Impaired Incretin Response After a Mixed Meal Is Associated With Insulin Resistance in Nondiabetic Men

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OBJECTIVE — To investigate whether features of the insulin resistance syndrome are associated with altered incretin responses to food intake.

RESEARCH DESIGN AND METHODS — From a population-based study, 35 men were recruited, representing a wide spectrum of insulin sensitivity and body weight. Each subject underwent a hyperinsulinemic-euglycemic clamp to determine insulin sensitivity. A mixed meal was given, and plasma levels of gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), as well as insulin, glucagon, and glucose were measured.

RESULTS — Insulin resistance was associated with impaired GIP and GLP-1 responses to a mixed meal. The total area under the curve (AUC) of the GIP response after the mixed meal was associated with insulin sensitivity ($r = 0.54$, $P < 0.01$). There was a significant difference between the highest and the lowest tertile of insulin sensitivity ($P < 0.05$). GLP-1 levels 15 min after food intake were significantly lower in the most insulin-resistant tertile compared with the most insulin-sensitive tertile. During the first hour, the AUC of GLP-1 correlated significantly with insulin sensitivity ($r = 0.47$, $P < 0.01$). Multiple linear regression analysis showed that insulin resistance, but not obesity, was an independent predictor of these decreased incretin responses.

CONCLUSIONS — In insulin resistance, the GIP and GLP-1 responses to a mixed meal are impaired and are related to the degree of insulin resistance. Decreased incretin responsiveness may be of importance for the development of impaired glucose tolerance.

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Insulin resistance is compensated by increased insulin secretion for the prevention of hyperglycemia (1). When insulin secretion is inadequately increased in relation to the degree of insulin resistance, impaired glucose tolerance (IGT) and diabetes ensue. To understand the mechanisms of this development, it is of importance to understand why insulin secretion is increased in insulin resistance, and why this increase fails in subjects developing diabetes. One potential mechanism is the release of incretin hormones from the gut, stimulating insulin secretion. Whether they contribute to the increased insulin secretion in insulin resistance or whether their secretion or action is impaired in subjects developing diabetes is not known.

One cause for impaired insulin secretion is a decreased capacity of pancreatic β-cells to secrete sufficient insulin. After food intake, insulin secretion depends not only on the degree of glycemia, but also on the secretion and insulinotropic effect of the gut hormones known as incretins, i.e., gastric inhibitory polypeptide (GIP, or glucose-dependent insulinotropic polypeptide) and glucagon-like polypeptide 1 (GLP-1). Normally, the incretins GLP-1 and GIP are responsible for as much as half of the glucose-dependent insulin release after food ingestion. Pretreatment of β-cells with GLP-1 in vitro enhanced the glucose sensitivity of the β-cell; and in a rat model of type 2 diabetes, GLP-1 improved the glucose sensitivity of previously resistant β-cells (2). The incretins are important in maintaining normal glucose tolerance, as evidenced by animal studies showing that both GLP-1– and GIP-receptor antagonists markedly reduce the insulin response to feeding, resulting in glucose intolerance (3). Similarly, mutations of either the GLP-1 or the GIP receptor gene are associated with glucose intolerance in mice (4,5). Whether the secretion and effect of incretins are of pathophysiological relevance for the development of IGT or diabetes in insulin resistance in humans is not known.

Previous studies have suggested that the insulin-releasing action of GLP-1 is normal in IGT/type 2 diabetes, because fasting hyperglycemia in such patients normalizes after GLP-1 administration (6). Consequently, GLP-1 has been proposed as a possible alternative treatment for type 2 diabetes. By contrast, the insulin-releasing action of GIP appears to be impaired in type 2 diabetes (7).

Disturbance of incretin secretion may also be present in insulin-resistant states, because a reduced GIP response after an oral glucose tolerance test has been demonstrated in middle-aged women with IGT (8). Furthermore, a reduced GLP-1 response after food intake occurs in obese individuals (9). In contrast to control subjects, no rise in plasma GLP-1 has been recorded in type 1 or type 2 diabetic patients after a mixed breakfast (10). How-

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CV, coefficients of variation; DPP-IV, dipeptidyl-peptidase IV; HOMA, homeostasis model assessment; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; IGT, impaired glucose tolerance; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; NEFA, nonesterified fatty acid; RIA, radioimmunoassay.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

ever, it is not clear whether insulin sensitivity per se is of regulatory importance for the release of the incretins, because in these studies there was no adjustment for insulin sensitivity. In contrast to these results, Nyholm et al. (11) found increased GIP but unaltered GLP-1 secretion after meals in female and male relatives of type 2 diabetic patients.

