*Original Article*

# Effects of Nonglucose Nutrients on Insulin Secretion and Action in People With Pre-Diabetes

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**To determine whether nonglucose nutrient–induced insulin secretion is impaired in pre-diabetes, subjects with impaired or normal fasting glucose were studied after ingesting either a mixed meal containing 75 g glucose or 75 g glucose alone. Despite comparable glucose areas above basal, glucose-induced insulin secretion was higher ($P < 0.05$) and insulin action lower ($P < 0.05$) during the meal than the oral glucose tolerance test (OGTT) in all subgroups regardless of whether they had abnormal or normal glucose tolerance (NGT). However, the nutrient-induced δ (meal minus OGTT) in insulin secretion and glucagon concentrations did not differ among groups. Furthermore, the decrease in insulin action after meal ingestion was compensated in all groups by an appropriate increase in insulin secretion resulting in disposition indexes during meals that were equal to or greater than those present during the OGTT. In contrast, disposition indexes were reduced ($P < 0.01$) during the OGTT in the impaired glucose tolerance groups, indicating that reduced glucose induced insulin secretion. We conclude that, whereas glucose-induced insulin secretion is impaired in people with abnormal glucose tolerance, nonglucose nutrient–induced secretion is intact, suggesting that a glucose-specific defect in the insulin secretory pathway is an early event in the evolution of type 2 diabetes. *Diabetes* 56: 1113–1119, 2007**

People with pre-diabetes are at increased risk of developing overt diabetes (1–3). Defects in insulin secretion and action contribute to this risk (4–14). The cause(s) of these abnormalities is an area of active investigation. A variety of factors modulate insulin secretion, including glucose and nonglucose nutrients (15–18). Numerous studies have shown that insulin secretion following intravenous glucose injection or glucose ingestion is impaired in people with pre-diabetes (4–13,19). We recently have reported that insulin secretion is also abnormal following ingestion of a meal containing both glucose and nonglucose nutrients (20). While such studies provide compelling evidence that glucose-induced insulin secretion is decreased in pre-diabetes, to our knowledge no study has specifically evaluated whether the ability of nonglucose nutrients to enhance glucose-induced insulin secretion is also abnormal.

Glucose and nonglucose nutrients stimulate insulin secretion, at least in part, via different mechanisms. Phosphorylation by glucokinase is believed to be the rate-limiting step for glucose-induced insulin secretion (15). In contrast, while the prevailing glucose concentration has a profound effect on nonglucose nutrient–induced insulin secretion, nutrients such as fat and amino acids modulate insulin secretion by multiple additional mechanisms (16–18). On the other hand, glucose and nutrient-induced insulin secretion presumably share common distal steps in the insulin secretory pathway (e.g., vesicle docking, priming, and exocytosis). Therefore, impaired glucose but intact nonglucose nutrient–induced insulin secretion in people with pre-diabetes would implicate a glucose-specific step in the insulin-secretory pathway. In contrast, a decrease in both glucose and nonglucose nutrient–induced insulin secretion could be due to a common process (e.g., decreased β-cell mass), a common distal defect (e.g., decreased number of primed docked granules), or a series of separate abnormalities.

The present study sought to address this question by determining whether the ability of nutrients to enhance glucose-induced insulin secretion is impaired in people with pre-diabetes. To do so, insulin secretion was measured in people with impaired fasting glucose (IFG) following ingestion of a mixed meal containing 75 g glucose and following ingestion of 75 g glucose alone. Insulin secretion and action were measured using the C-peptide and glucose oral minimal models. Subjects with IFG or normal fasting glucose (NFG) were subclassified as to whether they had abnormal or normal glucose tolerance (NGT). Results were compared with those observed in individuals who had both NFG and NGT. We report that, whereas glucose-induced insulin secretion is reduced in people with impaired glucose tolerance (IGT), the ability of nutrients to enhance glucose-induced insulin secretion is intact. In addition, the nutrient-induced increase in postprandial glucagon concentrations was the same in people with or without pre-diabetes. Insulin action was lower following ingestion of a mixed meal than following ingestion of an identical amount of glucose alone in all groups. However, the nutrient-induced enhancement of insulin secretion fully compensated for the decrease in

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DI, disposition index; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IGT-D, impaired glucose tolerance/diabetes; NFG, normal fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

