Low Circulating IGF-II Concentrations Predict Weight Gain and Obesity in Humans

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Results from experimental and gene-association studies suggest that IGF-II may influence body weight regulation and that individuals with low IGF-II levels may be more susceptible to weight gain and obesity. We therefore assessed the association between circulating concentrations of IGF-II and subsequent weight gain and progression to obesity. Participants in this study were 463 nonobese men and women aged between 45 and 60 years with normal glucose tolerance and with metabolic and anthropometric assessments at baseline and follow-up clinic visits. We examined the association between baseline concentrations of fasting serum IGF-II and risk of gaining ≥2.5 kg body wt or developing obesity using unconditional logistic regression. A total of 217 participants gained ≥2.5 kg body wt, and 29 developed obesity after >4 years of follow-up. In multivariate analysis, baseline IGF-II levels were significantly lower in participants who subsequently gained weight compared with individuals who remained stable or lost weight ($P = 0.010$). Similarly, individuals who developed obesity had lower baseline IGF-II levels ($P = 0.006$). Relatively higher IGF-II levels were also associated with a reduced risk of gaining weight (P for trend across quintiles of IGF-II = 0.006). Our data suggest that circulating IGF-II levels may play a role in body weight regulation and development of obesity in men and women with normal glucose tolerance. *Diabetes* 52: 1403–1408, 2003

IGF-I and IGF-II are peptide hormones that play an important role in the regulation of metabolism and growth (1). Although IGF-II is known to play a key role in fetal growth and development (2), the regulation and function of IGF-II in postnatal life is poorly understood (3,4). However, recent studies suggest that this hormone may be associated with lipid metabolism and body weight regulation (4).

Observational and experimental investigations in humans have shown that a number of allelic variants within the IGF-II gene influence body weight and BMI (5–7). In an overfeeding study, the apaI polymorphism in the IGF-II gene was also associated with increased adiposity and related metabolic changes (8). Together, these data indicate that IGF-II may influence body weight regulation and that individuals with low IGF-II levels may be more susceptible to weight gain and obesity. We therefore assessed the association between circulating concentrations of IGF-II and subsequent weight gain and progression to obesity in a random sample of middle-aged men and women who had normal glucose tolerance.

RESEARCH DESIGN AND METHODS

Participants and protocol. The volunteers in this study all were participants in the Ely Study, a continuing population-based cohort study in Ely, Cambridgeshire, U.K. The detailed design of the study has been described previously (9). The original sample, comprising 1,122 people without known diabetes, was recruited between 1990 and 1992 at random from a population-based sampling frame consisting of all people in Ely aged between 40 and 65 years in 1990. The initial response rate was 74%. These individuals attended a morning clinic and underwent a standard 75-g oral glucose load, having fasted since 2200 h the previous evening.

Between 1994 and 1996, a 4.5-year follow-up study was undertaken of the 1,071 (95%) of the 1,122 individuals who did not have diabetes by 1985 World Health Organization (WHO) criteria at baseline (10). Twenty (2%) participants had died in the interim, and 937 (89%) of the remaining volunteers agreed to participate in the follow-up study. These 937 individuals aged 50–70 years attended a second morning clinic and underwent an additional glucose tolerance assessment in accordance with the previously described criteria.

Inclusion criteria. Of the 937 individuals who attended both clinic visits, 604 (64%) were normoglycemic at baseline assessment by current WHO and American Diabetes Association criteria, with a fasting plasma glucose of <6.1 mmol/l and a 2-h plasma glucose value of <7.8 mmol/l (11,12). Because of the possible effects of insulin resistance and type 2 diabetes on IGF-II and body weight (13,14), only these 604 participants were included in the analysis.

To assess the development of obesity in this population and because of possible compensatory metabolic changes in obese individuals (14), we excluded individuals who were obese at baseline according to current WHO guidelines (15). Thus, 52 participants who had BMI ≥30 were omitted from the analysis. Of the 552 eligible participants, 463 (84%) had blood available for assessment of baseline IGF-II concentrations. Mean overall weight change did not differ between individuals in this analysis and the 89 participants who did not have blood available for IGF-II assays ($P = 0.301$). Therefore, the study population for this investigation comprised 173 men and 290 women.

