Original Article

Pathogenesis of Pre-Diabetes

Mechanisms of Fasting and Postprandial Hyperglycemia in People With Impaired Fasting Glucose and/or Impaired Glucose Tolerance

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Thirty-two subjects with impaired fasting glucose (IFG) and 28 subjects with normal fasting glucose (NFG) ingested a labeled meal and 75 g glucose (oral glucose tolerance test) on separate occasions. Fasting glucose, insulin, and C-peptide were higher ($P < 0.05$) in subjects with IFG than in those with NFG, whereas endogenous glucose production (EGP) did not differ, indicating hepatic insulin resistance. EGP was promptly suppressed, and meal glucose appearance comparably increased following meal ingestion in both groups. In contrast, glucose disappearance (R_d) immediately after meal ingestion was lower ($P < 0.001$) in subjects with IFG/impaired glucose tolerance (IGT) and IFG/diabetes but did not differ in subjects with IFG/normal glucose tolerance (NGT) or NFG/NGT. Net insulin action (S_i) and insulin-stimulated glucose disposal (S_i*) were reduced ($P < 0.001$, ANOVA) in subjects with NFG/IGT, IFG/IGT, and IFG/diabetes but did not differ in subjects with NFG/NGT or IFG/NGT. Defective insulin secretion also contributed to lower postprandial R_d since disposition indexes were lower ($P < 0.001$, ANOVA) in subjects with NFG/IGT, IFG/IGT, and IFG/diabetes but did not differ in subjects with NFG/NGT and IFG/NGT. We conclude that postprandial hyperglycemia in individuals with early diabetes is due to lower rates of glucose disappearance rather than increased meal appearance or impaired suppression of EGP, regardless of their fasting glucose. In contrast, insulin secretion, action, and the pattern of postprandial turnover are essentially normal in individuals with isolated IFG. *Diabetes* 55:3536–3549, 2006

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DI, disposition index; EGP, endogenous glucose production; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NFG, normal fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

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Individuals with impaired fasting glucose (IFG) have a 20–30% chance of developing diabetes over the next 5–10 years (1–3). The risk is even greater if they have combined IFG and impaired glucose tolerance (IGT). Furthermore, IFG and IGT are associated with increased risk of cardiovascular events (4,5). Therefore, the pathogenesis of IFG alone or in combination with IGT has engendered considerable interest. Glucose concentration begins to increase when glucose appearance exceeds glucose disappearance and continues to increase until these two rates are once again equal. In the fasting state, glucose appearance is determined by the rate of glucose release from the liver with perhaps a small contribution by the kidney. Together, these processes are referred to as endogenous glucose production (EGP). The situation is more complex following food ingestion when glucose appearance equals the sum of EGP and the rate of appearance of the ingested glucose (6). There is currently limited data as to the contribution of these processes to fasting hyperglycemia (7,8) and no data regarding the regulation of postprandial glucose metabolism in individuals with IFG. The latter is of particular interest since while most individuals with IFG also have either IGT or diabetes, some have normal glucose tolerance (NGT). On the other hand, some individuals with normal fasting glucose (NFG) have IGT.

When considered in the light of the prevailing glucose and insulin concentration, EGP is increased in individuals with mild and severe type 2 diabetes (9). To our knowledge, Weyer et al. (7) are the only investigators who have measured EGP in individuals with IFG. In those studies, fasting EGP was increased in Pima Indians with IFG, regardless of whether they had NGT or IGT. In contrast, fasting glucose production was not elevated in Pima Indians with IGT and NFG concentrations. However, since fasting insulin concentrations were elevated in those subjects, this implies the presence of hepatic insulin resistance. On the other hand, since glucose and insulin suppress glucose production (10–13) and enhanced insulin secretion can potentially compensate for a defect in insulin action (14,15), hepatic insulin resistance does not necessarily mean that excessive EGP is the cause of postprandial hyperglycemia in individuals with IFG and/or

IGT. Conversely, effective compensation via these mechanisms could normalize postprandial suppression of EGP, thereby enabling some individuals with IFG to maintain normal postprandial glucose concentrations.

To our knowledge, the postprandial rate of meal glucose appearance has not been measured in individuals with IFG. However, Sacca et al. (16) reported that splanchnic glucose uptake during intravenous glucose infusion was greater in six subjects with combined IFG/IGT than in seven normoglycemic control subjects, implying enhanced rather than decreased hepatic glucose extraction. Unfortunately, these data are difficult to interpret since glucose concentrations were far higher in subjects with IFG than in control subjects, and glucose is a potent stimulus of hepatic glucose uptake (13,17). Therefore, the relative contribution of alterations in meal-derived glucose appearance, EGP, or glucose disappearance to the regulation of postprandial glucose concentrations in individuals with IFG is presently not known.

To address these questions, meal glucose appearance, EGP, and glucose disappearance were measured in 32 subjects with IFG before and after ingestion of a mixed meal using a validated triple-tracer approach (18). Insulin secretion and action were concurrently measured using the C-peptide (19,20) and "oral" minimal models (21–25) in order to gain insight regarding the etiology of alterations (if observed) in postprandial glucose metabolism. Results were compared with those observed in 28 control subjects with NFG. In addition, to gain insight regarding the heterogeneity of glucose tolerance in individuals with NFG or IFG, the subjects were subdivided according to whether they also fulfilled oral glucose tolerance test (OGTT) criteria for IGT or diabetes.

RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 32 subjects (17 women and 15 men) with IFG and 28 subjects (17 women and 11 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, hydroxymethylglutaryl-CoA reductase inhibitors, selective serotonin reuptake inhibitor antidepressants, or antihypertensives, which are metabolically neutral (e.g., no ACE inhibitors or β-blockers).

All subjects were instructed to follow a weight-maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the study date. Fasting plasma glucose concentration was measured after an overnight fast on two separate occasions at least 1 week apart. Subjects whose average fasting glucose level was <5.2 mmol/l or between 5.6 and 7.0 mmol/l were selected for the study and referred to as having NFG or IFG, respectively. Subjects with a fasting glucose between 5.2 and 5.6 mmol/l were excluded from the study, since despite having glucose concentrations within the normal range, previous studies have shown that such individuals have an ~8% risk of developing diabetes within the next 10 years and, therefore, possibly represent an early form of IFG (1–3). Eligible subjects were then admitted to the Mayo General Clinical Research Center on two subsequent occasions at 1700 the evening before the study and ate a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein) between 1830 and 1900. No additional food was eaten until the next morning. On one occasion, subjects ingested 75 g glucose after a 12-h overnight fast. Based on these results, subjects with either NFG or IFG were subclassified as having NFG/NGT (2-h plasma glucose <7.8 mmol/l), NFG/IGT (2-h plasma glucose between 7.8 and 11.1 mmol/l), IFG/IGT, or IFG/diabetes (2-h plasma glucose >11.1 mmol/l).

On another occasion, subjects ingested a labeled mixed meal as previously described (18). In brief, an 18-gauge cannula was inserted at 0600 into a forearm vein for tracer infusions. Another 18-gauge cannula was inserted in a retrograde fashion in a dorsal hand vein of the opposite arm, and the hand was placed in a heated box (~55°C) to enable sampling of arterialized venous blood. A primed (12 mg/kg) continuous ($0.12\ mg \cdot kg^{-1} \cdot min^{-1}$) infusion of [6,6-$^2H_2$]glucose (MassTrace, Woburn, MA) started at 0700. At time 0, i.e., 1000, subjects ingested a standard mixed meal within 15 min, which consisted of three scrambled eggs, 55 g Canadian bacon (or 47 g steak), and Jell-O containing 75 g glucose that was enriched (to ~4%) with [1-^{13}C]glucose, as previously described (18,26). An intravenous infusion of [6-^{3}H]glucose was started at the same time and infused in a pattern that minimized the change in the ratio of plasma tracer (i.e., [6-^{3}H]glucose) to plasma meal tracee (i.e., [1-^{13}C]glucose). In addition, the [6,6-$^2H_2$]glucose infusion was varied in a manner mimicking the anticipated pattern of change of EGP, thereby also minimizing the change in the ratio of plasma tracer (i.e., [6,6-$^2H_2$]glucose) to plasma tracee (i.e., concentration of endogenous plasma glucose), as previously described (18,27).

Analytical techniques. Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until the assay was complete. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; 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 percent body fat and visceral fat. Plasma [6,6-$^2H_2$]glucose and [1-^{13}C]glucose enrichments were measured using gas chromatography–mass spectrometry (Thermoquest, San Jose, CA) to simultaneously monitor the C-1, C-2, and C-3 to C-6 fragments, as described by Beylot et al. (28), and [6-^{3}H]glucose specific activity by liquid-scintillation counting following deproteinization and passage over anion and cation exchange columns.

