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Abdominal Visceral Fat and Fasting Insulin Are Important Predictors of 24-Hour GH Release Independent of Age, Gender, and Other Physiological Factors

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Numerous physiological factors modulate GH secretion, but these variables are not independent of one another. We studied 40 younger (20–29 yr.; 21 men and 19 women) and 62 older (57–80 yr.; 35 men and 27 women) adults to determine the contributions of several demographic and physiological factors to the variability in integrated 24-h GH concentrations. Serum GH was measured every 10 min for 24 h in an enhanced sensitivity chemiluminescence assay. The predictor variables included: age group (young or old), gender, abdominal visceral fat (by computed tomography), total body fat mass and percentage body fat by dual-energy x-ray absorptiometry, serum IGF-I, fasting serum insulin, 24-h mean estradiol and testosterone, and peak oxygen uptake by graded exercise (treadmill) testing. Multiple ordinary least squares regression analysis was used to quantitatively assess the individual contribution that each predictive measure made to explain the variability among values of integrated 24-h GH concentrations while in the presence of the remaining predictors. The model explained 65% of the variance in integrated 24-h GH concentrations. Abdominal visceral fat ($P < 0.002$) and fasting insulin ($P < 0.008$) were consistently important predictors of integrated 24-h GH concentrations independent of age group, gender, and all other predictor variables. Although serum IGF-I was an important overall predictor of integrated 24-h GH concentrations ($P = 0.002$), this relationship was present only in the young subjects and was modulated by gender. The remaining variables failed to contribute significantly to the model. We conclude that abdominal visceral fat and fasting insulin are important predictors of integrated 24-h GH concentrations in healthy adults, independent of age and gender. Serum IGF-I is an important predictor of integrated 24-h GH concentrations in young but not older subjects. Bidirectional feedback between each of these three factors and GH secretion may account for the strong relationships observed. (*J Clin Endocrinol Metab* 86: 3845–3852, 2001)

GROWTH HORMONE (GH) is secreted by the anterior pituitary gland in a pulsatile fashion under the regulation of two hypothalamic peptides: GHRH and somatostatin. GHRH stimulates GH synthesis and secretion and somatostatin inhibits GH release (1, 2). The reciprocal control of GH secretion by GHRH and somatostatin may be regulated by activation of the GH secretagogue receptor by its putative endogenous ligand, ghrelin (3, 4). GH secretion changes throughout the life span. Twenty-four-hour integrated GH concentrations (24-h IGHC) are greatest during the adolescent years and decline to relatively low levels by the fifth decade of life (5). Although maturational and chronological age have been strongly associated with the amount of GH released, multiple physiological factors have been found to regulate GH secretion. These include age, gender, pubertal status, nutrition, sleep, body composition, regional distribution of body fat, stress, fitness, gonadal steroids, insulin, and IGF-I (1, 2).

In concert with the many factors that regulate GH, GH has diverse metabolic actions that regulate body composition, fluid homeostasis, glucose and lipid metabolism, bone metabolism, and cardiac function. These actions result in favorable metabolic effects and improve the quality of life in adults with GH deficiency who are treated with GH replacement (6). These complex relationships suggest that feedback loops govern the relationships among GH secretion, the central nervous system and the metabolic milieu.

Although multiple physiological factors have been reported to influence GH secretion, these predictors of GH secretion are not independent of one another, because there are significant correlations among several of these variables. For example, the amount of abdominal visceral fat (AVF) may be correlated with body fat mass, aerobic exercise capacity, and fasting insulin concentrations (7, 8, 9). Changes in estrogen levels may also regulate body fat distribution, because postmenopausal women have more AVF than premenopausal women (10). The relative importance of these physiological factors in the regulation of GH secretion is not known. We hypothesized that, among the variables known to regulate GH secretion, it would be possible to determine the relative importance of the predictors that contribute to 24-h GH release in young and old men and women. Therefore, the purpose of this study was to examine the relation-

Abbreviations: AVF, Abdominal visceral fat; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; E2, estradiol; 4-comp, four-compartment body composition model; GCRC, General Clinical Research Center; IGHC, integrated 24-h GH concentration(s); T, testosterone; VO_2 peak, peak oxygen consumption.

ship between suggested mediators of GH release and 24-h IGHC. The independent variables examined included: age (young and old), gender, total body fat mass, total body percentage body fat, AVF, aerobic fitness [peak oxygen consumption (VO_2 peak)], fasting insulin and IGF-I concentrations, and pooled estradiol (E2) and testosterone (T) concentrations. Multiple ordinary least squares regression was used to quantitatively assess the individual contribution that each predictive measure made to explain the variability among values of 24-h IGHC while in the presence of the remaining predictors.

