Plasma Glucose, Insulin, and Glucagon Before and After Long-Term Overfeeding in Identical Twins

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Plasma glucose, insulin, and glucagon levels were measured before and after long-term overfeeding (4.2 MJ/d during a 100-day period) in 24 lean adults (12 pairs of monozygotic twins). Fasting plasma glucose, insulin, and glucagon were significantly increased by overfeeding. During a 75-g oral glucose tolerance test (OGTT), no major alteration in glucose tolerance was observed and insulin area under the curve was increased. During a meal test, insulin and glucagon areas under the curve were increased. The pre-overfeeding values for glucose, insulin, and glucagon (fasting and areas) were not correlated with the gains in body weight and in fat mass. However, fasting glucagon before overfeeding was positively correlated with the gains in abdominal visceral fat and in femoral fat. The changes with overfeeding in insulin area during the OGTT were positively correlated with the changes in total subcutaneous fat, even after adjustment for total body fat gain. Significant twin intrapair similarity was observed for fasting plasma glucagon before overfeeding and for the changes in fasting insulin and glucagon with overfeeding. These results indicate that (1) in response to long-term overfeeding, both fasting insulin and glucagon are increased; (2) initial levels of glucose, insulin, and glucagon do not predict the gains in body weight and total body fat during overfeeding, but are related to changes in indicators of fat topography; (3) the changes in total subcutaneous fat represent an important correlate of insulin changes with overfeeding; and (4) the genotype could be an important determinant of insulin and glucagon responses to a prolonged positive–energy-balance period.

BODY WEIGHT VARIATIONS are known to influence substantially plasma insulin levels and glucose tolerance. More than 20 years ago, the overfeeding study of Sims et al[1] in Vermont demonstrated that body weight gain was associated with decreased glucose tolerance, hyperinsulinemia, and insulin resistance. Since then, high fasting levels of insulin have been repeatedly observed in several other overfeeding studies.[2-9] Together with insulin, pancreatic glucagon is involved in fuel homeostasis.[10] However, there is no report about glucagon concentrations during overfeeding.

In the last decade, emphasis has been placed on regional fat distribution and body fat topography.[11-15] In this field, computed tomography (CT) is an accurate method that allows precise measurement of cross-sectional areas of deep and subcutaneous adipose tissue at any site of the body.[16] A recent US National Institutes of Health Workshop concluded that if total body fat was the primary component associated with increased plasma insulin, increased subcutaneous truncal-abdominal fat was also correlated with increased insulin resistance, and increased visceral fat exacerbated insulin resistance and glucose intolerance even further.[17] The relationships between changes in indicators of body fat topography and insulin-glucose homeostasis during overfeeding are not known.

There is strong evidence that genetic factors contribute to the development of metabolic alterations leading to non–insulin-dependent diabetes mellitus.[18] The role of inheritance in the development of excess body fat and in the fat distribution profile is well recognized.[19,20] This suggests that the response of plasma glucose and insulin when body composition is altered through a nutritional challenge could also be genotype-dependent. One way to investigate this question is to challenge several genotypes in a similar manner, submitting both members of several monozygotic twin pairs to a standardized overfeeding protocol inducing an adaptive response.[21] Using such a design with six pairs of monozygotic twins, plasma glucose and insulin responses following 22 days of overfeeding were previously reported.[7,8,22] However, the metabolic effects of the short-term overfeeding were small in that experiment, and the results were not entirely conclusive.

Therefore, the aims of the present study were to compare plasma glucose, insulin, and glucagon levels before and after a long-term (100-day) overfeeding protocol conducted with 12 pairs of monozygotic twins, to assess their relationships with body fat changes evaluated by CT scans and to investigate the role of genotype in the observed metabolic and hormonal changes.

SUBJECTS AND METHODS

Subjects

Twenty-four sedentary young men (aged 21 ± 2 years, mean ± SD) gave their written consent to participate in this study, which was approved by the Laval University Medical Ethics Committee and the Office for Protection from Research Risks of the National Institutes of Health, Bethesda, MD. These subjects constituted 12 pairs of identical twins. The twins had been reared together and had been living together before this study.

The monozygosity of the twins was established on the basis of a questionnaire, their physical appearance, the similarity of 12 polymorphic red blood cell antigens and enzymes, the A, B, and C loci of the HLA antigen system, and 10 polymorphic adipose tissue proteins visualized by two-dimensional gel electrophoresis. Since

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the subjects were accepted in the study, the monozygosity has been confirmed by a large number of DNA markers. None of the men had a history of recent illness, obesity, diabetes, hyperlipidemia, hypertension, or endocrinopathy, and each had a normal physical examination, normal glucose tolerance,[23] and normal triglyceride and cholesterol concentrations. Subjects whose parents were obese or had diabetes or lipid disorders were not accepted in the study. Additional details about this study and the overfeeding protocol can be found in the first report of the series.[24]

Experimental Protocol

Eight subjects at a time were tested over a period of 18 months: the first subgroup started in August 1986, the second in February 1987, and the third the following August. The experimental protocol was exactly the same for each subgroup. For the duration of the study, the subjects lived on the campus of Laval University. They were housed in a closed section of a dormitory adjacent to the cafeteria where they took their meals. The subjects were under 24-hour supervision by members of the project staff who lived with them. Each subject stayed in the unit for 120 consecutive days—14 days for the baseline observation period, 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.

Baseline Period

During the baseline period, subjects were instructed to eat freely as if they had been maintaining their usual food habits. Foods were prepared by university staff and put aside by the dietitians involved in the study. All foods were weighted before meals and reweighed when not consumed to determine precisely the food consumption of the subjects. The energy content and macronutrient composition of the food were calculated using a computerized version of the Canadian food composition tables.[25] During the baseline period, body weight was measured daily and body density measurements were performed three times. Each subject's habitual daily energy intake was calculated from the 14-day record of food intake and was generally based on the entries for the last 12 days. The variations in body weight and changes in body composition, if any, were determined from the body density measurements. The average macronutrient composition of the food eaten during the baseline period was 52% ± 6% carbohydrate, 34% ± 6% lipid, and 14% ± 6% protein (mean ± SD).