Calculations. The systemic rates of meal appearance (R_{aMEAL}), EGP, and glucose disappearance (R_d) were calculated using Radziuk's two-compartment model (29), as previously described (18). In brief, R_{aMEAL} was calculated by multiplying the rate of appearance of [1-^{13}C]glucose (obtained from the infusion rate of [6-^{3}H]glucose and the clamped plasma ratio of [6-^{3}H]glucose and [1-^{13}C]glucose) by the meal enrichment (i.e., the ratio of total glucose to tracer in the meal). EGP was calculated from the infusion rate of [6,6$^2H_2$]glucose and the clamped plasma ratio of [6,6$^2H_2$]glucose to endogenous glucose concentration. Glucose disappearance was calculated by subtracting the change in glucose mass from the overall rate of glucose appearance (i.e., R_{aMEAL} + EGP).

Insulin sensitivity (S_i), which measures the overall effect of insulin to stimulate glucose disposal and inhibit glucose production, was estimated from plasma glucose and insulin concentrations using the oral glucose minimal model (24,25). The model assumes that insulin action on glucose production and disposal emanates from a compartment remote from plasma, which is usually identified with the interstitium. Similarly, the selective effect of insulin on glucose disposal (S_i*) was estimated from oral-ingested glucose tracer and insulin concentration by using the labeled oral glucose minimal model (23).

β-Cell responsivity indexes were estimated from plasma glucose and C-peptide concentrations measured during the test by using the oral C-peptide minimal model (19), incorporating age-associated changes in C-peptide kinetics as measured by Van Cauter et al. (30). The model assumes that insulin secretion is made up of two components. The dynamic component is likely to represent secretion of promptly releasable insulin and is proportional to the rate of increase of glucose concentration through a parameter, $Phi_{dynamic}$, which defines the dynamic responsivity index. The static component is derived from the provision of new insulin to the releasable pool and is characterized by a static index and by a delay time constant, T. The meaning of Phi_{static} and T can be made clear with reference to a response to an above-basal step increase of glucose; provision tends, with time constant T, toward a steady state, which is linearly related to the glucose step size through parameter Phi_{static}. To determine if insulin secretion indexes were appropriate for the prevailing level of insulin action, the disposition indexes (DIs) DI_{total}, $DI_{dynamic}$, and DI_{static} were calculated by multiplying Phi_{total}, $Phi_{dynamic}$, and Phi_{static}, respectively, by S_i.

Values from −30 to 0 min were averaged and considered as basal. The area above basal was calculated using the trapezoidal rule. Parameters of all models were estimated by using the SAAMII software (31). Measurement errors were assumed to be independent and Gaussian, with zero mean and variance for glucose and tracer glucose (22) and for C-peptide (32).

Statistical analysis. All data are presented as means ± SE. Rates of glucose turnover are expressed as micromoles per kilogram lean body mass. Two sample comparisons between subjects with IFG and NFG were made using Student's t test or rank-sum test for data that were nonnormally distributed. Analyses among the NFG and IFG subgroups were made using ANOVA followed, where appropriate, by Student's two-tailed nonpaired t test. A P value <0.05 was considered statistically significant.

TABLE 1
Characteristics of study subjects according to fasting and 2-h glucose tolerance status

	NFG/NGT	NFG/IGT	IFG/NGT	IFG/IGT	IFG/diabetes	IFG total
n	16	12	7	17	8	32
Sex (female/male)	10/6	7/5	4/3	8/9	5/3	17/15
Age (years)	49.9 ± 2.1	52.9 ± 2.6	53.1 ± 3.0	53.8 ± 2.0	54.3 ± 2.3	53.8 ± 1.3
BMI (kg/m^2)	27.5 ± 0.9	29.1 ± 1.3	30.9 ± 2.3	31.1 ± 1.3*	31.9 ± 1.2*	31.3 ± 0.9*
Lean body mass (kg)	48.6 ± 2.9	48.4 ± 3.3	52.6 ± 5.1	53.7 ± 3.4	49.4 ± 3.4	52.4 ± 2.2
Body fat (%)	35.3 ± 2.2	37.5 ± 3.3	39.1 ± 3.5	37.4 ± 2.2	41.1 ± 3.6	38.7 ± 1.6
Visceral fat (cm^2)	114.8 ± 21.6	120.7 ± 9.1	187.3 ± 34.5*	176.5 ± 24.6*	196.0 ± 17.3*	183.7 ± 15.3*
FPG (mmol/l)	5.0 ± 0.1	5.1 ± 0.1	5.9 ± 0.1†	6.1 ± 0.1†	6.5 ± 0.1†	6.2 ± 0.5†
2-h PPG (mmol/l)	6.8 ± 0.2	9.3 ± 0.3†	6.9 ± 0.3	9.5 ± 0.2†	12.8 ± 0.5†	9.8 ± 0.4†
Family history (%)	0	0	0	60	50	45

Data are means ± SE unless otherwise indicated. *$P < 0.05$ vs. NFG/NGT; †$P < 0.001$ vs. NFG/NGT. 2-h PPG, 2-h glucose concentration on the oral glucose tolerance test; FPG, fasting plasma glucose at screening.

RESULTS

By design, the fasting plasma glucose levels at screening were higher ($P < 0.001$) in subjects with IFG than in those with NFG/NGT (Table 1). Age, lean body mass, and body fat did not differ statistically between groups. On the other hand, BMI and visceral fat were greater ($P < 0.05$) in subjects with versus NFG/NGT. None of the above parameters differed in subjects with IFG with or without IGT or diabetes or in subjects with NFG with or without IGT. Sixty percent of the subjects with IFG/IGT and 50% of subjects with IFG/diabetes had a history of diabetes in a first-degree relative. No subject with IFG/NGT had a family history of diabetes. By selection, none of the subjects with NFG had a family history of diabetes.

Plasma glucose, insulin, C-peptide, and glucagon concentrations. Fasting plasma glucose concentrations were higher ($P < 0.001$) in the total group with IFG than in subjects with NFG/NGT (Fig. 1) and increased to a higher peak ($P < 0.002$) following meal ingestion (11.9 ± 0.4 vs. 9.9 ± 0.2 mmol/l). On the other hand, the glucose area above basal did not differ statistically between these two groups. When analyzed according to OGTT status, fasting plasma glucose concentrations were higher ($P < 0.001$) in subjects with IFG/diabetes but did not differ in the those with IFG/NGT and IFG/IGT (Fig. 2). Fasting glucose also did not differ in the subjects with NFG/IGT and NFG/NGT. Following meal ingestion, glucose concentration increased to a higher ($P < 0.001$) peak in the subjects with IFG/IGT (11.2 ± 0.4 mmol/l) and IFG/diabetes (14.5 ± 0.4 mmol/l) compared with subjects with NFG/NGT. On the other hand, peak glucose concentration did not differ in subjects with IFG/NGT and NFG/NGT (10.6 ± 0.6 vs. 9.9 ± 0.2 mmol/l, respectively), resulting in a lower ($P < 0.05$) postprandial increment in glucose in subjects with IFG/NGT. Peak glucose concentrations also did not differ in the subjects with NFG/IGT and NFG/NGT (10.4 ± 0.4 vs. 9.9 ± 0.2 mmol/l, respectively); however, it took longer for glucose concentrations to return to preprandial levels in the former, resulting in a greater ($P < 0.05$) glucose area above basal in the subjects with NFG/IGT. Glucose area above basal was also greater ($P < 0.001$) in the subjects with IFG/diabetes than in those with NFG/NGT.

Fasting plasma insulin concentrations were higher ($P < 0.05$) in the total group with IFG than in the subjects with NFG/NGT (Fig. 1) but rose to a comparable peak after meal ingestion (628 ± 45 vs. 531 ± 86 pmol/l, respectively) (Table 2). However, the time to peak was longer ($P < 0.01$) in the total group with IFG than in the subjects with NFG/NGT (88 ± 7 vs. 57 ± 6 min, respectively). Subgroups analysis (Fig. 2) indicated that the higher fasting plasma insulin concentrations in the total group with IFG were primarily due to higher insulin concentrations in subjects with IFG/IGT and IFG/diabetes ($P < 0.05$ vs. NFG/NGT) since insulin concentrations did not differ among the other groups. The insulin area above basal did not differ among the groups during the 1st hour after meal ingestion (i.e., when glucose concentrations were diverging in the subjects with IFG/IGT and IFG/diabetes). The insulin area above basal during the entire 6-h observation also did not differ among the subjects with NGT and IGT but was higher ($P < 0.05$) in the subjects with IFG/diabetes than in those with NFG/NGT. However, the time to peak insulin concentration was longer ($P < 0.01$) in the subjects with IFG/IGT and IFG/diabetes (92 ± 8 and 115 ± 11 min, respectively) than in those with NFG/NGT.