Materials and Methods

Subjects

The study was approved by the Human Investigation and General Clinical Research Center (GCRC) Advisory Committees of the University of Virginia, and each subject provided written informed consent. Forty healthy young (21 men and 19 women; 20–29 yr old) and 58 older (35 men and 23 women; 57–80 yr old) adults served as subjects. All subjects were Caucasian, with the following exceptions: five were African Americans (two young men, one young woman, one older woman, and one older man); two were Asian Americans (one young woman and one young man); two were Hispanic Americans (one young woman and one young man); one young woman was of Middle-Eastern origin; and one young woman was of Native American origin. Subjects were recruited by local media advertisement. All subjects completed a detailed medical history and underwent a physical examination before participation. None of the subjects were taking medications known to affect GH release or body composition measures including exogenous use of estrogen (*i.e.* oral contraceptives or estrogen replacement therapy). All of the women in the older age group were postmenopausal. The women in the younger age group were all eumenorrheic and were studied during the early follicular phase of the menstrual cycle (d 1–5 of the menstrual cycle). All subjects were nonsmokers, had not undertaken transmeridian travel for at least 4 wk, and had normal biochemical indices of hematological, renal, hepatic and thyroid function.

Testing procedures

Graded exercise testing. VO_2 peak (ml/kg·min) was determined using a graded maximal exercise treadmill (Quinton Q65; Quinton Instrument Co., Seattle, WA) test. Subjects were instructed to begin a walking warm-up on the treadmill at an initial velocity that varied between 60–100 m/min (self-selected). Velocity was increased every 3 min by 10 m/min until the subject reached volitional exhaustion. Metabolic measures were collected during the exercise test via standardized indirect calorimetry procedures using a SensorMedics 2900-Z metabolic cart (SensorMedics, Yorba Linda, CA). Heart rate (12 lead electrocardiogram), blood pressure, ratings of perceived exertion, and blood lactate (model 2700 Select; Yellow Springs Instruments, Yellow Springs, OH) measures were also collected at the end of each exercise stage.

AVF. AVF (cm^2) was determined by single-slice computed tomography scans. Scans were performed using a Picker PQ 5000 and analyzed with a newly developed tissue quantification analysis package using a Picker Voxel Q 3D imaging station (Picker International, Cleveland, OH) as described previously (11). The scanning was performed with 140 kV and a slice thickness of 0.5 cm. The subjects were clothed only in a loose gown and examined in a supine position with their arms stretched above their head. An abdominal scan at the level of the L4–L5 intervertebral space was performed with no angulation using a lateral pilot for location. AVF cross-sectional area (cm^2) was calculated by delineating with a mouse computer interface the anatomical boundary landmarks and then computing the adipose tissue area using an attenuation range from −190 to −30 Hounsfield units. All computed tomography scan analyses were performed by a single trained investigator (J.L.C.)

Total body fat mass and total body percentage fat measurements. Total body dual-energy x-ray absorptiometry (DXA) scans were performed in the pencil beam mode using a Hologic QDR 2000 (Hologic, Inc., Watham, WA) bone densitometer to determine the total body fat mass (fat mass; kg) and total body percentage fat (% fat) for each subject. The subjects were instructed to remove all objects such as jewelry or eyeglasses and wore only a standard hospital gown during the standardized scan procedure (12). All scans were subsequently analyzed by a single trained investigator (J.L.C.) using the Hologic enhanced whole body software version 5.64.

Blood sampling procedures

GH release. 24-h IGHC ($min \cdot \mu g/liter^{-1}$) was determined for each subject. Subjects were admitted to the GCRC the evening before blood sampling. The following morning, a venous cannula was inserted into a forearm vein of each arm at 0630 h and blood samples were obtained every 10 min for 24 h (0800–0800 h) for measurement of GH. Standardized meals (30% fat, 15% protein, and 55% carbohydrate) were served between 0800 and 0900 h, at 1200 h, and at 1800 h. These meals were based on total daily caloric requirements determined from resting (basal) bedside calorimetry measurements (Delta Trac I; SensorMedics) performed for 30 min (0600–0630 h). Total daily caloric requirements were calculated by multiplying the basal metabolic rate by an activity factor of 1.3–1.5, depending on the individual. Volunteers were permitted to ambulate, but were not allowed to nap or sleep until 2200 h during the blood sampling. During the sleeping hours, blood samples were obtained via a 12-ft double lumen catheter kept patent by a heparinized (5000 U/liter) saline solution infused at 30 ml/h as described previously (13).

Fasting insulin, IGF-I, and steroid concentrations. Fasting insulin and serum IGF-I concentrations were determined from blood samples drawn at approximately 0700 h during the first day of blood sampling. In addition, blood samples were drawn at 6-h intervals (0800, 1400, 2000, and 0200 h) for the measurement of E2 and total T; the means of these four measurements were used for statistical analysis.