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TABLE 1
Volunteer characteristics according to fasting and 2-h glucose tolerance status

| | NFG/NGT group | NFG/IGT group | IFG/NGT group | IFG/IGT-D group | IFG total |
|---|---|---|---|---|---|
| *n* | 18 | 12 | 7 | 25 | 32 |
| Sex (female/male) | 11/7 | 7/5 | 4/3 | 13/12 | 17/15 |
| Age (years) | 50.0 ± 1.9 | 52.9 ± 2.6 | 53.1 ± 3.0 | 53.9 ± 1.5 | 53.8 ± 1.3 |
| BMI (kg/m$^2$) | 27.7 ± 0.8 | 29.1 ± 1.3 | 30.9 ± 2.3 | 31.4 ± 0.9* | 31.3 ± 0.9* |
| LBM (kg) | 48.2 ± 2.7 | 48.4 ± 3.3 | 52.6 ± 5.1 | 52.3 ± 2.6 | 52.4 ± 2.2 |
| Body fat (%) | 35.3 ± 2.1 | 37.5 ± 3.3 | 39.1 ± 3.5 | 38.5 ± 1.9 | 38.7 ± 1.6 |
| Visceral fat (cm$^2$) | 116.9 ± 19.2 | 120.7 ± 9.1 | 187.3 ± 34.5* | 182.7 ± 17.5* | 183.7 ± 15.3* |
| FPG (mmol/l) | 5.0 ± 0.1 | 5.1 ± 0.1 | 5.9 ± 0.1† | 6.2 ± 0.1† | 6.2 ± 0.5† |
| 2-h PPG (mmol/l) | 6.7 ± 0.2 | 9.3 ± 0.3† | 6.9 ± 0.3 | 10.6 ± 0.4† | 9.8 ± 0.4† |
| Family history | No | No | No | 56% | 45% |

Data are means ± SE unless otherwise indicated. FPG, fasting plasma glucose at screening; LBM, lean body mass; PPG, postprandial glucose concentration at the OGTT. *$P < 0.05$ vs. NFG/NGT; †$P < 0.001$ vs. NFG/NGT.

insulin action resulting from equivalent postprandial glucose concentrations and unchanged or higher disposition indexes. The presence of IGT but intact nutrient-induced insulin secretion suggests that pre-diabetes is associated with a defect that alters glucose modulation of insulin secretion and occurs early in the evolution of diabetes.

## RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 32 subjects (17 women, 15 men) with IFG and 30 subjects (18 women, 12 men) with NFG gave informed written consent to participate in the study. All subjects were Caucasian, in good health, at a stable weight, and did not engage in regular vigorous physical exercise. At the time of study, subjects were on no medications other than a stable dose of thyroid hormone, low-dose aspirin, HMG-CoA reductase inhibitors, SSRI (selective serotonin reuptake inhibitor) antidepressants, or blood pressure medications that were metabolically neutral (e.g., no ACE inhibitors or β-blockers).

Details of the study have been described in detail and the results of the mixed-meal studies published in part elsewhere (20). In brief, subjects were instructed to follow a weight-maintenance diet containing a similar nutrient composition for at least 3 days before the study. Fasting plasma glucose concentrations were measured after an overnight fast on two separate occasions at least a week apart. Subjects with an average fasting glucose level <5.2 mmol/l (NFG) or between 5.6 and 7.0 mmol/l (IFG) were eligible for study. Subjects with a fasting glucose between 5.2 and 5.5 mmol/l were excluded from study because, despite having glucose concentrations within the normal range, previous studies have shown that such individuals have ~8% risk of developing diabetes within the next 10 years and, therefore, such glucose concentrations possibly represent an early form of IFG (1–3).

Eligible subjects were admitted on three separate occasions to the Mayo General Clinical Research Center at 1700 n and ate a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein) between 1830 and 1900 n. They remained fasting thereafter except for water. On the following morning, an 18-gauge cannula was inserted in a retrograde fashion into a dorsal hand vein, and the hand was placed in a heated box (~55°C) to enable sampling of arterialized venous blood. Subjects ingested in random order either a mixed meal consisting of three scrambled eggs, 16 g butter, 55 g Canadian bacon (or 47 g steak), and sugar-free Jell-O containing 75 g glucose (containing ~690 kcal) or 75 g glucose alone (oral glucose tolerance test [OGTT]). Blood samples were collected for glucose, insulin, and C-peptides at 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min. Blood samples for glucagon were collected at 0, 30, 60, 120, 180, and 240 min. Subjects were also admitted on a third occasion as part of a separate protocol that examined the mechanism by which the liver regulates fasting glucose concentrations. For the purpose of analysis, the OGTT data were used to classify subjects as having either NFG and NGT (NFG/NGT, 2-h plasma glucose <7.8 mmol/l), NFG and IGT (NFG/IGT, 2-h plasma glucose between 7.8 and 11.1 mmol/l), IFG and NGT (IFG/NGT, 2-h plasma glucose <7.8 mmol/l), or both IFG and IGT/diabetes, IFG/IGT-D, including subjects with either IGT ($n = 17$) or OGTT diabetes ($n = 8$). Since 2-h glucose concentrations during an OGTT are a continuum, and in order to increase our power, we chose to analyze the IFG/IGT-D subjects as a single group that was considered to have the greatest abnormality in glucose tolerance. The NFG/NGT subjects were considered as the reference group.