Testing Before and After the Overfeeding Period

On the first 2 days of the 3-day test period before the overfeeding period, blood samples were obtained in the morning after an overnight fast for the determination of plasma glucose, insulin, and glucagon concentrations. On day 1, a meal test was performed. The subjects consumed in 15 minutes a 4.2-MJ (1,000-kcal) meal with the following composition: 50% carbohydrate, 35% lipid, and 15% protein. Blood samples were collected every 15 minutes during the first hour and every 30 minutes during the next 3 hours following the ingestion of the meal for determination of plasma glucose, insulin, and glucagon. On day 2, a 75-g oral glucose tolerance test (OGTT) was performed. Blood samples were obtained every 15 minutes during the first hour and every 30 minutes during the next 2 hours for determination of plasma glucose, insulin, and glucagon. On day 3, densitometric measurements were repeated. Approximately 5 days before the overfeeding period, a CT scan was performed. The same measurements were repeated during the 3-day test period after the overfeeding period.

Plasma Glucose, Insulin, and Glucagon Determinations

The plasma glucose level was measured enzymatically,[26] and the plasma insulin level was measured by radioimmunoassay.[27] Glucagon was determined in unextracted plasma by radioimmunoassay with the 30K antibody of Unger.[28] Glucose, insulin, and glucagon total areas under the curve during the OGTT and the meal test were determined with the trapezoid method. Unless otherwise indicated, areas in the text refer to total areas under the curve.

Body Weight and Body Fat Measurements

Body weight was measured with the men wearing light exercise shorts in the morning before breakfast. Body density was determined by the hydrostatic weighing technique.[29] Percent body fat was calculated from body density with the equation of Siri.[30] Fat mass was obtained from percent body fat and body weight. Pulmonary residual volume was measured before immersion in the water tank by the helium-dilution technique.[31] The skinfold thickness was measured at 10 sites (subscapular, suprailiac, abdominal, midaxillary, chest, biceps, triceps, front midthigh, suprapatellar, and medial calf) according to the procedures recommended at the Airlie Conference.[32]

CT was performed on a Siemens Somatom DRH scanner (Erlangen, Germany) with the methodology described by Sjöström et al.[33] The men were examined in the supine position with both arms stretched above their heads. The scans were obtained at the abdominal (between L4 and L5 vertebrae) and midthigh (midpoint distance between the knee joint and the iliac crest) levels using a radiograph of the skeleton to establish the position of the scan to the nearest millimeter. Total fat areas were calculated by delineating these areas with a graph pen and then computing the adipose tissue surfaces with an attenuation range of −190 to −30 Hounsfield units. The abdominal visceral fat area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous fat area was calculated by subtracting the abdominal visceral fat area from the total abdominal fat area.

Overfeeding Period

After the baseline period, the subjects were fed a dietary regimen containing 4.2 MJ (1,000 kcal) per day over their established baseline energy intake, 6 days a week for 100 days. On the remaining day of each week, energy intake corresponded to baseline energy needs. Subjects were thus overfed during 84 of the 100 days, with the total excess energy intake being 353 MJ (84,000 kcal). Foods were served in three meals plus an evening snack, the content of which was adjusted to meet the prescribed daily energy intake. The macronutrient composition of the food consumed each day was as follows: 50% carbohydrate, 35% lipid, and 15% protein.

As for the pre-overfeeding period, the subjects were requested to remain sedentary throughout the study. Their schedule included activities such as playing videogames, reading, playing cards, watching television, and walking 30 minutes per day. The staff involved in the study constantly supervised the subjects. Therefore, we believe that their compliance with a sedentary mode of life was perfect. Because subjects were sedentary under free-living conditions, we believe that the activity program maintained during the experimental period did not impose a significant change in their activity habits.

Statistical Analysis

The effects of overfeeding and the interactions between genotype and overfeeding were assessed with a two-way ANOVA for repeated measures on one factor (time) as previously described.[24]

The twins were considered nested within the pair, whereas the treatment effect was considered a fixed variable. The intraclass correlation coefficient, which quantifies the similarity within pairs in the response to overfeeding, was computed from the between-pairs and within-pairs means of squares. The Pearson correlation coefficient was calculated to estimate the association between the overfeeding-induced changes in body weight and components of body fat and (1) the pre-overfeeding levels of metabolic variables and (2) the overfeeding-induced changes in the metabolic variables, with the 24 subjects considered independent persons. In some analyses, the results were adjusted for the gains in total fat mass. These adjustments were performed by regression of the variable to be adjusted on the gain in fat mass with overfeeding, with the 24 subjects considered unrelated persons. All statistical analyses were performed with the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

As previously reported,[24] overfeeding induced significant changes in body weight and body fat phenotypes (Table 1). Total fat mass, the amount of subcutaneous fat estimated on the basis of skinfold thickness, as well as CT-assessed abdominal fat areas (subcutaneous and visceral) and midthigh fat area, were all significantly increased ($P < .0001$).

Table 2 shows mean fasting plasma glucose, insulin, and glucagon before and after overfeeding. After the overfeeding period, fasting glucose was significantly increased ($P < .001$), although the magnitude of the mean change was relatively small (+6%). Insulin, as well as glucagon, were increased in the fasting state after the overfeeding period ($P < .05$ for both). Large variations were observed between subjects in the response to overfeeding, particularly with respect to fasting insulin and glucagon.

