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Adaptation of β -Cell and Endothelial Function to Carbohydrate Loading: Influence of Insulin Resistance



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High-carbohydrate diets have been associated with β -cell strain, dyslipidemia, and endothelial dysfunction. We examined how β -cell and endothelial function adapt to carbohydrate overloading and the influence of insulin resistance. On sequential days in randomized order, nondiabetic subjects (classified as insulin-sensitive [IS] [$n = 64$] or insulin-resistant [IR] [$n = 79$] by euglycemic clamp) received four mixed meals over 14 h with either standard (300 kcal) or double carbohydrate content. β -Cell function was reconstructed by mathematical modeling; brachial artery flow-mediated dilation (FMD) was measured before and after each meal. Compared with IS, IR subjects showed higher glycemia and insulin hypersecretion due to greater β -cell glucose and rate sensitivity; potentiation of insulin secretion, however, was impaired. Circulating free fatty acids (FFAs) were less suppressed in IR than IS subjects. Baseline FMD was reduced in IR, and postprandial FMD attenuation occurred after each meal, particularly with high carbohydrate, similarly in IR and IS. Throughout the two study days, higher FFA levels were significantly associated with lower (incretin-induced) potentiation and impaired FMD. In nondiabetic individuals, enhanced glucose sensitivity and potentiation upregulate the insulin secretory response to carbohydrate overloading. With insulin resistance, this adaptation is impaired. Defective suppression of endogenous FFA is one common link between impaired potentiation and vascular endothelial dysfunction.

The physiologic response to a high carbohydrate load involves changes in glucose fluxes (1) and blood flow distribution (2) and an increase in insulin secretion (3). However, how the β -cell achieves such adaptation has not been investigated. Thus, the contribution of enhanced glucose sensing (4–6) and incretin-induced potentiation (7,8) to insulin hypersecretion are not established in vivo. Furthermore, it is not known whether the metabolic responses to an augmented carbohydrate challenge are different in individuals with obesity/insulin resistance (9), who have already undergone long-term changes in pancreatic islet mass (10,11), structure (12), and function (3,12). The first aim of the current work therefore was to characterize the β -cell response to lower versus higher carbohydrate loading in nondiabetic subjects classified as insulin sensitive (IS) or insulin resistant (IR) (based on an insulin clamp) under controlled conditions simulating free living.

Another important potential consequence of high carbohydrate loading is vascular endothelial dysfunction, an early step toward atherosclerosis (13–16). Insulin activation of the phosphatidylinositol 3-kinase–Akt pathway also activates endothelial nitric oxide (NO) synthase, resulting in vasodilation (17). Insulin resistance of the glucose pathway is associated with resistance of the NO-mediated vasodilation in healthy subjects (18,19), in subjects with type 2 diabetes (20), and in subjects with essential hypertension (21). In insulin-resistant states, plasma glucose and

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free fatty acids (FFAs) are among the potential mediators of endothelial dysfunction (19). In fact, in man, acutely raising plasma glucose (by an intravenous glucose infusion [22–24]) or plasma FFA (by an intravenous infusion of a lipid emulsion [25,26]) induces endothelial dysfunction. Conversely, reduction of postchallenge hyperglycemia prevents acute endothelial dysfunction in subjects with impaired glucose tolerance (27). However, whether and how these negative influences operate under free-living conditions of repeat meal ingestion and carbohydrate loading and whether their impact differs by level of insulin sensitivity are not known.

Thus, the second aim of the present work was to measure endothelial function in response to meals with low or high carbohydrate content in IS and IR subjects.

RESEARCH DESIGN AND METHODS

Participants

Participants were recruited via flyer advertisement. Study eligibility criteria included the following: age 18–55 years, no nicotine use in the past year, no history of or current dependency on substance or alcohol, no prescribed medication, and negative pregnancy testing. The protocol was approved by the institutional review board of the University of Miami, and written informed consent was obtained from participants prior to inclusion.

Procedures

The protocol involved three visits: a screening session to confirm study eligibility, a euglycemic-hyperinsulinemic clamp, and a 3-day/night inpatient laboratory stay with two meal challenge days (standard vs. double carbohydrate load).

Visit 1

Urine was spot tested with a standard toxicology screen, waist circumference was measured at the level of the umbilicus, and participants were scheduled for visit 2 within 2–4 weeks.