Hormonal assays

GH concentrations in all serum samples were measured with an immunometric chemiluminescence assay (Nichols Luma Tag hGH; Nichols Institute Diagnostics, San Juan Capistrano, CA) modified to enhance the sensitivity to 0.002 μg/liter (14). This assay detects predominately the 22 kDa form of GH, with 34% cross-reactivity for 20 kDa GH (methionylated). The median intra-assay and interassay coefficients of variation (CV) were 4.7% and 8.6%, respectively.

Fasting insulin concentrations were measured in the University of Virginia Diabetes Core Laboratory by a RIA method with a sensitivity of 11 pmol/liter (15); the interassay CV was 11% at 49 pmol/liter and 5.9% at 100 pmol/liter. Serum IGF-I concentrations were measured by RIA after acid-ethanol extraction (Nichols Institute Diagnostics). The median intra-assay and interassay CV were 2.7% and 6.8%, respectively. The pooled E2 and total T concentrations were determined by RIA (Diagnostic Products, Los Angeles, CA). The E2 assay is known to have 10% cross-reactivity with estrone. Intra- and interassay CV were 3.9% and 9.5% for E2 and 6.9% and 10.3% for total T, respectively. The GH, IGF-I, E2, and total T assays were performed in the University of Virginia Health Sciences GCRC Core Laboratory. Hematology, serum chemistries, thyroid function tests, and urinalyses were performed in the University of Virginia Health Sciences Center Clinical Laboratories using routine methods.

Statistical analyses

Group differences in aerobic fitness, body composition, and serum hormone concentrations were determined by two-way ANOVA (gender X age group). Multiple ordinary least squares regression was used to quantitatively assess the individual contribution that each predictive measure (age group, gender, VO_2 peak, AVF, fat mass, % fat, fasting insulin, IGF-I, E2, and total T) made to explain the variability among values of 24-h IGHC while in the presence of all remaining variables. Because age group (young and old) rather than actual chronological age was used in this analysis, the model specified that the values of the intercept and slope parameters were free to change across age group and gender. An extra sum of squares principles (16) were used to measure the marginal reduction in the residual error sum of squares attributed

to each predictor being added to the regression model in the presence of the remaining variables. Variable ranking with respect to the overall importance to predicting 24-h IGHC was based on the contribution that each predictor made to the total regression sum of squares, a quantitative measure of the variation explained by the regression model (17). Global contributions were summarized by means of a χ^2 statistic adjusted by the total degrees of freedom associated with the terms of the predictors (18). Probabilities associated with tests of statistical inference were obtained from a χ^2 distribution with the appropriate number of degrees of freedom. Extra sum of squares tests were used to determine whether or not the slope parameters were equal across age and gender with respect to predicting 24-IGHC as a function of AVF, IGF-I, and fasting insulin. To stabilize residual variance across age group and gender required modeling the values of 24-h IGHC on their natural logarithmic scale.

Pearson's correlation was used to estimate the linear correlation between the values of each of the continuous predictors of 24-h IGHC and the values of the remaining continuous predictors and 24-h IGHC. Splus version 4.5 (Mathsoft Inc., Seattle, WA) was used to carry out the statistical analyses.

Results

Table 1 contains the mean (±SE) values for age, height, weight, body mass index (BMI), VO_2 peak, AVF, fat mass, % fat, IGF-I, fasting insulin, E2, and total T presented by age and gender groups. The age of the men and women did not differ within each age group (young *vs.* old). Compared with the young men, the young women had significantly lower values for weight ($P < 0.0001$), height ($P < 0.001$), VO_2 peak ($P < 0.006$), and total T ($P < 0.001$). In contrast, the values for fat mass ($P = 0.004$) and % fat ($P = 0.0001$) were significantly greater in the young women than in the young men and there was a trend for higher E2 concentrations in the young women ($P = 0.06$). There were no significant gender differences among the young subjects for BMI, AVF, fasting insulin, and IGF-I. Compared with the older men, the older women had significantly lower values for weight ($P < 0.001$), height ($P < 0.001$), VO_2 peak ($P < 0.001$), total T ($P < 0.001$), E2 ($P = 0.0001$), and IGF-I ($P = 0.02$). The values for fat mass ($P < 0.001$) and % fat ($P < 0.001$) were significantly greater in the older women than in the older men. There were no significant gender differences for BMI, AVF, and fasting insulin among the older subjects. Compared with the young women, older women had significantly greater values for BMI ($P = 0.009$), AVF ($P < 0.001$), fat mass ($P < 0.001$), and % fat ($P < 0.001$). In contrast, the values for height ($P = 0.006$), VO_2 peak ($P < 0.001$), total T ($P < 0.001$), E2 ($P < 0.001$), and IGF-I ($P < 0.001$) were significantly lower in the older women than in the young women. There were no significant differences by age group for weight and fasting insulin among the women. Compared with the young men, older men had significantly greater values for weight ($P = 0.03$), BMI ($P = 0.03$), AVF ($P < 0.001$), fat mass ($P < 0.001$), and % fat ($P < 0.001$). Serum E2 ($P = 0.007$), IGF-I ($P < 0.001$), and VO_2 peak ($P < 0.001$) were significantly lower in the older compared with the young men. There were no significant differences by age group for height, fasting insulin, and total T among the men.