Plasma glucose, insulin, and glucagon and total areas under the curve during the OGTT before and after overfeeding are depicted in Fig 1. After ingestion of glucose, plasma glucose levels during the test were not modified with the overfeeding protocol. Accordingly, none of the subjects developed impaired glucose tolerance according to National Diabetes Data Group criteria.[23] Overfeeding induced a significant increase in plasma insulin during the OGTT, resulting in a 30% increase in the insulin area under the curve ($P < .05$). The ratio of insulin area to glucose area was increased after overfeeding (72.3 ± 36.4 *v* 60.2 ± 27.1, mean ± SD, $P < .05$). No major change was observed in postglucose plasma glucagon after overfeeding. In addition, during the OGTT the area above baseline [(total area under the curve) − (pretest concentration × duration of the test)] for insulin was increased after overfeeding ($P < .02$), whereas the glucose and glucagon areas above baseline (which was negative for the latter) were not modified (data not shown).

Table 1. Effect of 100 Days of Overfeeding on Body Weight and Components of Body Fat

Variable	Before Overfeeding	After Overfeeding*
Body weight (kg)	60.3 ± 8	68.4 ± 8.2
Fat mass (kg)	6.9 ± 3.5	12.3 ± 4.5
Subcutaneous fat (mm)†	75.9 ± 21.1	129.4 ± 32.9
CT-derived adipose tissue areas (cm^2)		
Abdominal		
Total	106 ± 46	199 ± 50
Subcutaneous	72 ± 40	141 ± 46
Visceral	34 ± 9	58 ± 15
Midthigh	87 ± 36	151 ± 42

NOTE. Values are the mean ± SD. Statistical significance was determined by a two-way ANOVA for repeated measures on one factor (time).

*$P < .0001$ for all variables as compared with values obtained before overfeeding.

†Represents the sum of 10 skinfold thickness measurements.

Table 2. Effect of 100 Days of Overfeeding on Fasting Plasma Glucose, Insulin, and Glucagon

Variable	Before Overfeeding	After Overfeeding
Glucose (mmol/L)	4.4 ± 0.2 (4.0-5.0)	4.7 ± 0.2* (4.2-5.3)
Insulin (pmol/L)	45 ± 10 (24-62)	67 ± 29† (26-141)
Glucagon (ng/L)	92 ± 45 (44-254)	118 ± 47† (56-241)

NOTE. Values are the mean ± SD (range). Statistical significance was determined by a two-way ANOVA for repeated measures on one factor (time).

*$P < .001$.

†$P < .05$.

Figure 2 shows plasma glucose, insulin, and glucagon and total areas under the curve during the meal test before and after overfeeding. No detectable change was observed in the glucose area under the curve. Similar to what was observed during the OGTT, significant increases in postprandial insulin concentrations were observed after overfeeding, with a 30% increase in the insulin area under the curve ($P < .01$). The ratio of insulin area to glucose area was increased after overfeeding (66.3 ± 25.3 *v* 53.2 ± 13.8, mean ± SD, $P < .05$). Postprandial glucagon concentrations were also increased after overfeeding. However, the total postprandial area above baseline was increased for insulin ($P < .02$) but not for glucagon (data not shown).

No modification in the fasting insulin to glucagon ratio was observed after the long-term caloric surplus (0.64 ± 0.35 *v* 0.55 ± 0.19, mean ± SD, $P = .22$). There was also no change in the ratio of insulin total area to glucagon total area during the OGTT and the meal test after overfeeding (data not shown).

Table 3 shows the within–twin-pair resemblance before overfeeding and in the absolute response to overfeeding for plasma glucose, insulin, and glucagon. Before overfeeding, significant within–twin-pair resemblance was observed for glucose areas during the OGTT and the meal test. Significant similarity within pairs was also found before overfeeding for fasting glucagon and for glucagon areas after ingestion of glucose or a meal, with approximately three times more variance between pairs than within pairs and intraclass coefficients clustered around 0.5. In response to overfeeding, there was significant similarity within pairs for the absolute change in glucose area during the meal test and for the absolute change in fasting insulin. For fasting insulin, there was approximately six times more variance between pairs than within pairs in the response to overfeeding, with an intraclass coefficient of approximately 0.7

