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Insulin-like growth factor 2 (*IGF2*) and IGF-binding protein 1 (*IGFBP1*) gene variants are associated with overfeeding-induced metabolic changes

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Abstract

Aims/hypothesis. The aim of this study was to investigate the role of insulin-like growth factor 1 (*IGF1*), *IGF2*, *IGF* binding protein 1 (*IGFBP1*) and *IGFBP3* gene variants on the metabolic changes observed in response to a 100-day overfeeding protocol conducted with 12 pairs of monozygotic twins.
Methods. Genotyping was done by PCR-RFLP and DNA sequencer methods. Body fat measurements included hydrodensitometry and abdominal fat from computed tomography. Plasma glucose and insulin during fasting and in response to an OGTT were assayed. Plasma lipids were measured enzymatically.
Results. In response to caloric surplus, fasting plasma insulin ($p < 0.05$) and OGTT insulin ($p = 0.004$) but not glucose area, increased more among the subjects with *IGF2 Apa I GG* ($n = 12$) than those with AA + AG ($n = 12$). The changes were independent of changes in total fatness. The subjects with *IGFBP1 Bgl II AA* ($n = 8$) showed greater increases in abdominal visceral fat ($p < 0.01$), OGTT insulin area ($p = 0.05$) and total cholesterol ($p < 0.03$) with overfeeding than the subjects with AG + GG ($n = 16$). *IGFBP3 Nde I* and the *IGF1* $(CT)_n$ markers were not associated with responsiveness to overfeeding.
Conclusion/interpretation. Insulin sensitivity decreased in the subjects with *IGF2 Apa I* GG and the subjects with *IGFBP1 Bgl II* AA showed an accumulation of abdominal visceral fat and the early symptoms of the metabolic syndrome after long-term caloric surplus. Genetic variation at the *IGF2* and *IGFBP1* loci could be among the factors responsible for the inter-individual differences observed in the response to long-term alterations in energy balance and should be further investigated in larger cohorts. [Diabetologia (2001) 44: 2231–2236]

Keywords *IGF1*, *IGF2*, *IGFBP1*, *IGFBP3*, gene, polymorphism, insulin, abdominal fat, lipids, overfeeding.

Insulin-like growth factors 1 and 2 (*IGF1* and *IGF2*) are important for regulating growth and metabolism [1]. In biological fluids, *IGFs* are normally bound to specific binding proteins, insulin-like growth factor binding proteins, *IGFBPs* [2]. *IGFs* exert several insulinomimetic effects such as reducing plasma concentrations of insulin and glucagon and stimulating lipid oxidation [3]. An inverse association between total *IGF1* concentrations and visceral adipose depot has been reported [4, 5]. Thus the *IGF* and *IGFBP* genes could be involved in insulin resistance and other obesity-related phenotypes as well as in the responsiveness to dietary changes. A microsatellite polymorphism at the *IGF1* gene showed both linkage and association with body composition phenotypes in the sedentary state and in response to endurance training [6]. In addition, an *IGF2* gene variant has been shown to affect body weight variability and

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Abbreviations: IGFBP, Insulin-like growth factor binding protein; IGF, insulin-like growth factor; CT, computed tomography

IGF2 concentrations [7]. The aim of this study was to assess whether genetic variations at the *IGF1*, *IGF2*, *IGFBP1* and *IGFBP3* gene loci explained the observed large inter-individual variations in the morphological and metabolic changes [8] brought about by a long-term (100 days) overfeeding protocol carried out on 12 pairs of monozygotic twins.

Subjects and methods

Twelve pairs of young (aged 21 ± 2 years) male identical twins provided written consent to participate in an overfeeding study approved by the Laval University Medical Ethics Committee and the Office for the Protection from Research Risks of the National Institutes of Health (Bethesda, Md., USA). The men were housed in a closed section of a dormitory on the campus of Laval University. During the overfeeding period the men ate a 4.2 MJ per day (1000 kcal) energy surplus, six days a week, over 100 days. The subjects were under 24-h supervision by members of the project staff who lived with them. The specific aims, study design, and methodology of this overfeeding protocol have been described [8]. In this study, the twins are considered as 24 subjects and as 12 pairs. Each man stayed in the unit for 120 consecutive days: 14 days for the assessment of baseline daily energy intake, 3 days for testing before the period of overfeeding, 100 days for the period of overfeeding, and 3 days for testing after the period of overfeeding.