**Analytical techniques.** Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assay was done. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay with reagents (Access Assay) obtained from Beckman (Chaska, MN). Plasma glucagon and C-peptide were measured by radioimmunoassay using reagents supplied by Linco Research (St. Louis, MO). Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI) and computerized absorption tomography with cuts at L2/3 and T11/12 to determine percentages of body fat and visceral fat.

**Calculations.** Insulin secretion was measured as previously described using the oral–C-peptide minimal model incorporating age-associated changes in C-peptide kinetics as measured by Van Cauter et al. (20–22). A total responsivity-to-glucose insulin secretion index ($Phi_{total}$) was calculated from model indexes $Phi_{dynamic}$ and $Phi_{static}$ (21). To enable comparison between the mixed-meal and glucose-alone study days, the threshold $h$ was set equal to the preprandial glucose concentration in both datasets. Insulin sensitivity was determined by using the oral glucose minimal model (23). Disposition indexes were calculated as previously described to determine whether insulin secretion was appropriate for the prevailing level of insulin action (24–26). Parameters of all models were estimated by using SAAM II software (27). Measurement errors were assumed to be independent and Gaussian, with zero mean and variance for glucose (23) and C-peptide (28) as previously described.

Values from −30 to 0 min were averaged and considered as basal. The area above basal was calculated using the trapezoidal rule and, for the purposes of presentation, is referred to as the postprandial excursion. The difference in response on the meal- and OGTT-study days was calculated by subtracting the area above basal observed on the OGTT-study day from the area above basal observed in the same subject on the mixed-meal study day.

**Statistical analysis.** Data are presented as means ± SE. Comparisons between meal and OGTT days were made using Student's paired $t$ test or Wilcoxon's rank-sum test for variables that were not normally distributed (i.e., $S_i$, $Phi_{total}$, and $DI_{total}$ ). Analyses among groups were made using ANOVA followed by the least-significant-difference test for variables for which the $F$ test was significant. $S_i$, $Phi_{total}$, and $DI_{total}$ were log transformed to obtain a normal distribution of the data for ANOVA analysis. A $P$ value <0.05 was considered statistically significant.

## RESULTS

**Volunteer characteristics.** By design, fasting glucose concentrations at screening were higher ($P < 0.001$) in the IFG than in the NFG groups (Table 1). Age, lean body mass, and body fat did not differ among groups. However, BMI was greater ($P < 0.05$) in the IFG/IGT-D group and visceral fat greater ($P < 0.05$) in the IFG/NGT and IFG/IGT-D groups than in the NFG/NGT group. By selection, none of the NFG/NGT subjects had a family history of diabetes in a first-degree relative.