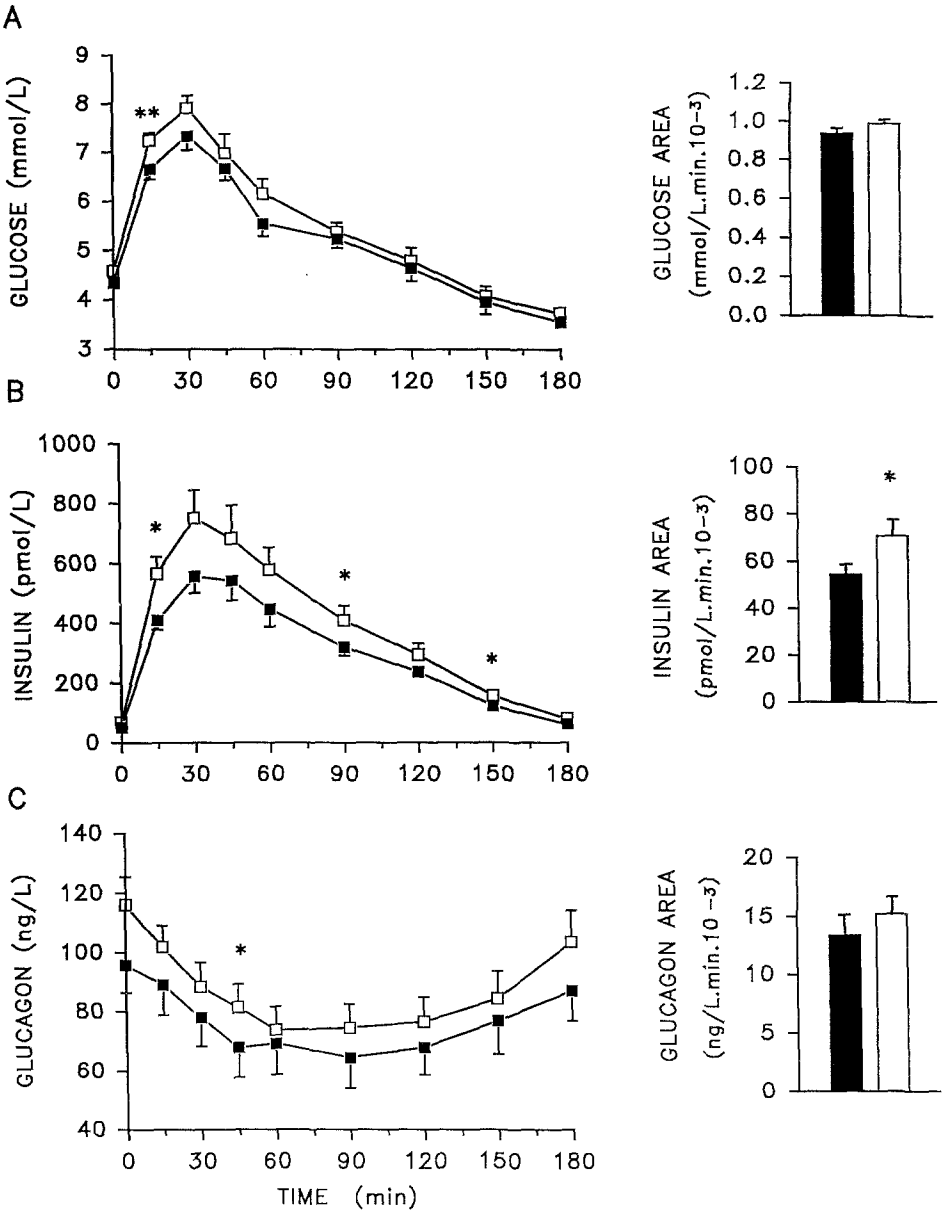

Fig 1. Plasma (A) glucose, (B) insulin, and (C) glucagon concentrations and total areas during a 75-g OGTT before (■) and after (□) 100 days of overfeeding. Values are the mean ± SEM. Areas are total areas under the curve. *$P < .05$; **$P < .01$.

($P < .01$). Within-pair resemblance was also noted for the changes in fasting glucagon and in the glucagon area after a meal. The within-pair similarity for the changes in fasting glucose, insulin, and glucagon are illustrated in Fig 3.

The relations between metabolic variables and changes in body weight and body fat phenotypes were investigated by calculating the correlation coefficients with the 24 men considered as independent persons, without and with control over the gain in total body fat. First, we assessed the associations between the absolute changes in body weight and body fat phenotypes and the pre-overfeeding levels of plasma glucose, insulin, and glucagon. No association was noted between pre-overfeeding glucose, insulin, and glucagon fasting concentrations or areas, and body weight and total body fat changes with overfeeding. With respect to regional fat topography and after adjustment for the gain in fat mass, changes in midthigh fat area were negatively correlated with pre-overfeeding insulin area and the insulin area to glucose area ratio during the OGTT ($r = -.46$ and $r = -.44$, respectively, $P < .05$). As indicated in Table 4, fasting glucagon before overfeeding was positively correlated with changes in abdominal visceral fat and midthigh fat area. After adjustment for the gain in total fat mass, all these correlations remained significant. Glucagon areas during the OGTT and the meal test before overfeeding were both positively correlated with changes in midthigh fat area. When comparing the high and low gainers for visceral abdominal fat with overfeeding (n = 6 in each subgroup, 35 ± 8 *v* 14 ± 6 cm^2, mean ± SD, $P < .00001$), fasting plasma glucagon was significantly higher before overfeeding in the high gainers (112 ± 53 *v* 73 ± 25 ng/L, mean ± SD, $P < .05$; Fig 4).

Second, we investigated the relations between changes in body weight and body fat phenotypes and the overfeeding-