Body composition and regional fat distribution measurements. The data on body weight represent the mean measurements of 3 days, the day on which the underwater weighing was done and the days before and after. Body density was analysed by the hydrostatic weighing technique [9] and fat mass was calculated with a standard equation [10]. Abdominal computed tomography (CT) scanning was done before and after the overfeeding period with a Siemens Somatom DRH scanner (Erlangen, Germany) [11].

Plasma glucose, insulin and glucagon measurements. The plasma glucose concentration was measured enzymatically [12], and the plasma insulin by radioimmunoassay (RIA) [13]. Glucagon was analysed in unextracted plasma by radioimmunoassay with the 30 K antibody [14]. Glucose and insulin total areas under the curve during the OGTT were assessed with the trapezoid method.

Plasma lipid measurements. Total plasma cholesterol was analysed using the enzymatic kit CHOD-PAP from Boehringer (Mannheim, Germany). High-density lipoprotein cholesterol was assayed with the same method after separation of HDL from low-density and very-low-density lipoproteins. Plasma triglycerides were analysed enzymatically with the A-Gent kit (Abbott Laboratories, South Pasadena, Calif., USA).

Plasma testosterone and IGF1 analysis. The testosterone concentrations were measured by RIA [15]. *IGF1* concentrations were determined with the commercial RIA SM-C kit from Nichols Institute Diagnostics (San Juan Capistrano, Calif., USA). The samples were fasting plasma and all the assays were done shortly after the overfeeding protocol was completed.

Genotype measurements. Genomic DNA was isolated from lymphoblastoid cell lines [16]. *IGF1* $(CT)_n$ polymorphism and PCR and DNA sequencer techniques by which it was uncovered have been described [6]. The primer sequences were as follows: forward primer, 5'-TTG-TGT-CAA-CTG-CTG-ATA-TG-3'; reverse primer, 5'-AAC-CAA-AAC-ATC-ATT-CCC-TA-3'. The *IGF2 Apa I* polymorphic site is located in the 3' untranslated region at the *IGF2* gene. The primers were derived from previously published data [17]: forward primer, 5'-CTT-GGA-CTT-TGA-GTC-AAA-TTG-G-3'; reverse primer, 5'-CCT-CCT-TTG-GTC-TTA-CTG-GG-3'. The amplification product was digested at 25 °C for 16 h with 10 U of *Apa I (BRL)* enzyme. The fragments were separated on a 2 % agarose gel and visualized under UV light after staining with ethidium bromide. The *Bgl II* restriction site is in the first intron of the *IGFBP1* gene and PCR analysis of the polymorphism was carried out in a volume of 15 µl containing 100 ng DNA, 0.3 µmol/l of each primer, 0.2 mmol/l of each of the dNTPs, 0.5 unit Taq polymerase in a standard buffer and Q solution (Qiagen, Mississauga, Ontario, Canada). The primers for the *IGFBP1 Bgl II* polymorphism were as follows: forward, 5'-CAC-GTA-GTT-GGG-GAA-GGA-GC-3'; reverse, 5'-GGC-AAT-GAA-TGG-AAG-TGA-AGC-3'. The PCR was started at 94 °C for 5 min, 55 °C and 72 °C for 1 min followed by 30 cycles at 94 °C, 55 °C and 72 °C for 30 s and 1 cycle at 72 °C for 10 min using a thermal cycler (model 9600: Perkin-Elmer Cetus Instruments, Branchburg, N.J., USA). The amplified product was digested at 37 °C for 18 h with 20 units of *Bgl II*. The fragments were separated on a 2.5 % agarose gel. The *Nde I* restriction site is in the third intron of the *IGFBP3* gene and genotyping of this polymorphism was done as described earlier [18]. The primer sequences were as follows: forward primer, 5'-CTC-CGA-GTC-ACT-GGC-ATT-TC-3'; reverse primer, 5'-ACC-AGC-CCT-TGT-AGA-ACC-TC-3'.