Anthropometric and metabolic assessment. At both visits, height and weight were measured with the participant in light clothing. BMI was estimated as weight (in kilograms) divided by height (in meters) squared. Waist and hip circumferences were measured in duplicate using a metal tape. Blood samples were taken at fasting and 120 min after a 75-g oral glucose load. All samples were permanently stored at −70°C within 4 h. Plasma glucose was measured in the routine National Health Service Laboratory at Addenbrooke's Hospital using the hexokinase method (16). Plasma insulin was measured by two-site immunometric assays with either ^{125}I or alkaline phosphatase labels (17,18). Cross-reactivity with intact proinsulin was <0.2%, and interassay coefficients of variation (CVs) were <7%.

Cholesterol and triglycerides were measured using the RA 1000 (Bayer Diagnostics, Basingstoke, U.K.), with a standard enzymatic method. Nonest-

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WHO, World Health Organization.

TABLE 1
Baseline characteristics of study participants by subsequent weight change status, the Ely study, 1990–1996

Baseline variables	Weight stable/loss (n = 246)	Weight gain (n = 217)	P value‡
Women (%)	154 (63)	136 (63)	0.855
Age (years)	53.1 (52.1–54.0)	51.7 (50.7–52.7)	0.049
Former/current smokers (%)*	108 (45)	112 (53)	0.116
Antihypertensive medication (%)	21 (9)	25 (12)	0.284
Family history of diabetes (%)	40 (16)	38 (17)	0.720
Height (cm)	167.4 (166.7–168.2)	167.5 (166.7–168.3)	0.877
BMI (kg/m^2)	24.2 (23.9–24.5)	24.2 (23.9–24.5)	0.855
Weight (kg)	68.1 (67.1–69.2)	68.2 (67.1–69.3)	0.939
Waist-to-hip ratio	0.80 (0.79–0.80)	0.80 (0.80–0.81)	0.250
Waist circumference (cm)	78.7 (77.7–79.6)	79.6 (78.6–80.6)	0.168
2-h glucose (mmol/l)†	5.4 (5.3–5.6)	5.5 (5.4–5.7)	0.372
0-h insulin (pmol/l)†	33.2 (31.3–35.2)	35.5 (33.3–37.8)	0.123
0-h IGF-I (ng/ml)†	149 (142–155)	146 (140–153)	0.652
0-h cholesterol (mmol/l)†	6.3 (6.1–6.4)	6.1 (6.0–6.3)	0.113
0-h NEFA (mmol/l)†§	0.37 (0.34–0.40)	0.39 (0.35–0.42)	0.525
0-h triglycerides (mmol/l)†	1.07 (1.01–1.12)	1.03 (0.97–1.09)	0.370
0-h leptin (ng/ml)†*	6.5 (5.8–7.2)	6.9 (6.1–7.7)	0.453
0-h IGF-II (ng/ml)	598 (572–624)	542 (514–569)	0.004

Data shown are age- and sex-adjusted means and 95% CIs where applicable, unless otherwise indicated. *n = 452 because of missing values; †age- and sex-adjusted geometric means and 95% CIs; ‡obtained from χ^2 tests and stratified linear regression analyses; §n = 457 because of missing values. NEFA, nonesterified fatty acid.

erified fatty acid concentrations were determined enzymatically on the basis of the activity of acyl-CoA synthetase (Boehringer Mannheim, Lewes, Sussex, U.K.). Plasma leptin levels were measured using a two-site immunometric assay with a detection limit of 0.1 ng/ml (Department of Clinical Biochemistry, University of Cambridge, U.K.). Inter- and intra-assay CVs were <7.5%. Baseline plasma concentrations of fasting IGF-I and IGF-II were measured by previously reported antibody-based assays (19). All interassay CVs were <10%. The Cambridge Local Research Ethics Committee, U.K., granted ethical permission for the study, and informed consent was obtained from all participants.