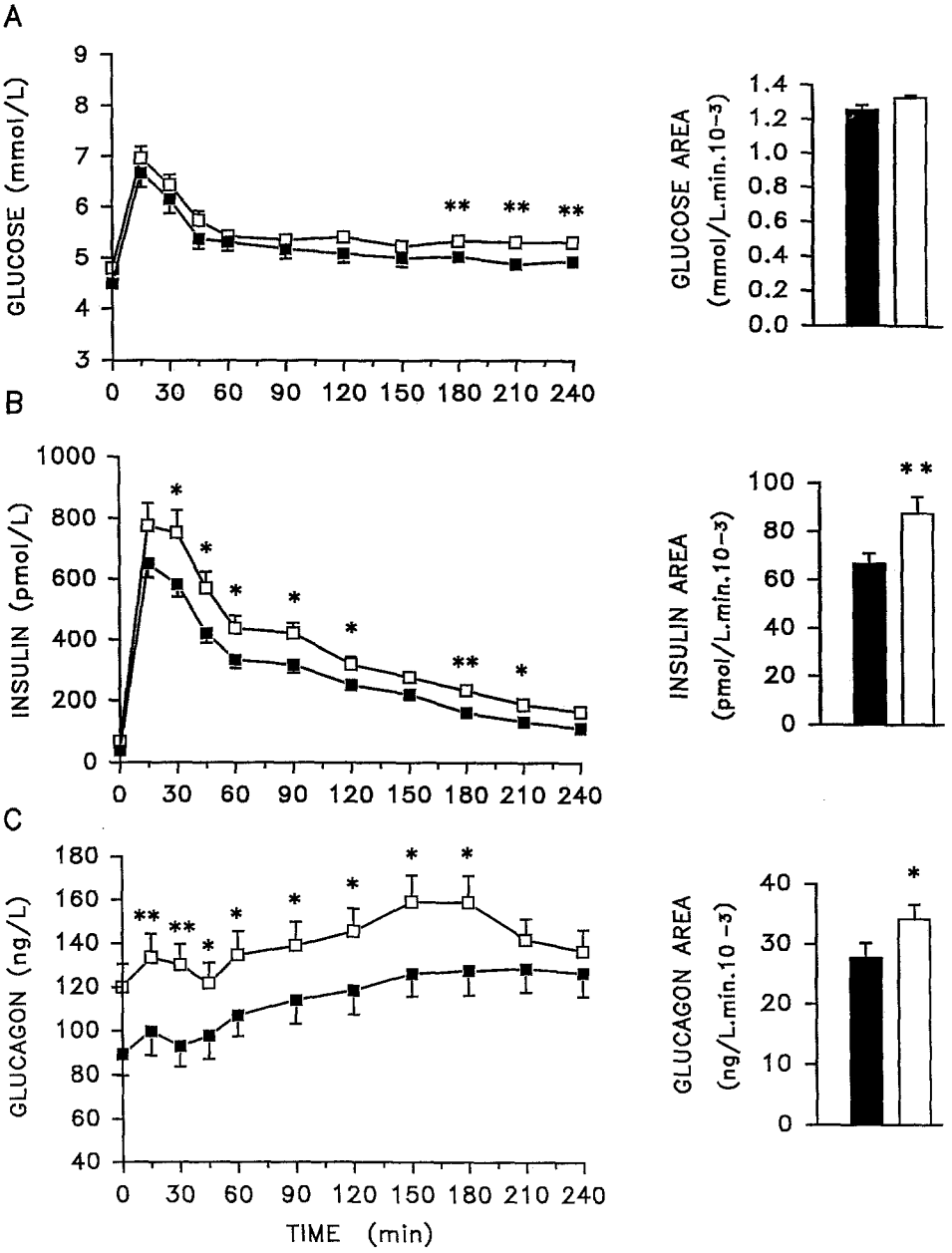

Fig 2. Plasma (A) glucose, (B) insulin, and (C) glucagon concentrations and total areas during a 4.2-MJ meal test before (■) and after (□) 100 days of overfeeding. Values are the mean ± SEM. Areas are total areas under the curve. *$P < .05$; **$P < .01$.

induced changes in metabolic variables. No association was found between changes in fasting glucose and body weight or total body fat changes with overfeeding, and no correlation was found between changes in glucagon values and changes in body weight and total body fat (data not shown). As indicated in Table 5, changes in fasting insulin levels and insulin areas during the OGTT and the meal test were positively correlated with those in body weight and total fat mass. With respect to fat distribution, without control over the gain in fat mass, gains in total subcutaneous fat were positively correlated with changes in fasting insulin, insulin area, and insulin area to glucose area ratio during the OGTT and the meal test. Changes in CT-assessed subcutaneous fat at the abdominal level were also associated with changes in insulin area and insulin area to glucose area ratio during the OGTT and the meal test. After adjustment for the gain in total fat mass, two of the relations between changes in insulin values and indicators of changes in fat topography remained significant (Table 5): changes in insulin area and in the insulin area to glucose area ratio during the OGTT were positively correlated with changes in total subcutaneous fat ($r = .41$, $r = .48$, respectively, $P < .05$). No associations were observed between changes in glucose levels and indicators of changes in fat topography (data not shown). Changes in fasting glucagon concentrations and glucagon areas during the OGTT and the meal test were negatively correlated with changes in midthigh scan area after adjustment for the gain in fat mass ($r = -.45$, $r = -.42$, $r = -.47$, respectively, $P < .05$).

DISCUSSION

This study shows that a long-term overfeeding protocol with a mixed diet induced significant changes not only in

Table 3. Within-Pair Similarity Before Overfeeding and in the Absolute Response to Overfeeding for Plasma Glucose, Insulin, and Glucagon Variables

Variable	Twin Resemblance Before Overfeeding		Twin Resemblance in Response to Overfeeding	
	F Ratio	Intraclass Coefficient	F Ratio	Intraclass Coefficient
Fasting glucose	2.42	.41	0.68	−.19
Glucose area				
OGTT	2.82	.48*	2.54	.43
Meal test	6.10	.72†	5.38	.69†
Fasting insulin	1.29	.13	5.92	.71†
Insulin area				
OGTT	2.53	.43	2.59	.44
Meal test	2.12	.36	1.81	.29
Fasting glucagon	2.76	.47*	3.21	.53*
Glucagon area				
OGTT	3.20	.52*	2.24	.38
Meal test	3.12	.51*	5.14	.67†

NOTE. Statistical significance was determined by a two-way ANOVA for repeated measures on one factor (time). The F ratio was the ratio of the variance between pairs to that within pairs. The intraclass coefficient was used to assess the similarity within pairs.

*$P < .05$.

†$P < .01$.

glucose and insulin levels but also in glucagon concentrations. Fasting plasma glucose was slightly but significantly increased by overfeeding. This is in agreement with the results of the long-term overfeeding study by Sims et al[1] and of other overfeeding studies of shorter duration.[2,6] No major change was observed in glucose concentrations after a carbohydrate load or a mixed meal. In a study by Olefsky et al,[2] who gave their subjects a high energy excess (8.4 MJ/d) during a 3-week period inducing a mean 4.4-kg body weight gain, the glucose area during an OGTT was unchanged, whereas the glucose area after ingestion of a mixed meal was increased. In a previous overfeeding protocol performed in our laboratory (4.2-MJ/d energy excess for 22 days with a small increase in body weight), there was no modification in the postglucose or postprandial glucose levels.[7,8] Welle and Campbell even reported a decrease in postprandial glucose concentrations following 17 days of carbohydrate overfeeding.[3] Finally, in the long-term Vermont study, increased glucose levels were noted after an oral glucose load during the period of weight gain, but the values remained within the accepted normal range,[1] as in our data. Thus, it appears that increasing the duration of overfeeding or the intensity of the nutritional stimulus for a short period of time, may lead to some decrement in glucose tolerance. However, this phenomenon is of moderate magnitude in healthy men, at least in subjects with no family history of diabetes or obesity as in the present study.