Figure 1 depicts the 24-h IGHC results for the four groups. Young women had 1.6-fold higher 24-h IGHC than young men (2375 ± 260 *vs.* 1444 ± 230 $min \cdot \mu g \cdot liter^{-1}$, $P = 0.01$). Among the older subjects, the women had 1.4-fold higher 24-h IGHC than the men (1012 ± 126 *vs.* 733 ± 95 $min \cdot \mu g \cdot liter^{-1}$, $P = 0.08$). For both genders, the older subjects had 24-h IGHC that were approximately 50% of those observed in the young subjects ($P < 0.002$).

Table 2 displays Pearson's correlation coefficients for the relationships among the physiological factors used to predict 24-h IGHC. The multiple ordinary least squares regression model explained 65% of the variance in 24-h IGHC when age group, gender, VO_2 peak, AVF, fat mass, % fat, IGF-I, fasting insulin, total T, and E2 concentrations were included as predictor variables. The statistical model ranked the predictor variables based on the contribution that each variable made to the total regression sum of squares while in the presence of the remaining predictors. Variables with higher predictive value for 24-h IGHC have greater values of χ^2 minus the degrees of freedom than variables with lower predictive value. These results are shown in Table 3. AVF ($P < 0.002$) and fasting insulin ($P < 0.008$) were the most important predictors of 24-h IGHC independent of age group, gender, and all other predictor variables. Significant age ($P < 0.011$) and gender ($P < 0.016$) interactions were observed with serum IGF-I, indicating that the relationship between 24-h IGHC and IGF-I changed depending on the age and gender

TABLE 1. Physical characteristics, aerobic fitness (VO_2 peak), AVF, total body fat mass (fat mass), total body percentage fat (% fat), fasting serum IGF-I and insulin concentrations, and pooled serum E2 and total T concentrations of the subjects

Mean ± SE	Young		Old	
	Men (n = 21)	Women (n = 19)	Men (n = 35)	Women (n = 27)
Age (yr)	24.4 ± 0.6	23.7 ± 0.6	66.6 ± 0.8[a]	65.7 ± 1.2[a]
Weight (kg)	78.9 ± 2.0	66.8 ± 2.1[b]	85.2 ± 1.9[a]	71.5 ± 2.7[b]
Height (cm)	176.8 ± 1.1	167.6 ± 1.5[b]	177.0 ± 1.1	162.2 ± 1.2[a,b]
BMI (kg/m^2)	25.3 ± 0.7	23.8 ± 0.7	27.2 ± 0.5[a]	27.1 ± 0.9[a]
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	42.6 ± 1.4	35.0 ± 2.0[b]	30.0 ± 1.0[a]	21.7 ± 1.1[a,b]
AVF (cm^2)	44.8 ± 4.8	37.0 ± 5.5	128 ± 8.9[a]	107 ± 8.0[a]
Fat mass (kg)	13.7 ± 1.2	20.0 ± 1.7[b]	22.8 ± 1.3[a]	30.9 ± 2.0[a,b]
% fat	17.4 ± 1.4	29.8 ± 1.9[b]	26.3 ± 1.1[a]	42.4 ± 1.4[a,b]
IGF-I ($\mu g \cdot liter^{-1}$)	289.5 ± 24.6	292.8 ± 25.7	144.2 ± 10.2[a]	110.9 ± 8.5[a,b]
Fasting insulin ($\mu U \cdot ml^{-1}$)	11.7 ± 1.5	10.6 ± 1.3	12.4 ± 0.9	10.7 ± 1.1
E2 ($pg \cdot ml^{-1}$)	29.2 ± 1.5	38.7 ± 4.8	23.0 ± 1.4[a]	12.8 ± 1.3[a,b]
Total T ($ng \cdot dl^{-1}$)	471.1 ± 18.8	33.6 ± 4.5[b]	423.7 ± 19.1	17.7 ± 1.6[a,b]

[a] $P < 0.05$ compared with young subjects of the same gender.
[b] $P < 0.05$ compared with men within the same age group.

