Obesity-Independent Hyperinsulinemia in Nondiabetic First-Degree Relatives of Individuals With Type 2 Diabetes

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A close association between obesity and hyperinsulinemia is well recognized, but it is not known whether this relationship is affected by the genetic susceptibility to type 2 diabetes. Insulin response to a 75-g oral glucose load was evaluated in healthy nondiabetic Caucasians with first-degree family history of diabetes (relatives, n = 55) and those without family history (nonrelatives, n = 33). A significant correlation between the BMI and insulin response (area under the curve [AUC] during the 2-h period) was seen in nonrelatives (r = 0.68, P < 0.0001) but not in the relatives (r = 0.12, P = 0.37). Multivariate analysis revealed that obesity (BMI) was the primary determinant of insulin response in nonrelatives (P < 0.001), whereas among the relatives, BMI had no significant impact (P = 0.28). Thus, these distinctions between the relatives and nonrelatives remained after adjusting for glucose level, age, and gender. Among first-degree relatives, the commonly observed association between BMI and insulin response is lost, and hyperinsulinemia is present even in the absence of obesity. First-degree family history of diabetes may confer insulin resistance that is independent of obesity. Alternatively, this could suggest a pathological regulation of an obesity-insulin feedback loop, e.g., a defective recognition of adiposity. *Diabetes* 47:788–792, 1998

Obesity and type 2 diabetes are closely related, but the biological basis of this link remains elusive. Diabetes is frequently accompanied by obesity (1), and obesity greatly increases the risk of diabetes (2,3). Because obesity and diabetes are both characterized by insulin resistance (4,5), it is understandable that the obesity-insulin interaction is one of the areas of intense investigation.

Nondiabetic relatives of individuals with diabetes are genetically highly susceptible to develop diabetes (6,7). Because their insulin secretion or insulin action may not yet be affected by the secondary effects of hyperglycemia (8,9), many investigators evaluated these individuals in search of early metabolic changes. Numerous attempts to describe the primary pathogenic events of diabetes—β-cell dysfunction or insulin resistance—have yielded no consistent results as yet (10,11), possibly because of the heterogeneous nature of diabetes or because of ethnical variability (12,13).

We evaluated this diabetes-susceptible population from the viewpoint of the association of hyperinsulinemia with obesity. This report consists of the glucose and insulin profiles after an oral glucose challenge in nondiabetic Caucasians with and without first-degree family history of diabetes. While characterizing the difference between the relatives and nonrelatives, an interesting feature was discovered.

RESEARCH DESIGN AND METHODS

Healthy Caucasian adult volunteers who responded to on-campus advertisements for diabetes screening were enrolled in this study. Subjects with normal glucose tolerance after the oral glucose tolerance test (OGTT) by World Health Organization criteria were included in the analysis, and those with prior diagnosis of diabetes were not eligible. None of the participants had hypertension or chronic medical conditions or were taking medications known to affect glucose tolerance. Written consent was obtained from all participants. After an overnight fast of at least 12 h, all subjects received 75 g of oral glucose load. Venous blood samples were drawn before and at 30-min intervals after the oral glucose load for 2 h.

Plasma glucose concentrations were measured enzymatically using a Ciba-Corning Express Plus Chemistry Analyzer. Serum insulin levels were measured in duplicate using a sensitive radioimmunoassay kit provided by Diagnostic. The sensitivity for this assay is 7 pmol/l (1.2 μU/ml). The inter- and intra-assay variability is <5%.

Statistical methods. The statistical analysis was performed using BMDP statistical software (SPSS, Chicago, IL) on the General Clinical Research Center's Digital VAXstation 3100. Differences between baseline variables were assessed with the Wilcoxon's rank-sum test. Repeated-measures analysis of variance (ANOVA) models with a grouping factor for family history (first-degree relatives, nonrelatives) were used to assess glucose and insulin response at five time points during the 2-h OGTT. Subjects were also separated into tertiles based on BMI, and the BMI factor was included in the ANOVA model. Pearson correlation coefficients were computed to assess the relationship between insulin and glucose levels and BMI. Partial correlation coefficients and multiple regression analysis were used to examine the effects of variables such as age, gender, and glucose levels on the serum insulin (fasting level and area under the curve response). Area under the curve (AUC) and incremental AUC (area above the baseline measurement) were computed using the trapezoidal rule. Results are expressed as means ± SD unless otherwise indicated.

