73.6 ± 2.4% above VT.

However, when reported in absolute intensity, these values were different for Tr and Sed: respectively, 27.17 ± 1.2 vs. 17.59 ± 0.9 ml·kg^{-1}·min^{-1} and 84 ± 3.9 vs. 130 ± 5.4 W below VT ($P < 0.05$) and 35.3 ± 1.4 vs. 23.41 ± 1.3 ml·kg^{-1}·min^{-1} and 118 ± 5.1 vs. 189 ± 7.1 W above VT ($P < 0.05$).

S_I. Fasting values of plasma glucose and plasma insulin were lower in Tr than in Sed (0.84 ± 0.01 vs. 0.90 ± 0.02 g/l and 6.85 ± 0.7 vs. 10.5 ± 1.36 μU/ml, $P < 0.03$). Compared with Sed, Tr had higher S_I {15.2 ± 3.1 vs. 2.99 ± 0.6 [×10^{-4}(μU·ml^{-1}·min^{-1})], $P < 0.001$}.

Substrate concentrations and plasma hormones at rest and during exercise. Hematocrit values were not significantly different during exercise tests. Although baseline plasma glucose concentrations were not different between Tr and Sed, glucose concentrations were higher at 30, 45, and 60 min in Tr when exercise was performed above VT ($P < 0.05$; Fig. 1).

Baseline plasma lactate concentrations did not differ between Tr and Sed. The lactate response to exercise was lower in both groups when exercise was performed below VT ($P < 0.05$; Fig. 1). However, the lactate concentrations were lower in Tr than in Sed at 5, 30, 45, and 60 min during exercise below VT and at 5, 45, and 60 min during exercise above VT ($P < 0.05$; Fig. 1).

Baseline plasma insulin was lower in Tr than in Sed ($P < 0.05$), whereas insulin kinematic responses were not different between groups (Fig. 1). However, insulin concentrations were decreased during exercise above VT in Tr only ($P < 0.05$; Fig. 1).

Baseline plasma GH was higher in Tr than in Sed, but GH concentrations were substantially higher in Tr than in Sed during exercise both below and above VT ($P < 0.05$; Fig. 2).

Baseline plasma IGF-I was higher in Tr than in Sed ($P < 0.05$; Fig. 2). However, although IGF-I concentration was higher in Tr at 30 min and at the end of exercise performed below VT, no significant difference was found above VT (Fig. 2).

Baseline plasma IGFBP-1 was higher in Tr than in Sed ($P < 0.05$; Fig. 2).

Baseline plasma IGFBP-3 was higher in Tr than in Sed ($P < 0.05$; Fig. 2). The IGFBP-3 level of Tr during exercise was higher above than below VT ($P < 0.05$; Fig. 2).
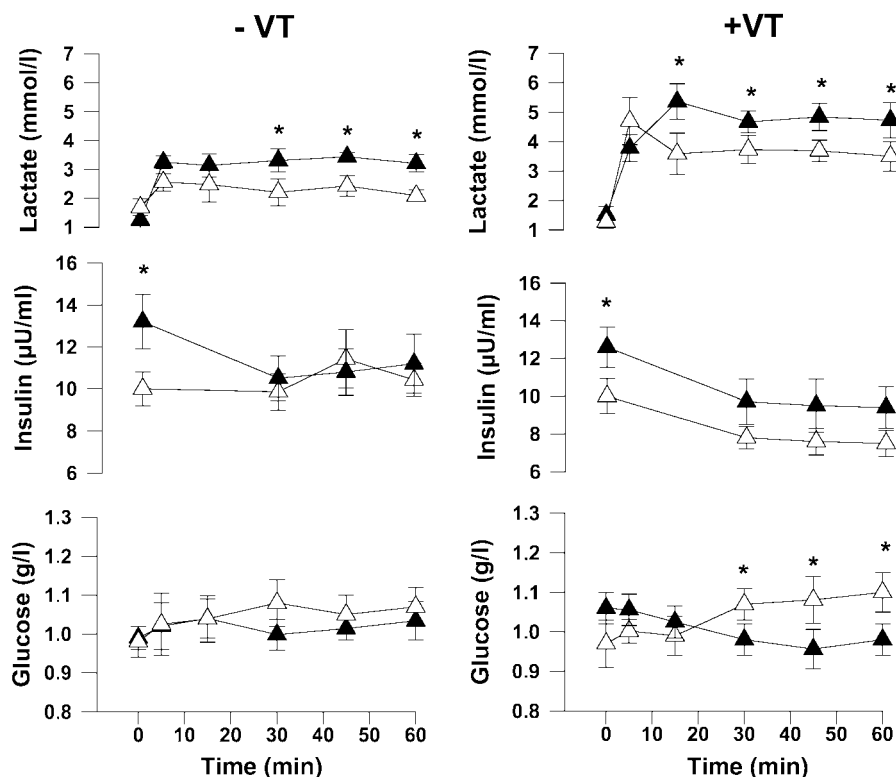
Fig. 1. Plasma lactate, insulin, and glucose concentrations at rest and during 60 min of cycle ergometer exercise below ($-$VT) and above ($+$VT) the ventilatory threshold in middle-aged endurance-trained cyclists (Tr; $n = 7$; $\triangle$) and middle-aged sedentary men (Sed; $n = 7$; $\blacktriangle$). Values are means $\pm$ SE; $*P < 0.05$ Tr vs. Sed. The absolute intensity was for Tr and Sed, respectively: 27.17 ± 1.2 vs. 17.59 ± 0.9 ml$\cdot$kg$^{-1}\cdot$min^{-1} and 84 ± 3.9 vs. 130 ± 5.4 W below VT ($P < 0.05$) and 35.3 ± 1.4 vs. 23.41 ± 1.3 ml$\cdot$kg$^{-1}\cdot$min^{-1} and 118 ± 5.1 vs. 189 ± 7.1 W above VT ($P < 0.05$).