Fasting insulin levels were increased, as well as the insulin areas, during the OGTT and the meal test. An increased insulin concentration in the fasting state is a general feature in overfeeding studies.[1-7,9] An increase in the sum of serum insulin concentrations during the first 3 hours of an OGTT was reported by Sims et al[1] in their initial long-term experiment of mixed overfeeding. Olefsky et al[2] showed that an increase in postprandial insulin area was induced by overfeeding on a shorter period but with a large nutritional stimulus (8.4 MJ/d, 2,000 kcal/d). Our data also show an increase in the ratio of insulin area to glucose area, which was observed after ingestion of glucose and also after ingestion of a meal, suggesting that the prolonged nutritional challenge was associated with the development of some degree of insulin resistance.

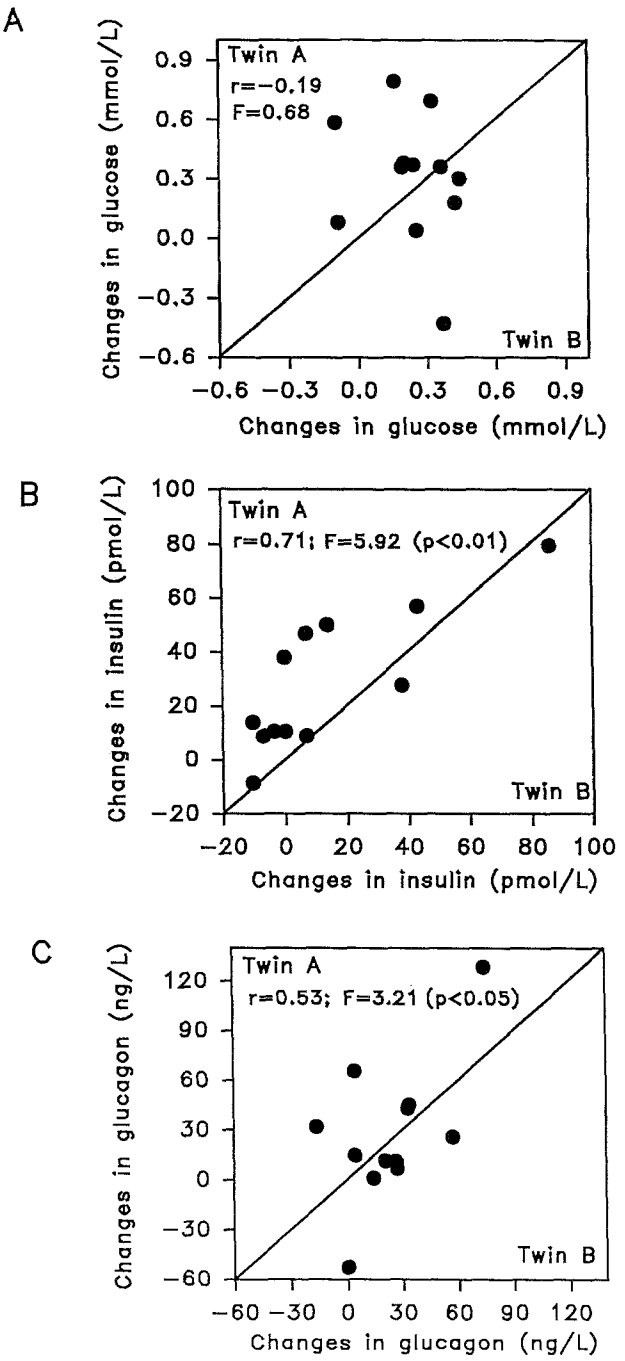

Fig 3. Twin resemblance with respect to changes in fasting plasma (A) glucose, (B) insulin, and (C) glucagon concentrations in 12 pairs of twins in response to 100 days of overfeeding. Each point represents the changes for the two brothers of one pair of twins (A and B). The closer the points are to the diagonal line, the more similar the twins are to each other in response to overfeeding. Adjustment on the gain in body weight or total fat mass with overfeeding did not modify the level of the correlations.

Table 4. Correlations Between Glucagon Values Before Overfeeding and Changes (Δ) in CT-Derived Body Fat Phenotypes During the Period of Overfeeding, Before and After Adjustment for the Gain in Total Fat Mass

	ΔAbdominal Fat Areas			ΔMidthigh Fat Area
	Total	SC	V	
Fasting glucagon	.43*	.29	.42*	.45*
Adjusted	.45*	.33	.41*	.47*
Glucagon area (OGTT)	.34	.26	.30	.44*
Adjusted	.37	.32	.29	.48*
Glucagon area (meal test)	.32	.28	.22	.42*
Adjusted	.34	.34	.22	.46*

NOTE. Correlations were computed from the absolute changes measured in the 24 subjects considered as independent persons.

Abbreviations: SC, subcutaneous; V, visceral.

*$P < .05$.

To our knowledge, there is no report about plasma glucagon levels during an overfeeding experiment. In the present study, we observed increased fasting plasma glucagon concentrations after the 100-day protocol. The insulin to glucagon ratio in the fasting state was not changed, indicating that insulin and glucagon were increased to the same extent. After ingestion of a mixed meal containing 15% protein, increased plasma glucagon concentrations were observed following the long-term caloric surplus. However, this effect was explained by the increase in fasting plasma glucagon levels, since the glucagon area above baseline during the meal test was unchanged. There was no evidence of a change in glucagon concentrations after an oral glucose load. Additional studies will be needed to understand potential changes in pancreatic glucagon secretion or metabolism elicited by overfeeding. Among other hypotheses, a decrease in intraislet somatostatin release could be a contributing factor for the simultaneous increase in fasting insulin and glucagon levels.