Statistical analysis. The differences in absolute changes between genotypes at a given marker were assessed by a Student's *t* test. The 24 subjects considered as unrelated persons and the phenotype mean of each of the 12 pairs were analysed. Statistical analyses were carried out with the SAS statistical package (SAS institute, Cary, N.C., USA). A p value of less than 0.05 was considered statistically significant.

Results

The effects of overfeeding on measures of body composition in this sample of identical twins have been reported [8]. In this study, the *IGF1* dinucleotide $(CT)_n$ polymorphism showed six alleles, the 189/189 bp homozygotes (189/189 bp) being the most common genotype ($n = 14$). Because of the low number of rare allele homozygotes, all other genotypes ($n = 10$) were pooled together (non-189/189 bp) for the purpose of comparison. Thus, *IGF2 Apa I* A allele homozygotes ($n = 2$) were pooled together with AG heterozygotes ($n = 10$) (AA + AG) and compared with G allele homozygotes ($n = 12$) (GG). In addition, *IGFBP1 Bgl II* G allele homozygotes ($n = 2$) were pooled together with GA heterozygotes ($n = 14$) (GG + GA). 189/189 bp subjects of the *IGF1* $(CT)_n$ polymorphism had higher IGF1 concentrations before [1144.5 (288.0); $p = 0.019$] and after [1195.9 (419.6); $p = 0.011$] overfeeding than subjects who were non-189/189 bp [before: 801.3 (374.4) and

Table 1. Effect of overfeeding on obesity-related phenotypes in male twins in relation to the *IGF2 APA I* and *IGFBP1 BGL II* genotypes

	IGF2 APA I genotypes		*IGFBP1 BGL II* genotypes	
	AA/AG (n = 12)	GG (n = 12)	GG/GA (n = 16)	AA (n = 8)
Body weight (kg)				
Before	59.9 (2.2)	60.7 (2.54)	61.3 (2.2)	58.2 (1.9)
After	67.8 (2.3)	69.1 (2.5)	69.1 (2.3)	67.2 (2.4)
Change	7.9 (0.7)	8.4 (0.7)	7.7 (2.2)	8.9 (2.7)
Fat mass (kg)				
Before	5.5 (0.9)	8.4 (1.0)*	6.7 (0.8)	7.5 (1.5)
After	10.2 (1.3)	14.4 (0.9)**	12.0 (0.9)	12.9 (2.2)
Change	4.7 (0.6)	6.1 (0.5)	5.4 (0.4)	5.4 (0.9)
Subcutaneous fat (mm)				
Before	69.1 (3.9)	82.8 (7.4)	79.1 (6.0)	69.6 (4.4)
After	114.9 (7.2)	144.0 (9.9)**	130.3 (8.1)	127.6 (12.6)
Change	45.8 (5.7)	61.2 (4.2)*	51.2 (3.5)	58.0 (9.4)
Abdominal visceral fat (cm^2)				
Before	32.0 (2.0)	36.0 (3.3)	32.2 (2.2)	37.7 (3.7)
After	58.7 (3.7)	58.1 (5.2)	51.5 (2.2)	72.1 (5.8)***
Change	26.7 (3.3)	22.1 (4.0)	19.3 (2.7)	34.4 (3.5)***
Total cholesterol (mmol/l)				
Before	4.56 (0.24)	4.43 (0.23)	4.63 (0.20)	4.20 (0.27)
After	4.77 (0.17)	5.07 (0.45)	4.79 (0.23)	5.18 (0.56)
Change	0.21 (0.17)	0.64 (0.31)	0.16 (0.16)	0.97 (0.39)**

Values are means (SE). Changes are post-overfeeding minus pre-overfeeding values. p values based on 24 subjects considered as unrelated persons are as follows:
*$p < 0.05$
**$p \leq 0.03$
***$p < 0.01$ between genotype groups
p values when phenotype means of each pair are compared (n = 12) are as follows:
* and ** NS, ***$p = 0.019$ for the difference in the change in abdominal visceral fat

after 771.1 (288.8)]. However, the variant was not associated with any of the phenotype changes in response to overfeeding.