Visit 2

In the overnight-fasted state, participants received fasting blood draws and the clamp procedure. Subjects were then scheduled for visit 3 within 1–3 weeks.

Visit 3

On day 1, subjects received a standard 75-g, 3-h oral glucose tolerance test (OGTT). At mid-afternoon, subjects underwent a computed tomography (CT) scan, had dinner at 6:00 P.M. and a light snack at 9:00 P.M., and then fasted overnight. On this day and throughout the inpatient days, subjects remained in their private room, free to move about, read, watch television, or take naps; water was provided ad libitum. Subjects were not permitted to leave the laboratory and had no access to external food sources. On days 2 and 3, subjects were given a meal every 3.5 h over 14 h, totaling four meals per day (at 8:00 A.M., 11:30 A.M., 3:00 P.M., and 6:30 P.M.). At 7:00 A.M. on each day, an indwelling intravenous catheter was inserted for serial

blood sampling (at –15, 0, 15, 30, 60, 90, 120, 150, and 180 min after subjects completed the meal within 15 min). Ultrasound assessment of flow-mediated dilation (FMD) was performed at –15 min premeal and 60 min after each meal.

The D-glucose content of each meal was the same as the OGTT (i.e., 75 g, 300 kcal) on one day and double (600 kcal) on the other day in randomized order. Fat and protein calories were kept identical across meals but adjusted on the basis of body weight and sex, approximating the U.S. national criteria ($38\text{--}41 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for adult men and $35\text{--}38 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for adult women, 35% fat, 15% protein). Thus, on one day a 75-kg man would receive 1,200 kcal as glucose, 300 kcal as rice, and the remaining 1,500 kcal as fat and protein; on the double carbohydrate-loading day, this person would receive 2,700 carbohydrate kcal and the same 1,500 kcal from fats and proteins. Fats and proteins were provided by hamburger and sausage. Food consumption was verified by staff observation, and any uneaten food was weighed and logged.

Euglycemic Clamp

As described previously (15), steady-state hyperinsulinemia was achieved by a primed constant infusion of Humulin-R (Lilly, Indianapolis, IN) at a rate of $40 \text{ mU} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ for 150 min. Plasma glucose was clamped at within 5% of the fasting value by feedback-controlled infusion of 20% dextrose. Whole-body glucose disposal rate (M) (in milligrams per minute per kilogram) was calculated as the mean of consecutive 20-min periods and applying a space correction factor. An insulin sensitivity level of $M = 4.5 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ was chosen to index insulin resistance (3).

Abdominal Fat Measurement

Abdominal fat was quantitated by multislice CT (five slices every 5 cm above and two slices at 5 and 10 cm below the L4–5 interspace using 10-mm-thick slices) using a Siemens Somatom-Sensation-16 scanner (Siemens, Malvern, PA). Quantitation used a seeding program with a Siemens WorkStream Wizard workstation. Subcutaneous (subcutaneous adipose tissue) and visceral (visceral adipose tissue) fat, in kilograms, was derived using a triangulation formula multiplied by 0.9391 mg/mL.

Blood Assays

Serum cholesterol and triglycerides were measured enzymatically using an autoanalyzer (Cobas Mira Plus; Roche Diagnostics, Branchburg, NJ). HDL cholesterol was measured after precipitation with dextran sulfate, and LDL cholesterol was calculated by the Friedewald formula. C-reactive protein was measured by particle-enhanced immunoturbidimetry (cobas 6000 analyzer; Roche Diagnostics). Plasma glucose was obtained using a YSI 2300 STAT Plus analyzer (Yellow Springs Instruments, Yellow Springs, OH). Serum insulin was determined by a radioimmunoassay insulin-specific kit (Linco Research, St. Charles, MO), with sensitivity of $2 \mu\text{U}/\text{mL}$, intra-assay coefficient of variation (CV) 4–8%, and interassay CV 6–11%. C-peptide

was assayed by chemiluminescence (cobas e 411; Roche Diagnostics).