Fasting plasma C-peptide concentrations also were higher ($P < 0.05$) in the total group with IFG than in the subjects with NFG/NGT and rose to a comparable peak after meal ingestion (3.4 ± 0.2 vs. 3.0 ± 0.3 nmol/l, respectively). As with insulin, the higher fasting C-peptide concentrations in the total group with IFG were primarily due to higher C-peptide concentrations in subjects with IFG/diabetes ($P < 0.001$ vs. NFG/NGT). On the other hand, the C-peptide area above basal during the first 60 min after meal ingestion, and during the entire 6 h of observation, did not differ among subjects with NGT and IGT. In contrast, the C-peptide area above basal over the 6 h of observation was higher ($P < 0.05$) in subjects with IFG/diabetes than in those with NFG/NGT.

Fasting plasma glucagon concentrations were higher ($P < 0.02$) in the total group with IFG than in the subjects with NFG/NGT before meal ingestion (75 ± 4 vs. 57 ± 4 pg/ml, respectively) but did not differ after meal ingestion. While fasting plasma glucagon concentrations were somewhat higher in the subjects with IFG/IGT and IFG/diabetes compared with those with NFG/NGT, the differences were not significant. Plasma glucagon concentrations remained constant or tended to fall immediately after meal ingestion in all subgroups, then subsequently rose as glucose concentrations fell back toward preprandial values.

Meal glucose appearance, EGP, and glucose disappearance. The systemic rate of appearance of meal-derived glucose reached a comparable peak within ~30–60 min after meal ingestion in subjects with IFG and NFG/NGT. While the area above basal did not differ among groups, the area above baseline over the 6 h of observation

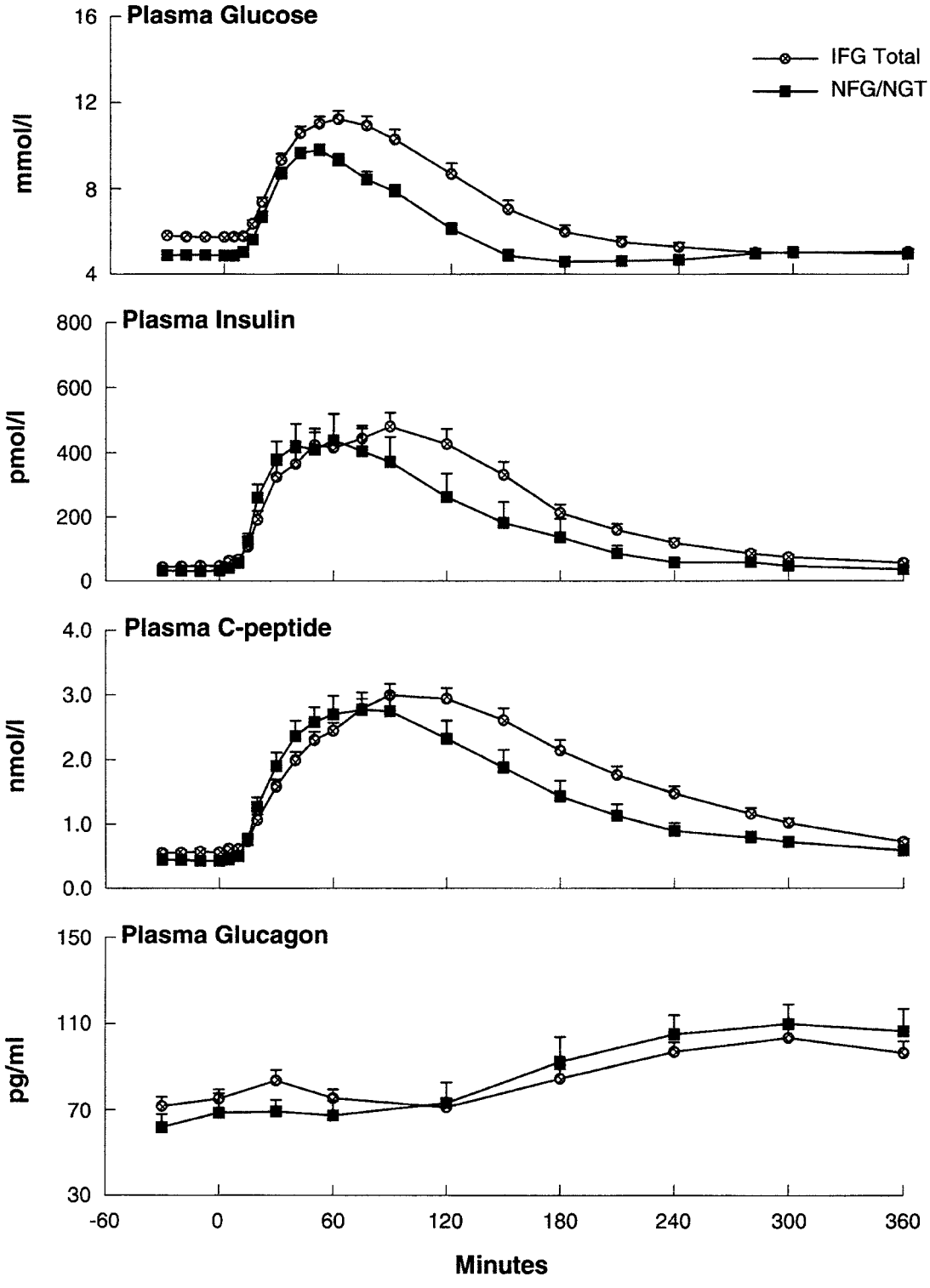

FIG. 1. Plasma glucose, insulin, C-peptide, and glucagon concentrations observed in subjects with IFG (IFG total) and in subjects with NFG and NGT on an OGTT (NFG/NGT). A mixed meal was ingested at time 0 min.

tended ($P = 0.08$) to be lower in the subjects with IFG/IGT and IFG/diabetes compared with the subjects with NFG/NGT.

Despite increased fasting glucose and insulin concentrations, fasting EGP did not differ in subjects with IFG and NFG. Subgroup analysis indicated that EGP also did not differ in subjects with or without IGT and was slightly higher ($P = 0.08$) in subjects with IFG/diabetes. In addition, postprandial suppression of endogenous glucose (area below basal) following meal ingestion also did not differ between groups.

Glucose disappearance before meal ingestion also did not differ in the total goup with IFG and the subjects with NFG/NGT or in the various subgroups. On the other hand, the increment above basal during the 1st hour after meal ingestion was smaller ($P < 0.002$) in the total group with

