Serum Levels of Insulin-like Growth Factor-I (IGF-I), and IGF-Binding Proteins-1 and -3 in Middle-Aged and Young Athletes Versus Sedentary Men: Relationship With Glucose Disposal

J. Manetta, J.F. Brun, C. Fedou, L. Maimoun, C. Prefaut, and J. Mercier

The goal of this study was to characterize the respective effects of aging and endurance training on serum insulin-like growth factor I (IGF-I), as well as IGF-binding proteins (IGFBP)-1 and -3 in relationship with glucose disposal. Thirty-two subjects (16 middle-aged men: 8 cyclists and 8 sedentary men; and 16 young men: 8 cyclists and 8 sedentary men) were compared in this study. Insulin sensitivity (SI) and glucose effectiveness (Sg) were assessed by the minimal model. Endurance training increased SI, Sg, and IGFBP-1 and -3 in both age groups (P < .05), but the older group showed a greater increase in SI and IGFBP-1 than the younger group (P < .05). IGF-I was increased only in the middle-aged trained men (P < .05). An effect of aging was found in the sedentary subjects, who presented lower IGF-I and SI (P < .05) when older. This effect disappeared with training since IGF-I and SI were nearly identical in young and middle-aged trained subjects. SI was correlated with IGFBP-1 (P < .01). These data suggest that (1) endurance training increases SI, Sg, and IGFBP-1 and -3 in men and, for SI and IGFBP-1, this increase becomes more pronounced with age; (2) endurance training may attenuate the aged-related decline in SI and IGF-I; and (3) IGFBP-1 may protect against the risk of hypoglycemia by counteracting the hypoglycemic effect of IGF-I in such situations of high SI.

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modifies the effect of endurance training on IGF-I, IGFBP-1, and IGFBP-3 levels in connection with glucose homeostasis.

SUBJECTS AND METHODS

Subjects

Sixteen male cyclists (8 young [24.7 ± 1.4 years] cyclists [Ycy] and 8 middle-aged [51.6 ± 1.2 years] cyclists [Macy]) and 16 sedentary males (8 young [23.9 ± 0.8 years] men [Ysed] and 8 middle-aged [52.3 ± 1.1 years] men [MASed]) participated in the study. None had a family history of diabetes or hypertension. Smokers or those currently using medication for the control of blood pressure or lipid or carbohydrate metabolism were excluded. No subject exhibited electrocardio-gram abnormalities at rest or during a maximal ergocycle test. Physical characteristics of all subjects are shown in Table 1. The training program for the middle-aged cyclists was carried out as a group activity and amounted to almost 16 hours of training per week. All had followed this training schedule for the past 11 ± 1.2 years. None of the sedentary subjects participated in competitive sports or organized leisure time activities. Prior to study enrollment, a brief interview was conducted to ascertain that all subjects had approximately the same dietary habits. After a complete and accurate verbal description of the procedure, risks, and benefits associated with the study, subjects provided their written consent.

Methods

Protocol. The subjects came to the laboratory on 2 separate occasions for a maximal aerobic capacity test and an intravenous glucose tolerance test. All subjects were requested to refrain from exercise for 3 days before the glucose tolerance test. The subjects were asked to fast overnight before the intravenous glucose tolerance test until their arrival in our hospital unit at 8 AM so that the different tests of the protocol could be performed at the same time of day.

Body composition. Body composition was assessed with a 4-terminal impedance plethysmograph (Dietosystem Human IM-Scan, Milan, Italy).28

Frequently sampled intravenous glucose tolerance test (FSIVGTT) and blood samples. A cannula was placed in the cephalic fossa for blood sampling at various times, while glucose was administered via the contralateral cephalic vein. Glucose (0.5 g · kg⁻¹, solution at 30%) was slowly injected over 3 minutes. Insulin (0.02 units/kg⁻¹ body weight, ie, 1 to 2 units) was injected into the vein contralateral to the one used for sampling, immediately after 19 minutes. 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 minutes following glucose injection. Other basal blood samples were pooled for determination of basal plasma concentrations of IGF-I, IGFBP-1, and IGFBP-3.

Measurement of SI and Sg. Minimal model analysis of FSIVGTT was according to Bergman et al.29 with TISPAG software from the Department of Physiology, University of Montpellier I,29 which uses a nonlinear least square estimation. This program gave the values of SI and Sg. SI is an index of the influence of plasma insulin to change glucose’s own effect on glucose concentration. Sg is the fractional disappearance rate of glucose, independent of any insulin response.

Incremental maximal exercise test. The subject’s VO₂max was measured during 8 to 12 minutes 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 using flow rates similar to subject ventilation. Heart rate was monitored throughout the exercise test. Exercise testing started with a 3-minute 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 O₂ consumption (VO₂) stability.

