Free Insulin-Like Growth Factors in Human Obesity

J. Frystyk, E. Vestbo, C. Skjærbæk, C.E. Mogensen, and H. Ørskov

It is well established that spontaneous and stimulated growth hormone (GH) secretion is diminished in human obesity. In contrast to classic GH deficiency, obesity is not associated with hypopituitary levels of circulating total (extractable) insulin-like growth factor-I (IGF-I) and reduced somatic growth. Thus, the riddle of "normal growth without GH" in obese children and the mechanisms behind the GH suppression have remained unsolved. Insulin reduces hepatic production of IGF-binding protein-1 (IGFBP-1), an in vitro inhibitor of IGF bioactivity, and it has been suggested that the obesity-related hyperinsulinemia may increase free (bioactive) IGF in vivo by reducing the concentration of IGFBP-1. We have recently developed a method that during near in vivo conditions isolates the free, unbound fractions of IGF-I and IGF-II in human serum. Using this method, we have determined overnight fasting serum levels of free IGFs in obese subjects and compared the results with levels of total (extractable) IGFs, IGFBPs, GH, and insulin. The study included 92 healthy subjects (56 males and 36 females) allocated to three age-matched groups depending on body mass index (BMI): 31 controls (BMI ≤ 25), 33 subjects with moderate obesity (25 < BMI < 30), and 28 subjects with severe obesity (BMI ≥ 30). Fasting serum insulin correlated positively ($r = .61$, $P < .0001$) with BMI and was significantly elevated in moderate and severe obesity ($P < .05$). In contrast, levels of serum GH and IGFBP-1 were suppressed in both obese groups ($P < .05$), and the latter inversely correlated ($r = -.60$, $P < .001$) with BMI. Serum free IGF-I was 470 ± 50 ng/L (mean ± SEM) in controls, and was elevated in moderate obesity by 47% (690 ± 90 ng/L, $P < .05$) and in severe obesity by 72% (810 ± 90 ng/L, $P < .05$), whereas levels of total IGF-I were unaltered. In addition, serum free IGF-I was inversely correlated with IGFBP-1 ($r = -.47$, $P < .001$). Serum IGFBP-3 and total IGF-II were both increased ($P < .05$) in obese subjects, whereas serum free IGF-II was unaltered. All phenomena were more pronounced in males than in females. We conclude that in obesity, the concentration of free IGF-I in fasting serum is increased. This is likely a result of decreased circulating IGFBP-1, again caused by hyperinsulinemia. Elevated serum free IGF-I may, by feedback, explain the low levels of GH and may be responsible for the normal growth without GH in obese children. Increased levels of IGFBP-3 and normal levels of serum total IGF-I support the interpretation that obese subjects are hypersensitive to the actions of GH.

IN HUMAN OBESITY, spontaneous 24-hour growth hormone (GH) secretion is diminished, response to GH secretagogues is blunted, and GH clearance is accelerated. This GH hyposecretion is reversible upon weight reduction and is believed to be a consequence of obesity.[1-4] Based on the well-documented inverse relation between obesity and GH secretion, circulating levels of insulin-like growth factor-I (IGF-I) would be expected to be subnormal. However, the results have been conflicting, and concentrations of serum total (extractable) IGF-I are reported to be low,[5-9] unchanged,[10-12] or increased,[13-16] as have measurements of IGF bioactivity.[17-19]

However, the diminished GH secretion in obesity is not accompanied by decreased somatic growth. On the contrary, obese children are tall, as well as heavy, for age.[17,20] This may be explained by the hyperinsulinemia present in obesity[21]: insulin is an important regulator of hepatic IGF-I production[22] and also suppresses IGF-binding protein-1 (IGFBP-1),[23] which inhibits IGF-I bioactivity in vitro.[24] Thus, it has been suggested that decreased circulating levels of IGFBP-1 accentuate the growth-promoting effects of IGF-I and its feedback regulation of GH secretion.[11] One way the biological effects of IGF-I may be modified is by altering the level of the free fraction.

In the present study, we investigated the effect of obesity on circulating levels of free and total (extractable) IGF-I and IGF-II; free IGFs were determined using a recently developed ultrafiltration method that allows free fractions to be isolated during in vivo conditions.[25] The results were related to circulating levels of insulin, GH, and IGFBPs.