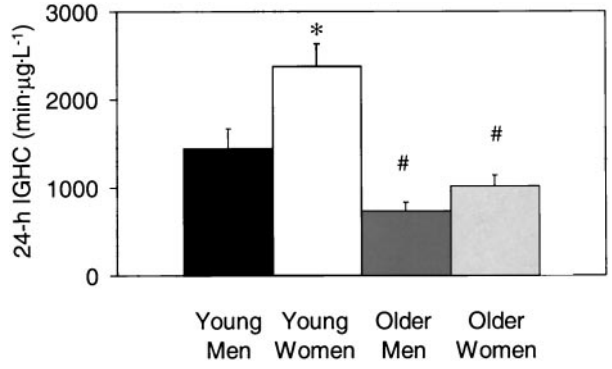

FIG. 1. Mean (±SE) 24-h IGHC for the four groups of subjects studied. Serum GH was measured using a chemiluminescence immunometric assay in blood samples collected every 10 min for 24 h. *, $P = 0.01$ *vs.* young men. #, $P < 0.002$ *vs.* young subjects of same gender.

of the subject. Thus, although serum IGF-I was the best overall predictor of 24-h IGHC (greatest global effect, $P = 0.002$), it was only useful if the age and gender of the subject was known. Global effects were observed for age ($P = 0.038$) and gender ($P = 0.079$); however, the main effects for age and gender were not significant. This suggests that the major impact of age and gender in the model was to vary the relationship between serum IGF-I and 24-h IGHC. The remaining variables (VO_2 peak, fat mass, % fat, and total T and E2 concentrations) and remaining interactions failed to contribute significantly to the model.

The relationship between 24-h IGHC and the three most important variables (AVF, fasting insulin, and IGF-I) are presented in Figs. 2–4, respectively, by age group and gender. Figure 2 graphically demonstrates that the relationship between 24-h IGHC and AVF was similar in each of the four groups of subjects. The slopes for the four regression lines were statistically equivalent. Among the young and older subjects with overlapping AVF areas (33.0–123.9 cm^2), the 24-h IGHC were not significantly different [1320 ± 202 (young) *vs.* 1150 ± 122 (older) $min \cdot \mu g \cdot liter^{-1}$, $P = 0.32$]. This provides further evidence that the relationship between AVF and 24-h IGHC was independent of age and gender. Figure 3 illustrates that the relationship between 24-h IGHC and fasting insulin was also similar among the four groups. In contrast, the relationship between 24-h IGHC and IGF-I was significantly different between the young and older subjects (Fig. 4). A positive relationship between these two variables was present in the young men and women as the slopes of the regression lines were significantly different from zero ($P < 0.002$). A trend was present for a gender difference in the slopes ($P = 0.060$). Conversely, a relationship between 24-h IGHC and IGF-I was not observed in the older men and women, as evidenced by the fact that the slopes of the regression lines were not significantly different from zero.

We have recently reported that total body fat mass and % fat measurements by DXA show significant quantifiable differences from the same measurements determined using a four-compartment body composition model (4-comp) using measurements of body density, total body water, and total body bone mineral (19). These data were available for a subset of the subjects (n = 78). We calculated the contribution that fat mass and % fat by 4-comp made to the variability in 24-h IGHC in this subgroup. These results demonstrated that regardless of the method used to determine fat mass and % fat (DXA or 4-comp), these variables failed to contribute significantly to the prediction of 24-h IGHC beyond that explained by AVF, fasting insulin, and serum IGF-I.

Discussion

Many physiological factors influence GH secretion by modulating GHRH and somatostatin secretion, and/or by altering biologically available serum IGF-I levels. However, the precise mechanisms that mediate the effect of a particular factor on GH secretion in humans are difficult to determine because of the interrelationships that exist among several of these regulators of GH secretion. The present study determined that among 10 physiological factors previously reported to regulate GH secretion, AVF and fasting serum insulin were the most important predictors of 24-h IGHC, independent of age group, gender and all other variables examined. Serum IGF-I was also an important predictor in young but not older subjects and its relationship with 24-h IGHC was significantly influenced by gender. These data do not imply that the other physiological factors examined (age, gender, fat mass, % fat, VO_2 peak, E2, and total T concentrations) are unimportant in the regulation of GH secretion. However, these variables did not contribute additional information to the prediction of 24-h IGHC beyond that explained by AVF, fasting insulin and IGF-I.

An inverse relationship between the BMI (kg/m^2) and 24-h GH release has been demonstrated in men but not consistently in studies including both men and women (9, 20–22). The relationship between percentage body fat and GH release also seems to be stronger in men than in women (9, 21–23). These variable findings suggest that percentage body fat (or fat mass) *per se* is not a primary determinant of GH secretion.

Recent studies have demonstrated a strong inverse relationship between the amount of AVF and both GH secretion and serum IGF-I concentrations (9, 23, 24). In the present analysis, AVF was the strongest predictor of 24-h GH release among the ten variables studied. An inverse, curvilinear relationship between AVF and 24-h GH release was demonstrated in both the young and old subjects, as well as in both men and women (Fig. 2). The slopes of these regression lines were very similar in all four groups studied, suggesting that this relationship is equally important in both genders and both age groups. This concept is reinforced by the observation that among young and older subjects with overlapping AVF areas there was no significant difference in 24-h IGHC. These data support the hypothesis that AVF is a more important determinant of GH secretion than age and gender, as suggested previously by Vahl and coworkers (9). The amount of AVF increases with aging as fat storage shifts from peripheral sc to intraabdominal adipose tissue depots (25). Whether this is a cause or an effect of declining GH secretion with aging remains to be determined.