RESULTS

Of 105 consecutive study volunteers, 88 subjects with normal glucose tolerance were identified, and the rest were excluded from the analysis because of abnormal glucose tolerance or incomplete data. Among those 88 subjects, 33 had no family history of diabetes (nonrelatives), and 55 had first-degree relatives with diabetes (relatives). Of the subjects, 22 had fathers with diabetes, 28 had affected mothers, and 15

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ANOVA, analysis of variance; AUC, area under the curve; OGTT, oral glucose tolerance test.

TABLE 1
Clinical characteristics of subjects with and without a first-degree relative with NIDDM

	Nonrelatives	First degree relative	*P* value
n	33	55	—
W/M	18/15	34/21	—
Age (years)	38.7 ± 12.0 (19.0–68.0)	42.4 ± 9.4 (28.0–69.0)	0.20
BMI (kg/m^2)	25.9 ± 5.0 (17.8–37.4)	27.7 ± 4.7 (17.8–38.4)	0.08
Systolic blood pressure (mmHg)	117.4 ± 16.2 (100.0–168.0)	118.4 ± 14.4 (80.0–156.0)	0.34
Diastolic blood pressure (mmHg)	74.6 ± 12.0 (58.0–100.0)	75.7 ± 9.2 (48.0–96.0)	0.49
Glycated hemoglobin (%)	5.14 ± 0.59 (4.2–6.4)	5.19 ± 0.57 (3.9–6.3)	0.44
Fasting plasma glucose (mmol/l)	4.87 ± 0.64 (3.83–6.55)	5.25 ± 0.52 (4.22–6.61)	0.0007
Fasting serum insulin (pmol/l)	43 ± 36 (6–169)	55 ± 27 (3–150)	0.02

Data are means ± SD (ranges). *P* values are from the Wilcoxon's rank-sum test.

had affected siblings; no one reported having children with diabetes. Oversampling (overrepresentation) of relatives, who have increased diabetes awareness from having the affected family members, was inevitable with our consecutive recruitment independent of family history status. The characteristics of these two study groups are shown in Table 1. The first-degree relatives had higher fasting glucose and insulin levels. Plasma glucose profiles (glucose levels from fasting to min 120) during the 75-g OGTT were similar between the first-degree relative group and the nonrelative group (repeated-measures ANOVA, $P = 0.32$). Serum insulin profile was substantially higher among the relatives ($P = 0.001$) than among the nonrelatives. However, repeated-measures ANOVA models assessing insulin and glucose response over time (at each time point) indicated a significant effect of BMI and a group × BMI interaction, suggesting a differential obesity effect within each group.

The effects of obesity on glucose and insulin profiles are demonstrated in Fig. 1, where the subjects are separated into tertiles of obesity: lean (BMI <25 kg/m^2), moderate (25–29 kg/m^2), and obese (>29 kg/m^2). Insulin response consistently increased with higher BMI categories in the nonrelative group, but this insulin–obesity association was not seen among the relatives. The insulin profiles (fasting through min 120) from each tertile were significantly different among the nonrelatives (ANOVA, $P < 0.0001$) but not statistically different among the relatives ($P = 0.13$). In nonrelatives, the BMI showed strong positive correlation with insulin levels at all measurement points, including the AUC during the 2-h period (Fig. 2). In the relatives, on the other hand, no significant correlations were present (Fig. 2) except for the fasting insulin level (see univariate correlations in Table 2).

The effects of obesity on glucose profiles were also somewhat different between the relatives and nonrelatives (Fig. 1). The correlation between BMI and glucose AUC was nonsignificant in the relatives ($r = -0.05$, $P = 0.73$) and stronger in nonrelatives ($r = 0.44$, $P = 0.009$). The correlation between insulin AUC and glucose AUC was nonsignificant in the relatives ($r = 0.12$, $P = 0.36$), whereas an association existed in nonrelatives ($r = 0.37$, $P = 0.03$).