Laboratory measurements. Samples were analyzed for plasma insulin by radioimmunoassay (kit SB-INSI-5 from International CIS, Vercelli, Italy). The within-assay coefficient of variation (CV) for insulin was determined by repetitive measurements of the same sample and was 6.6% and the between-assay CV was 6.2%. The sensitivity (lowest detectable value) was less than 1 μU/mL. Plasma glucose was measured with a Beckman glucose analyzer, with CVs of 8.3% (within-assay) and 7.9% (between-assay).

Serum somatomedin C/IGF-I was assayed with the kit from Medegenix (Brussels, Belgium). This is a double-antibody displacement assay which includes an ethanol acid extraction procedure from serum samples. After the extraction procedure, radioimmunoassay (RIA) is performed employing the addition of sample and rabbit anti-IGF-I, followed by a 2-hour incubation at 2 to 8°C. Iodine 125 IGF-I is then added, followed by a second incubation for 20 hours at 2 to 8°C. Pre-precipitated carrier, the second antibody, and polyethylene glycol are added in a single step. The assay is centrifuged after the second 2-hour antibody incubation at 2 to 8°C. The detection limit is 2 nmol/L. This assay does not cross-react (<1%) with IGF-2, human GH, fibroblast growth factor (FGF), or platelet-derived growth factor (PDGF).

Table 1. Baseline Characteristics for Cyclists and Sedentary Subjects

<table>
<thead>
<tr>
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<th>Sedentary Subjects</th>
<th>Cyclists</th>
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<tr>
<td></td>
<td>Middle-Aged</td>
<td>Young</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.1 ± 1.1</td>
<td>23.7 ± 0.74</td>
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<tr>
<td>Height (cm)</td>
<td>173.1 ± 1.4</td>
<td>178.8 ± 1.5</td>
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<tr>
<td>Weight (kg)</td>
<td>75.2 ± 2.4</td>
<td>70.8 ± 2.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.09 ± 0.6</td>
<td>22.1 ± 0.4</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>22.4 ± 1.5</td>
<td>15.1 ± 2.7</td>
</tr>
<tr>
<td>VO₂max (mL/min/kg)</td>
<td>33.1 ± 1.2</td>
<td>47.3 ± 2.24</td>
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NOTE. Values are means ± SE.
*Significant difference between trained v sedentary in young group, P < .05.
†Significant difference between trained v sedentary in middle-aged group, P < .05.
‡Significant difference between young v middle-aged in sedentary group, P < .05.
§Significant difference between young v middle-aged in trained group, P < .05.
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 kit (Diagnostic System Laboratories, Webster, TX). This is a 2-site immunoradiometric assay (IRMA) in which the analyte to be measured is “sandwiched” between 2 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 1% and 3.5%; 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 RIA kit (Diagnostic System Laboratories). This is a classical RIA with competition for a fixed number of antibody-binding sites. The separation of free and bound antigen is achieved 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-1, -2, and -4 has been detected.

Statistics

Data are expressed as means ± SE. To detect differences between training status and age groups, a 2-way analysis of variance (ANOVA) was performed. If the ANOVA indicated significant differences, these were located by a pairwise multiple comparison procedure (Student-Newman-Keuls). To detect differences between parameters represented by a single measurement, nonparametric tests for unpaired (Mann-Whitney) and paired (Wilcoxon) data were used as appropriate. Correlations were performed by Pearson analysis. P < .05 was considered significant.

RESULTS

Subjects were matched for height, weight, body mass index (BMI), and fat (%) in each category of age (Table 1). Vo2max was higher in the cyclists than in the sedentary subjects independently of age and it was higher in the younger than in the older subjects (Table 1).

Effect of Age

Sedentary group. Basal glucose was higher (+17%, P < .05; Table 2) in MAcy than in Ycy. Basal IGF-I was lower (-28%, P < .05; Table 2) in MAcy than in Ycy. Basal Vo2max was lower (-31%, P < .05; Fig 1) in MAcy than in Ycy.

Trained group. Basal insulin was lower (-154%, P < .05; Table 2) in MAcy than in Ycy. No difference in the minimal model parameters was found between MAcy and Ycy. Basal IGFBP-1 was higher (+26%, P < .05; Fig 1) in MAcy than in Ycy.

Effect of Training

Young subjects. SI and Sg were higher (+205% and 81%, respectively, P < .05; Table 2) in Ycy than in Ysedy. Basal
IGFBP-1 and IGFBP-3 were higher (+38 % and +17 %, respectively, \( P < .05 \); Fig 1) in Ycy than in Ysed.