β -Cell Function

β -Cell function was assessed from the OGTT and the multiple meal tests using a model that describes the relationship between insulin secretion and glucose concentration, which has been illustrated in detail previously (28). Briefly, the model expresses insulin secretion (in picomoles per minute per square meter) as the sum of two components. The first component represents the dependence of insulin secretion on absolute glucose concentration at any time point during the test through a dose-response function relating the two variables. A characteristic parameter of the dose response is the mean slope over the observed glucose range, denoted as β -cell glucose sensitivity. The dose-response is modulated by a potentiation factor, which accounts for differences in glucose-induced insulin secretion between early and late phases of the test. The potentiation factor thus encompasses several potentiating mechanisms (prolonged hyperglycemia, nonglucose substrates, gastrointestinal hormones, neural modulation). For quantification of the potentiation factor, the ratio between the average value between 1 and 14 h and the average value from 0 to 20 min was calculated. This ratio is denoted as the potentiation ratio. The second insulin secretion component represents the dependence of insulin secretion on the rate of change of glucose concentration. This component is termed derivative component, and is determined by a single parameter, denoted as rate sensitivity. Rate sensitivity is related to early insulin release. The model parameters were estimated from glucose and C-peptide concentrations by regularized least squares, as previously described (28). Insulin secretion rates were calculated from the model every 5 min with the use of C-peptide deconvolution analysis as previously described (29).

FMD

A B-mode scan of the right brachial artery was obtained in the longitudinal plane about 5 cm above the elbow by use of an 11-MHz linear array ultrasound transducer (Sonos 5500; Philips) held in place by stereotactic clamp. Fine image adjustments were made as needed by means of micromanipulators attached to the mount permitting horizontal and vertical axis and rotation movement. A referencing system was used to position the probe to ensure within and intersession consistency. Arterial flow was manipulated by a pneumatic cuff affixed to the forearm distal to the arterial segment being imaged. Cuff inflation (60 mm Hg suprasystolic) and deflation were performed by an electronic controller, and recordings were made for 1.5 min of baseline, 5 min of inflation, and 5 min after deflation. The image analysis employed in-house software (LabVIEW v8.2.1; National Instruments) that obtained ~ 150 paired measurements along a 30-mm arterial wall length at 30-ms intervals. This measurement frequency provided change in diameter through the cardiac cycle and, hence, reliable artifact rejection. The percent

change in arterial diameter during diastole at peak response was derived. Arterial diameter measurement reproducibility was previously assessed within session (2 h apart) and between sessions (1 day apart); reliabilities were very high for within session (diastolic $r = 0.997$, CV 1.6%) and between sessions (diastolic $r = 0.991$, CV 2.6%).

Statistical Analysis

Values are given as means \pm SE or median (interquartile range) for variables with a normal or nonnormal distribution, respectively. Group comparison (IS vs. IR) was carried out by Mann-Whitney test. ANCOVA for repeated measures (low vs. high carbohydrate loading) was used to compare variables across groups (IS vs. IR); this model was further adjusted for meal-loading sequence. ANCOVA for doubly repeated measures (over time and meal loading) was used for time course of continuous variables. Multiple regression was performed using standard methods. Cross-correlation was used to compare the time course of plasma FFA with those of FMD and potentiation as a function of a time shift (SPSS-20). Because both FMD and potentiation were strongly related to plasma glucose levels in a reciprocal fashion ($r = -0.44$ and $r = -0.19$, respectively; $P < 0.01$ for both), all cross-correlation analyses were also run between the respective residuals against glucose values. Statistical significance was set at $\alpha < 0.05$.

RESULTS

The final cohort consisted of 143 subjects who completed the full protocol (Supplementary Fig. 1). Of these, 79 persons (55%) were classified as IS (M value 7.23 [3.87] $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) and 64 as IR (M value 2.99 [1.47] $\text{mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$). Although well-matched for sex, age, ethnicity, socioeconomic status and smoking habits, the IR group differed from the IS group in all other clinical/metabolic characteristics (Table 1).

OGTT

Plasma glucose and insulin responses were higher in IR than IS subjects, and plasma FFAs were less suppressed (Table 2). Insulin secretion was $\sim 40\%$ higher in IR than IS both under basal conditions (i.e., insulin secretion at 5 mmol/L glucose) and after glucose ingestion. Thus, over the 3 h of the OGTT, IR subjects released a median 10.2 units/ m^2 of insulin vs. 6.9 units/ m^2 for IS subjects. Potentiation was significantly worse in IR than IS subjects.