Because variations in glucose levels and other factors may have influenced the relationship between BMI and insulin response, multivariate analyses were performed. The multiple regression results in Table 2 clarify the distinct characteristics between the two groups. BMI was found to be the primary predictor of insulin levels (either AUC, incremental AUC, or fasting levels, Table 2) in nonrelatives, but not a significant determinant of insulin response (AUC) for the relatives. Although there was wider variation in glucose profiles among nonrelatives and a weak correlation with insulin existed, as discussed earlier, the glucose was not

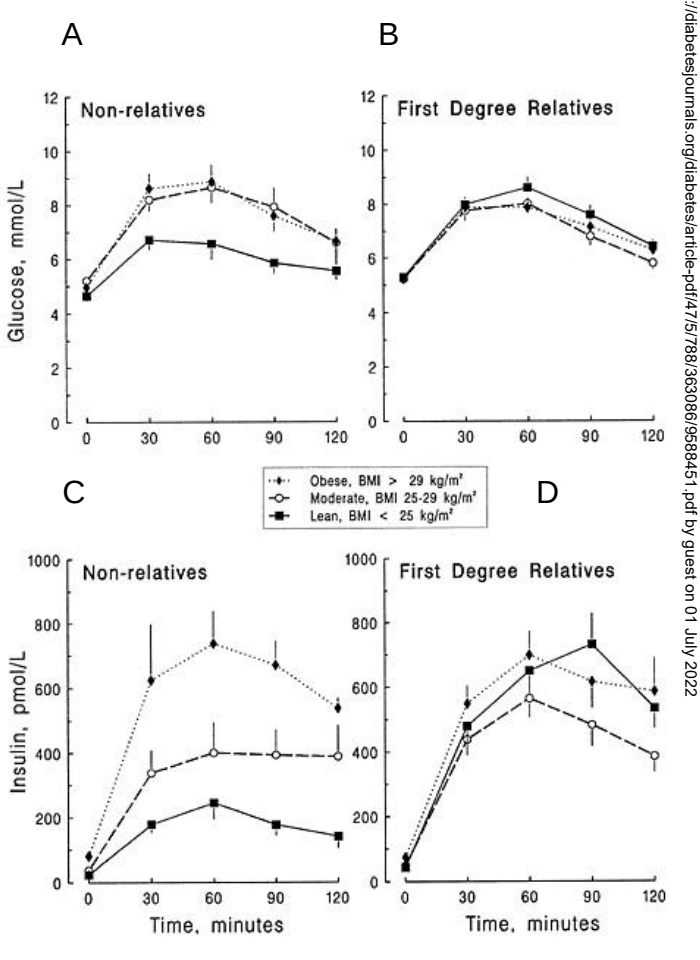

FIG. 1. Serum insulin and glucose profiles (mean and SE) for nonrelatives (*A* and *C*) and first-degree relatives (*B* and *D*) during the 2-h OGTT stratified by BMI. Lean, BMI <25 kg/m^2; obese, BMI >29 kg/m^2. The insulin profiles from these three BMI categories were significantly different among the nonrelatives (ANOVA, $P < 0.0001$), but not statistically different among the relatives ($P = 0.13$). The number of subjects in each BMI category is $n = 15$ lean, $n = 9$ moderate, and $n = 9$ obese for the nonrelatives and $n = 14$ lean, $n = 23$ moderate, and $n = 18$ obese for first-degree relatives.