Racial differences in the amount of AVF and the relationship between AVF and metabolic risk factors have been reported (26–28). Wright *et al.* (29, 30) reported that 24-h GH secretion was greater in black men compared with white

TABLE 2. Pearson's correlation coefficients for the relationships among the physiological factors used to predict 24 h IGHC

Variable	% fat	AVF	Fat mass	IGF-I	Insulin	T	E2	VO_2 peak	Age	24-h IGHC	Log (24-h IGHC)
% fat	1.000	0.505	0.906	−0.431	0.146	−0.635	−0.256	−0.749	0.423	−0.382	−0.327
AVF	0.505	1.000	0.656	−0.514	0.288	0.034	−0.279	−0.553	0.672	−0.641	−0.702
Fat mass	0.906	0.656	1.000	−0.419	0.278	−0.386	−0.194	−0.701	0.431	−0.521	−0.507
IGF-I	−0.431	−0.514	−0.419	1.000	−0.017	0.140	0.415	0.546	−0.705	0.536	0.461
Insulin	0.146	0.288	0.278	−0.017	1.000	0.051	0.128	−0.079	−0.011	−0.353	−0.412
T	−0.635	0.034	−0.386	0.140	0.051	1.000	0.126	0.408	−0.010	−0.197	−0.224
E2	−0.256	−0.279	−0.194	0.415	0.128	0.126	1.000	0.304	−0.523	0.280	0.177
VO_2 peak	−0.749	−0.553	−0.701	0.546	−0.079	0.408	0.304	1.000	−0.631	0.414	0.349
Age	0.423	0.672	0.431	−0.705	−0.011	−0.010	−0.523	−0.631	1.000	−0.484	−0.438
24-h IGHC	−0.382	−0.641	−0.521	0.536	−0.353	−0.197	0.280	0.414	−0.484	1.000	0.907
Log (24-h IGHC)	−0.327	−0.702	−0.507	0.461	−0.412	−0.224	0.177	0.349	−0.438	0.907	1.000

The natural logarithm (Log) of the 24-h IGHC was used in the regression model to stabilize the residual variance across age group and gender. See Table 1 for an explanation of other abbreviations.

TABLE 3. Adjusted χ^2 statistics and P values for tests of global effect, main effect, predictor by age interaction, and predictor by gender interaction with respect to predicting log (24-h IGHC)

Linear predictor	Global tests		Main effects		Age interaction		Gender interaction	
	Adjusted χ^2 χ^2observed-df	P $\chi^2 \geq \chi^2$observed	Adjusted χ^2observed-χ^2 df	P $\chi^2 \geq \chi^2$observed	Adjusted χ^2observed-χ^2 df	P $\chi^2 \geq \chi^2$observed	Adjusted χ^2observed-χ^2 df	P $\chi^2 \geq \chi^2$observed
IGF-1	11.88	**0.002**	4.80	**0.028**	6.51	**0.011**	5.83	**0.016**
Age	9.16	**0.038**	1.76	0.185			7.14	**0.007**
AVF	8.01	**0.012**	9.67	**0.002**	0.22	0.641	1.03	0.310
Gender	6.81	**0.079**	2.27	0.132	7.14	**0.007**		
Insulin	4.87	**0.049**	7.09	**0.008**	0.09	0.770	0.71	0.399
% fat	0.80	0.284	1.80	0.180	2.00	0.158	0.02	0.897
T	−0.57	0.488	0.82	0.365	0.64	0.424	1.15	0.283
VO_2 peak	−0.94	0.560	0.27	0.603	1.72	0.190	0.18	0.670
E2	−1.31	0.639	0.36	0.548	1.23	0.266	0.85	0.358
Fat mass	−1.68	0.724	0.04	0.841	1.04	0.308	0.17	0.676

P values less than 0.05 are highlighted in *bold*. See Tables 1 and 2 for an explanation of the abbreviations.

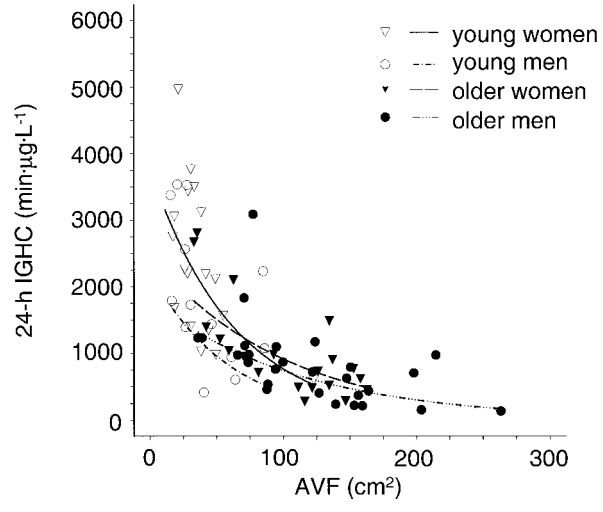

FIG. 2. The relationship between 24-h IGHC and AVF in the four groups of subjects studied. The individual data and regression line for each group is shown. The relationship between 24-h IGHC and AVF was similar in each of the four groups of subjects.