SUBJECTS AND METHODS

Subjects

Fasting blood samples obtained from 92 subjects (56 males and 36 females) were included in the study. All participants were from an outpatient clinic and underwent a routine medical examination including history (none of the subjects had a family history of diabetes) and medication and blood analyses. If fasting serum glucose was greater than 5.5 mmol/L, an oral glucose tolerance test was performed to preclude diabetes. Apart from obesity, no abnormalities were detected, and all participants were considered otherwise healthy subjects. The following age-matched groups were studied: (1) males + females, (2) males, and (3) females. They were divided a priori into three body mass index (BMI) classes: (1) controls (BMI ≤ 25, 17 males and 14 females), (2) subjects with moderate obesity (25 < BMI < 30, 21 males and 12 females), and (3) subjects with severe obesity (BMI ≥ 30, 18 males and 10 females).

The study was approved by the local ethics committee, and all subjects gave informed consent to participate in the study.

Reagents

All chemicals were analytical grade, and if not otherwise stated, were from Merck (Darmstadt, Germany). Human serum albumin was obtained from Behring (Marburg, Germany).

Serum Glucose and Insulin

Serum glucose level was measured by the glucose-oxidase method and insulin level by enzyme-linked immunosorbent assay

From the Institute of Experimental Clinical Research and Medical Department M, Aarhus University Hospital, Aarhus, Denmark.

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Address reprint requests to J. Frystyk, MD, Institute of Experimental Clinical Research, Aarhus Kommunehospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark.

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(ELISA).[26] This ELISA uses two monoclonal antibodies (Novo Nordisk A/S, Bagsværd, Denmark) directed against human insulin and does not cross-react with human proinsulin.

Serum GH, IGFBP-3, and IGFBP-1

These were measured by commercial assays: GH by a noncompetitive human GH time-resolved immunofluorometric assay (TR-IFMA) based on the dissociation-enhanced lanthanide fluorescence immunoassay principle (Wallac Oy, Turku, Finland); IGFBP-3 by radioimmunoassay (Diagnostic System Laboratories, Inc, Webster, TX); and IGFBP-1 by ELISA (Medix Biochemica, Kainiainen, Finland).

IGF-I and IGF-II

These were determined by two in-house, noncompetitive TR-IFMAs (dissociation-enhanced lanthanide fluorescence immunoassays) as previously described.[27] Two sets of monoclonal IGF-I and IGF-II antibodies were used: the first was immobilized on microtest-plate wells (IGF-I, MAB 41 from Novo Nordisk A/S; IGF-II, anti–rat IGF-II IgG from Upstate Biotechnology, NY), and the second was labeled with europium (Eu^{3+}) (IGF-I, clone 021 from Diagnostic System Laboratories Inc; IGF-II, MAB 73 from Novo Nordisk A/S). Biosynthetic human IGF-I (Amersham International, Amersham, Bucks, UK) and recombinant human (rh)IGF-II (Austral Biologicals, San Ramon, CA) served as standards. IGF-I and IGF-II cross-reactivity in heterologous assays was less than 0.0002%, and rh proinsulin and rh insulin cross-reacted less than 0.01% in the IGF-I assay. Detection limits were 0.0025 and 0.010 μg/L for IGF-I and IGF-II assays. The operating range of 0.005 to 2.5 μg/L (IGF-I) and 0.020 to 10.0 μg/L (IGF-II) and both calibration curves were linear in these intervals. Intraassay and interassay coefficients of variation (CVs) were less than 5% and 10%, respectively.

Serum Total (extractable) IGF-I and IGF-II

Acid ethanol[28] was used to remove IGFBPs: the present assays have been validated for determination of IGFs in serum obtained from normal subjects and acromegalic and GH-deficient patients using this extraction procedure.[27] Serum extracts were diluted in assay buffer (final dilution, 1 in 1,000) without previous neutralization or evaporation. These steps were unnecessary due to the high dilution, which in combination with the use of noncompetitive assays made the interference of possible remnants of IGFBPs undetectable. All measurements of serum total IGF-I and IGF-II levels were performed each in duplicate in one assay.