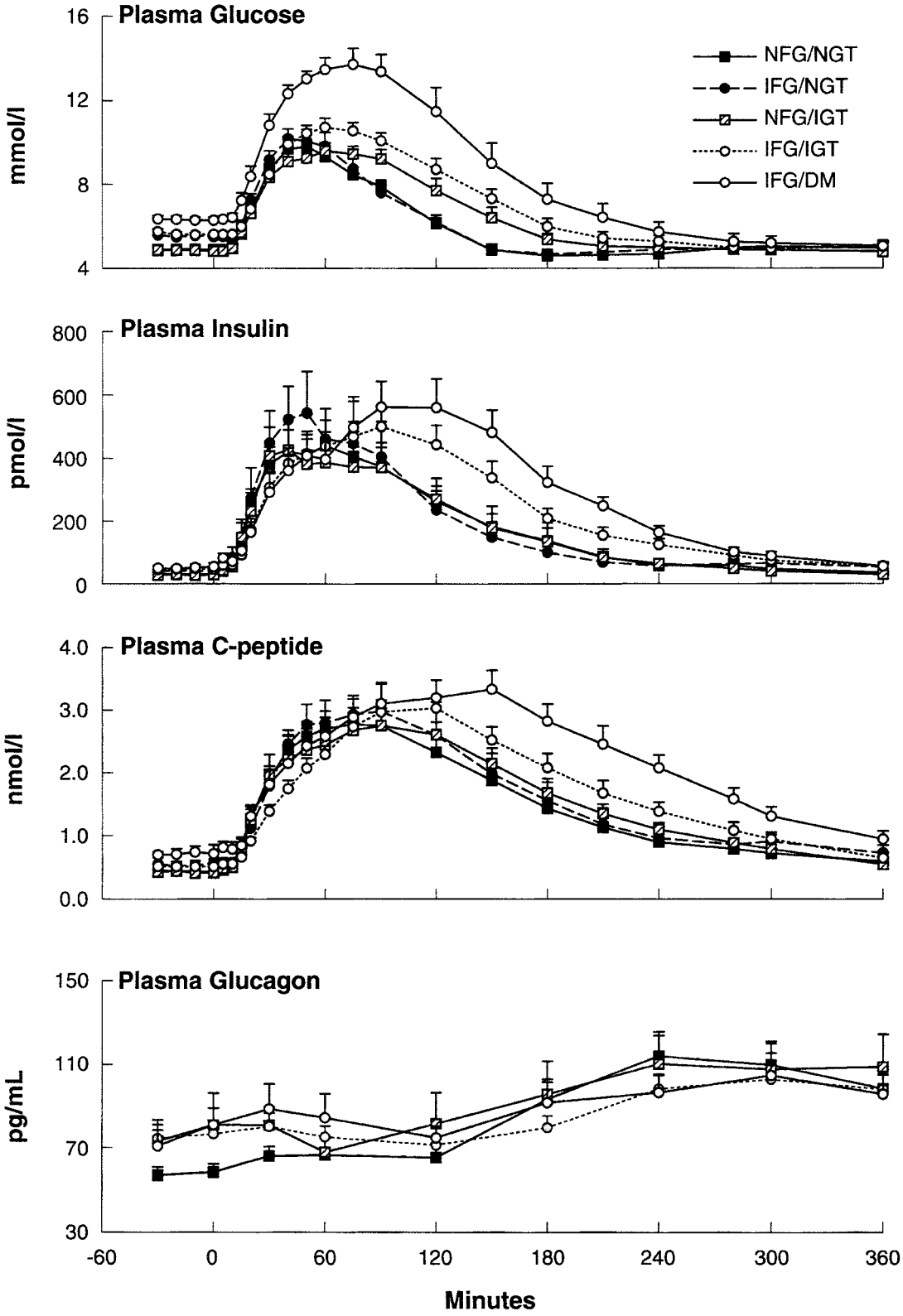

FIG. 2. Plasma glucose, insulin, C-peptide, and glucagon concentrations observed in subjects with NFG/NGT, NFG/IGT, IFG/IGT, or IFG/diabetes. A mixed meal was ingested at time 0 min.

IFG than in the subjects with NFG/NGT (Fig. 3). The blunted increase in glucose disappearance immediately after meal ingestion was primarily due to a smaller increase in the subjects with IFG/diabetes ($P < 0.001$) and IFG/IGT ($P < 0.001$) (Fig. 4). The increase in glucose disappearance during the 1st hour after meal ingestion tended to be lower ($P = 0.08$) in the subjects with NFG/IGT than in those with NFG/NGT. Glucose disappearance reached comparable rates in all groups by 2 h after meal ingestion and was slightly higher in the subjects with

TABLE 2
Hormone concentrations and glucose turnover rates

	NFG/NGT	NFG/IGT	IFG/NGT	IFG/IGT	IFG/diabetes	IFG total
Glucose (mmol/l)						
Basal	4.9 ± 0.1	4.9 ± 0.1	5.5 ± 0.1†	5.6 ± 0.1†	6.3 ± 0.1†	5.8 ± 0.1†
Area 0–60 min	170 ± 10	162 ± 15	164 ± 19	161 ± 15	228 ± 17*	178 ± 11
Area 0–360 min	339 ± 24	522 ± 61*	165 ± 36*	422 ± 56	687 ± 119†	432 ± 52
Insulin (nmol/l)						
Basal	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.05 ± 0.01*	0.05 ± 0.01*	0.05 ± 0.00*
Area 0–60 min	15.0 ± 2.3	15.4 ± 3.6	17.5 ± 3.5	12.9 ± 1.7	12.2 ± 2.5	13.7 ± 1.3
Area 0–360 min	49.5 ± 9.8	50.5 ± 8.3	49.8 ± 10.1	66.5 ± 7.0	82.3 ± 8.7*	66.8 ± 5.1
C-peptide (nmol/l)						
Basal	0.42 ± 0.04	0.42 ± 0.04	0.53 ± 0.07	0.51 ± 0.04	0.70 ± 0.09†	0.56 ± 0.04*
Area 0–60 min	73 ± 8	72 ± 13	74 ± 8	52 ± 5	54 ± 9	57 ± 4
Area 0–360 min	356 ± 40	412 ± 35	376 ± 51	434 ± 30	545 ± 32*	449 ± 23*
Glucagon (pg/ml)						
Basal	58 ± 3	77 ± 13	65 ± 7	77 ± 7	81 ± 8	75 ± 4*
Area 0–60 min	389 ± 131	35 ± 282	542 ± 215	47 ± 234	126 ± 624	175 ± 200
Area 0–360 min	10,955 ± 1,333	5,658 ± 1,380	7,104 ± 1,644	3,354 ± 1,666†	3,257 ± 2,355†	4,150 ± 1,126†
Meal appearance (μmol · kg^{-1} · min^{-1})						
Total area 0–60 min	3,390 ± 237	3,080 ± 198	3,155 ± 283	2,753 ± 296	3,105 ± 219	2,929 ± 177
Total area 0–360 min	10,708 ± 599	10,181 ± 580	9,832 ± 1,031	9,065 ± 778	8,727 ± 722	9,149 ± 495
Glucose production (μmol · kg^{-1} · min^{-1})						
Basal	12.8 ± 0.4	12.5 ± 0.9	13.2 ± 0.7	12.7 ± 0.5	14.5 ± 0.9	13.3 ± 0.4
Area 0–60 min	−529 ± 67	−420 ± 61	−430 ± 111	−429 ± 56	−478 ± 70	−442 ± 41
Area 0–360 min	−2,965 ± 202	−2,835 ± 241	−2,972 ± 354	−3,119 ± 193	−3,040 ± 340	−3,067 ± 149
Glucose disapperance (μmol · kg^{-1} · min^{-1})						
Basal	12.8 ± 0.4	12.5 ± 0.9	13.2 ± 0.7	12.7 ± 0.5	14.5 ± 0.9	13.3 ± 0.4
Area 0–60 min	1,256 ± 138	961 ± 102	1,164 ± 105	716 ± 108†	539 ± 101†	770 ± 76†
Area 0–360 min	7,638 ± 460	7,251 ± 422	7,073 ± 998	6,417 ± 637	7,107 ± 775	6,733 ± 437

Data are means ± SE. Area denotes above or below basal. *$P < 0.05$, †$P < 0.001$ vs. NFG/NGT.

IGT and diabetes than in those with NGT, resulting in a comparable total area above basal during the 6 h of observation.

Indexes of insulin action. Net insulin action (S_i) and the ability of insulin to stimulate glucose uptake (S_i*) were measured following meal ingestion with the respective unlabeled and labeled oral minimal models (Fig. 5). S_i ($P < 0.005$) and S_i* ($P < 0.001$) were lower in the total group with IFG than in the subjects with NFG/NGT. Subgroup analysis indicated that S_i and S_i* were lower ($P < 0.005$) in subjects with NFG/IGT, IFG/IGT, and IFG/diabetes than in those with NFG/NGT. S_i and S_i* were also lower ($P < 0.05$) in the subjects with IFG/diabetes than in those with IFG/NGT. On the other hand, while S_i and S_i* were numerically lower in the subjects with IFG/NGT versus those with NFG/NGT, the differences were not significant.

Indexes of insulin secretion. Indexes of insulin secretion were measured following meal ingestion with the C-peptide minimal model, which enabled concurrent assessment with insulin action (Fig. 6). The overall response to glucose (Phi_{total}), the response to a change in glucose ($Phi_{dynamic}$), and the response to a given glucose level (Phi_{static}) did not differ in the total group with IFG and those with NFG/NGT. However, when DIs were calculated to determine if insulin secretion was appropriate for the prevailing level of insulin action, DI_{total} ($P < 0.01$), DI_{static} ($P = 0.01$), and $DI_{dynamic}$ ($P < 0.001$) were all lower in the total group with IFG than in those with NFG/NGT.