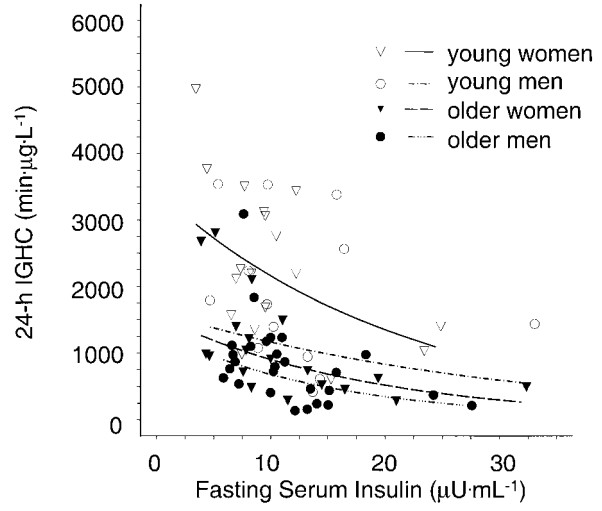

FIG. 3. The relationship between 24-h IGHC and fasting serum insulin concentration in the four groups of subjects studied. The individual data and regression line for each group is shown. The relationship between 24-h IGHC and fasting insulin was similar in each of the four groups of subjects.

men, but this racial difference was not present in women. Because 90% of the subjects recruited for the present study were Caucasian, we cannot determine whether race influences the relationship between AVF and 24-h IGHC.

The mechanisms that account for the dominant relationship between AVF and 24-h IGHC remain to be elucidated. Two plausible hypotheses could be considered: 1) increased plasma levels of insulin and free fatty acids associated with greater amounts of AVF (31) might result in negative feedback on GH secretion; or 2) reduced GH secretion might allow for accumulation of abdominal fat, as suggested by observations in adults with GH deficiency due to hypothalamic-pituitary disease (6, 32). Thus, the relationship be-

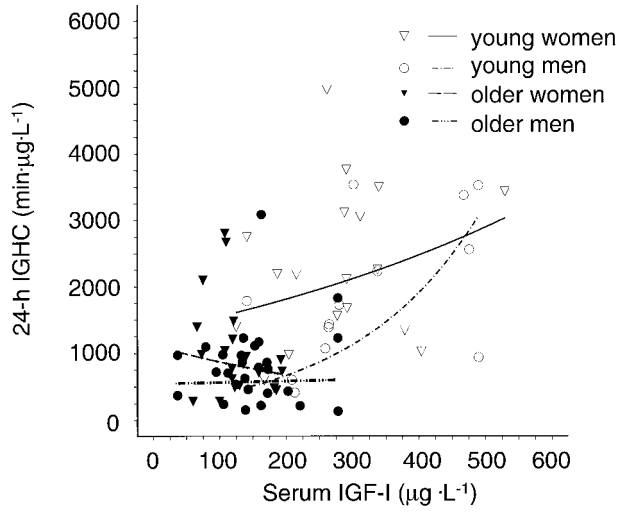

FIG. 4. The relationship between 24-h IGHC and serum IGF-I concentration in the four groups of subjects studied. The individual data and regression line for each group is shown. The relationship between 24-h IGHC and serum IGF-I was statistically significant in the young men and women but not in the older subjects.

tween AVF and 24-h IGHC may be bidirectional. Because the amount of AVF increases with increasing amounts of body fat (r = 0.66 in the present analysis) (7), this might account for the inverse association between percentage body fat and 24-h GH secretion reported in some studies that included subjects with higher percentage body fat (21, 22). Obese subjects usually have increased amounts of AVF (7), and this may account for the observation that obesity is associated with dramatic reductions of spontaneous and stimulated GH secretion (1, 2).

The observation that fasting plasma insulin concentration explained a significant portion of the variability in 24-h IGHC independent of all other variables suggests that neuroendocrine mechanisms controlling GH secretion receive negative feedback from the metabolic milieu associated with AVF. The positive association between increased AVF and hyperinsulinemia has been well established (31, 33, 34). In the present analysis, the relationship between AVF and insulin was stronger among older (r = 0.50 and 0.44 for women and men, respectively) than young subjects (r = 0.14 and 0.20 for women and men, respectively). The overall correlation between 24-h IGHC and fasting insulin was modest (r = -0.41), and insulin levels did not differ significantly among the four groups. These findings suggest that the inhibitory effect of greater amounts of AVF on GH secretion may be modulated in an individual by other variables influencing insulin sensitivity such as genetic and lifestyle factors (35). The present findings suggest that lifestyle changes that result in decreased fasting insulin levels, such as weight loss and increased physical activity, may increase 24-h IGHC in both young and older adults.