Serum Free IGF-I and IGF-II

Free IGF was determined using ultrafiltration by centrifugation as previously described.[25] In brief, Amicon YMT 30 membranes and MPS-1 supporting devices were used (Amicon Division, W.R. Grace and Co, Beverly, MA). Before centrifugation, serum samples were diluted (1 in 11) in Krebs-Ringer buffer that had been adjusted to pH 7.4 by airing with CO_2: we have previously shown that it is possible to dilute at least 20-fold human serum obtained from normal subjects and acromegalic and GH-deficient patients in this buffer without altering the equilibrium between free and IGFBP-complexed IGF. From each dilution, an aliquot of 600 μL was applied to the membranes, incubated (30 minutes at 37°C), and centrifuged (1,500 rpm at 37°C) (swinging-bucket rotor model Rotixa/RP; Hettich Zentrifugen, Tuttlingen, Germany). Ultrafiltrates were collected in 5 mL polyethylene tubes, which before centrifugation were pretreated with human serum albumin. Because less than one third of the sample volume is removed during the ultrafiltration process, only a modest increase of 16% to 43% in IGF above the filter is observed. In addition, recovery of added IGF to newborn calf serum is 80% to 100% in the ultrafiltrates. The possible presence of IGFBPs in the ultrafiltrates has been examined[25]: ultrafiltrates obtained from human serum contain no detectable IGFBPs as measured by Western ligand blotting and IGFBP-1 (detection limit, 0.4 μg/L) and IGFBP-3 (detection limit, 1.0 μg/L) immunoassays.

Because the maximum capacity of the rotor was 72 samples per centrifugation, samples obtained from males and females were processed separately as single determinations performed on 3 different days. Hence, six different centrifugations were required to process all samples, which were analyzed in IGF-I and IGF-II assays immediately after ultrafiltration: 10 μL ultrafiltrate was applied to the microtest-plate wells, which contained 200 μL assay buffer. In the ultrafiltrates, this dilution equaled a detection limit of 52.5 ng/L (IGF-I) and 210 ng/L (IGF-II), respectively. To estimate intraassay and interassay CVs following ultrafiltration and immunochemical analysis, two control sera were centrifuged and analyzed in triplicate simultaneously with the serum samples, ie, six times.

Statistical Analysis

Statistical analyses were performed using the Solo Statistical Software Packet (BMDP Statistical Software, Los Angeles, CA). Data were analyzed using one-way ANOVA followed by Student's unpaired *t* test. Otherwise, the Kruskal-Wallis nonparametric test was applied, followed by the Mann-Whitney unpaired test. All correlations (including multiple regression analysis) were performed on log-transformed data. *P* values less than .05 were regarded as significant. All data with a normal distribution are presented as the mean ± SEM, and otherwise as the median and 10th and 90th percentiles.

RESULTS

Clinical Data

The average age, BMI, body weight, waist to hip ratio (WHR), and fasting serum glucose level are summarized in Table 1. All groups had been matched for age, and for all BMI classes, the mean body weight and WHR of males were greater than those of females ($P < .05$). Fasting serum glucose levels were similar.

Serum Insulin

Increasing BMI was accompanied by elevated levels of fasting serum insulin (Fig 1A). In males + females, all BMI classes differed ($P < .05$). However, in males with moderate and severe obesity, insulin levels were identical but were significantly different from control levels ($P < .05$). In females, serum insulin was elevated ($P < .05$) in severe obesity only, as compared with controls and moderately obese subjects. In males + females, as well as in males and in females, log-transformed serum insulin was linearly correlated ($P < .0001$) with BMI ($r = .59$ to $.66$) and WHR ($r = .47$ to $.72$).

Serum IGFBP-1

Serum IGFBP-1 was inversely related to BMI and fasting serum insulin (Fig 1B). In males + females and in males, all three BMI classes differed ($P < .05$), whereas in females the difference was restricted to include severely obese subjects only ($P < .05$). In addition, serum IGFBP-1 was

Table 1. Clinical Data for All Subjects

Characteristic	Males + Females			Males			Females		
	Controls	Moderate Obesity	Severe Obesity	Controls	Moderate Obesity	Severe Obesity	Controls	Moderate Obesity	Severe Obesity
No. of subjects	31	33	28	17	21	18	14	12	10
Age (yr)	49.4 ± 1.4	49.9 ± 1.4	49.5 ± 1.4	50.2 ± 1.8	49.2 ± 1.8	48.8 ± 1.9	48.4 ± 2.2	51.3 ± 2.0	50.7 ± 2.1
BMI (kg/m²)	22.2 ± 0.3*	27.2 ± 0.2	33.0 ± 0.6†	22.6 ± 0.4*	27.5 ± 0.3‡	32.1 ± 0.4†‡	21.7 ± 0.3*	26.6 ± 0.4	34.6 ± 1.2†
Weight (kg)	66.8 ± 1.6*	81.2 ± 1.7	99.6 ± 1.9†	70.0 ± 1.8*‡	87.2 ± 1.4‡	102.5 ± 2.1†‡	62.9 ± 2.5*	70.8 ± 1.2	94.5 ± 3.2†
WHR	0.83 ± 0.02*	0.90 ± 0.02	0.96 ± 0.02†	0.87 ± 0.01*‡	0.94 ± 0.01‡	0.99 ± 0.02†‡	0.76 ± 0.02*	0.81 ± 0.02	0.90 ± 0.03†
Serum glucose (mmol/L)	4.4 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.2	4.5 ± 0.1	4.5 ± 0.2	4.2 ± 0.1	4.4 ± 0.2	4.6 ± 0.2