$Phi_{dynamic}$ did not differ among the subjects with NFG/NGT, IFG/NGT, and NFG/IGT but tended to be lower in those with IFG/IGT and IFG/diabetes. Phi_{total} and Phi_{static} also did not differ among the groups. However, the time required to reach Phi_{static} (37 ± 5 vs. 14 ± 2 min) was longer ($P < 0.001$) in those with IFG/diabetes than in those with NFG/NGT but did not differ among the other groups. On the other hand, when the appropriateness of insulin secretion for the prevailing level of insulin action was considered, DI_{total}, $DI_{dynamic}$, and DI_{static} all were lower in subjects with NFG/IGT ($P < 0.05$), IFG/IGT ($P < 0.01$), and IFG/diabetes ($P < 0.001$) than in those with NFG/NGT. Furthermore, DI_{total} and DI_{static} were also lower in subjects with NFG/IGT ($P < 0.01$), IFG/IGT ($P < 0.005$), and IFG/diabetes ($P < 0.001$) compared with those with IFG/NGT, and $DI_{dynamic}$ was lower ($P < 0.01$) in subjects with IFG/IGT and IFG/diabetes than in those with IFG/NGT. While DI_{total}, $DI_{dynamic}$, and DI_{static} did not differ in subjects with NFG/IGT and IFG/IGT, they tended to be lower ($P = 0.06$) in subjects with IFG/diabetes compared with those with NFG/IGT. On the other hand, none of the DIs differed in subjects with IFG/NFG and NFG/NGT.

DISCUSSION

People with pre-diabetes (i.e., IFG and/or IGT) are at increased risk of developing overt diabetes (1–3). The present study indicates that fasting EGP is inappropriately increased and glucose disappearance is inappropriately decreased in individuals with IFG when considered in light of the higher prevailing glucose and insulin concentrations. It therefore appears that both abnormalities contribute to IFG. On the other hand, EGP is promptly suppressed in individuals with IFG and/or IGT after meal ingestion.

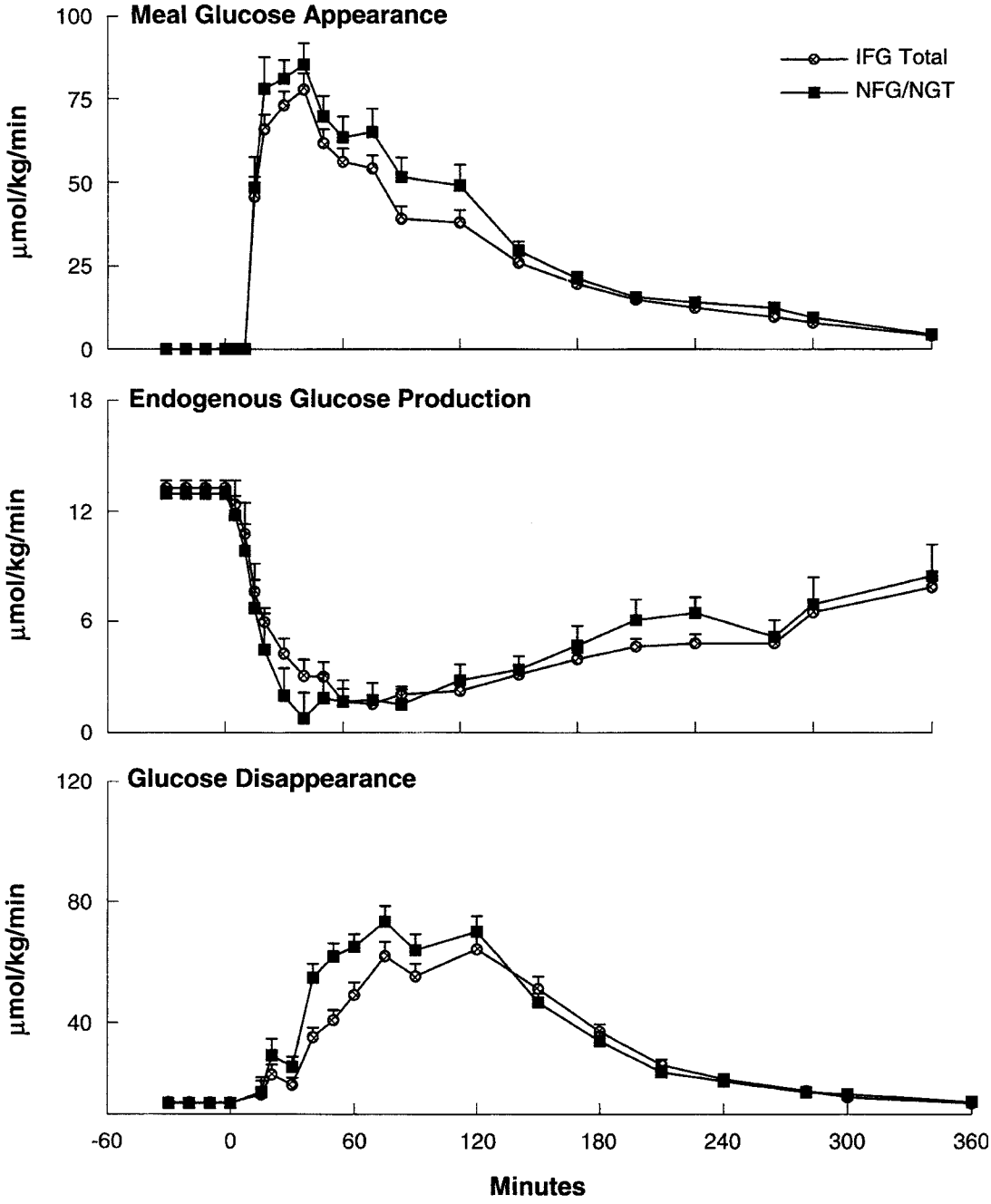

FIG. 3. Meal rates of appearance, EGP, and glucose disappearance observed in subjects with NFG/NGT or IFG. A mixed meal was ingested at time 0 min.

The rate of meal appearance also did not differ among groups, indicating that excessive glucose absorption and/or decreased hepatic glucose uptake does not cause postprandial hyperglycemia in individuals with pre-diabetes. In contrast, postprandial glucose disposal was decreased in individuals with IGT or diabetes, with the decrease being most evident in individuals who also had IFG. Both the defects in insulin secretion and the ability of insulin to stimulate glucose uptake contributed to the lower rates of disposal, since both were impaired in individuals with IGT or diabetes. On the other hand, postprandial EGP, meal appearance, glucose disposal, insulin secretion, and insulin action were all normal in individuals with isolated IFG, implying a set point abnormality with an intact β-cell response to food ingestion. Taken together, these data indicate that both the defects in insulin secretion and action contribute to postprandial hyperglycemia. They also indicate that the pattern of postprandial glucose metabolism is essentially normal in individuals with isolated IFG, perhaps presaging a lower risk of progression to overt diabetes.

Rates and disappearance of EGP did not differ in subjects with IFG and NFG before meal ingestion. However, since both glucose and insulin concentrations were higher in the former than in the latter, fasting EGP was inappropriately elevated and disappearance inappropriately reduced. Subgroup analysis indicated that the increase in fasting glucose production was most evident in subjects with the worst glucose tolerance (i.e., subjects with IFG/diabetes). This observation supports prior re-