Definition of outcomes. Weight change is a complex phenomenon that reflects a composite of negative, stable, and positive energy balance. These components may have distinct underlying biological processes (20). Weight loss is also associated with severe illness or preexisting disease (21,22). We therefore categorized participants a priori into exclusive categories of weight loss (lost ≥2.5 kg), weight stable (lost <2.5 kg or gained <2.5 kg), and weight gain (gained ≥2.5 kg). Using the WHO criterion of BMI ≥30 (15), we also examined progression to obesity as a secondary outcome variable.

Statistical analysis. To obtain near-normal distributions, we applied logarithmic transformations to all nonnormally distributed variables. For baseline risk factors and follow-up characteristics, means or proportions were calculated for categories of weight change status. Stratified linear regression and the χ^2 test were used to assess the statistical significance of associations between categories of weight change for continuous and categorical variables, respectively. Linear regression and the Pearson partial correlation coefficients were used to assess the association between baseline risk factors and concentrations of circulating IGF-II.

We used unconditional logistic regression analysis to estimate the relative risk of weight gain and obesity according to circulating levels of IGF-II. As well as using IGF-II as a continuous variable, we categorized participants according to quintiles (20th percentile cutoffs) determined by the distribution of IGF-II levels in the study population. Because there was only a small number of participants who developed obesity (n = 29), we assessed the association between IGF-II and progression to obesity using IGF-II as a continuous variable. In a secondary post hoc analysis, we also used a dichotomous IGF-II variable comparing the relative risk of obesity and weight gain above and below the 20th percentile. Adjusted estimates of relative risk and 95% CIs were obtained with multivariate models that controlled for baseline age, sex, BMI, length of blood storage, follow-up time, weight, cholesterol, insulin, and IGF-I concentrations.

To further exclude the potential for bias and confounding, sex-specific relative risks were also assessed and the analysis was repeated excluding participants in the weight loss group or the 28 (6%) individuals who developed glucose intolerance at follow-up. In sex-specific analysis, associations between levels of IGF-II and weight gain were similar, and there was no detectable interaction between sex and IGF-II with subsequent weight gain (P = 0.295). All data were therefore presented for men and women combined. An analysis excluding participants who lost weight or developed glucose intolerance showed similar results to those for all eligible participants. We therefore presented data for the whole study population.

Linear trends comparing continuous variables with their corresponding categorical or polynomial terms and possible interactions between covariates and IGF-II were assessed with log likelihood ratio tests. For continuous variables, values are given as arithmetic or geometric means and 95% CIs. All analyses were done with Stata 7.0 statistical package (Stata, College Station, TX).

RESULTS

Baseline and follow-up characteristics. A total of 41 (9%) study participants lost ≥2.5 kg body wt, 217 (47%) gained ≥2.5 kg body wt, and 205 (44%) maintained a stable weight (gained or lost <2.5 kg) after >4 years of follow-up (mean ± SD, 4.4 ± 0.3). In a cross-sectional investigation, baseline IGF-II levels were significantly correlated with cholesterol (r = 0.14, P = 0.002) and IGF-I (r = 0.11, P = 0.022), which remained statistically significant in multivariate analysis. However, IGF-II did not show any substantial associations with BMI (r = 0.01, P = 0.803), waist-to-hip ratio (r = −0.04, P = 0.333), waist circumference (r = −0.05, P = 0.384), or body weight (r = 0.01, P = 0.898). By contrast, in a prospective analysis, circulating IGF-II was inversely correlated with overall weight change (r = −0.10, P = 0.025) and change in BMI (r = −0.09, P = 0.046).