*$P < .05$, *v* moderate obesity and severe obesity of the same group.
†$P < .05$, *v* moderate obesity of the same group.
‡$P < .05$, males *v* females of the same BMI.

significantly lower in moderately obese males versus females ($P < .05$). In males + females, as well as in males and in females, log-transformed serum IGFBP-1 was linearly correlated ($P < .001$) with BMI ($r = -.58$ to $-.64$), insulin ($r = -.61$ to $-.64$), and WHR ($r = -.53$ to $-.55$).

Serum GH

In all obese groups (BMI > 25), fasting serum GH was suppressed to a similar degree as compared with control levels ($P < .05$; Fig 1C). In addition, a marked difference between males and females was observed ($P < .05$), with the latter having higher GH levels for all BMI classes.

Serum IGFBP-3

With the exception of males with severe obesity ($P = .06$), levels of IGFBP-3 were elevated by 20% to 35% in all obese subjects ($P < .05$) as compared with the respective controls (Fig 1D). In addition, in males + females and in males with moderate obesity, IGFBP-3 levels were significantly elevated as compared with levels in severely obese subjects ($P < .05$).

Serum Total IGF-I and IGF-II

No differences in circulating levels of total IGF-I were observed (Fig 2A). In contrast, serum total IGF-II tended to be higher ($P < .10$) with increasing obesity in males + females (Fig 2B) and became significant ($P < .05$) after separation of males and females: on average, total IGF-II increased by 18% and 36% in moderately and severely obese subjects. For all three groups, serum total IGF-II was linearly correlated with IGFBP-3 ($r = .57$ to $.62, P < .0001$).

Serum Free IGF-I and IGF-II

The two control sera used for estimation of interassay CV contained 800 and 1,750 ng/L free IGF-I and 1,050 and 1,150 ng/L free IGF-II, respectively. At these levels, interassay CV averaged 20% for free IGF-I and 22% for free IGF-II (including both ultrafiltration and immunochemical analyses).

In contrast to serum total IGF-I, free IGF-I was increased in obesity (Fig 2C): for males + females, free IGF-I was 470 ± 50 ng/L in controls. This was significantly increased in moderate obesity by 47% (690 ± 90 ng/L, $P < .05$) and in severe obesity by 72% (810 ± 90 ng/L, $P < .05$). Similar results were observed in males: free IGF-I was 470 ± 70 ng/L in controls and was increased by 51% in moderate obesity (710 ± 80 ng/L, $P < .05$) and by 85% in severe obesity (870 ± 130 ng/L, $P < .05$). Although elevated levels of free IGF-I were observed in obese females (470 ± 70 [controls] *v* 660 ± 80 [$P = .12$, moderate obesity] *v* 680 ± 90 ng/L [$P = .06$, severe obesity]), these differences did not reach significance. This was due to a lesser increase of free IGF-I levels in obese females, since no difference in control levels between males and females was recognized. However, when comparing the relative fractions of free IGF-I (free over total IGF-I), the percentage was increased from approximately 0.3% in controls to approximately 0.5% to 0.6% in all obese groups ($P < .05$; Fig 2E).

When considering males + females, free IGF-II was unaltered in obesity, although levels tended to increase ($P < .10$) from 1,140 ± 70 ng/L in controls to 1,410 ± 90 ng/L in moderate obesity and 1,370 ± 90 ng/L in severe obesity (Fig 2D). In males, serum free IGF-II tended to be elevated in moderate obesity (1,090 ± 90 [controls] *v* 1,450 ± 130 ng/L [$P = .053$, moderate obesity]) and reached significance in severe obesity (1,460 ± 130 ng/L, $P < .05$). In contrast, no difference was observed in females: free IGF-II was 1,200 ± 90 ng/L in controls, 1,330 ± 100 ng/L in moderate obesity, and 1,190 ± 110 ng/L in severe obesity. The relative fraction (free over total IGF-II) was similar (~0.15%) in all groups, with the exception of severely obese females, who showed a decreased ratio (~0.1%, $P < .05$; Fig 2F).