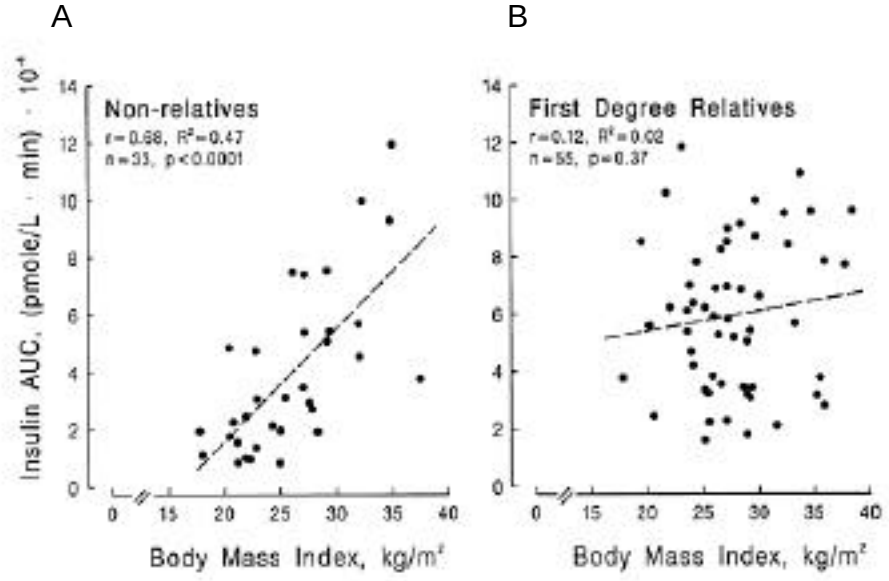

FIG. 2. Relationship between BMI and serum insulin AUC response during the 2-h OGTT for nonrelatives (*A*) and first-degree relatives (*B*).

an independent predictor of insulin response (AUC, Table 2). BMI remained the only significant independent predictor of the insulin response to the OGTT among nonrelatives. Inclusion of age and gender in the models did not affect the results. Therefore, after controlling for these potential confounding variables, the characteristic distinction between nonrelatives and relatives of a BMI-insulin correlation remained significant.

DISCUSSION

Obesity is generally associated with hyperinsulinemia (14,15). That was also the case in our study subjects who had no family history of diabetes. However, we discovered that among first-degree relatives, the commonly observed correlation between BMI and postglucose insulin response was lost, and hyperinsulinemia was present even in the absence of obesity. This interesting phenomenon may provide some clues to the understanding of diabetes and obesity.

Insulin resistance hypothesis. One interpretation of this finding is that the first-degree relatives have uniformly increased insulin resistance. Assuming hyperinsulinemia as a surrogate marker for insulin resistance, one could hypothesize that the first-degree family history of diabetes may confer insulin resistance status that is independent of the degree of obesity.

In support of this view are several studies that found the nondiabetic first-degree relatives to be insulin resistant compared with the control population. In each study, insulin resistance was specifically assessed by minimal model (16), glucose clamp (8), or insulin tolerance test (17). These reports included ethnically diverse population (African-Americans, Mexican-Americans, and Asian Indians).

However, others found no difference in the insulin sensitivity between the relatives and nonrelative control subjects (18). Two Caucasian studies, one evaluated by glucose clamp method (19) and another by insulin tolerance test (17), revealed similar results.

Heterogeneity of the study populations, particularly the ethnic difference in insulin sensitivity (13,17), could partly explain those conflicting results. Also, heterogeneity within the first-degree relatives could be a major confounding factor. Although first-degree relatives are considered susceptible to diabetes, not all of them will inherit the "susceptibility" gene. Nondiabetic first-degree relatives are preferable for studies into the pathogenesis of diabetes, because the responses to be studied are not influenced by the effect of hyperglycemia. On the other hand, by only selecting nondiabetic individuals, susceptibility gene carriers are more likely to be excluded from the study. Further, cross-sectional study of the "truly" susceptible relatives would inevitably have involved various disease stages of preclinical metabolic alterations preceding the clinical onset of diabetes. Because there is no means of identifying the truly susceptible yet purely metabolically intact individuals at present, clinical investigations involving the first-degree relatives are prone to these confounding factors.

Adipoinsular axis dysregulation hypothesis. Although insulin resistance may be regarded as a single clinical entity, the obesity-independent hyperinsulinemia that we found in this subpopulation may indicate pathology in one of the mechanisms of insulin action or its secretion. We propose an alternative "adipoinsular axis dysregulation" hypothesis to explain our findings. A feedback regulation of insulin secretion relative to an individual's adiposity has been suggested because of a linear correlation between insulin production and the degree of obesity (20) and the reversibility of hyperinsulinemia after weight loss (21–24). In addition to this circumstantial evidence, the adipose tissue may play an important role in the genesis of insulin resistance or hyperinsulinemia through various secretory products, such as free fatty acids (25), tumor necrosis factor-α (26), and leptin (27). It is plausible that the obesity-independent hyperinsulinemia among the first-degree relatives that we found in this study may indicate pathology in adipoinsular axis, e.g., a faulty recognition of adiposity.