The correlations between pre-overfeeding glucose, insulin, and glucagon and the changes in body weight and body fat phenotypes showed that body weight and total body fat responses to long-term overfeeding could not be predicted from the initial levels of any of these metabolic parameters. Although insulin resistance was not precisely assessed in the present study, these results contrast with those of Swinburn et al,[34] who provided evidence that increased insulin sensitivity predicted weight gain in Pima Indians. In their longitudinal study over 3.5 years in 192 subjects covering a wide range of body fat and of insulin sensitivity relative to body weight, the glucose disposal rate during a euglycemic-hyperinsulinemic clamp at entry was positively correlated with percent weight change per year ($r = .19$ to .34). Body weight change was also negatively correlated with initial total area under the insulin curve during an OGTT ($r = -.21$), but not with fasting insulin ($r = -.11$), fasting glucose ($r = -.05$), or glucose area under the curve during the OGTT ($r = -.11$). These investigators thus speculated that the degree of insulin sensitivity or resistance might modulate the impact on body weight of a major energy imbalance. Our results do not seem to be in accordance with this hypothesis. However, it should be noted that our normal-weight subjects were likely to represent a more homogeneous group regarding insulin sensitivity than the population studied by Swinburn et al.[34]

Interestingly, we observed that changes in regional fat distribution indicators were related to some metabolic indices after adjusting for the gain in total fat mass. A negative correlation was found between the gain in midthigh fat and the insulin area and insulin area to glucose area ratio measured during the OGTT before overfeeding. Fasting glucagon levels were predictors of the subsequent gain in abdominal visceral fat and femoral fat, even after controlling for the increase in total body fat. Pre-overfeeding fasting glucagon concentrations were also increased in the group of high gainers for visceral fat. Although it is not a consistent finding,[35] elevated glucagon levels in the presence of increased adiposity have been observed by some investigators[36-38] and recently in our laboratory.[39,40] Although glucagon resistance has not been proven in humans, one could speculate that increased glucagon concentrations represent a compensatory phenomenon to diminished sensitivity to glucagon. In the hyperglucagonemic ob/ob mouse, hepatic insensitivity to glucagon has been documented.[41] By increasing the flow of substrates derived from glucose into the lipogenic pathway, glucagon resistance could be a contributing factor to the development of obesity.[42]

Dominant inheritance of possible large-molecular-weight immunoreactive precursors of glucagon has been suggested.[43] In the present study, significant intrapair resemblance was observed with respect to fasting glucagon levels, as well as for the glucagon areas during the OGTT and the meal test before overfeeding (intraclass coefficients ≥ 0.47, $P < .05$). It is known that glucagon levels exhibit large interindividual variation in healthy subjects.[44] Our results suggest that the genotype could account for a significant portion of the variance of basal glucagon levels and of the glucagon response to oral glucose and to a mixed meal.

Insulin changes with overfeeding were significantly related to the gains in body weight and fat mass (Table 5). However, the positive correlations between body weight gain and increased fasting insulin, increased insulin area during the OGTT, and increased insulin area during the meal test were all accounted for by the gain in total body fat. These results are in agreement with the notion that increased total adiposity is associated with insulin hypersecretion.[45] In addition, there is evidence that total body fat mass is also a primary determinant of tissue sensitivity to insulin in subjects within a normal range of body mass index.[46,47] After adjustment for the gain in total fat mass, we found that the changes in total subcutaneous fat remained positively correlated with the changes in the insulin area and insulin area to glucose area ratio after a glucose load. Although insulin resistance was not assessed precisely in our study, this suggests an association between the gain in subcutaneous fat and the modification of tissue insulin sensitivity during prolonged overfeeding. Subcutaneous fat cell hypertrophy was previously reported at the abdominal (umbilicus level) and femoral (midthigh level) sites after