The influence of the long-term caloric surplus on obesity-related phenotypes and hormonal changes by *IGF2 Apa I* and *IGFBP1 Bgl II* genotypes is shown (Tables 1, 2 and Fig. 1). *IGF2 Apa I* GG subjects had higher fat mass before ($p < 0.05$) and after ($p < 0.03$) overfeeding as well as higher subcutaneous fat ($p < 0.03$), leptin ($p < 0.05$) but lower total testosterone concentrations ($p < 0.01$) after overfeeding compared with the AA/AG subjects. The *IGF2 Apa I* GG subjects also experienced greater increases in the amount of subcutaneous fat ($p < 0.05$; Table 1), leptin ($p < 0.05$; Table 2) and fasting insulin ($p < 0.05$; Table 2) in response to overfeeding. Furthermore, the OGTT insulin area increased more among subjects with the genotype GG ($p = 0.004$; Fig. 1). The increase in OGTT insulin area in the *IGF2 Apa I* GG subjects remained significant after adjustment for the change in fat mass. However, the changes in OGTT glycaemic area did not differ by *IGF2 Apa I* genotype. In contrast, total ($p < 0.01$) and free testosterone (NS) showed less increase among subjects with the *IGF2 Apa I* GG. Finally, the IGF1 concentrations tended to decrease in GG but increase in AA/AG subjects (Table 2).

Homozygotes for the *IGFBP1 Bgl II A* allele had higher glucagon values both before ($p < 0.03$) and after overfeeding ($p < 0.03$) and a higher amount of abdominal visceral fat ($p < 0.01$) than homozygotes or heterozygotes for the G allele (Table 2). IGF1 concentrations were lower before ($p < 0.05$) and after ($p < 0.03$) the caloric surplus among the *IGFBP1 Bgl II AA* homozygotes. In addition, *IGF1* concentrations tended to decrease among AA but increase in GG/GA subjects (Table 2). The *IGFBP1 Bgl II* A allele homozygotes showed greater increases in abdominal visceral fat ($p < 0.01$), OGTT insulin area and total cholesterol ($p < 0.03$) with overfeeding than G allele homozygotes or heterozygotes (Tables 1, 2). Increases in OGTT insulin area and cholesterol in the *IGFBP1 Bgl II* A allele homozygotes were not statistically significant after adjustment for the change in abdominal visceral fat (data not shown). The increase in abdominal visceral fat was marginally associated with the increase in cholesterol among A allele homozygotes ($r = 0.67$, $p = 0.07$). Among the 24 subjects, the changes in abdominal visceral fat correlated negatively with post-overfeeding IGF1 concentrations ($r = –0.42$, $p = 0.039$). The number of carriers for the rare allele of the *IGFBP3 Nde I* ($n = 4$) polymorphism was low and no differences in response to overfeeding were found between genotypes.

Table 2. Effects of overfeeding on blood levels of hormones in male twins in relation to the *IGF2 APA I* and *IGFBP1 BGL II* genotypes

	IGF2 APA I genotypes		*IGFBP1 BGL II* genotypes	
	AA/AG ($n = 12$)	GG ($n = 12$)	GG/GA ($n = 16$)	AA ($n = 8$)
Leptin (ng/ml)				
Before	0.7 (0.1)	1.6 (0.4)	1.2 (0.3)	0.9 (0.2)
After	2.8 (0.6)	7.0 (1.8) *	5.0 (1.4)	4.7 (1.0)
Change	2.1 (0.5)	5.4 (1.3)*	3.8 (1.1)	3.7 (0.9)
Plasma insulin (pmol/l)				
Before	52.8 (3.3)	49.9 (3.7)	49.3 (2.9)	55.5 (4.2)
After	54.8 (8.8)	79.8 (9.0)	65.7 (8.8)	70.6 (10.2)
Change	2.0 (7.6)	29.9 (9.7)*	16.4 (9.5)	15.1 (7.0)
OGTT insulin area (10^3 pmol/l · min)				
Before	50.2 (5.8)	58.8 (5.8)	56.1 (5.1)	51.2 (7.3)
After	54.5 (8.6)	86.6 (8.6) **	66.3 (8.4)	79.1 (11.9)
Change	4.3 (5.2)	27.8 (5.2)***	10.2 (5.0)	27.9 (7.1)*
Plasma glucagon (ng/l)				
Before	103.1 (14.0)	88.2 (12.0)	77.3 (5.7)	132.2 (20.0) **
After	108.2 (7.4)	124.0 (16.9)	95.7 (5.0)	156.8 (19.1) **
Change	5.1 (10.1)	35.8 (11.3)	18.4 (6.1)	24.6 (21.9)
Total testosterone (ng/dl)				
Before	661.9 (27.6)	614.4 (33.5)	619.6 (26.3)	675.4 (37.9)
After	800.1 (37.8)	640.0 (34.8)***	717.7 (39.0)	724.6 (49.5)
Change	138.2 (33.2)	25.6 (19.2)***	98.2 (27.0)	49.2 (38.6)
Insulin like growth factor I (IU/l)				
Before	982.2 (123.0)	1020.7 (88.0)	1091.9 (98.9)	820.6 (71.5) *
After	1087.3 (155.0)	950.4 (78.2)	1125.7 (119.1)	805.1 (49.5) **
Change	105.1 (74.8)	–70.3 (80.9)	33.8 (80.4)	–15.5 (63.1)