Using multiple linear regression analysis on log-transformed data, free IGF-I and free IGF-II were analyzed versus BMI, WHR, fasting serum insulin, IGFBP-1, IGFBP-3, and GH. Of all variables, only IGFBP-1 was significantly associated with free IGF-I: in males + females, as well as in males and in females, IGFBP-1 was inversely correlated with free IGF-I ($r = -.46$ to $-.53, P < .001$). In addition, in all three groups significant correlations were found between free IGF-I and free IGF-II ($r = .53$ to $.70$, $P < .0001$) and between free IGF-I and total IGF-I ($r = .38$ to $.43$, $P < .01$), whereas free and total IGF-II were only weakly correlated in males and in females, respectively ($r = .27$ to $.30, P < .05$).

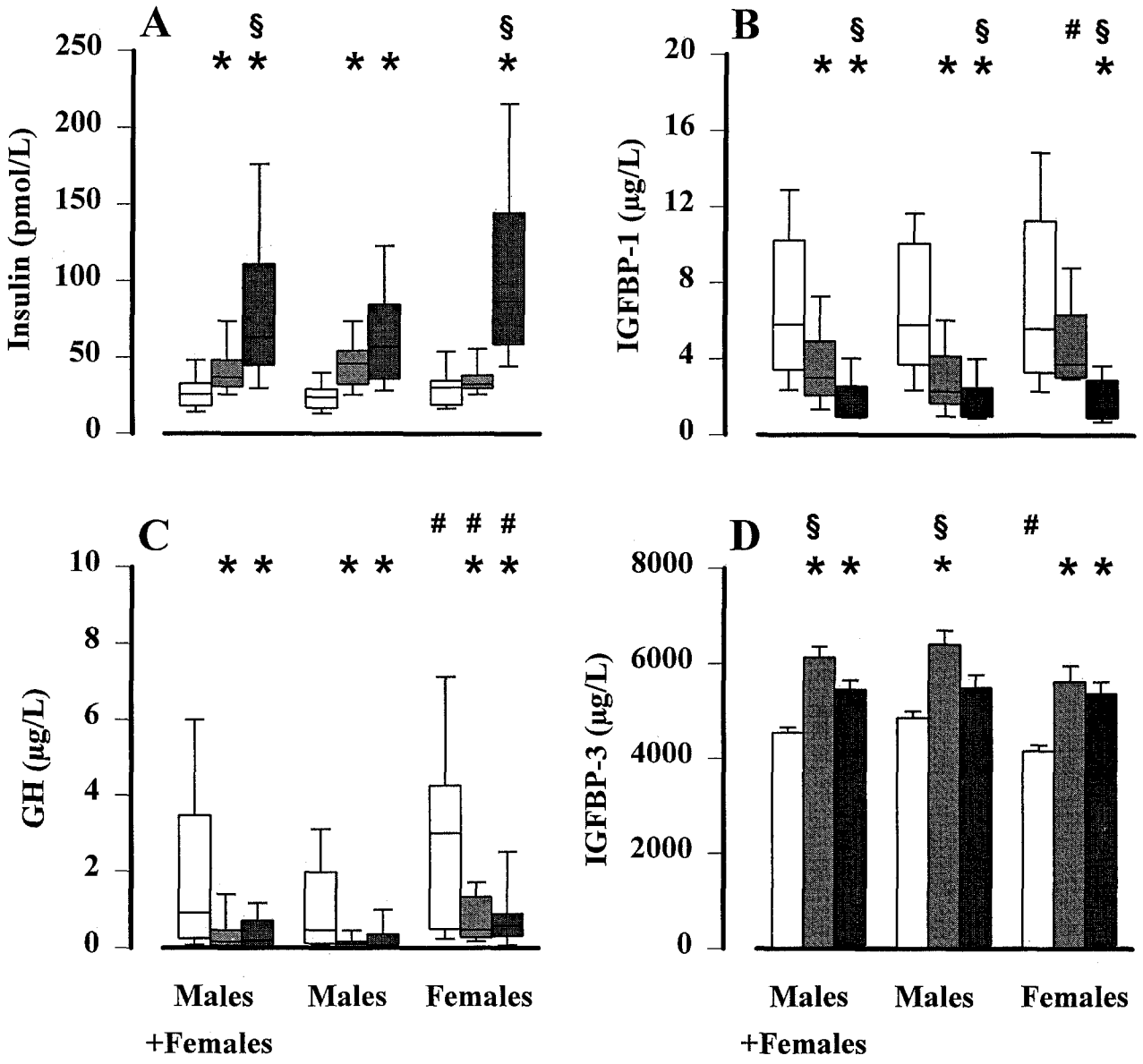

Fig 1. Levels of fasting serum insulin (A), serum IGFBP-1 (B), GH (C), and IGFBP-3 (D) in controls (BMI ≤ 25, □), moderate obesity (25 < BMI < 30, ▨), and severe obesity (BMI ≥ 30, ■). Data are shown as box plots (median with 10th and 90th percentiles, A, B, and C) and as the mean ± SEM (D). *P < .05, obese subjects v controls of the same group; §P < .05, moderate obesity v severe obesity of the same group; #P < .05, males v females of the same degree of obesity.