Baseline plasma epinephrine (Epi) and norepinephrine (NE) concentrations were not different in Tr and Sed (respectively, 60 ± 20 vs. 45 ± 12 pg/ml for Epi and 606 ± 270 vs. 449 ± 149 pg/ml for NE), but Epi concentration was higher in Tr than Sed at the end of exercise when performed above VT ($1,330 \pm 238$ vs. 669 ± 189 pg/ml, $P < 0.05$). No significant difference was found above VT (103 ± 60 vs. 282 ± 146 pg/ml). Concerning NE during exercise, no significant difference was found between Tr and Sed at both intensities (respectively, $1,330 \pm 698$ vs. $1,479 \pm 604$ pg/ml below VT and $3,707 \pm 1,806$ vs. $2,232 \pm 1,320$ pg/ml above VT).

Correlation studies. Basal IGFBP-1 was correlated with S_I in Tr ($r = 0.75$, $P < 0.03$). Basal IGF-I was correlated with W_{max} in Tr ($r = 0.84$, $P < 0.01$). When we grouped all the middle-aged men together, basal IGFBP-3 was positively correlated with $\dot{V}o_{2\,max}$ ($r = 0.55$, $P < 0.05$), and S_I was correlated positively with basal IGFBP-1 and negatively with the percentage of fatness (respectively, $r = 0.89$, $P < 0.01$ and $r = -0.91$, $P < 0.01$; Fig. 3). Basal IGFBP-1 was not significantly correlated with the percentage of fatness ($r = 0.29$, $P = 0.28$).

There was no positive correlation between Epi and blood glucose at exercise, but rather an unexpected negative one ($r = -0.63$, $P < 0.01$ in all subjects and $r = -0.71$, $P < 0.05$ in trained subjects).

DISCUSSION

This study was undertaken to investigate the effects of training at middle age on both the GH/IGF-I axis and glucose homeostasis (both at rest and during pro-

longed exercise) and to look for possible relationships between these two functions. Results show that middle-aged trained subjects have increased baseline levels of plasma GH, IGFBP-1, IGFBP-3, and IGF-I. Both IGFBP-3 and IGF-I exihibit correlations with aerobic working capacity. GH response during prolonged exercise either below or above VT is higher in trained compared with sedentary subjects. On the other hand, S_I is higher in trained subjects and is correlated positively with IGFBP-1 and negatively with fat mass.

This study was designed as a cross-sectional comparison of carefully matched subjects. It is clear that an effect of training would have been demonstrated with more statistical strength with a longitudinal study. Therefore, we tried hard to control for all of the possible confusion factors such as body composition, age, weight, height, gender, nutritional status, pattern of physical activity, GH and IGF status, and glucoregulation. Concerning our trained population, training was performed in the same cycling team, and subjects exercised together, were given the same diet guidelines, and performed at exactly the same intensity and volume of exercise. Our sedentary controls were carefully matched with these trained subjects with regard to age, gender, BMI, and percentage of fat mass, i.e., all major factors of confusion for the parameters investigated in this experiment. Thus our results very likely reflect mainly the effect of training.