**Middle-aged subjects.** Basal insulin and glucose were lower (−39 % and −10%, respectively, \( P < .05 \); Table 2) in MAcy than in MA sed. SI and Sg were higher (+510 % and +56 %, respectively, \( P < .05 \); Table 2) in MAcy than in MA sed. Basal IGF-I, IGFBP-1, and IGFBP-3 were higher (+61%, +127%, and 21%, respectively, \( P < .05 \); Fig 1) in MAcy than in MA sed.

However, the increase in SI and IGFBP-1 was higher in older than younger subjects (+250% and +330%, respectively, \( P < .05 \); Table 2 and Fig 1).

**Relationships Between Glucose Disposal, GH/IGF-I Axis, and Fitness Level**

When we considered all subjects together (\( n = 32 \)), we found a positive correlation between SI and IGFBP-1 \( (r = 0.46, P < .001) \) (Fig 2) and a negative correlation between fasting insulin and IGFBP-1 \( (r = -0.6, P < .001) \). In addition, \( \text{Vo}_{2 \text{max}} \) was positively correlated with IGFBP-3 \( (r = 0.49, P < .01) \).

**DISCUSSION**

In the present study, we delineated the effect of an interaction between aging and endurance training on IGF-I and its binding proteins IGFBP-1 and -3 in relationship with glucose disposal. The main results of this cross-sectional investigation were that endurance training increased IGFBP-1 and IGFBP-3 in both middle-aged and young men in association with an improvement in SI and Sg. However, the increase in SI and IGFBP-1 was higher in the older subjects. IGF-I was increased by endurance training in the middle-aged men but not in the young subjects, in accordance with a recent study. The effect of age was quite pronounced in the sedentary population, with a decrease in IGF-I in the older subjects in association with impaired SI, whereas in the trained population, these parameters were identical in the 2 age groups. Further, when we considered all subjects together, we found a positive correlation between SI and IGFBP-1 and between \( \text{Vo}_{2 \text{max}} \) and IGFBP-3, and a negative correlation between fasting insulin and IGFBP-1.

Endurance training increased IGFBP-1 and IGFBP-3 independently of age. Although this finding agrees with the reports of others who investigated in a young population, our data contrast with those of a study that found no improvement in IGFBP-1 and -3 after training in healthy aged men. A possible explanation for this difference is that their population was older (66 ± 52 years); in fact, aging itself may affect the potential of physical training to enhance IGFBP-1 and IGFBP-3. Another explanation is a difference in training status (ie, intensity and duration), particularly when it concerns comparison with an aged population. For example, 2 weeks of endurance training at moderate intensity did not increase IGFBP-1 in an aged sedentary population, whereas specific intense training in elite athletes performed over several months increased this binding protein. In the latter case, the positive effects on the IGF system could be supported by an increased gene expression of these proteins in skeletal muscular tissue after long periods of training, as previously demonstrated for IGF-I from an animal study.

Although aerobic exercise is generally acknowledged to be effective for increasing GH and IGF-I levels, little attention has been paid to the kind of training that yields the best results and, therefore, optimal exercise programs have not been defined. Greater intensity and duration during training, for example, would be sufficient to improve somatotropic activity, especially with endurance sports such as cycling. However, circulating IGF-I and its binding proteins seem to affect previously trained and untrained individuals differently during prolonged physical training, with more extensive increases in the untrained subjects. Moreover, if training is intense and the expenditure of energy exceeds energy intake, this calorie restriction can cause IGF-I to decrease in the circulation. In addition, Poehlman et al showed that the increased fasting level of IGF-I by endurance training was more pronounced in older men than women. This last finding explains in part why the secretion activity of the GH/IGF-I axis was not enhanced after exercise and/or training in studies with exclusively female populations or with non-inclusion of women.