In this study, we investigated the relation between insulin sensitivity and food-induced secretion of GIP and GLP-1 by performing a meal challenge to a group of nondiabetic men who were well characterized regarding insulin sensitivity and obesity.

RESEARCH DESIGN AND METHODS

— Subjects were recruited from a population-based study, the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project (12). In the 1990 and 1994 survey, 2,815 women and men participated. Of these, 40 men living in the health care district of Umeå or Luleå were selected from individuals in the highest and lowest quartiles of fasting plasma insulin levels, as measured in the MONICA study. None of the subjects had diabetes. Nine subjects had a relative with known type 2 diabetes, and these subjects were equally represented in the tertiles of insulin sensitivity.

Anthropometric measurements included height to the nearest centimeter, weight to the nearest 0.2 kg, waist circumference at the level of the umbilicus to the nearest 0.5 cm, and hip circumference, which was measured as the maximum circumference over the buttocks to the nearest 0.5 cm, according to the MONICA project manual. Blood pressure was measured in the supine position with a mercury sphygmomanometer. Body composition was estimated by bioelectrical impedance analysis using a BIA 101F Akern-RJL System bioelectrical impedance instrument (EL-Dot K/S, Fredriksvaerk, Denmark).

Insulin sensitivity was estimated by a hyperinsulinemic-euglycemic clamp (13) performed in the morning after an overnight fast. The subjects were placed in a supine position, and a catheter was inserted in the right antecubital vein for infusion of insulin and glucose. Another catheter was inserted in a dorsal vein on the left hand for sampling of venous blood, which was arterialized by heating to reduce glucose loss. Synthetic human insulin (Actrapid 40 IE/ml; NovoNordisk, Malmö, Sweden) was infused in a priming dose for the first 10 min and then as a continuous infusion for 110 min to maintain steady state hyperinsulinemia. The continuous insulin infusion rate was 56 mU/min per meter squared of body surface area, resulting in a mean plasma insulin concentration of 97.7 ± 26.6 mU/l. The chosen blood glucose level during the clamp was 4.5 mmol/l, and this was obtained by adjusting the infusion rate of a 20% glucose solution after blood glucose measurements every 5 min. Serum insulin was measured before the clamp and after 60, 90, and 120 min. The amount of glucose metabolized by the individual, *M*, was calculated on the basis of the amount of glucose infused during the second hour of the clamp, expressed as milligrams × (kilograms body weight × minute)$^{-1}$. Insulin sensitivity was calculated as the amount of glucose metabolized per unit of plasma insulin (expressed as the mean insulin concentration during the second hour of the clamp) × 100, i.e., milligrams × (kilograms body weight × minute)$^{-1}$ per milliunits/liter, multiplied by 100 (14).

Mixed meal

At 6–12 months after the clamp study, 35 subjects agreed to attend the outpatient clinic in the morning after an overnight fast and avoidance of tobacco. An indwelling catheter was inserted in an antecubital vein for venous sampling. Between 0900 and 0930, a mixed meal containing 424 kcal was served and ingested within 10 min. The meal consisted of three slices of bread, butter, one slice of cheese, one small portion of marmalade, and coffee or tea without sugar. The fat, protein, and carbohydrate content were 161 kcal, 55 kcal, and 208 kcal, respectively, which corresponds to 38, 13, and 49% of the total energy content. Venous samples were then taken 5 and 2 min before the meal and 15, 30, 45, 60, 90, 120, and 180 min after the meal. The mean of the two first samples is referred to as the basal level in the data presentation. Two subjects were excluded from data analysis because of concomitant steroid medication and an earlier colectomy, respectively. The study was approved by the Ethics Committee of Umeå University, and written informed consent was obtained from the participants.