Another methodological aspect of this study that needs comment is the exercise protocol. We chose 60-min steady-state exercise, i.e., a situation where blood glucose levels need to be maintained by regula-
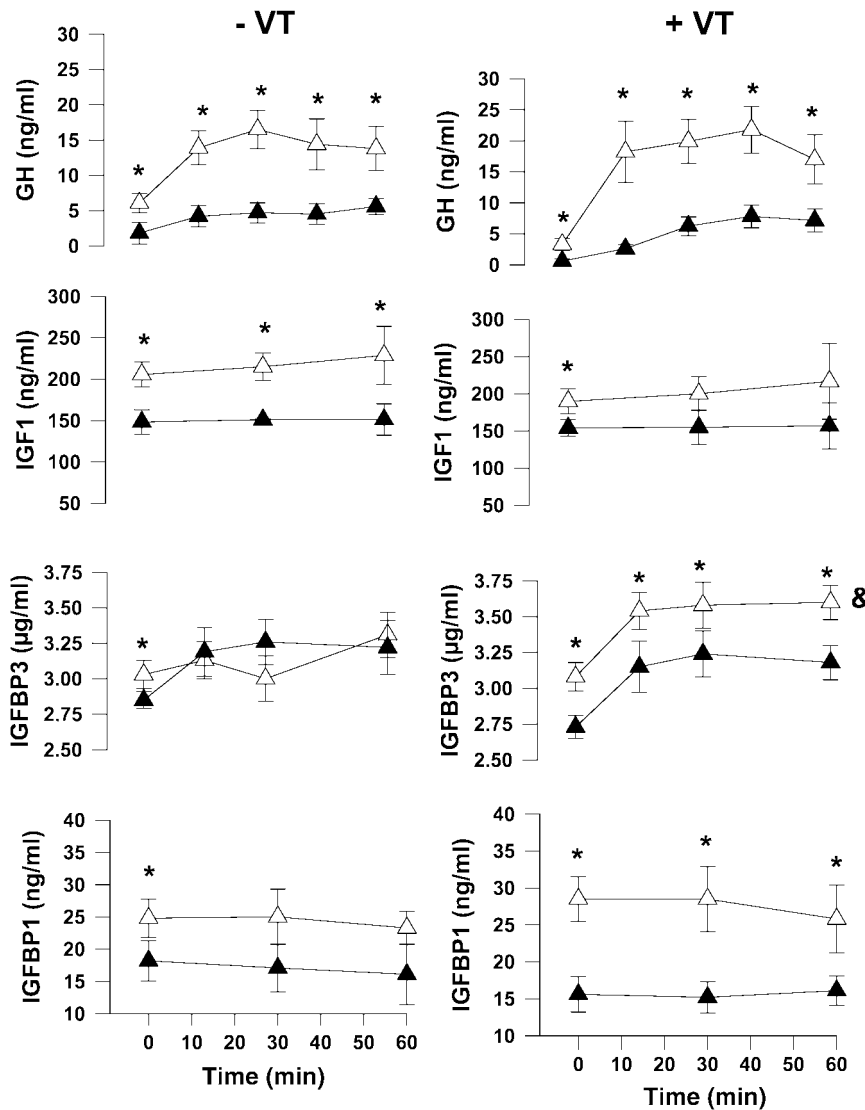
Fig. 2. Plasma growth hormone (GH), IGF-I, and IGF-binding protein (IGFBP)-3 and -1 concentrations at rest and during 60 min of cycle ergometer exercise performed below ($-$VT) and above ($+$VT) the VT in middle-aged endurance-trained cyclists (Tr; $n = 7$; $\triangle$) and middle-aged sedentary men (Sed; $n = 7$; $\blacktriangle$). Values are means $\pm$ SE; *$P < 0.05$ Tr vs. Sed. and &$P < 0.05$ $-$VT vs. $+$VT. The absolute intensity was for Tr and Sed, respectively: 27.17 ± 1.2 vs. 17.59 ± 0.9 ml·kg^{-1}·min^{-1} and 84 ± 3.9 vs. 130 ± 5.4 W below VT ($P < 0.05$) and 35.3 ± 1.4 vs. 23.41 ± 1.3 ml·kg^{-1}·min^{-1} and 118 ± 5.1 vs. 189 ± 7.1 W above VT ($P < 0.05$).

tion of hormonal mechanisms whose failure may result in hypoglycemia. There was a paucity of studies related to this kind of exercise, especially at middle age. In particular, we were not aware of reports of prolonged exercise above VT in sedentary middle-aged individuals. Although they found this quite difficult, our untrained subjects were able to complete this protocol and thus provide us a picture of what occurs in this condition.