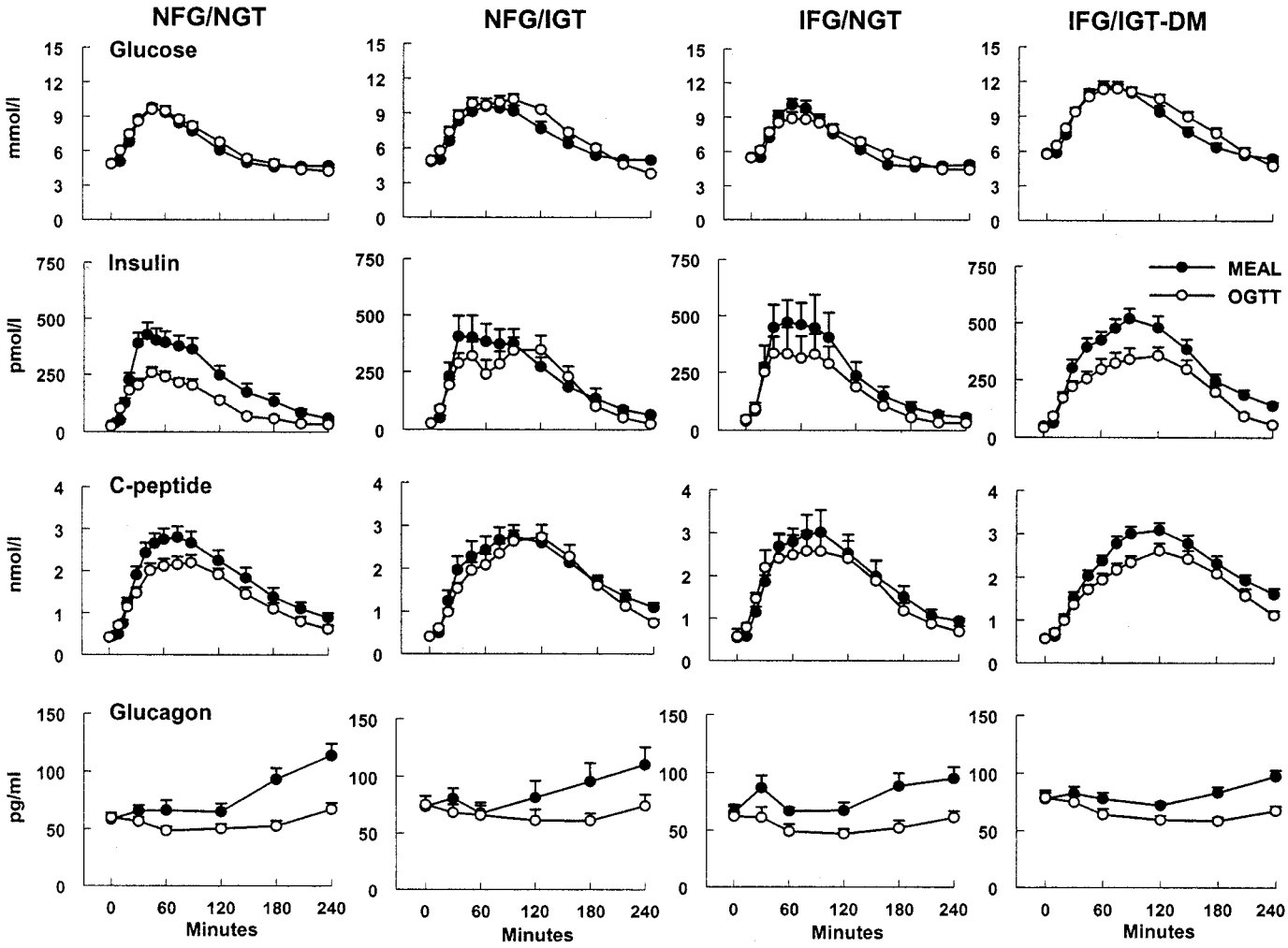


**FIG. 1. Plasma concentrations of glucose, insulin, C-peptide and glucagon observed in subjects with NFG/NGT, NFG/IGT, IFG/NGT, and IFG/IGT-D following ingestion of a mixed meal containing 75 g glucose and after ingestion of 75 g glucose alone.**

**Plasma glucose, insulin, C-peptide, and glucagon concentrations.** Fasting glucose concentrations did not differ on the mixed-meal and OGTT study days in any of the groups (Fig. 1, Table 2). Peak glucose concentrations (NFG/NGT 10.2 ± 0.2 vs. 10.0 ± 0.4 mmol/l, NFG/IGT 10.4 ± 0.4 vs. 10.7 ± 0.4 mmol/l, IFG/NGT 10.6 ± 0.6 vs. 9.4 ± 0.4 mmol/l, and IFG/IGT-D 12.3 ± 0.4 vs. 11.9 ± 0.4 mmol/l, respectively) and postprandial glycemic excursion (Table 2) also did not differ following ingestion of the mixed meal or the glucose drink in any group. On the other hand, the increment in glucose concentrations during the first 30 min was lower ($P < 0.05$) after ingestion of the mixed meal than after the ingestion of the glucose drink in all groups. The postprandial glycemic excursion was higher in both the NFG/IGT and IFG/IGT-D groups than in the NFG/NGT group following ingestion of glucose both during meals (ANOVA; $P < 0.001$) and alone (ANOVA; $P < 0.001$).

Fasting insulin concentrations did not differ on the mixed-meal and OGTT study days in any of the groups. The postprandial insulin excursion was greater following the mixed meal than the OGTT in the NFG/NGT ($P < 0.05$) and IFG/IGT-D ($P < 0.01$) groups but did not differ in the NFG/IGT and IFG/NGT groups. In contrast, the nutrient-induced δ (area above basal mixed meal minus area above basal OGTT) in the postprandial insulin excursion between study days did not differ among groups.