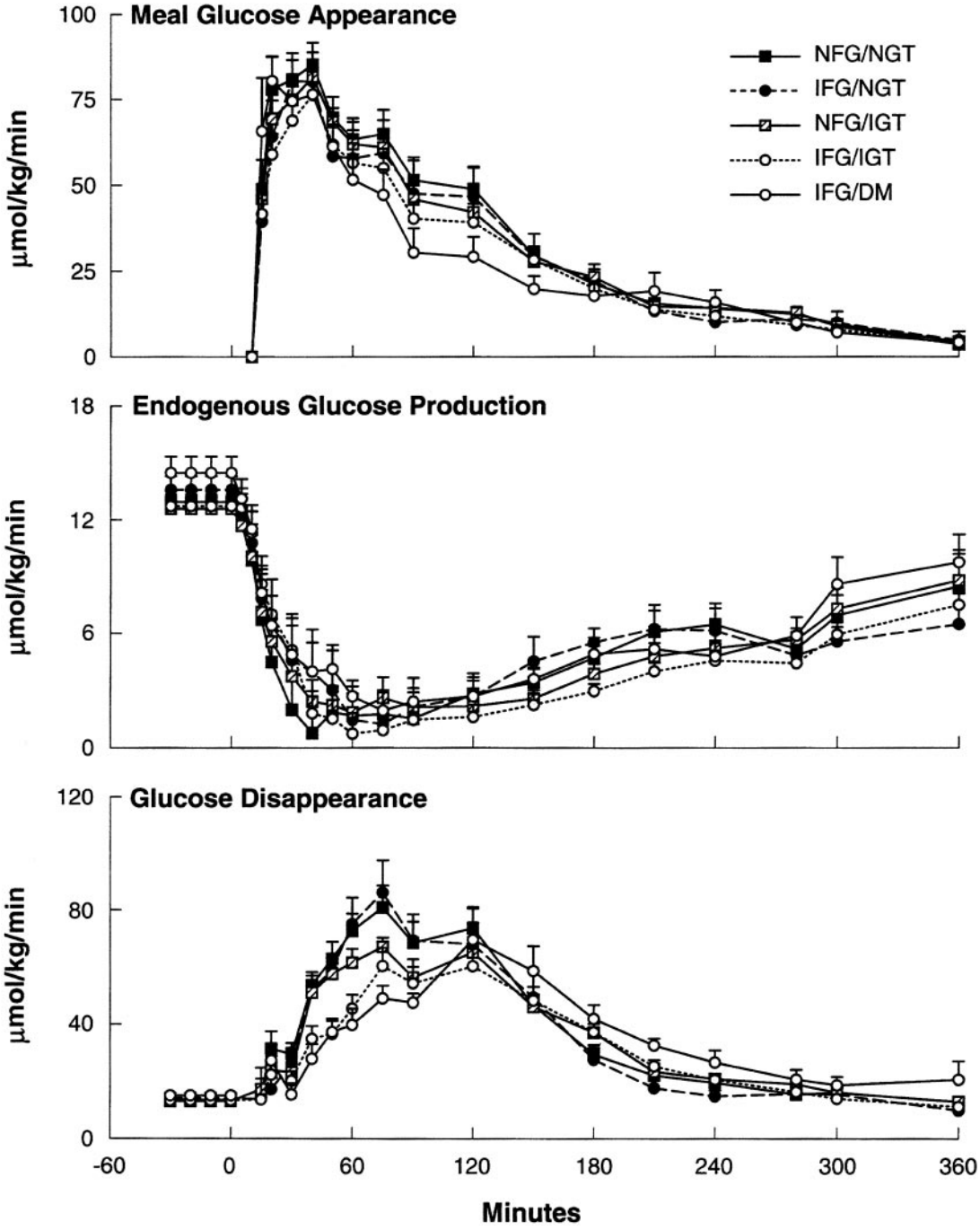

FIG. 4. Meal rates of appearance, EGP, and glucose disappearance observed in subjects with NFG/NGT, NFG/IGT, IFG/IGT, or IFG/diabetes. A mixed meal was ingested at time 0 min.

ports by Weyer et al. (7) and Lillioja et al. (33) that Pima Indians with IFG have both hepatic and extrahepatic insulin resistance. Of interest, fasting glucagon concentrations were higher in subjects with IFG than in subjects with NFG/NGT. The increase in fasting glucagon appeared to be most evident in the subjects with IFG/IGT and IFG/diabetes; however, the number of subjects in these subgroups was relatively small, and the differences were not significant. Nevertheless, these data suggest that glucagon may contribute to increased fasting glucose concentrations in individuals with IFG.

EGP rapidly suppressed in all groups, following meal ingestion, presumably reflecting the combined suppressive effects of the concurrent increases in glucose and insulin (9–13). While the rate of suppression during the 1st hour after food ingestion tended to be slower in subjects with IFG/diabetes, it did not differ significantly from that observed in subjects with NFG. This pattern is not dissimilar to the situation observed in individuals with overt diabetes who also ultimately suppress EGP to normal following food ingestion (34–37). However, since the preprandial rates are increased and defects in insulin secretion are more marked in individuals with "severe" diabetes, the time to suppression is delayed and excess amounts of glucose enter circulation. Taken together, these data lend additional support to the concept that hepatic insulin

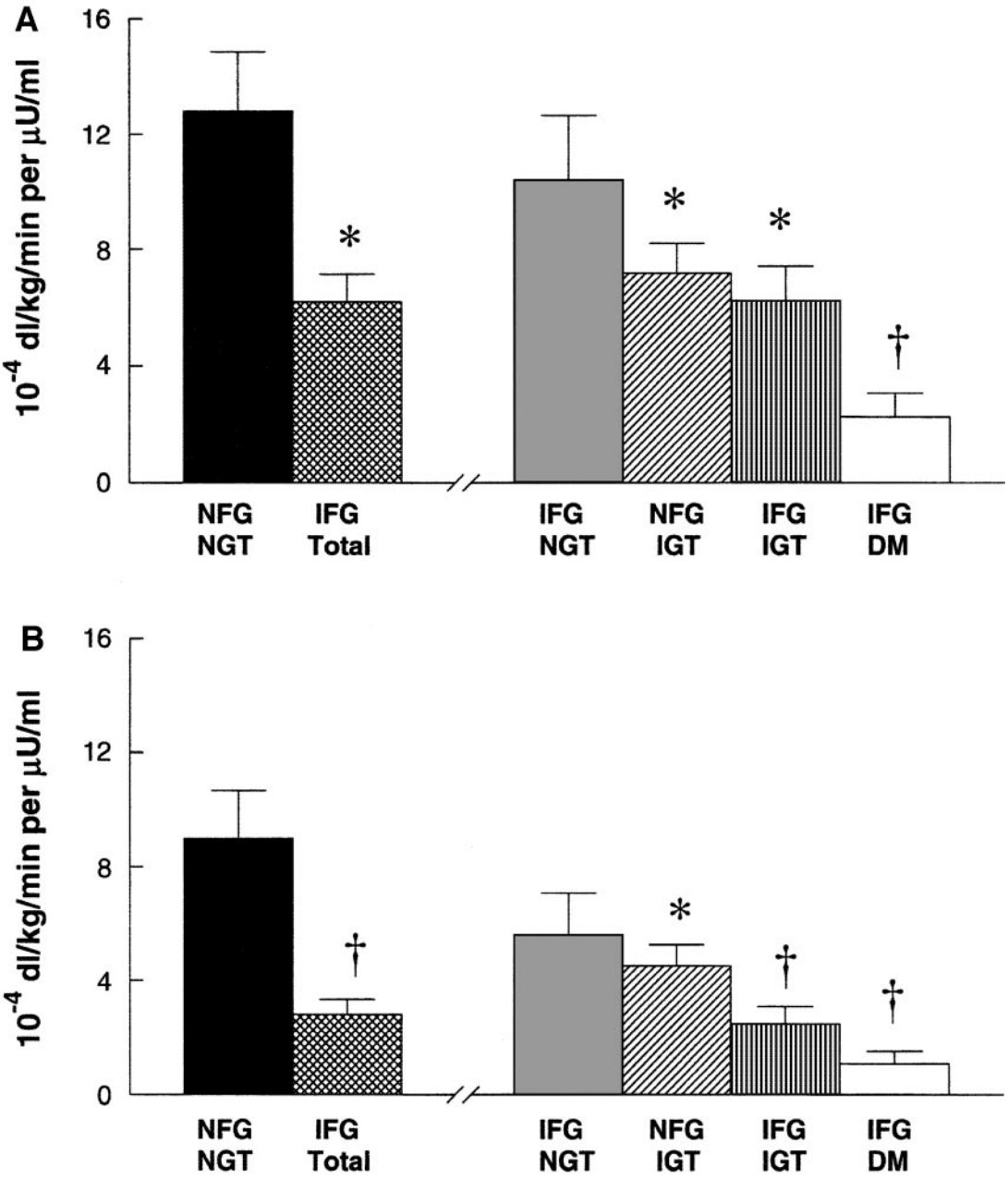

FIG. 5. Net insulin action (S_i) (*A*) and effect of insulin on glucose disposal (S_i*) (*B*) observed in subjects with NFG/NGT, IFG total, IFG/NGT, NFG/IGT, IFG/IGT, or IFG/diabetes. *$P < 0.05$, †$P < 0.001$ vs. NFG/NGT.

resistance occurs early in the evolution of type 2 diabetes. On the other hand, failure to appropriately suppress EGP is not the cause of postprandial hyperglycemia in individuals with IGT or early diabetes.