The effects of exercise and training on IGF-I and its binding proteins IGFBP-1 and -3 remain incompletely understood, more especially at the critical period that is middle age, when GH and IGF-I decline (16, 39). Several authors, however, have described an increase in plasma IGF-I after endurance training (21, 34), whereas others have reported an opposite effect (14, 15). Moreover, exercise and training have been reported to increase IGFBP-1 (18, 30) and IGFBP-3 (21, 30), but there is not general agreement about this finding (13). The discrepancies among these studies are likely to be explained in great part by differences in either subject training level, body composition status, or age. The literature contains few studies at any exercise intensity, particularly on middle-aged subjects and the possible involvement of these factors in exercise-induced modifications in glucose disposal. To our knowledge, ours is the first study to investigate GH, IGF-I, and IGFBP-1 and -3 during prolonged exercise at both moderate and high intensities in relation to glucose homeostasis in middle-aged trained and sedentary men.

One of the main determinants of the magnitude of GH response to endurance-type exercise is the intensity of exercise (10). Thus GH response increases progressively during exercise up to 100% V̇o$_{2\,max}$ (29). Aging, however, decreases GH levels (22, 28, 32) after 40 yr of age (16, 39). The present study shows that, during exercise, the rise in plasma GH was almost threefold higher in the middle-aged trained men than in the sedentary men at both intensities. We can explain this result by the positive effect of physical fitness on GH response during exercise (10). It is inter-
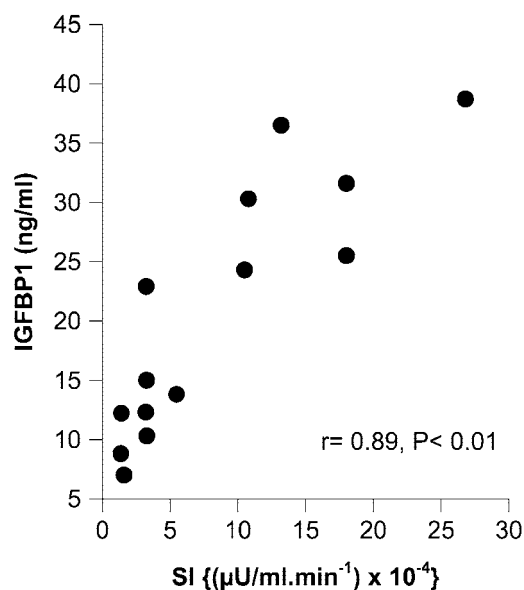
Fig. 3. Relationship between insulin sensitivity (S_I) and IGFBP-1 in middle-aged subjects ($n = 14$).

esting to point out that, as can be clearly observed in Fig. 2, prolonged exercise induces a sustained GH response both below and above VT in the group of trained subjects, resulting in a large amount of GH physiologically delivered in blood. These levels of GH are similar to those observed after exercise in 20-yr-old subjects. Because they are associated with higher resting values of IGF-I and IGFBP-3, they can be assumed to exert an anabolic effect. It is interesting to notice, as shown in Fig. 2, that no clear difference can be detected between GH responses below and above VT, consistent with a previous report (24) that indicated that, above 75% $\dot{V}O_{2 \, max}$, GH response to exercise no longer increased. Therefore, a long-duration exercise session 15% below the VT appears to be sufficient to achieve, in trained subjects, this large and sustained GH response, whereas exercising above this level does not appear to markedly improve this response.

On the whole, therefore, it appears obvious that training is responsible for an increase in secretory activity of the GH/IGF-I axis in middle-aged men. This increase is reflected by higher GH response but also by higher baseline values of IGF-I and IGFBP-3. The levels of these two proteins remain unchanged during exercise. All this suggests that the age-associated decline in somatotropic system activity (28, 32) may be attenuated by endurance training. In accord with this finding, Poehlman et al. (34) concluded that endurance training increases the fasting level of IGF-I in older individuals. However, a recent study did not support this finding and showed that only IGFBP-1 was higher in marathon runners than in age-matched sedentary controls (13). One explanation for this finding may be the significant difference in BMI between groups. Thus the low body fat percentage in these runners may have modified the GH/IGF-I response (8). In the present

study, we matched subjects for body composition (i.e., BMI and percentage of fatness) to avoid the impact of this parameter. Another explanation could be the specific interactions of duration and intensity during different types of endurance exercise (e.g., running vs. cycling).