DISCUSSION

In this study, we investigated the influence of nondiabetic obesity on circulating levels of free and total (extractable) IGF-I and IGF-II and on IGFBPs. The most noteworthy finding is the increased free IGF-I level in obese subjects. This is probably due to the inherent hyperinsulinemia and resulting low levels of circulating IGFBP-1, and may explain the well-known GH hyposecretion in human obesity.

In the circulation, the vast majority of IGF-I and IGF-II is bound to six specific binding proteins (IGFBP-1 to -6). Following size-exclusion chromatography on serum in neutral buffer, two fractions elute: a minor fraction (25%) of approximately 30 to 50 kd made of binary IGF/IGFBP complexes, and a major fraction (75%) of approximately 150 kd composed of IGF bound to IGFBP-3, which subsequently has formed a ternary complex with the acid-labile subunit.[29,30] The free, unbound fraction of IGF has also been estimated using different chromatographic techniques (size-exclusion chromatography, high-performance liquid chromatography, and reverse-phase chromatography); however, results have varied considerably, from undetectable levels up to 19% of total circulating IGF.[31-37] This diversity is probably caused by different degrees of disturbance of equilibrium in the columns between free and IGFBP-complexed peptide and by laboratory conditions distinct from the in vivo milieu. Recently, an immunoradiometric assay for direct determination of free IGF-I in human serum has been reported.[38] This assay uses an antibody