Fasting C-peptide concentrations did not differ on the mixed-meal and OGTT study days in any group. The postprandial C-peptide excursion was greater following the mixed meal than the glucose drink in the NFG/NGT ($P < 0.05$) and IFG/IGT-D ($P < 0.01$) groups but did not differ in the NFG/IGT and IFG/NGT groups. As with plasma insulin, the nutrient-induced δ in the postprandial C-peptide excursion between study days did not differ among groups.

Fasting glucagon concentrations were different among groups (ANOVA; $P < 0.05$) on the mixed-meal study day primarily because of higher concentrations in the IFG/IGT-D group. Fasting glucagon concentrations did not differ on the OGTT study day in any group. While the postprandial glucagon excursion was greater ($P < 0.05$) on the mixed-meal than the OGTT study day in all groups, the nutrient-induced δ in the postprandial glucagon excursion between the mixed-meal and OGTT study days did not differ among groups.

**Indexes of insulin action.** Insulin sensitivity index, measured with the oral minimal model, was lower ($P < 0.05$) following ingestion of the mixed meal than following the glucose drink in all groups (Fig. 2, Table 2). As

TABLE 2
Hormone concentrations, insulin action, and insulin secretion indexes following ingestion of mixed meal and glucose alone

| | NFG/NGT group | | | NFG/IGT group | | | IFG/NGT group | | | IFG/IGT-D group | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Meal | OGTT | *P* | Meal | OGTT | *P* | Meal | OGTT | *P* | Meal | OGTT | *P* |
| Glucose (mmol/l) | | | | | | | | | | | | |
| Fasting | 4.9 ± 0.1 | 4.9 ± 0.1 | 0.501 | 4.9 ± 0.1 | 5.0 ± 0.1 | 0.423 | 5.5 ± 0.1 | 5.5 ± 0.1 | 0.520 | 5.8 ± 0.1 | 5.8 ± 0.1 | 0.540 |
| Area 0–240 | 343 ± 22 | 414 ± 29 | 0.060 | 519 ± 58 | 628 ± 42 | 0.145 | 231 ± 28 | 272 ± 33 | 0.365 | 594 ± 55 | 724 ± 58 | 0.109 |
| Insulin (nmol/l) | | | | | | | | | | | | |
| Fasting | 0.03 ± 0.0 | 0.03 ± 0.0 | 0.439 | 0.03 ± 0.0 | 0.03 ± 0.0 | 0.858 | 0.04 ± 0.0 | 0.04 ± 0.0 | 0.798 | 0.05 ± 0.0 | 0.04 ± 0.0 | 0.403 |
| Area 0–240 | 46 ± 9 | 24 ± 2 | 0.022 | 48 ± 8 | 43 ± 7 | 0.599 | 47 ± 10 | 30 ± 6 | 0.091 | 67 ± 5 | 45 ± 5 | 0.005 |
| C-peptide (nmol/l) | | | | | | | | | | | | |
| Fasting | 0.42 ± 0.04 | 0.41 ± 0.04 | 0.846 | 0.42 ± 0.04 | 0.40 ± 0.04 | 0.794 | 0.53 ± 0.07 | 0.58 ± 0.06 | 0.597 | 0.57 ± 0.05 | 0.56 ± 0.06 | 0.889 |
| Area 0–240 | 334 ± 34 | 251 ± 21 | 0.044 | 366 ± 32 | 339 ± 34 | 0.565 | 338 ± 50 | 255 ± 50 | 0.142 | 404 ± 21 | 320 ± 24 | 0.011 |
| Glucagon (pg/ml) | | | | | | | | | | | | |
| Fasting | 57 ± 3 | 60 ± 5 | 0.608 | 77 ± 13 | 75 ± 7 | 0.906 | 65 ± 7 | 62 ± 8 | 0.777 | 78 ± 5 | 79 ± 7 | 0.913 |
| Area 0–240 | 4,879 ± 1,305 | −1,548 ± 777 | <0.005 | 2,145 ± 1,053 | −1,855 ± 1,583 | 0.047 | 3,015 ± 672 | −2,164 ± 812 | <0.005 | 643 ± 938 | −3,486 ± 731 | <0.005 |
| Insulin action | | | | | | | | | | | | |
| $S_I$ | 23 ± 3 | 29 ± 3 | 0.020 | 11 ± 2 | 17 ± 4 | 0.034 | 17 ± 4 | 26 ± 4 | 0.043 | 8 ± 2 | 11 ± 2 | 0.048 |
| Insulin secretion | | | | | | | | | | | | |
| Total | 51 ± 5 | 35 ± 3 | <0.001 | 50 ± 6 | 36 ± 4 | <0.005 | 68 ± 5 | 53 ± 5 | 0.018 | 49 ± 3 | 30 ± 2 | <0.001 |
| Disposition indexes | | | | | | | | | | | | |
| Total | 958 ± 132 | 929 ± 78 | 0.680 | 471 ± 40 | 547 ± 121 | 0.270 | 1,191 ± 266 | 1,358 ± 231 | 0.170 | 401 ± 71 | 324 ± 61 | 0.022 |