Analytical methods

For the insulin assay, guinea pig anti-human insulin antibodies, human insulin standard, and mono-^{125}I-Tyr-human insulin (Linco, St. Charles, MO) were used. The assay was specific for insulin, with no cross-reactivity (<0.2%) with intact proinsulin or des-31,32-proinsulin. The intra- and interassay coefficients of variation (CVs) of the insulin assay were <3%. Samples for analysis of glucagon were obtained in prechilled test tubes containing 0.084 ml EDTA (0.34 mol/l) and aprotinin (450 kallikrein-inhibiting units/ml blood), (Bayer, Leverkusen, Germany). The analysis of glucagon concentration was performed with double-antibody radioimmunoassay (RIA) using guinea pig anti-human glucagon antibodies specific for pancreatic glucagon, ^{125}I-glucagon as tracer, and glucagon standard (Linco). The interassay CV for this assay was <9%. GIP levels were analyzed with a double-antibody RIA technique using rabbit anti-human GIP antibodies, ^{125}I-labeled human GIP, and human GIP standard, as previously described (15), with an intra-assay CV of <6%. The antibody used cross-reacts fully with human GIP but not with the 8-kDa GIP, the nature of which —and relationship to the synthesis or secretion of GIP—is still unclear. GLP-1 was determined with RIA after extraction with ethanol, as previously described (16). The antiserum is directed against the amidated COOH-terminus of GLP-1, and it therefore measures primarily the GLP-1 of intestinal origin. The intra-assay CV was <6%. Plasma glucose concentrations were analyzed using the glucose oxidase method, and plasma samples were used for all of the above-stated measurements. All samples were analyzed in duplicate.

Statistics

Statistical analyses were performed with a commercial statistical program, SPSS for Macintosh, version 6.1.1. Data are given as means ± SD or as medians with the interquartile range, if not normally distributed. The area under the curve (AUC) responses were estimated according to the trapezoid rule. Variables not normally distributed were naturally log-transformed before they were included in the statistic calculations.

Pearson correlation coefficients and partial correlations were calculated (Table 2). Subjects were divided into tertiles ac-

Table 1—*Characteristics of the participants*

	Insulin sensitivity				
	Lowest tertile	Medium tertile	Highest tertile	*F* probability	Tukey B post hoc test
n	11	11	11		
Age (years)	53.1 ± 11.1	49.1 ± 9.6	47.6 ± 8.6	0.37	—
BMI (kg/m^2)	30.4 (27.3–32.7)	26.4 (26.3–28.6)	23.9 (22.6–25.6)	<0.001	*,†
Fat mass (kg)	28.9 (25.1–45.5)	23.6 (19.1–25.9)	17.1 (13.2–18.7)	<0.001	*,†,‡
Waist circumference (cm)	108.0 (103.0–117.4)	98.0 (95.0–103.0)	90.0 (81.0–93.0)	<0.001	*,†,‡
Fasting glucose (mmol/l)	4.8 ± 0.4	4.6 ± 0.5	4.7 ± 0.2	0.52	—
Fasting insulin (pmol/l)	97.0 (66–116)	72.5 (50.5–76.5)	44.0 (40.5–52.5)	<0.01	*,†
Insulin sensitivity§	3.1 (2.1–3.2)	7.2 (5.1–7.8)	10.1 (9.4–13.8)	<0.001	*,†,‡
Systolic blood pressure (mmHg)	141 ± 22	129 ± 12	133 ± 24	0.33	—
Diastolic blood pressure (mmHg)	84 ± 11	83 ± 8	78 ± 11	0.46	—

Data are means ± SD, or, if not normally distributed, median (interquartile range). §Mg glucose × body wt × (kg min)$^{-1}$ per mU/l of insulin × 100. Group differences tested with one-way ANOVA. *Lowest versus medium insulin sensitivity tertile; †lowest versus highest insulin sensitivity tertile; ‡medium versus highest insulin sensitivity tertile.

cording to their insulin sensitivity, and one-way analysis of variance (ANOVA) followed by Tukey's B post hoc test were used to test differences between groups. Multiple linear regression analysis was used to further evaluate predictors of incretin responses. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Anthropometric data and insulin sensitivity

The subjects showed a continuous range of insulin sensitivity according to the hyperinsulinemic-euglycemic clamp. Table 1 shows the clinical characteristics of the subjects divided into tertiles according to insulin sensitivity. During the clamp, steady-state plasma insulin levels were 105 ± 24, 98 ± 34, and 91 ± 22 mU/l, respectively. High insulin resistance was significantly associated with increased BMI and fat mass. However, neither fasting nor steady state "clamped" glucose levels (4.5 ± 0.1, 4.6 ± 0.1, and 4.6 ± 0.1 mmol/l, respectively) differed between the insulin sensitivity groups, and blood pressure levels were similar in all groups. The groups did not differ significantly in terms of steady state insulin, but showed a distinct separation in insulin sensitivity (Table 1). Fasting insulin levels had a strong negative correlation to insulin sensitivity ($r = -0.73$, $P < 0.001$). The homeostasis model assessment (HOMA) index was calculated based on fasting insulin and fasting glucose levels from both visits. A small but statistically significant increase in HOMA index between visits was noted (median 8.2 vs. 11.0), and this was attributable to an increase in fasting insulin levels among the most insulin-sensitive men (median 3.0 vs. 6.1 mU/l in this subgroup).