Some of the recent important discoveries around the leptin biology are in agreement with this adipoinsular axis dysregulation hypothesis. First came the identification of a defective leptin receptor gene in obese diabetic animal models (i.e., *db/db* mice and *fa/fa* rats) (28–30). These animals represent the actual disease model for progressive obesity and the eventual development of diabetes due to

TABLE 2
Multivariable analysis to identify variables contributing to the variance in serum insulin for three regression models (AUC, incremental AUC, and fasting insulin)

Model	Nonrelatives (n = 33)			First-degree relatives (n = 55)		
	Univariate Correlation		Contribution to R^2 after the other three variables are in the model	Univariate Correlation		Contribution to R^2 after the other three variables are in the model
	r	P		r	P	
Insulin AUC						
BMI	0.68	<0.0001	0.266*	0.12	0.37	0.026
Glucose (AUC)	0.37	0.03	0.011	0.12	0.36	0.018
Sex	–0.16	0.37	0.014	–0.15	0.27	0.027
Age	0.33	0.06	0.0001	0.07	0.62	0.003
All four variables			Model R^2 = 0.49, P = 0.0007			Model R^2 = 0.07, P = 0.47
Incremental Insulin AUC						
BMI	0.66	<0.0001	0.238*	0.06	0.63	0.011
Glucose (AUC)	0.38	0.02	0.016	0.12	0.39	0.016
Sex	–0.15	0.40	0.013	–0.17	0.21	0.031
Age	0.32	0.07	0.0001	0.08	0.56	0.002
All four variables			Model R^2 = 0.46, P = 0.001			Model R^2 = 0.06, P = 0.56
Fasting Insulin						
BMI	0.69	<0.0001	0.336*	0.50	0.0001	0.255*
Glucose (AUC)	0.24	0.18	0.002	0.11	0.43	0.013
Sex	–0.19	0.29	0.010	0.09	0.49	0.001
Age	0.32	0.07	0.000	–0.07	0.62	0.006
All four variables			Model R^2 = 0.49, P = 0.0007			Model R^2 = 0.28, P = 0.002

Independent variables are BMI, glucose AUC, sex, and age. Dependent variables are insulin AUC, incremental insulin AUC, and fasting insulin for models 1,2, and 3, respectively. *P < 0.002 for the BMI regression coefficient; all other coefficients are nonsignficant. R^2 and P values refer to the multiple regression model with the four independent variables included.

inability to recognize growing body fat mass. Faulty recognition of adiposity in a diabetes-susceptible human population is compatible with this leptin receptor disease model.

Subsequent demonstration of the direct inhibitory effect of leptin on insulin secretion (31,32) substantiated the presence of an adipoinsular endocrine axis, at least in animals. As both leptin-deficient (*ob/ob*) and leptin-resistant (*db/db*) mice lack the tonic inhibition of insulin secretion by leptin, they provide a mechanism for the early development of hyperinsulinemia, as well as the resulting predisposition for diabetes syndrome (32) . The same mechanism—the lack of insulin inhibition due to adiposity signal deficit—comfortably explains our observation of hyperinsulinemia among lean first-degree relatives.

Although neither leptin deficiency nor its receptor defects is the likely pathogenesis of human obesity and diabetes, signaling interference downstream from the leptin receptors or the involvement of similar adipoinsular axis pathophysiology via other mediators (adipostat) remain possibilities. Our observation in humans does not prove but suggests such pathophysiology by demonstrating a resemblance to the animal model of obesity/diabetes. Validation of this adipoinsular axis dysregulation hypothesis could lead to the identification of some of the major genetic determinants of both human obesity and diabetes.

Our findings provide several interesting clues to the understanding of human obesity and diabetes. Verification of our hypotheses could lead to significant progress in this area that affects many of our society. Further studies should incorporate specific measurements of adipose size, insulin secretion, and insulin action, as well as investigation into the adiposity signaling system and insulin regulatory mechanism.

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