Our athletes were well-nourished men who belonged to the same cycling club. Training intensity and duration were almost the same in the 2 groups (an average of 14 hours per week), and because the study design ensured that no subject fasted for more than 12 hours, we do not believe that an altered energy balance could have modulated IGF-I under the present experimental conditions. Our study was cross-sectional, however, and the role of genetics is important in determining hormonal responses. A recent study that investigated elderly twins showed that approximately 60% of IGF-I levels are genetically determined. To limit this impact and control for all possible confounding factors, we carefully matched our subjects in terms of body composition, age, weight, height, gender, nutritional status, and pattern of physical activity. Our results are thus very likely a reflection of the effect of endurance training.
The increase in IGF-I through exercise training was seen only in the middle-aged subjects. This agrees with previous studies\(^9\) and suggests that physical activity may reverse the age-related decline in this hormone.\(^9\) In this case, the exercise-induced increase in IGF-I would stimulate protein synthesis in muscle\(^9\) and thus correct the loss of lean body mass observed in aged subjects.\(^9\) On the other hand, few data on IGFBP-3 are available in the current literature, especially in older subjects. IGFBP-3 is considered to be an integrated index of GH secretion\(^9\) and, although we did not measure plasma GH, the training-related increase in both IGFBP-3 and IGF-I in our middle-aged subjects indicates increased GH and IGF-I secretion. In fact, it appears likely that endurance training amplified the secretory activity of the GH/IGF-I axis in these men. This suggests that the age-associated decline in somatotropic system activity\(^\) may be attenuated by endurance training. A recent study that investigated older marathon runners compared with age-matched sedentary controls\(^37\) did not support this hypothesis, but it should be noted that there was a significant difference in BMI between the 2 groups and the low body fat percentage in their runners may have modified the GH/IGF-I response.\(^38\) In the present study, we matched subjects of each age group for fat percentage to control for this parameter. As seen in Table 1, there was no significant difference in the middle-aged subjects and only a minimal one (2%) in the young subjects. The higher somatotropic response in our trained groups is therefore not likely to be explained by differences in fat mass. Moreover, since VO\(_{2\text{max}}\) is in relationship with the GH/IGF-I response,\(^10\) the lack of physical fitness data in their report\(^37\) limited the interpretation of their result. We showed a relationship between IGFBP-3 and VO\(_{2\text{max}}\) which confirms that the level of physical activity is an important modulator of the GH/IGF-I axis\(^1\) and that IGFBP-3 is a marker of physical fitness.\(^2\) However, lower levels of IGF-I in aging men are more strongly related to the decline in maximal aerobic power than to an increase in adiposity.\(^8\) In the present study, we did not find a negative relationship between IGF-I and VO\(_{2\text{max}}\).

Consistent with previous studies,\(^5,8,9\) our data showed that aging decreased IGF-I and SI in sedentary individuals. On the other hand, training resulted in high values of IGF-I, which in fact were quite the same in the young and middle-aged cyclists. Given that IGF-I and SI were both completely preserved in our older trained subjects, it thus appears that middle-age in men is a beneficial period for exercise training (ie, cycling) to counteract the decrease in SI and GH/IGF-I responses associated with aging.

Because aging has been demonstrated to impair both glucose disposal\(^5,10,11\) and this axis,\(^5,7\) we investigated SI and Sg in relationship with the GH/IGF-I axis. In agreement with several studies,\(^3,5\) we showed that training improved SI and Sg. Interestingly, it has been shown both this study and a previous one of other group\(^9\) that endurance training decreased fasting insulin concentration and increased IGFBP-1. Although noted in both groups, the increase in SI and IGFBP-1 was higher in the older than in the younger men. Hopkins et al\(^22\) proposed that IGFBP-1 directs circulating IGFs to skeletal muscle, in which IGF-I receptors are abundant and IGFs could facilitate glucose uptake into muscle. In agreement with this, IGFBP-1 is also thought to be an insulin counter-regulator, blocking “free” insulin-like activity during hypoglycemia.\(^23\) In addition, a direct effect of IGF-I on SI and specifically on glycogen storage in skeletal muscle tissue has been evidenced.\(^40\) The higher glucose uptake in middle-aged trained men, due to an increase in the insulin-dependent component rather than non-insulin-dependent glucose uptake,\(^5\) might therefore be controlled by the IGFBP-1 level. If this is so, the effect of IGFBP-1 on glucose metabolism by counteracting the hypoglycemic effect of both insulin\(^23,27\) and IGF-I\(^16\) would be more pronounced in our well-trained middle-aged population, who showed the higher IGFBP-1 level. In addition, we found a negative relationship between fasting insulin level and IGFBP-1 and a positive relationship between SI and IGFBP-1. This last correlation has been interpreted as reflecting a homeostatic loop aimed at preventing hypoglycemia in subjects whose SI is high and in whom IGF-I may result in hypoglycemia.\(^2\) These results indicate a role for IGFBP-1 in regulating glucose disposal and, more particularly, insulin action.

On the whole, the observation of higher concomitant increases in SI and IGFBP-1 through endurance training in middle-aged men suggests an physiological role for IGFBP-1 in the regulation of glucose metabolism in this age population. However, this requires further investigation.

In summary, our results show that endurance training improved glucose disposal (ie, SI and Sg) and increased serum levels of IGFBP-1 and -3 in men independently of age, whereas IGF-I was increased only in middle-aged men. Although the effect of age was quite pronounced in the sedentary population, with decreases in SI and IGF-I, these parameters were identical in the 2 trained populations. Thus, endurance training may attenuate the age-related decline in both the GH/IGF-I axis and insulin sensitivity. Higher IGFBP-1 levels due to training seem to be involved in glucose regulation in relationship with insulin sensitivity and may provide protection against hypoglycemia. This phenomenon seems to be more pronounced in middle-aged trained men.

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