Mixed meal responses

Mean plasma insulin levels differed significantly between groups even before food ingestion (Table 1) (Fig. 1*A*), and the most insulin-resistant men had a markedly exaggerated insulin increase after the meal compared with the more insulin-sensitive subjects. In all groups, the peak level of plasma glucose occurred 30 min after ingestion of the meal (data not shown).

Basal levels of GIP did not differ between the groups. However, in the most insulin-resistant men, plasma GIP levels 60 min after food ingestion were 60% of the levels of the most insulin-sensitive men ($P < 0.01$) (Fig. 1*B*). Similarly, the total AUC GIP response in the most insulin-resistant men was 66% of that observed in the most insulin-sensitive men ($P < 0.05$). The AUC of GIP (mean ± SD) in the tertiles were 8,043 ± 3,276, 8,314 ± 2,680, and 12,215 ± 3,970 pmol/l · min, respectively.

Total AUC GIP and GIP levels 60 min after the meal were significantly associated with insulin sensitivity (Table 2). Multiple linear regression models, including age, fat mass, and insulin sensitivity as independent variables and the GIP level at 60 min or the total AUC GIP as the dependent variable, showed that insulin sensitivity was an independent predictor for both variables ($P < 0.01$). Because different measures of obesity are strongly interrelated, only one of these measures (i.e., fat mass) was chosen for the linear regression model in order to diminish multi-colinearity. Hence, insulin resistance was accompanied by a lowered GIP response to the ingestion of mixed meals, and the degree of reduction correlated with the degree of insulin resistance.

Fasting plasma levels of GLP-1 did not differ significantly between the groups (Fig. 1*C*). The early GLP-1 response to food ingestion was impaired in insulin-resistant subjects. Thus, at 15 min after food ingestion, the mean GLP-1 level among the most insulin-resistant men was 44% of the GLP-1 level in the most insulin-sensitive group (8.0 ± 5.7, 12.9 ± 6.5, and 18.3 ± 5.4 pmol/l, respectively; $P < 0.01$). Similarly, AUC GLP-1 among the most insulin-resistant men at 30 min was 55% (298 ± 183 vs. 542 ± 157 pmol/l · min, $P < 0.05$), and at 45 min it was 63% (482 ± 274 vs. 771 ± 231, $P < 0.05$) of the AUC GLP-1 response among the most insulin-sensitive individuals.

Neither the total AUC for GLP-1 nor the incremental change after the meal differed between groups. The GLP-1 levels at 15 min after food ingestion and the AUC GLP-1 during the first 30 min after the mixed meal correlated significantly with insulin sensitivity (Table 2), and it correlated inversely with the total AUC insulin response to the mixed meal ($r = 0.47$, $P < 0.01$; and $r = -0.38$, $P < 0.05$; respectively). Using a multiple linear regression model with GLP-1 as the de-