Data are means ± SE.

anticipated, insulin action was lower in the IFG/IGT-D ($P < 0.001$) and NFG/IGT ($P < 0.01$) groups than in the NFG/NGT group following both ingestion of the mixed meal and the glucose drink. However, insulin action did not differ in the IFG/NGT and NFG/NGT groups. Thus, regardless of the fasting glucose concentration, subjects with IGT were insulin resistant, whereas those with NGT were not.

**Indexes of insulin secretion.** The overall response to glucose ($Phi_{total}$) was greater ($P < 0.05$) following meal ingestion than the glucose drink in all groups (Fig. 2, Table 2), indicating that the presence of nonglucose nutrients in the meal enhanced the ability of glucose to stimulate insulin secretion. However, the nutrient-induced increase in insulin secretion (i.e., $Phi_{total}$ meal − $Phi_{total}$ OGTT) did not differ among the NFG/NGT, NFG/IGT, IFG/NGT, and IFG/IGT-D groups (15.9 ± 3.2 vs. 14.1 ± 3.4 vs. 14.9 ± 4.7 vs. 18.8 ± 2.8 $10^{-9}$ $min^{-1}$, respectively). In addition, when the prevailing level of insulin action was taken into account by calculation of disposition indexes, meal and OGTT disposition indexes were virtually identical in the NFG/NGT, NFG/IGT, and IFG/NGT groups and, if anything, higher on the meal than OGTT study day in the IFG/IGT-D group (Fig. 2), indicating that, on both occasions, the amount of insulin secreted was appropriate for the prevailing level of insulin action.

Consistent with previous reports, disposition indexes were lower ($P < 0.01$) in the NFG/IGT and IFG/IGT-D group than in the NFG/NGT group during the OGTT, confirming that subjects with IGT have a defect in glucose-induced insulin secretion. In contrast, the disposition indexes during the OGTT did not differ in the NFG/NGT and IFG/NGT groups (Fig. 2, bottom panels). As previously reported (20), they also did not differ following ingestion of a mixed meal.

## DISCUSSION

The present study yielded four novel findings. First, whereas glucose-induced insulin secretion is impaired in people with IGT, nonglucose nutrient–induced insulin secretion is intact. Second, nonglucose nutrient–induced glucagon secretion is not enhanced in pre-diabetes. Third, insulin action following ingestion of a mixed meal containing protein and fat is lower than that observed following ingestion of an identical amount of glucose alone. Fourth, nonglucose-induced enhancement of insulin secretion appropriately compensates for the lower insulin action that occurs following ingestion of a mixed meal, resulting in unchanged glucose tolerance in people with NGT and in those with pre-diabetes.

Although glucose is the primary regulator of insulin secretion, nonglucose nutrients also stimulate insulin secretion, with the magnitude of stimulation dependent upon the prevailing glucose concentration (16–18,29). The present study used a C-peptide model to evaluate nonglucose nutrient–induced insulin secretion (21). We reasoned that, since identical amounts of glucose were ingested on both study days, differences in insulin secretion (if observed) would be due to the effects of the additional nonglucose nutrients (e.g., protein and fat) present in the mixed meal. We are unaware of any previous studies that have used this experimental design. We anticipated that insulin secretion would be greater after the mixed meal in the subjects who had both NFG and NGT. This outcome was observed. However, we did not anticipate that glucose