Table 1 shows baseline characteristics of the 463 study participants according to follow-up weight gain or weight stable/loss status. Weight gainers were slightly younger than those who maintained a stable weight or lost weight (P = 0.049) and had slightly longer mean follow-up time (4.4 ± 0.3 years vs. 4.5 ± 0.3 years; P = 0.011). In addition, IGF-II levels at baseline were significantly lower among people who gained weight than among those who remained stable or lost weight (P = 0.004). Figure 1 shows that in multivariate analysis, the difference in baseline IGF-II concentrations between groups remained statisti-

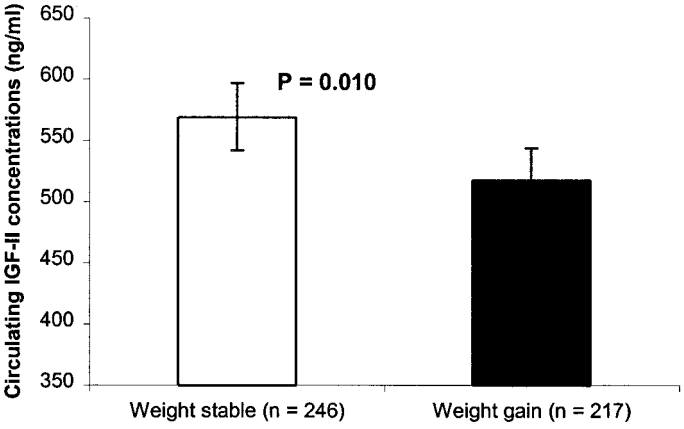

FIG. 1. Fasting IGF-II concentrations at baseline in participants who subsequently maintained a stable weight or lost weight compared with weight gainers. Data are means and 95% CIs adjusted for follow-up time, length of blood storage, baseline age, sex, weight, BMI, IGF-I, 0-h cholesterol, and 0-h insulin.

cally significant ($P = 0.010$). Similarly, Fig. 2 shows that levels of IGF-II were much lower in the 29 participants who later developed obesity compared with nonobese participants (mean [95% CI]; 466 [386 to 546] ng/ml vs. 580 [560 to 599] ng/ml; $P = 0.006$).

Levels of IGF-II and risk of weight gain and obesity. As a continuous variable, the relative risk of weight gain was 0.88 (95% CI, 0.80–0.97; $P = 0.010$) for each 100 ng/ml increase in the level of IGF-II. Adding a quadratic term for IGF-II did not improve the fit of the model ($P = 0.097$), and the linear term remained statistically significant ($P = 0.027$), suggesting that the relation was linear. In a similar analysis, the relative risk of developing obesity was 0.75 (95% CI, 0.60–0.92; $P = 0.007$), which was not materially altered in multivariate analysis (relative risk = 0.76 [0.59–0.98]; $P = 0.037$).

To examine whether there was a threshold level of IGF-II associated with weight gain, we examined the risk of weight gain across categories of IGF-II concentrations (Table 2). An inverse association was still evident across categories of IGF-II concentrations in both univariate analysis (P for trend = 0.001) and multivariate analysis (P for trend = 0.006). However, relative risk estimates above the 20th percentile were broadly similar, suggesting that there might be a threshold level of circulating IGF-II concentrations and risk of weight gain or that the relation was hyperbolic. Compared with participants with levels below the 20th percentile (IGF-II <400 ng/ml), the risk of gaining weight in multivariate analysis was 0.42 (95% CI, 0.25–0.68; $P = 0.001$) among participants with IGF-II levels above the 20th percentile. Likewise, the corresponding relative risk of developing obesity for individuals above the 20th percentile was 0.39 (95% CI, 0.14–1.11; $P = 0.078$), compared with individuals with IGF-II levels below the 20th percentile. However, because of the small number of cases, this finding did not reach conventional statistical significance.