The systemic rate of appearance of the ingested glucose did not differ in subjects with IFG and NFG and, if anything, tended to be lower in the subgroups with IFG/IGT and IFG/diabetes, perhaps reflecting increased hepatic glucose uptake due to higher portal glucose concentrations. Therefore, increased meal glucose appearance was not the cause of the higher postprandial glucose concentrations in any of the groups. In contrast, postprandial glucose disappearance was lower in subjects with IFG immediately after meal ingestion, particularly during the 1st hour when the excessive rise in glucose occurred in subjects with IFG/IGT and IFG/diabetes. Of note, glucose disappearance in subjects with NFG/IGT during the 1st hour after meal ingestion only slightly reduced, perhaps accounting for the fact that the peak glucose concentration in this group did not differ from that in subjects with NFG/NGT. However, it took longer for glucose to return to preprandial concentrations, resulting in higher glucose concentrations at 2 h in subjects with NFG/IGT. Since insulin secretion was relatively intact in these individuals, whereas insulin action was markedly decreased, this postprandial pattern of change in glucose concentrations (and the OGTT pattern, since that is why subjects in this group were classified as having IGT) is consistent with previous reports indicating that a delay and decrease in early insulin secretion results in a higher peak glucose concentration, whereas a defect in insulin action results in an increased duration of hyperglycemia (38,39). Of interest, subjects

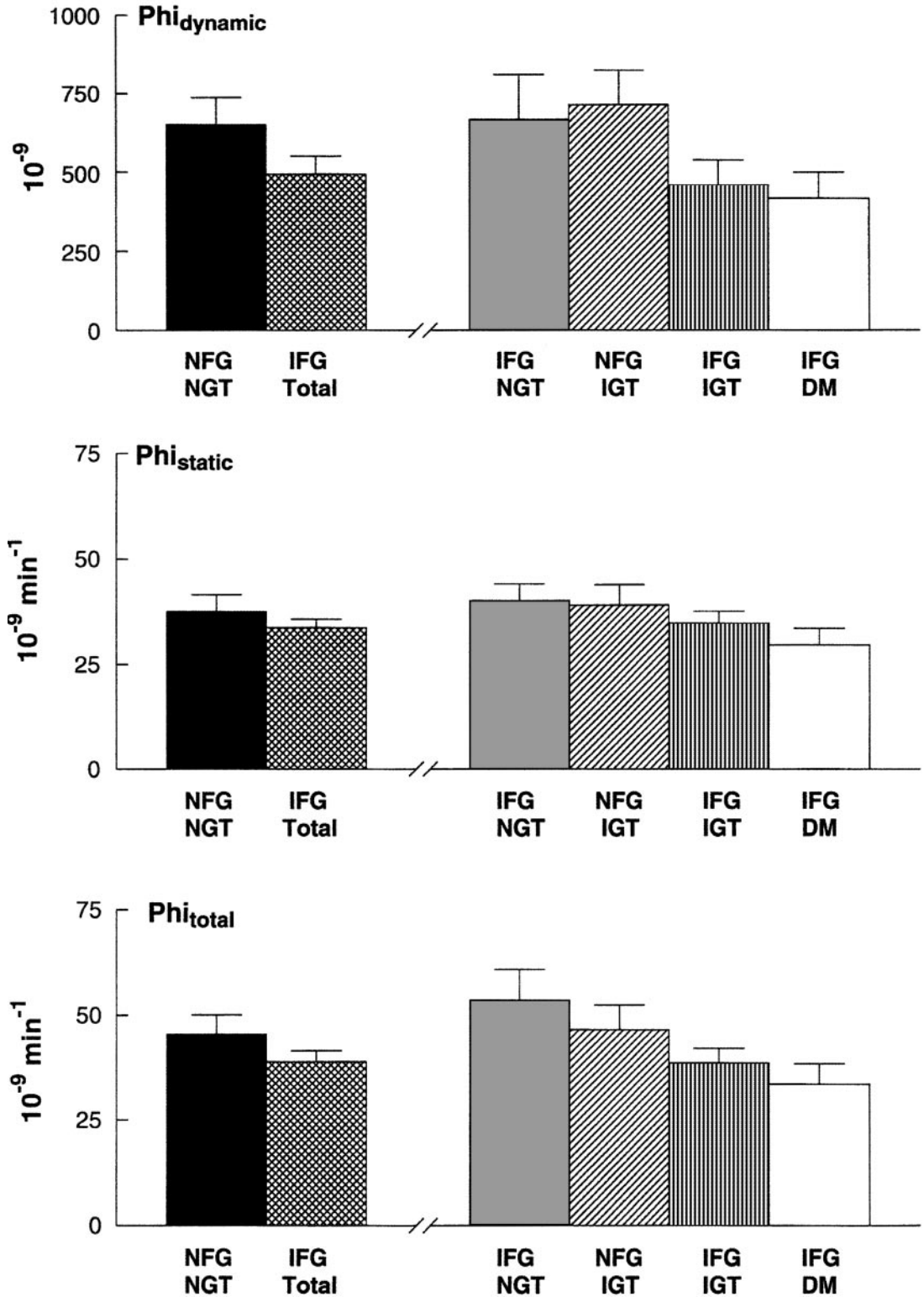

FIG. 6. Insulin secretion indexes observed in subjects with NFG/NGT, IFG total, IFG/NGT, NFG/IGT, IFG/IGT, or IFG/diabetes. *$P < 0.05$, †$P < 0.001$ vs. NFG/NGT.

with IFG/IGT and IFG/diabetes had both a higher peak glucose concentration and a more prolonged duration of hyperglycemia. This occurred since they were both insulin resistant and had a severe defect in their ability to secrete insulin in response to the rapid increase in glucose, which occurred immediately after meal ingestion.

Combined use of the oral minimal and C-peptide models enabled indexes of insulin action and insulin secretion to be simultaneously measured under physiologic conditions in the same individual. This is important since insulin action is context dependent in that it differs depending on how much insulin is given and the pattern in which it is given (40–42). Therefore, the observation that net insulin action (S_i) was decreased in subjects with IFG following

meal ingestion is reassuring and consistent with previous studies that have measured net insulin action in the fasting state using the homeostasis model assessment method (43–48), following intravenous glucose injection or during insulin infusion (7,49). The labeled oral minimal model established that the decrease in net insulin action was due, at least in part, to a decrease in the ability of insulin to stimulate glucose uptake (S_i*). Subgroup analysis indicated that this was primarily due to reduced insulin action in subjects with IGT or diabetes, irrespective of their fasting glucose concentration. On the other hand, insulin action did not differ significantly in subjects with NFG/NGT and IFG/NGT.

$DI_{dynamic}$, which assesses the appropriateness of insulin secretion in response to a change in glucose, was impaired in all of the subjects with IGT, with the severity of the defect increasing as glucose tolerance deteriorated. To our knowledge, the only other study examining a similar aspects of insulin secretion is that of Ferrannini et al. (50). The authors used a model to evaluate insulin secretion in individuals with IGT during an OGTT, which was similar to the model used in the present experiments. They reported that while the static response to insulin was decreased in their subjects with IGT, the dynamic response to glucose was intact. However, this discrepancy is likely more apparent than real since the appropriateness of the dynamic response was not considered in light of the ~50% reduction in insulin action in subjects with IGT. Furthermore, their IGT group contained subjects with both NFG and IFG. As is evident in the present studies, the impairment in $DI_{dynamic}$ is more marked in individuals with both IFG and IGT than in those with IGT alone, which likely accounts for the higher peak postprandial glucose concentrations in the former than in the latter (see Fig. 2).

The static response was decreased in all subjects with IGT. This observation is consistent with the previous report of Ferraninni et al. (50), which showed that the static response to glucose is decreased during an OGTT in individuals with IGT. The static response to glucose evaluates the amount of insulin that is secreted at any given level of glucose and therefore has been referred to as "glucose sensitivity." Since this response occurs throughout the entire 6 h of study, it presumably is influenced by insulin synthesis and processing, as well as more distal steps in the insulin secretion pathway. The model used in the current experiments indicates that there was a delay between the time when glucose reaches a given level and when the static response achieves a steady state. This time (t in the model) averaged ~10 min in subjects with NFG/NGT and tended to be slightly increased (~15 min) in subjects with IGT. In contrast, it was markedly prolonged in subjects with IFG/diabetes, averaging ~35 min. Of interest, in vitro studies suggest that ~8–10 min are required for insulin granules in the storage pool to move to the plasma membrane, dock, and become primed for exocytosis (50–53). It is interesting to speculate that the activation of this process is required for the static response to glucose to achieve a steady state and that the development of diabetes either is exacerbated by or causes a delay in the rate at which insulin granules in the storage pool are primed for secretion.