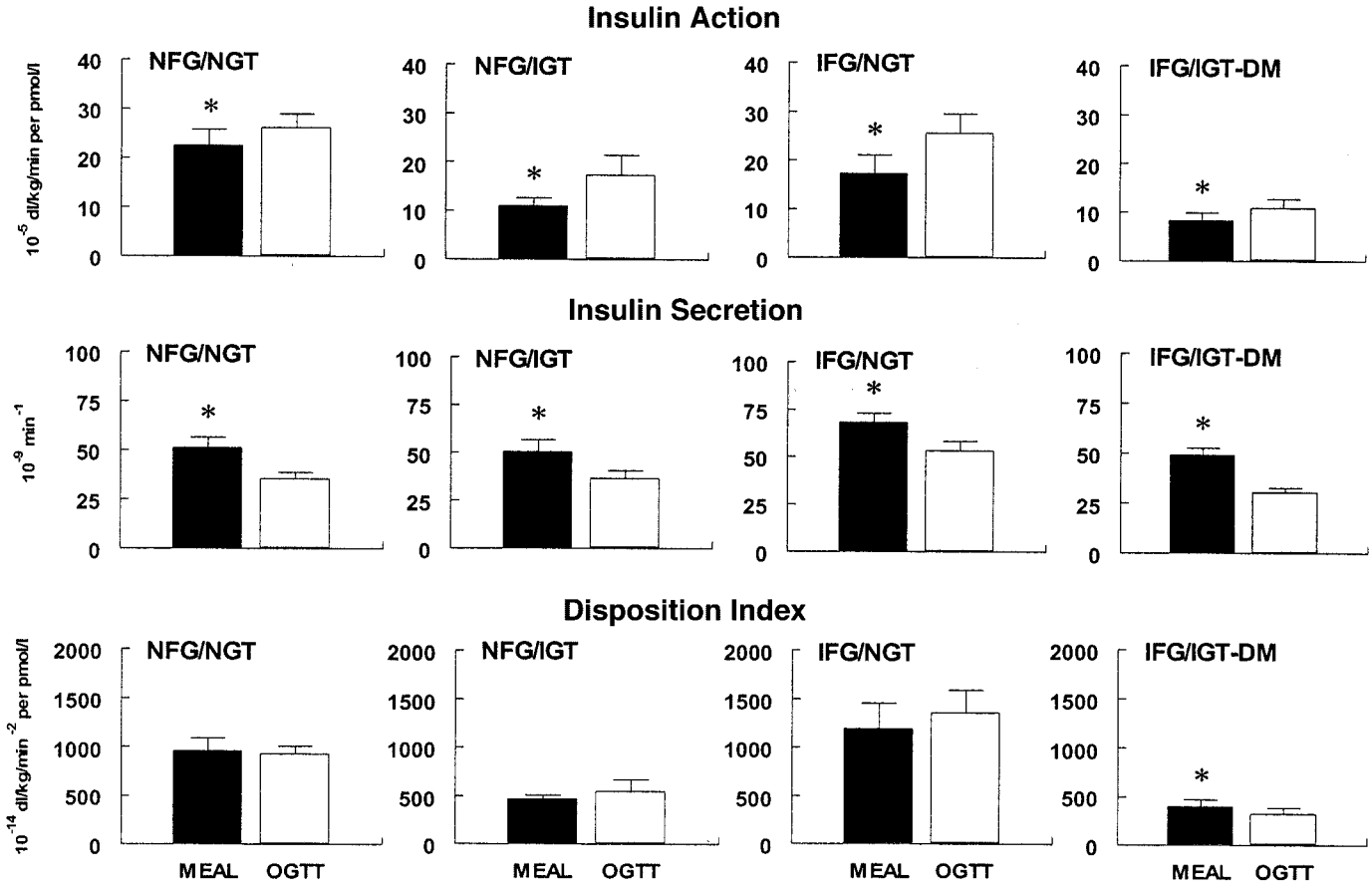


**FIG. 2. Insulin action ($S_I$), insulin secretion indexes ($Phi_{total}$), and disposition indexes ($DI_{total}$) observed in subjects subclassified as NFG/NGT, NFG/IGT, IFG/NGT, and IFG/IGT-D following ingestion of a mixed meal containing 75 g glucose and after ingestion of 75 g glucose alone. *$P$ < 0.05 vs. OGTT.**

concentrations would be the same on the mixed-meal and OGTT study days in both the NFG and IFG groups. The equivalent glucose concentrations facilitated assessment of the ability of nonglucose nutrients to stimulate insulin secretion. C-peptide as well as insulin concentrations were greater following the mixed meal than the OGTT in the NFG/NGT and IFG/IGT-D groups, confirming that insulin secretion was increased. To our surprise, the nonglucose nutrient–induced increase in secretion (i.e., C-peptide meal minus C-peptide OGTT) was virtually identical. A similar pattern also was observed in the NFG/IGT and IFG/NGT groups but did not reach statistical significance. On the other hand, when insulin secretion was quantitatively assessed using the oral C-peptide model, the identical pattern was observed in all groups. It has been long known that even a minimal elevation in plasma glucose concentration is associated with a decreased β-cell response to glucose (30,31). The observation in the present study that OGTT disposition indexes were reduced in both the NFG/IGT and IFG/IGT-D groups lends further support to the concept that abnormal glucose tolerance rarely occurs unless there is an underlying defect in glucose-induced insulin secretion.