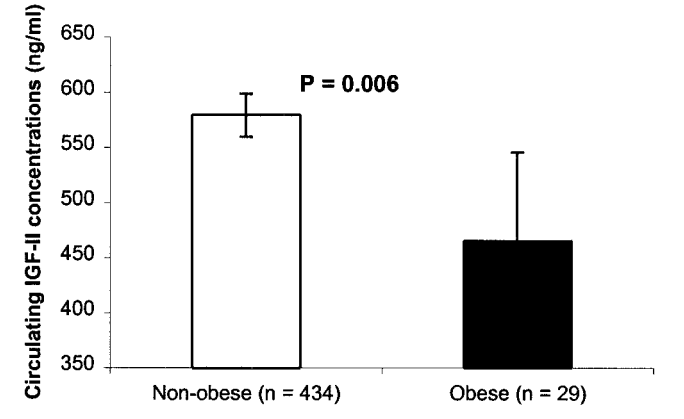

FIG. 2. Fasting IGF-II concentrations at baseline in participants who subsequently developed obesity compared with nonobese participants at follow-up. Data are means and 95% CIs adjusted for follow-up time, length of blood storage, baseline age, sex, weight, BMI, IGF-I, 0-h cholesterol, and 0-h insulin.

DISCUSSION

In this prospective study of men and women with normal glucose tolerance, relatively low concentrations of circulating IGF-II were associated with an increased risk of gaining weight and developing obesity. There was also some evidence to suggest that the association between IGF-II levels and weight gain may be nonlinear or that there may be a threshold level of IGF-II that confers the greatest risk of gaining weight.

The limitations of these observational data merit consideration. It is possible that unidentified correlates of IGF-II and risk factors for obesity could explain or modify our observations. For example, circulating concentrations of IGF-binding proteins may modify the association between IGF-II and weight gain. Measurement error as a result of variability in levels of IGF-II and other biological variables might have led to underestimation of the effect of IGF-II on weight gain and residual confounding. However, the pronounced inverse association between IGF-II and weight gain or obesity was not materially changed after controlling for correlates of IGF-II, possible confounders and putative risk factors for obesity.

Circulating concentrations of IGF-II may be elevated in individuals with underlying disease, such as cancer (23)—a condition that may also be associated with weight loss (22). We therefore conducted a secondary analysis excluding participants who subsequently lost weight and found similar inverse associations, indicating that these findings are unlikely to be biased by a subset of individuals with underlying disease. Furthermore, excluding from the analysis 28 participants who subsequently developed glucose intolerance did not materially alter the reported associations.

The results from this study concur with findings from gene-association studies examining the relation between allelic variants in the IGF-II gene and body weight (5,6). The imprinted IGF-II gene lies in close proximity to the insulin gene on chromosome 11p in humans. Accumulating data suggest that this genomic region may be important in the regulation of childhood and adult body weight and fat mass (7,24–28). More recently, a 12-kb deletion of a possible intergenic control region of the IGF-II gene was associated with decreased IGF-II expression and increased adiposity in mice (29).

At least four polymorphisms within the IGF-II gene have shown strong associations with body weight and BMI in men (6). One of these variants has also been associated

TABLE 2
Relative risk of weight gain according to levels of circulating IGF-II concentrations at baseline, the Ely Study, 1990–1996

IGF-II (ng/ml)	Percentile	Weight change category: Weight stable/loss (n = 246)	Weight change category: Weight gain (n = 217)	Unadjusted odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
<400	<20th	33 (13)	60 (28)	1.00	1.00
400–492	20th–40th	50 (20)	43 (20)	0.47 (0.26–0.85)	0.49 (0.26–0.90)
493–610	41st–60th	54 (22)	38 (17)	0.39 (0.21–0.70)	0.36 (0.19–0.66)
611–736	61st–80th	54 (22)	39 (18)	0.40 (0.22–0.72)	0.44 (0.23–0.82)
>736	>80th	55 (23)	37 (17)	0.37 (0.20–0.67)	0.40 (0.21–0.74)
P for trend				0.001	0.006

Data are n (% of total) unless otherwise indicated. *Adjusted for follow-up time, length of blood storage, baseline age, sex, weight, BMI, IGF-I, 0-h cholesterol, and 0-h insulin.

with circulating concentrations of the hormone. Consistent with the results from the present study, in heavier wild-type (GG) homozygotes, circulating IGF-II levels were found to be statistically significantly lower than lighter rare (AA) homozygotes (5). These results may explain why individuals with IGF-II levels below the 20th percentile had the greatest risk of weight gain in the current investigation. More notable, the apaI IGF-II gene variant has also been related to overfeeding-induced anthropometric and metabolic changes. Specifically, wild-type (GG) carriers of the apaI polymorphism gained significantly more subcutaneous fat mass than rare (AA) allele carriers (8), suggesting that in an environment of caloric excess, individuals with low circulating IGF-II levels may be more likely to gain weight and develop obesity.