Values are means (SE). p values based on 24 subjects considered as unrelated persons are as follows:
*$p \leq 0.05$
**$p < 0.03$
***$p < 0.01$ between genotype groups.
p values when phenotype means of each pair are compared ($n = 12$) are as follows: * and ** NS and ***$p \leq 0.05$

Discussion

The data of this study suggest that although the 189/189 bp genotype of the *IGF1* $(CT)_n$ polymorphism has higher IGF1 concentrations, it is not associated with the responsiveness to the caloric surplus. However, *IGF2 Apa I* G allele homozygotes showed greater increases in OGTT insulin area, fasting insulin, leptin and subcutaneous fat concentrations than the A allele carrier in response to long-term overfeeding. Fat mass and amount of subcutaneous fat were lower before and after the overfeeding protocol in A allele carriers than in the homozygous subjects with the G allele. These observations correspond with the recently reported association of the *IGF2 Apa I* AA genotype with a lower mean body weight in a Caucasian population [7]. In the latter study, the *IGF2 Apa I* AA genotype was also associated with a higher serum IGF2 concentration [7], which was not measured in our study.

The increases in fasting insulin and OGTT insulin area in the male twins homozygotes for the *IGF2 Apa* G allele remained statistically significant after adjustment for the change in fat mass. Because the OGTT glycaemic area did not change, the *IGF2 Apa I* G allele homozygotes probably had a decrease in insulin sensitivity with overfeeding. We speculate that the *IGF2 Apa I* G allele homozygotes could have had a decrease in IGF2 concentration or activity after exposure to the caloric overload. The latter could lead to alterations in glucose transport and glucose utilization in skeletal muscle thereby increasing peripheral insulin resistance [19]. The *IGF2 Apa I* GG subjects also showed less increase in total testosterone concentrations compared with the AA + AG subjects. IGF1 has been shown to suppress sex-hormone binding protein production [20], a major determinant of free sex hormone concentrations. Therefore, the observed trend for an increase in IGF1 in the AA + AG subjects could at least partly explain their greater increase in testosterone concentrations.

In the present study, the *IGFBP1 Bgl II* A allele homozygotes gained more abdominal visceral fat and showed greater increases in OGTT insulin area and total cholesterol with overfeeding than G allele homozygotes or heterozygotes. In addition, IGF1 concentrations among the *IGFBP1 Bgl II* A allele homozygotes were lower before and after overfeeding and decreased slightly in response to overfeeding. Thus the *IGFBP1 Bgl II* AA genotype could increase IGFBP1 concentrations resulting in a decrease in the plasma concentration or metabolic activity of IGF1.