A bidirectional relationship exists between serum IGF-I and GH levels, and this may account for the emergence of serum IGF-I as one of the important predictors of 24-h IGHC in the present model. GH is a major determinant of serum IGF-I concentration because it stimulates the production of IGF-I by the liver and other tissues (2). In addition, increases in serum IGF-I rapidly decrease GH secretion by decreasing the mass of GH secreted per pulse, as has been demonstrated in both young and older subjects using iv infusion of recombinant human IGF-I (36, 37). The recovery of GH secretion from suppression by recombinant human IGF-I infusion is closely related to the decline in free but not total IGF-I concentrations (38). Plasma IGF-binding protein-1 concentrations are decreased rapidly by increases in insulin levels (39). Thus, an increase in serum insulin concentrations may increase the amount of free IGF-I in the circulation (40) and this may be one of the mechanisms that accounts for the strong influence of AVF, serum insulin, and serum IGF-I on 24-h IGHC.

Serum IGF-I concentrations are known to decline with aging and are influenced by gender (2). Thus, it was not surprising that age and gender altered the relationship between serum IGF-I and 24-IGHC in the present study. However, although a previous smaller study suggested that this relationship was weaker in older subjects (41), we are unaware of any prior reports demonstrating no relationship between serum IGF-I and 24-h IGHC in healthy subjects over the age of 55 yr (Fig. 4). Hilding *et al.* (42) have reported that the percentage of GH-deficient adults with normal serum IGF-I levels increases with age, suggesting that regulators of serum IGF-I other than GH secretion may become more important with aging.

In the present analysis, VO_2 peak and E2 and total T concentrations did not contribute additional information to the prediction of 24-h IGHC beyond that explained by AVF, fasting insulin, and IGF-I. However, positive associations between each of these variables and 24-h IGHC have been reported previously. Acute exercise is a known stimulus for GH secretion (1, 2). Both 24-h GH release and the GH response to pharmacological stimuli have been positively correlated with VO_2 peak (9, 22, 23). In the present analysis, VO_2 peak was positively related to 24-h IGHC in both the overall group (r = 0.35) as well as within the four subgroups (r values ranging from 0.17–0.77). The results of the present regression analysis suggest that aerobic fitness may influence 24-h GH release via effects on AVF and fasting insulin concentrations. In support of this hypothesis, vigorous exercise training has been reported to decrease AVF (43) and improve glucose tolerance in older subjects (44).

Gender differences in 24-h GH release have been reported previously (9, 45, 46), and gonadal steroids are thought to regulate GH secretion (1, 2). The decline in serum GH concentrations with age in men and women correlates with changes in gonadal steroid levels (20, 21, 45). In the present study, young women had significantly greater 24-h IGHC than young men and a similar trend was present among the older subjects (Fig. 1). However, the relationships between 24-h IGHC and serum concentrations of E2 and T were weak both in the overall group (r = 0.18 and −0.22, respectively) as well as within each of the four subgroups (r values ranging from −0.44 to 0.36). It is possible that we did not find a major influence of E2 on GH release because all of the young women were studied during the early follicular phase of the menstrual cycle when E2 levels are lowest (47). The present analysis suggests that gonadal steroids may influence 24-h GH release in adults, at least in part, via effects on AVF,

serum IGF-I, and serum insulin. This hypothesis is supported by three observations from prior studies. First, estrogen replacement therapy in menopausal women increases 24-h GH release only when such therapy also decreases serum IGF-I levels, as occurs with oral and high-dose transdermal estrogen administration (48–50). This suggests that estrogen may influence GH secretion by modulating IGF-I-negative feedback. Second, the impact of gonadal steroids on 24-h GH secretion may be attenuated with increasing amounts of body fat (and presumably AVF) as was reported in men (21). Third, gonadal steroids may have an influence on the regional distribution of body fat (7, 10).

We conclude that AVF and fasting serum insulin are important predictors of 24-h GH release in healthy adults, independent of age and gender. Serum IGF-I is an important predictor of 24-h IGHC in young but not older subjects and gender modulates this relationship. Bidirectional feedback between each of these three factors and GH secretion may account for the strong relationships observed. Age, gender, percentage body fat, body fat mass, aerobic fitness, and gonadal steroid concentrations all appear to be less important regulators of 24-h GH release in adults and may exert their influence on GH secretion via effects on AVF and serum levels of insulin and IGF-I.

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