Intact nonglucose nutrient–induced enhancement of insulin secretion in people with pre-diabetes is intriguing. Amino acids such as arginine can stimulate insulin secretion in people with overt diabetes (24,32–34). However, the magnitude of the response is blunted compared with that in nondiabetic subjects studied at the same glucose concentration (29). This differs from the present study, since the magnitude of the response to nonglucose nutrients was the same in subjects with NFG and in the subjects with pre-diabetes. In fact, the nutrient-induced increase in insulin secretion measured as the disposition index was, if anything, slightly increased in the group with the most abnormal glucose tolerance (IFG/IGT-D). Thus, reduced nonglucose nutrient–induced insulin secretion likely occurs later in the evolution to overt diabetes. Since glucose- but not nonglucose nutrient–induced insulin secretion is impaired, and since glucose phosphorylation appears to be the rate-limiting step in glucose-induced insulin secretion, it is interesting to speculate that a decrease in β-cell glucokinase activity contributes to this abnormality. On the other hand, since both glucose- and nonglucose nutrient–induced insulin secretion utilize the same distal steps in the insulin secretory pathway (e.g., docking, priming, and exocytosis), the results from the current study imply that these processes appear to be relatively intact in pre-diabetes. However, since this conclusion can only be inferred from the present data, and since glucose can exert a direct effect on exocytosis and other distal steps (35), future studies will be required to specifically address this hypothesis.

Insulin action was lower following ingestion of a mixed meal than following ingestion of an identical amount of glucose alone. A greater requirement for insulin could have occurred for several reasons. First, glucagon concentrations were higher after the mixed meal than after the glucose drink, presumably due to the stimulatory effects of protein contained within the meal. We have previously

shown that in the absence of a compensatory increase in insulin secretion, an increment in glucagon comparable with that observed after the mixed meal causes substantial hyperglycemia (36,37). This did not occur in the present study, presumably since subjects in both the IFG and NFG groups secreted sufficient additional insulin to offset the effects of the higher glucagon concentrations. Second, chronic elevation of free fatty acids or amino acids can decrease glucose uptake via both direct and indirect mechanisms (38–41). We did not measure amino acids or lipid concentrations on the two study dates, so we do not know whether the concentrations differed sufficiently that substrate competition would be plausible. Furthermore, even if free fatty acid and amino acid concentrations were markedly different, we do not know whether the effects would be rapid enough to cause the increase in insulin need that was evident within the first hour after ingestion of the mixed meal. Amino acids and free fatty acids are known stimulants of gluconeogenesis (42–46), and higher insulin concentrations are required to suppress gluconeogenesis than glycogenolysis (47–49). Therefore, the protein and fat contained within the mixed meal may have increased the amount of insulin required to suppress glucose production. Regardless of the cause, lower insulin action after a mixed meal could be potentially advantageous from a teleological point of view, since protein synthesis occurs primarily in the postprandial state, and a concurrent increase in both insulin and amino acids is a more potent stimulus for protein synthesis than an elevation of either insulin or amino acids alone (50).

Fasting plasma glucagon concentrations have been reported to be elevated in people with pre-diabetes by some (51) but not all (52) investigators. In the present experiments, fasting glucagon concentrations were higher on the meal study day when all of the IFG subjects were considered as a single group rather than the NFG/NGT group primarily because of higher concentrations in the IFG/IGT-D group. This suggests, at most, a subtle abnormality in the regulation of glucagon. Consistent with this premise, Larsson et al. (52) reported an increase in the glucagon response to intravenous arginine at a glucose concentration of 14 mmol/l in subjects with IGT but no difference at a glucose concentration of either 5 or 28 mmol/l. In addition, Henkel et al. (51) reported that glucagon was higher in IGT and NGT subjects 30 min after ingestion of a mixed meal. On the other hand, while glucagon concentrations were higher on the meal than the OGTT study days in both groups, the nonglucose nutrient–induced increase in glucagon did not differ between groups. Thus, while glucagon regulation may be abnormal in the fasting state and following glucose ingestion in some individuals with pre-diabetes, under the conditions of the current experiments, glucagon stimulation by nonglucose nutrients is not excessive and therefore unlikely to be the cause of postprandial hyperglycemia in these individuals.

In summary, insulin action is lower and glucagon concentration higher following ingestion of a nonglucose nutrient–containing meal than it is following ingestion of the same amount of glucose alone. This is accompanied by a compensatory increase in insulin secretion, resulting in glucose tolerance being maintained unchanged in people with NGT or pre-diabetes. In addition, whereas glucose-induced insulin secretion is impaired in people with glucose intolerance, nonglucose nutrient–induced secretion is intact, suggesting that a glucose-specific defect in the insulin secretory pathway is an early event in the evolution of type 2 diabetes.

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