The results of subjects with IFG/NGT are particularly intriguing. Despite fasting hyperglycemia, postprandial glucose concentrations were virtually identical to those in subjects with NFG/NGT. Since preprandial glucose concentrations were higher, the postprandial increment in glucose concentration was lower than in subjects with NFG/NGT (Fig. 2 and Table 2). This was not due to reduced glucose absorption or increased hepatic glucose uptake, since the rate of appearance of ingested glucose was virtually identical in both groups, as were postprandial changes in EGP and disposal. Insulin action was slightly, but not significantly, lower in subjects with IFG/NGT compared with those with NFG/NGT, perhaps because of greater visceral adiposity in the former. Indexes of insulin secretion including the dynamic and static responses to glucose were normal whether assessed as actual responses (Fig. 6) or when corrected for the degree of insulin resistance by calculating DIs (Fig. 7). Preprandial rates of EGP and disappearance did not differ in subjects with IFG/NGT and NFG/NGT. However, in contrast to subjects with IFG/IGT and IFG/diabetes, fasting insulin concentrations were not increased, making it difficult to invoke insulin resistance as the cause of fasting hyperglycemia. On the other hand, since glucose production and disappearance did not differ in subjects with IFG/NGT and NFG/NGT and since, as noted above, even small increases in glucose concentrations result in suppression of glucose production (14,15), this implies that glucose concentration continued to increase until what has recently been referred to as "allostasis" (54) was again achieved at a higher glucose concentration.

The observations that fasting insulin concentration was normal, despite a higher fasting glucose concentration, and that insulin secretion in response to a meal-related rise in glucose concentration was also normal argue for a higher "set point" in the subjects with IFG/NGT. A decrease in β-cell glucokinase activity in subjects with IFG/NGT could provide an explanation for this pattern of response (39). However, none of subjects with IFG/NGT had a family history of diabetes, which is in contrast to the subjects with IFG/IGT and IFG/diabetes, whereas 50–60% of the subjects had a first-degree relative with diabetes. Since subjects with a defect in glucokinase activity generally have a family history of diabetes, the lack of family history in the subjects with IFG/NGT reduces the likelihood of such a defect. Nevertheless, the cause and potential consequences (e.g., relative risk of subsequently developing diabetes) of elevated fasting but normal postprandial glucose concentrations in these individuals clearly warrant further study.

The present study has certain limitations. The subjects were healthy, had no history of vascular disease, and were only modestly obese (BMI ~31 kg/m^2). More marked abnormalities in insulin secretion, action, and postprandial glucose turnover may be present in more obese individuals or in those with other comorbid conditions. Insulin secretion and action were significantly reduced in subjects with IGT and diabetes but not in subjects with IFG/NGT compared with those with NFG/NGT. Lack of statistical difference always raises the issue of power. Insulin action as reflected by S_i and S_i* was 20 and 35% lower in subjects with IFG/NGT than in those with NFG/NGT. Assuming the variability in insulin action in these groups would be the same in subsequent studies, we estimate that 143 and 53 additional subjects with IFG/NGT would have to be studied for this difference to become statistically significant. Therefore, if subjects with IFG/NGT have a defect in insulin action, it appears to be subtle. This of course does not exclude the possibility that defects in insulin action and/or insulin secretion subsequently will develop if glucose tolerance deteriorates over time.

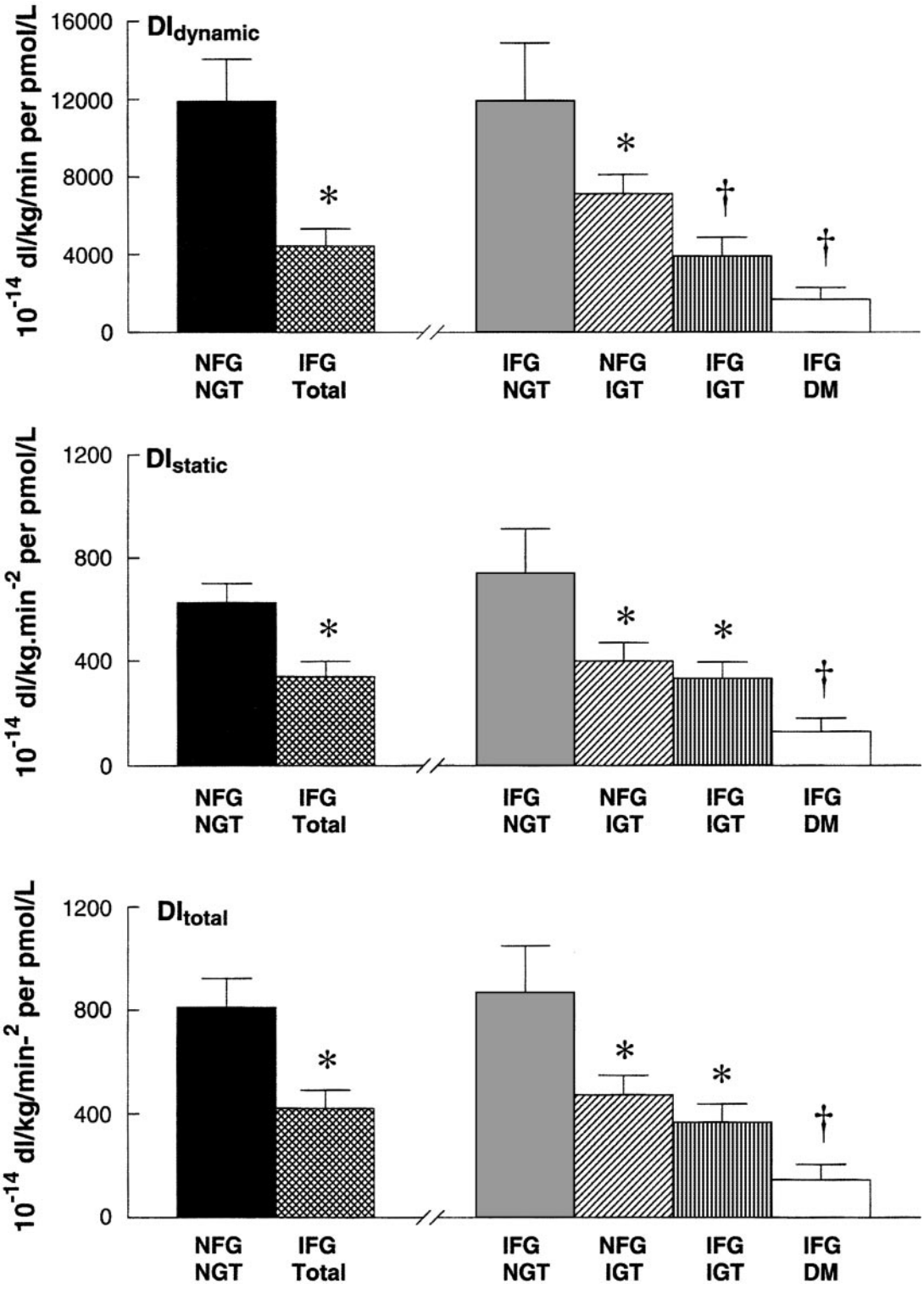

FIG. 7. Disposition indexes observed in subjects with NFG/NGT, IFG total, IFG/NGT, NFG/IGT, IFG/IGT, or IFG/diabetes. *$P < 0.05$, †$P < 0.001$ vs. NFG/NGT.

In summary, when considered as a group, glucose increased to higher concentrations in individuals with IFG following ingestion of a carbohydrate-containing mixed meal than in individuals with NFG. The excessive rise in glucose was due to lower rates of glucose disposal, since postprandial suppression of EGP and the systemic rate of appearance of the ingested glucose did not differ in individuals with IFG or NFG. Subgroup analysis indicated that postprandial glucose disappearance progressively decreased as glucose tolerance deteriorated, being lowest in individuals with IFG/IGT or IFG/diabetes. Insulin secretion and action were most impaired in these individuals, presumably accounting for the reduction in disposal. Insulin secretion and action also were impaired in individuals with NFG/IGT; however, the defects were less severe and therefore resulted in a smaller reduction in postpran-

dial glucose disposal. On the other hand, insulin secretion, action, and the postprandial pattern of glucose turnover were virtually normal in individuals with isolated IFG, suggesting that the set point but not the subsequent response to glucose was abnormal in these individuals. Thus, it appears that there is substantial heterogeneity in the regulation of postprandial glucose metabolism in individuals with IFG and/or IGT. This implies there are differences in the pathogenesis of pre-diabetes and therefore differences in the risk of subsequently developing diabetes and/or differences in response to therapeutic agents that seek to prevent diabetes.

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