Population studies assessing the cross-sectional association among circulating IGF-II concentrations and indexes of body weight or obesity are sparse. One investigation in three ethnic groups found no association with IGF-II levels and BMI (30) and only weak inverse associations with measures of central adiposity, such as waist-to-hip ratio. A more recent cross-sectional study found that obese men and women had statistically significantly lower mean IGF-II levels compared with leaner individuals (31). However, in comparison with lean controls, at least two clinical studies have reported higher IGF-II concentrations in people with obesity (14,32).

The inconsistent cross-sectional associations between IGF-II and indexes of adiposity and possibly elevated IGF-II levels in obese individuals may be due to compensatory changes as a result of weight change. For example, weight recuperation in female patients with anorexia nervosa is associated with significant increases in serum levels of IGF-II (33). However, it is difficult to draw any firm conclusions from these clinical observations because of the associated metabolic disturbances related to prolonged periods of fasting and caloric deficit. In addition, by altering levels of circulating IGF-binding proteins, the degree of hyperinsulinemia may also influence levels of IGF-II in obesity (14). Furthermore, propensity to obesity may depend not only on initial levels of circulating IGF-II but also on how IGF-II levels change in response to changes in body weight and adiposity. Hence, metabolic adaptation to caloric excess and subsequent weight change may also be important in body weight regulation (34).

Alternatively, IGF-II may be associated with other correlates of energy balance that have been shown to predict weight gain, such as muscle mass, energy expenditure, or the ratio of fat to carbohydrate oxidation (35,36). IGF-I and IGF-II play a critical role in muscle regeneration, and relatively higher IGF-II levels may prevent the age-related decline in muscle mass and metabolic function (37–39). Evidence from transgenic experimental studies also suggest that IGF-II may be involved in fat metabolism. Circulating IGF-II concentrations in humans are nearly fourfold higher than levels of circulating IGF-I, peaking at puberty and showing only a modest decline with age, whereas systemic levels of IGF-II decline soon after birth in rodents (3). Nevertheless, transgenic mice overexpressing IGF-II are lighter, exhibiting reduced fat mass and lipid content of adipose tissue (40–42). Oxidation of oral lipids is also increased in IGF-II transgenic animals, whereas rates of lipogenesis and lipolysis are similar to control animals, indicating that IGF-II may influence the metabolic utilization of ingested lipids (42).

The relation between IGF-II and body weight might also be due to a central-acting role of IGF-II on the regulation of feeding behavior and body weight. In both humans and rodents, IGF-I, IGF-II, and insulin and their receptors are expressed in hypothalamic regions implicated in adiposity signaling and regulation of food intake (43). Similar to insulin, experimental studies have shown that intracerebroventricular injections of IGF-II induce hypophagia and weight change in rodents, although data are inconclusive (44–46). Furthermore, in a manner analogous to insulin, IGF-II attenuates the release of neuropeptide Y, a potent orexigenic peptide, from the hypothalamic paraventricular nucleus in vitro (47). These central IGF-II actions may be mediated through the IGF-I receptor or via the insulin receptor isoform A. The latter is the only insulin receptor isoform expressed in central nervous tissue and has high affinity for IGF-II (48). Systemic and central administration of insulin has also been shown to increase IGF-II expression in the ventromedial and paraventricular nuclei of the hypothalamus (49,50), suggesting that insulin-mediated changes in IGF-II may have a neuroendocrine function in regulating feeding behavior.

In summary, these prospective data demonstrate that low levels of circulating IGF-II are associated with an increased risk of weight gain and obesity in a population with normal glucose tolerance. Investigations of the regulation and physiological activity of IGF-II in postnatal life may help to clarify these observations.

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