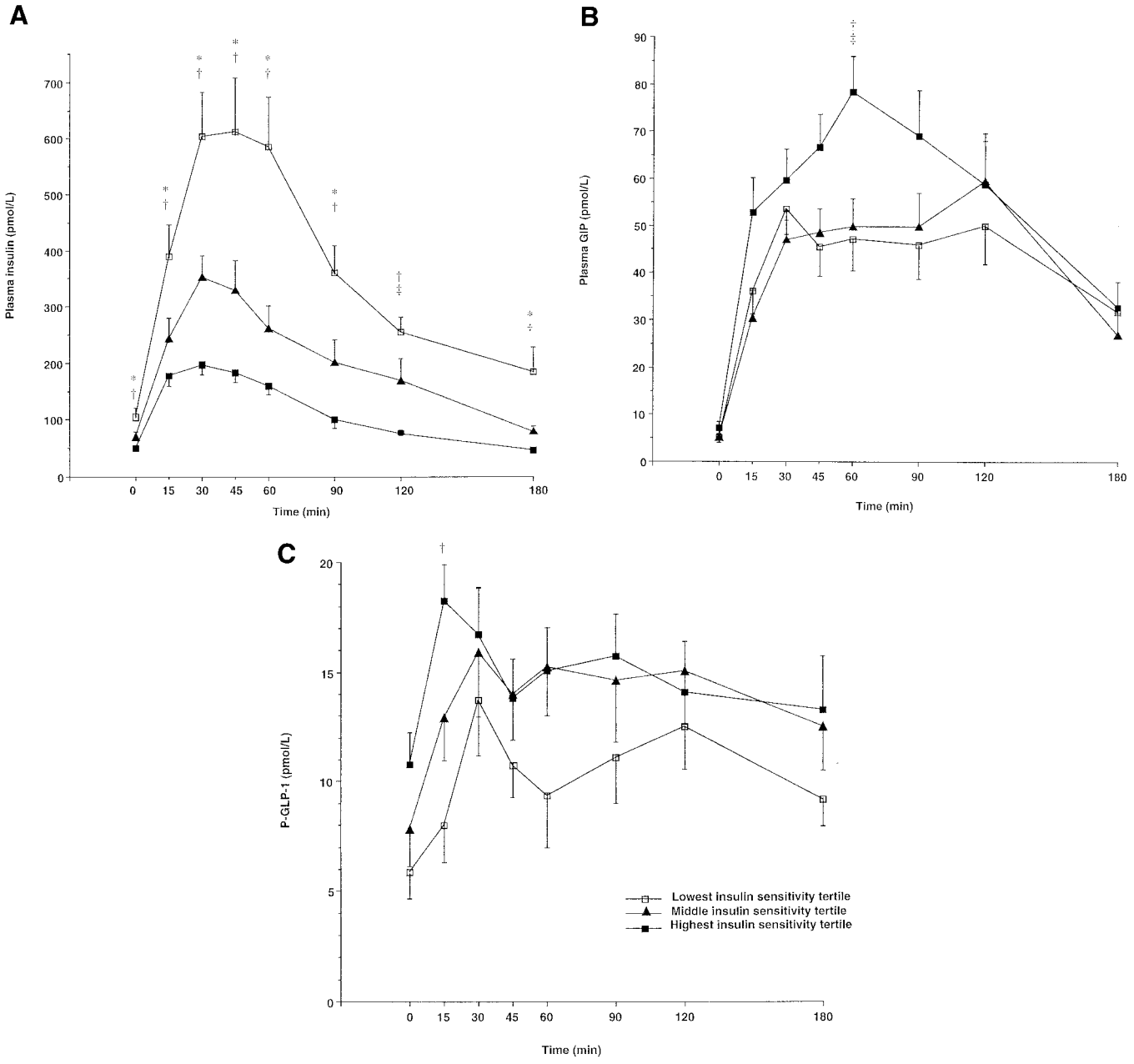

Figure 1—A: *Plasma insulin levels (mean ± SEM) before and after a mixed meal.* B: *Plasma GIP levels (mean ± SEM) before and after a mixed meal. C: Plasma GLP-1 levels (mean ± SEM) before and after a mixed meal. –□–, Lowest insulin sensitivity tertile; –▲–, middle insulin sensitivity tertile; –■–, highest insulin sensitivity tertile. Group differences tested with one-way ANOVA followed by Tukey's B post hoc test.* *P < *0.05 for lowest versus medium insulin sensitivity tertile;* †P < *0.05 for lowest versus highest insulin sensitivity tertile;* ‡P < *0.05 for medium versus highest insulin sensitivity tertile.*

pendent variable, insulin sensitivity and age—but not fat mass—were found to be independent predictors of the GLP-1 level at 15 min ($P < 0.01$, $P < 0.05$, and $P = 0.51$, respectively). Using the same model for AUC GLP-1 during the first 30 min, insulin sensitivity and age were independent predictors of this response ($P < 0.05$). Hence, insulin resistance per se was also accompanied by a decreased early GLP-1 response to ingestion of a mixed meal.

The most insulin-resistant men had increased basal levels of plasma glucagon compared with the most insulin-sensitive group (66.4 ± 15.3, 60.5 ± 17.3, and 47.6 ± 13.4 pg/ml, respectively; $P < 0.05$). All three groups showed a small peak in glucagon levels 15 min after ingestion of a meal, but the magnitude of this peak did not differ significantly among the groups.