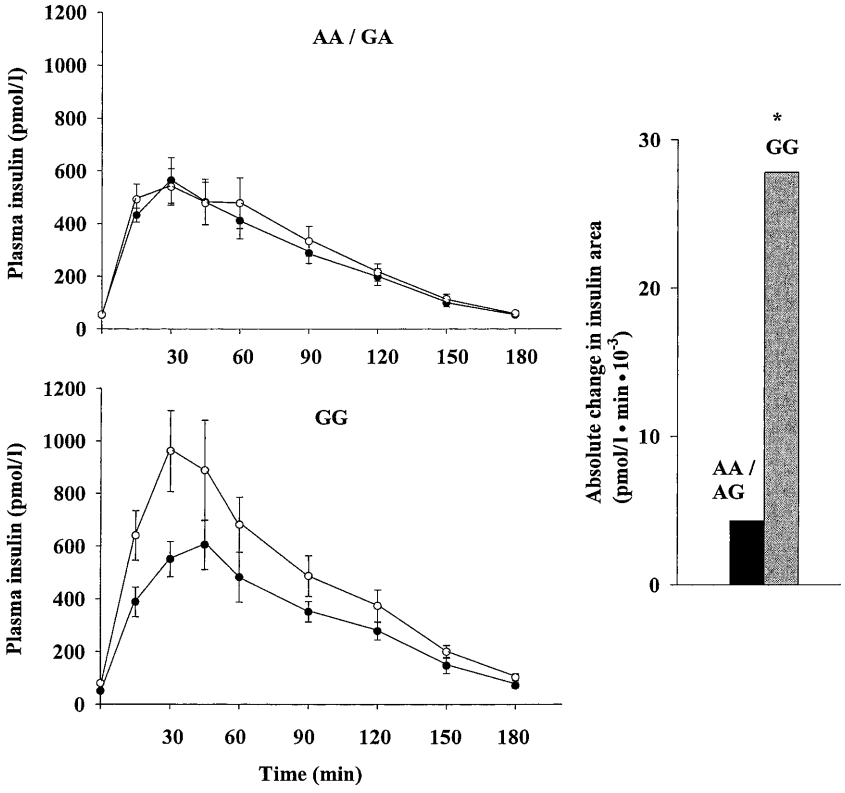

Fig. 1. Plasma insulin concentrations in the AA/GA and GG genotypes of the *IGF2 Apa I* polymorphism before (—○—) and after (—●—) overfeeding as well as the absolute changes of the total insulin areas under the curve during the OGTT. *$p = 0.004$ between the AA/AG and GG genotypes for the insulin area changes (24 cases) and $p = 0.018$ when the mean of each pair ($n = 12$) is used

Low IGF1 concentrations in abdominal obesity have been reported previously [4]. In our study, the increase in abdominal visceral fat correlated negatively with post-overfeeding IGF1 concentrations. However, the hypothesis that IGFBP1 concentrations would be increased in homozygotes for the A allele does not agree with some studies showing lower *IGFBP1* concentrations in obesity [21]. The greater increase in the amount of abdominal visceral fat in the *IGFBP1 Bgl II* A allele homozygotes could explain some of the changes observed in OGTT insulin area and cholesterol. The latter hypothesis is supported by the notion that the increase in abdominal visceral fat correlated positively with the change in cholesterol among A allele homozygotes. Moreover, subjects who were *IGFBP1 Bgl II* A allele homozygotes had higher glucagon concentrations before and after overfeeding. The reason for the difference between A allele carriers and non-carriers is not known. Of note, *IGF1* has inhibitory effects not only on the secretion of insulin but also on glucagon [3]. Therefore, the higher glucagon concentrations in AA subjects could be caused by the lower IGF1 concentration. Glucagon is also able to increase IGFBP1 concentration [20] and theoretically could change the fraction concentration of free circulating IGFs.

Both *IGF2 Apa I* and *IGFBP1 Bgl II* polymorphisms are located in non-coding regions and do not cause changes in the amino acid sequence of the IGF2 or IGFBP1 proteins. However, they could be in linkage disequilibrium with other mutations that have a more functional significance.

In summary, the *IGF2 Apa I G* allele homozygotes had decreased insulin sensitivity independently of the increase in fatness in response to chronic overfeeding. The *IGFBP1 Bgl II* A allele homozygotes had a greater accumulation of abdominal visceral fat which could have contributed to the overfeeding-induced early symptoms of the metabolic syndrome. Genetic variation at the *IGF2* and *IGFBP1* loci could thus be among the factors responsible for the inter-individual differences observed in response to long-term alterations in energy balance and should be further investigated in large cohorts.

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