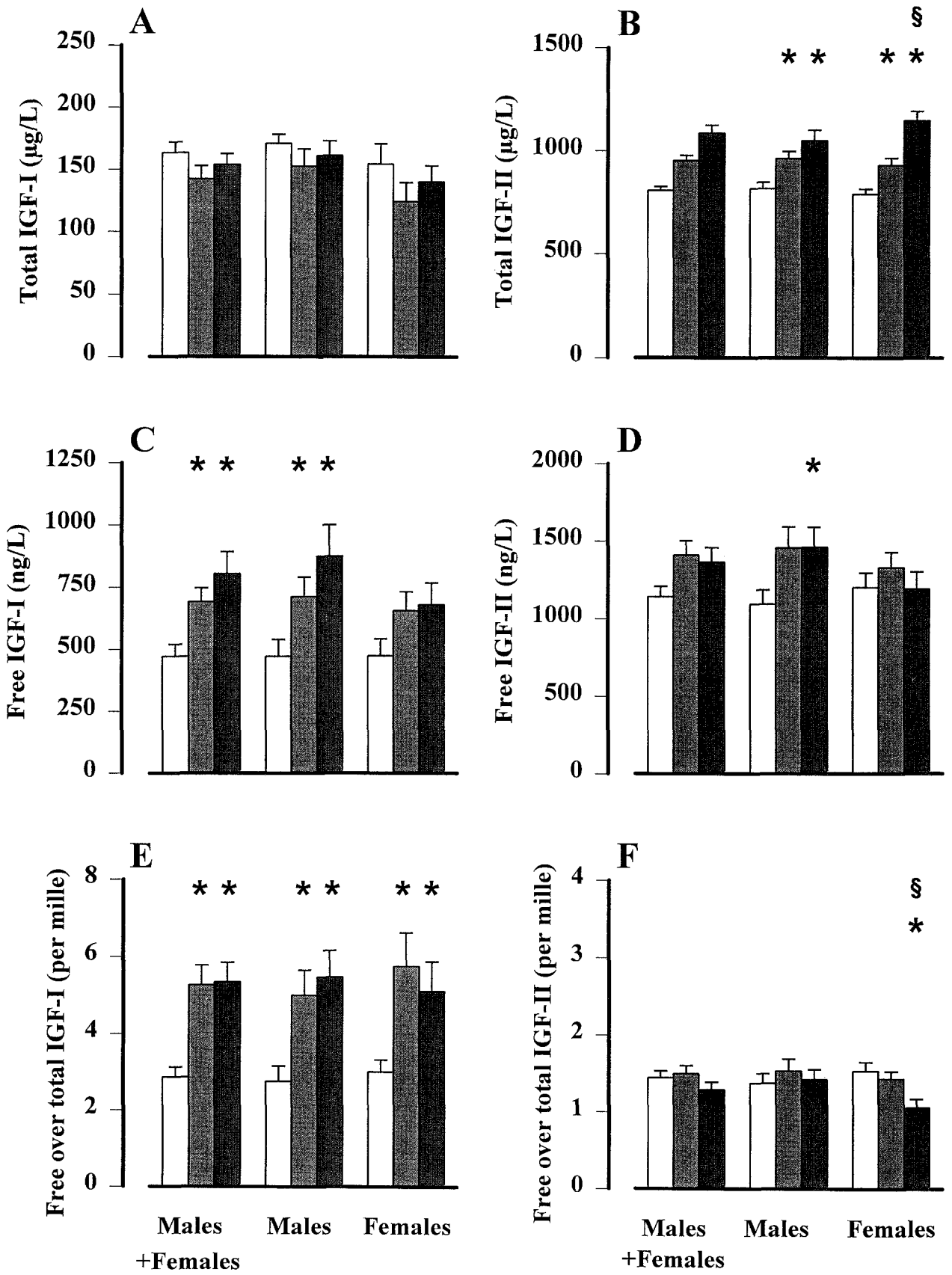

Fig 2. Levels of fasting serum total IGF-I (A), serum total IGF-II (B), serum free IGF-I (C), serum free IGF-II (D), serum free IGF-I over serum total IGF-I (E), and serum free IGF-II over serum total IGF-II (F) in controls (BMI ≤ 25, □), moderate obesity (25 < BMI < 30, ▨), and severe obesity (BMI ≥ 30, ■). Data are the mean ± SEM. *$P < .05$, obese subjects *v* controls of the same group; §$P < .05$, moderate obesity *v* severe obesity of the same group; #$P < .05$, males *v* females of the same degree of obesity.

directed against the free, unbound IGF-I peptide, and when performed at 37°C, free IGF-I averaged 0.7% of the total IGF-I concentration. However, the influence of the antibody on the equilibria between free and IGFBP-complexed peptides remains unknown. We have recently described a method based on ultrafiltration by centrifugation to separate free from bound IGF.[25] In this way, near in vivo conditions (temperature, pH, and ionic milieu) can be reestablished and maintained during ultrafiltration. Using this method, free IGF-I and IGF-II averaged approximately 0.4% and 0.2% of total IGF-I and IGF-II, respectively. In addition, highly significant changes in circulating free IGF-I and IGF-II were detectable (during an oral glucose tolerance test and after short-term fasting) without concomitant changes in the total concentrations. These changes in free IGF levels were in opposition to changes in IGFBP-1, suggesting that IGFBP-1 is important in the regulation of free IGF.

The concentration of serum total IGF shows little or no diurnal variation, as is the case for IGFBP-3, when complexed in the ternary complex.[2,39] In contrast, IGFBP-1 exhibits marked diurnal variations: it binds IGF-I and IGF-II with approximately identical affinity[40] and is meal-related, with low levels during the daytime and several fold increased levels during the night.[39,41] Insulin is the principal regulator of IGFBP-1 and is reported to inhibit hepatic IGFBP-1 synthesis[42] and to increase IGFBP-1 clearance.[43] Accordingly, increased levels of IGFBP-1 have been observed following nutritional deprivation (fasting[25,44]) and in insulin-dependent diabetes mellitus.[45,46] Since most in vitro studies have reported that IGFBP-1 acts as an inhibitor of IGF bioactivity (for review, see Lee et al[24]), it has been suggested that IGFBP-1 in vivo regulates IGF bioactivity and coordinates insulin and IGF action with respect to substrate availability.[11,40]

Increased peripheral insulin resistance, hyperinsulinemia, and hyposomatotropinemia are endocrine characteristics of human obesity.[1-4,21] However, metabolic insulin resistance apparently does not involve regulation of IGFBP-1; others have reported on decreased circulating levels of IGFBP-1 in human obesity,[47] in accordance with our findings. In a recent report, suppression of IGFBP-1 was found to be maximal when serum insulin (after an overnight fast) was greater than 70 to 90 pmol/L.[11] Since measurement of IGFBP-1 in the present study was also performed in fasting serum samples, it is likely that levels of IGFBP-1 in obese subjects are chronically depressed even at night, with blunted diurnal variation.