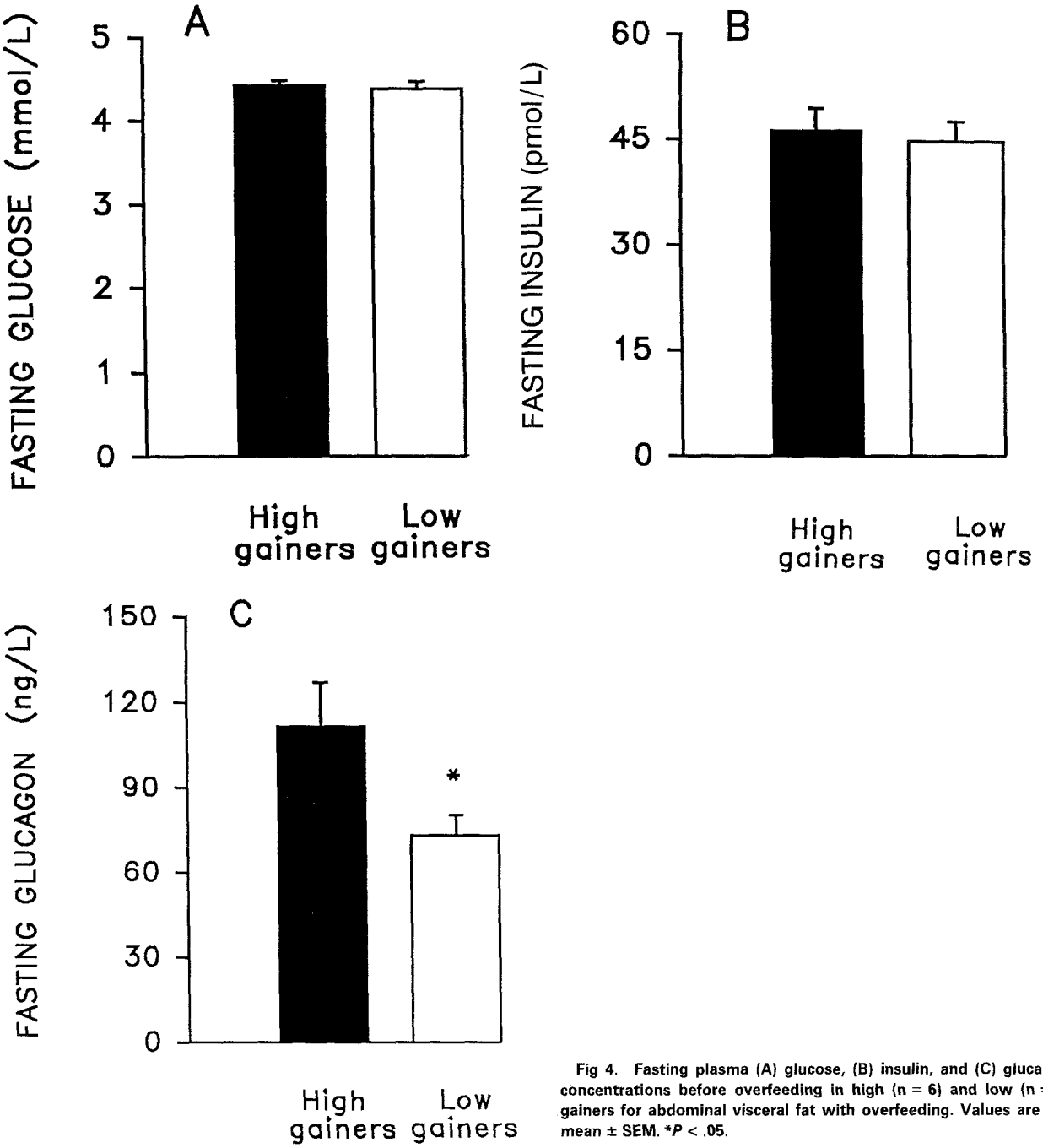

Fig 4. Fasting plasma (A) glucose, (B) insulin, and (C) glucagon concentrations before overfeeding in high (n = 6) and low (n = 6) gainers for abdominal visceral fat with overfeeding. Values are the mean ± SEM. *$P < .05$.

this 100-day overfeeding period.[24] Hypertrophy of adipocytes is known to be related to insulin resistance,[48] and diminished sensitivity to insulin of subcutaneous adipose tissue was demonstrated in vitro by Salans et al[49] after the long-term overfeeding study in Vermont. In addition, the level of subcutaneous trunk fat and the extent of abdominal fat cell hypertrophy were two important correlates of insulin levels in a previous study in obese women.[50] No association was found here between changes in insulin values and changes in midthigh fat area. This finding is in agreement with the notion that a high accumulation of femoral fat might be neutral or even protective regarding some adverse effects of increased adiposity.[40] We did not find any association between changes in indices of insulin-glucose homeostasis and changes in abdominal visceral fat. Although the implications of excessive abdominal visceral fat in insulin-glucose metabolism are well recognized,[17] it should be kept in mind that subjects in the present study were normal-weight men with relatively low levels of visceral fat even after overfeeding. In addition, during the overfeeding protocol more fat was gained in the subcutaneous fat depots than in the visceral depot.[24]

The design of the current study allowed us to test for the presence of genotype-overfeeding interaction effects by

Table 5. Correlations Between Changes (Δ) in Insulin and Insulin/Glucose Ratios and Changes in Body Weight and Body Fat Phenotypes During the Period of Overfeeding, Before and After Adjustment for the Gain in Total Fat Mass

	ΔBody Weight	ΔFat Mass	ΔSC Fat*	ΔAbdominal Fat Areas			ΔMidthigh Fat Area
				Total	SC	V	
ΔFasting insulin	0.58†	0.46‡	0.44‡	0.07	0.25	−0.22	−0.02
Adjusted	0.36	—	0.14	−0.22	−0.10	−0.26	−0.29
OGTT							
ΔInsulin area	0.48‡	0.39	0.56†	0.43‡	0.42‡	0.26	0.20
Adjusted	0.28	—	0.41‡	0.26	0.20	0.23	0.01
ΔInsulin area/glucose area	0.40	0.41‡	0.62†	0.51†	0.49‡	0.31	0.31
Adjusted	0.13	—	0.48‡	0.35	0.28	0.28	0.12
Meal-test							
ΔInsulin area	0.45‡	0.48‡	0.48‡	0.24	0.42‡	−0.13	0.05
Adjusted	0.13	—	0.17	−0.02	0.12	−0.16	−0.22
ΔInsulin area/glucose area	0.46‡	0.50‡	0.45‡	0.26	0.40	−0.07	−0.03
Adjusted	0.13	—	0.11	−0.02	0.07	−0.11	−0.32

NOTE. Correlations were computed from the absolute changes measured in the 24 subjects considered as independent persons.
*Represents the sum of 10 skinfold thickness measurements.
†$P < .01$.
‡$P < .05$.

comparing the within-pair variance and the between-pair variance in response to overfeeding. There were important differences between subjects for the changes in fasting insulin. However, these individual differences were not distributed randomly among the 24 men, as shown by the high within-pair similarity in the absolute response to overfeeding. There was approximately six times more variance between the pairs than within the pairs with respect to fasting insulin concentration changes. Thus, some individuals were more prone than others to modify their insulin levels. Since prospective longitudinal studies have shown that hyperinsulinemia in nondiabetic individuals was a significant predictor of the development of non–insulin-dependent diabetes mellitus,[51] this may be of clinical significance. Interestingly, fasting glucagon concentrations were also characterized by a significant similarity within pairs in the response to overfeeding. These results suggest that the genotype could be an important determinant of fasting insulin and glucagon responses to a prolonged positive–energy-balance period.

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