In the present study, we found a correlation between basal IGFBP-3 and $\dot{V}O_{2 \, max}$, as prevously described in young subjects (4, 5). Because IGFBP-3 is considered to be an integrated index of GH action (3), this increase is likely to reflect the training-induced amplification of the secretory activity of the GH/IGF axis that has been previously reported (10). In view of these remarks, we suggest that IGFBP-3 is an endocrine marker of physical fitness. Two other studies (22, 34) have demonstrated a positive relationship between IGF-I and $\dot{V}O_{2 \, max}$. Poehlman et al. (34), however, showed that this association is less robust in older men. The authors explained this, in part, by a tendency toward less frequent and less intense exercise in older men, which may explain why our correlation between IGF-I and W_{max} was found only in the middle-aged trained individuals.

This study evidenced several effects of training on glucose tolerance mechanisms. First, trained subjects at both ages have a markedly increased S_I. This finding is consistent with previous reports (26, 27) and shows that regular exercise is able to counteract the decline in S_I that occurs at this age. Because insulin resistance is a major factor involved in the development of metabolic and circulating disturbances that occur at this age (11, 12), the strong beneficial effect of training on this parameter may contribute to an explanation of the recently demonstrated efficiency of regular exercise in the prevention of type 2 diabetes (38). Training appears also to be associated with alterations in the glucoregulation adaptation to exercise. As shown in Fig. 1, trained subjects exhibit both a better stability of blood glucose during prolonged exercise and a significant decrease of insulinemia when cycling above VT. This response indicates a metabolic adaptation to prolonged exercise that may to some extent prevent the decline in blood glucose that occurs during prolonged exercise and is assumed to induce fatigue and decreased performance. Such long-duration exercise sessions at high-power intensity (above VT) are relevant to some usual situations in middle-aged subjects, e.g., intense and prolonged leisure cycling sessions. Thus it is interesting to notice that trained subjects exhibit a metabolic adaptation that seems to be worthwhile for coping with this situation. However, the mechanism for this effect of training is not fully explained by the information collected in this study.

One could suggest a first explanation related to the absolute power output. Although the trained men worked at the same relative intensity (i.e., 70% of $\dot{V}O_{2 \, max}$), they had a higher absolute intensity. However, the difference between groups was not important (+50 W below VT and +70 W above VT). Theoretically, catecholamines may be involved in this mechanism, more especially above VT. In fact, the trained men had

a higher Epi response than the sedentary men at the end of exercise performed above VT, but there was also no clear workload-related difference in catecholamine response and glycemia. Moreover, there was no positive correlation between catecholamines and blood glucose, but rather a negative one (between Epi and blood glucose), which is likely to reflect more the effect of these hormones on glycolysis than their hyperglycemic action. Thus neither differences in absolute workload nor differences in catecholamine responses appear to provide a satisfactory explanation for differences between trained and sedentary men.

The GH/IGF-I axis can also be hypothesized to play a role in this specific pattern observed in the trained men, keeping in mind that in these subjects it has also undergone very marked modifications. There is clearly a link in this study between the GH/IGF-I axis and glucoregulation, as shown in Fig. 3, with the positive correlation between S_I and IGFBP-1. It is assumed to reflect a homeostatic loop aimed at preventing hypoglycemia in subjects whose S_I is high and in whom IGF-I may thus result in hypoglycemia. A more specific role of IGFBP-1 as a factor preventing hypoglycemia at exercise has also been suggested (19, 30). Although in our study the trained men had both a higher IGFBP-1 concentration in blood and a better stability of glucose at exercise, there is no obvious relationship between these two phenomena, and we do not provide additional evidence for this so-called "antihypoglycemic role of IGFBP-1" (19, 30).

In summary, the present study shows that training increases both the secretory activity of the somatotrope axis (GH/IGF-I) and S_I in middle-aged men. These findings further support the notion that age-related declines in activity of the GH/IGF-I axis and glucose tolerance in middle-aged men can be improved by endurance training. During 1-h endurance sessions, both below and above VT, there is a sustained GH response, which is markedly amplified in trained men. In addition, basal IGFBP-3 was correlated with $\dot{V}O_{2 \, max}$, as previously reported in young subjects (4, 5), further suggesting IGFBP-3 as an endocrine marker of physical fitness. On the other hand, basal IGFBP-1 is higher in trained men and is correlated with S_I. Finally, training is also associated with a stronger insulin decrease and a lack of decrease in blood glucose during high-intensity exercise. Therefore, endurance training in middle-aged men induced several metabolic and hormonal alterations that are associated with an improvement in glucose homeostasis at rest and during exercise and with an increased activity of the GH/IGF-I axis. However, the causal relationships among all these modifications remain unclear and will require additional studies.

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