In the present study, circulating total IGF-I was unaltered but nevertheless positively correlated with free IGF-I, which was significantly elevated by approximately 50% to 75% in obese males + females and in males, and tended to be elevated in obese females. Several factors may explain why free IGF-I was not significantly elevated in obese females: fewer females included in the study, differences in BMI, WHR, and fasting serum GH and IGFBP-1, and the influence of sex hormones. In addition, since the relative fraction of free IGF-I (free over total IGF-I) was increased in all obese groups, lower levels of free IGF-I may be explained by the slightly lower levels of total IGF-I observed in obese females. However, based on multiple regression analysis, it followed that circulating IGFBP-1, rather than obesity (BMI), fat mass distribution (WHR), fasting serum insulin, GH, and IGFBP-3, was correlated with serum free IGF-I in all three groups. Thus, our findings support the hypothesis that IGFBP-1 is a dynamic regulator of IGF-I bioavailability.[11,40]

Systemic administration of IGF-I inhibits pituitary GH secretion, presumably by stimulating hypothalamic somatostatin secretion,[48-50] and although single-sample measurement of GH secretory activity must be interpreted with caution, we found a highly significant depression of fasting GH levels in all groups of obese subjects. Whether it is the free rather than the total concentration of IGF-I that regulates GH secretion is unknown. However, the unchanged levels of total IGF-I and the increased levels of free IGF-I indicate that the free fraction may be the GH-regulating moiety of IGF-I. This is further supported by a recent study performed in healthy subjects.[48] Using a 2-hour infusion of insulin (0.8 mU/kg/min) and IGF-I (0.7 μg/kg/min), a similar degree of hypoglycemia was induced but GH release following IGF-I was significantly attenuated as compared with insulin. In this situation, a large proportion of administered IGF-I must be present as free IGF-I, again indicating that it is the free fraction that regulates pituitary GH secretion. If one assumes that free IGF-I regulates GH secretion, this may also (at least partly) explain the observation that in all three BMI groups serum GH was significantly lower in males than in females, whereas levels of free IGF-I tended to be higher.

Despite the well-known GH hyposecretion in human obesity confirmed in the present report, serum levels of total IGF-I and IGFBP-3, both under strict GH regulation,[51] were present at normal and increased concentrations, respectively. This is not a novel observation,[10-12] and it emphasizes that GH hyposecretion in obesity and in classic GH deficiency has different effects on the IGF system. It has been suggested that the nutritional excess in obese subjects is capable of substituting for GH action in the stimulation of hepatic IGF-I production.[52] However, studies in GH-deficient subjects have not supported this hypothesis.[2] It may therefore be that in obesity the responsiveness to GH is enhanced sufficiently to maintain normal circulating levels of total IGF-I and IGFBP-3, as previously suggested.[20,53]

In this study, serum levels of total IGF-II were significantly increased in obese males and females and correlated with IGFBP-3. This is a novel observation, but the significance is unknown. It may merely be an effect of increased IGF-binding capacity in serum, but the molar ratio of total IGF-I and IGF-II as compared with the molar ratio of IGFBP-3 did not add any further information (data not shown). Serum free IGF-II tended to be elevated in moderately obese males ($P = .053$) and reached significance in severe obesity. In contrast, no difference was observed in either males + females or in females. Although IGFBP-1 binds IGF-I and IGF-II with equal affinity, we did

not observe any significant correlation between free IGF-II and IGFBP-1, but did see a highly significant positive correlation between free IGF-I and free IGF-II. Further studies are required to investigate the relationship between free IGF-I and IGF-II.

It is obvious to try to "translate" the concentration of free IGF-I and IGF-II obtained with the present method into "insulin-like activity." On a molar basis, the potency of insulin on glucose disposal is approximately 16 times that of IGF-I; when the substances are infused intravenously.[34] However, it is probably less, since not all IGF-I in these experiments would have been in the free form. If a factor of 16 is used, the free IGF-I + IGF-II concentrations in the present study ranging from 200 to 300 pmol/L would thus "correspond" to insulin concentrations of 13 to 19 pmol/L. In comparison, the median fasting serum insulin ranged from 26 to 64 pmol/L.

In conclusion, circulating fasting free IGF-I is increasingly elevated with increasing obesity, whereas serum total IGF-I is normal. The elevated serum free IGF-I may be caused by insulin resistance, inducing hyperinsulinemia, which suppresses IGFBP-1. This view is supported by the inverse correlation between IGFBP-1 and free IGF-I observed in all groups. In addition, the elevated serum free IGF-I may be partly or solely responsible for the suppressed spontaneous and stimulated GH secretion in obese subjects.

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