CONCLUSIONS — A major finding in this study is the impaired secretion of GIP and GLP-1 in response to the ingestion of a mixed meal in insulin-resistant men. This suggests that the insulin-resistant state is associated with an impaired secretory response of incretins,

Table 2—*Correlation coefficients for incretins versus BMI and insulin sensitivity*

	BMI	Adjusted for age and insulin sensitivity	Insulin sensitivity	Adjusted for age and BMI
GIP_{60}	−0.33	0.19	0.61*	0.59*
Total AUC GIP	−0.34	0.08	0.54†	0.48†
GLP-1_{15}	−0.43‡	0.04	0.60*	0.57†
AUC GLP-1_{30}	−0.36‡	0.05	0.53†	0.50†

Pearson correlation coefficients and partial correlation coefficients for GIP 60 min after mixed meal (GIP_{60}), total AUC for GIP, GLP-1 15 min after meal (GLP-1_{15}), and AUC of GLP-1 at 30 min (GLP-1_{30}); all variables are with and without adjustment for insulin sensitivity, age, and BMI. *$P < 0.001$; †$P < 0.01$; and ‡$P < 0.05$.

with an attenuated early GLP-1 response and a diminished GIP secretion somewhat later in the postprandial period. The reduction in the incretin responses was significantly linked to the degree of insulin resistance, even after adjustment for possible confounding factors.

It is well known that enteral glucose stimulates incretin secretion, but whether incretin secretion is regulated only by the glucose level, or whether insulin per se might negatively control incretin secretion, has not been established. The hypothesis that insulin resistance per se is of importance for the impaired endogenous incretin response among insulin-resistant men is supported by our data, and this is also in line with earlier studies showing that insulin has a negative feedback on the enteroinsular axis (17). One animal study has also supported this hypothesis, demonstrating that exogenous administration of insulin reduces the GIP response to duodenal administration of glucose (18). Delayed gastric emptying could be a possible alternative explanation for the decreased incretin response in our study. We have not specifically investigated this, but the simultaneous peak in plasma glucose 30 min after meal in all three groups argues against this interpretation (data not shown).

As for the converse hypothesis, i.e., the possible influence of incretins on insulin sensitivity? In vitro, adipocytes incubated with GLP-1 have enhanced glucose disposal (2). GLP-1 infusion in healthy subjects enhances glucose disposal and the insulin response to an intravenous glucose tolerance test (19), and it can also reduce glucose delivery from the liver (20). However, no effect of GLP-1 on glucose disappearance, measured using tracer techniques, has been reported in healthy subjects (21). Moreover, acute treatment with GLP-1 during a euglycemic-hyperinsulinemic clamp has been reported to have no influence on insulin sensitivity in subjects with type 2 diabetes (22).

Incretin responses are also of interest in obesity. Animal as well as human studies have revealed disturbances in the enteroinsular axis in obesity (23). However, despite efforts to control for insulin resistance, studies have indicated both impaired (24) and enhanced (25) incretin responses to test meals in obese subjects. Comparisons are hampered by different hormone assays and different compositions of test meals in terms of the size of the meal and the content of protein, fat, or carbohydrate. Another explanation for diverging results could be the heterogeneity in the subjects studied with regard to age, sex, and, possibly, genetic factors (26,27). In this study, we included only men who were matched for age and selected based on insulin sensitivity.

In the present study, plasma insulin levels in the insulin-resistant group were elevated throughout the study. Therefore, the expected reduction in insulin secretion as a consequence of the impaired incretin response has to be overridden by other insulin-stimulating factors. The most important factor was probably the increase in plasma glucose after the meal, although other factors, such as increased levels of nonesterified fatty acids (NEFAs), cannot be excluded.

The insulin-resistant state includes peripheral resistance to insulin, with impaired glucose uptake and disposal, continued endogenous/hepatic glucose production, and an increased outflow of NEFAs from the adipose tissue—all factors necessitating a compensatory higher insulin secretion in order to maintain normal blood glucose levels. Diabetes will develop if the hyperinsulinemia becomes inadequate. The attenuated incretin response, as observed in this study in insulin-resistant but nondiabetic men, may therefore be of pathophysiological relevance as an early event. This suggests that treatment with incretins may be of potential therapeutic value in the early stages of type 2 diabetes. However, the therapeutic utility is limited by its short half-life. Another possible method to enhance the attenuated incretin response is to inhibit the enzyme dipeptidyl-peptidase IV (DPP-IV), which degrades and inactivates both GLP-1 and GIP, or to use DPP-IV–resistant GLP-1 analogs (28).

In conclusion, we have found an impaired incretin secretion after a mixed meal in insulin-resistant but nondiabetic men. This impairment includes both the integrated GIP response and the early GLP-1 response after the meal, and appears to be a distinct feature of the insulin-resistant state, possibly contributing to the development of type 2 diabetes.

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