Meals

Plasma glucose and insulin responses were higher on the 600-g than 300-g carbohydrate/day regimen and higher in IR than IS subjects (Table 2; Fig. 1). Of note, the glucose responses to the high-carbohydrate meals showed peaks almost simultaneous with those for the low-carbohydrate meals in both groups but slower declines after the peaks, with a shoulder-like pattern. In addition, the glucose response to the first morning meal was higher than the responses to subsequent meals. The daily profiles of insulin secretion closely paralleled the plasma glucose time

Table 1—Clinical characteristics of the IS and IR groups

	IS (n = 64)	IR (n = 79)	P†
M value (mg · min ⁻¹ · kg ⁻¹)	7.78 ± 0.24	2.71 ± 0.08	***
Age (years)	37 ± 1	40 ± 1	ns
Sex (% men)	64	66	ns
Ethnicity (%)			ns
Black	14.4	17.7	
Hispanic white	70.3	77.2	
Non-Hispanic white	10.9	2.5	
Former smoker (%)	10.9	19.0	ns
Annual income (\$)‡	13.5 ± 1.8	14.6 ± 2.1	ns
Prediabetes (%)	20	47	***
Education (years)	13.1 ± 0.3	13.4 ± 0.3	ns
BMI (kg · m ⁻²)	26.3 ± 0.4	31.2 ± 0.5	***
Waist girth (cm)	88 ± 1	104 ± 1	***
VAT (kg)	2.6 ± 0.2	4.4 ± 0.2	***
SAT (kg)	5.6 ± 0.3	9.3 ± 0.4	***
Systolic/diastolic blood pressure (mmHg)	114 ± 2/78 ± 2	120 ± 2/84 ± 1	**
Triglycerides (mmol/L)	1.2 ± 0.1	1.9 ± 0.1	***
LDL-C (mmol/L)	3.0 ± 0.1	3.2 ± 0.1	ns
HDL-C (mmol/L)	1.3 ± 0.04	1.1 ± 0.03	***
hs-CRP (mg/L)	1.4 ± 0.2	3.7 ± 0.5	*
Fasting FFA (μmol/L)	443 ± 17	533 ± 21	**
HbA _{1c} (%) (mmol/mol)	5.3 ± 0.04 (34 ± 1)	5.5 ± 0.05 (37 ± 1)	*

Data are means ± SE unless otherwise indicated. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; ns, not significant by Mann-Whitney test; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. † χ^2 or *t* test for group differences. ‡In thousands. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

courses (Fig. 2), with significantly higher insulin secretion rates with high- than low-carbohydrate meals and higher secretion in IR than IS participants (Table 3). In particular, insulin secretion at 5 mmol/L glucose—a concentration that approximates fasting insulin secretion rate—was similar across OGTT and low-carbohydrate

and high-carbohydrate meals in either group and consistently higher in IR than IS. In both groups, the insulin output elicited by each of the low-carbohydrate meals over 3-h periods was roughly similar to that of the 3-h OGTT (Supplementary Fig. 2), indicating a limited contribution of the noncarbohydrate content of the meals to

Table 2—Metabolic parameters during the OGTT in the IS and IR groups

	IS (n = 64)	IR (n = 79)	P†
FPG (mmol/L)	4.83 ± 0.05	5.07 ± 0.07	**
FPI (pmol/L)	52 (30)	86 (54)	***
AUC _G (mol · L ⁻¹ · h)	1.02 ± 0.02	1.26 ± 0.03	***
AUC _I (mol · L ⁻¹ · h)	40.9 ± 2.6	86.3 ± 5.9	***
AUC _{FFA} (mol · L ⁻¹ · h)	44.2 ± 1.7	54.2 ± 2.5	**
ISR _{5mM} (pmol · min ⁻¹ · m ⁻²)	85 (44)	117 (52)	**
Total IS (nmol · m ⁻²)	46 (22)	68 (25)	***
β-GS (pmol · min ⁻¹ · m ⁻² · [mmol/L] ⁻¹)	108 (63)	102 (56)	ns
Rate sensitivity (nmol · m ⁻² · [mmol/L] ⁻¹)	1.16 (0.84)	1.26 (0.87)	ns
Potential (ratio)	1.52 (0.70)	1.33 (0.42)	**

Data are means ± SE or median (interquartile range). Potential (ratio) = (1–14 h)/(0–20 min). AUC_G, glucose AUC; AUC_I, insulin AUC; β-GS, β-cell glucose sensitivity; FPG, fasting plasma glucose; FPI, fasting plasma insulin; ISR_{5mM}, insulin secretion at 5 mmol/L glucose; ns, not significant; total IS, total insulin secretion. † χ^2 or Mann-Whitney test for group differences; ***P* < 0.01; ****P* < 0.001.

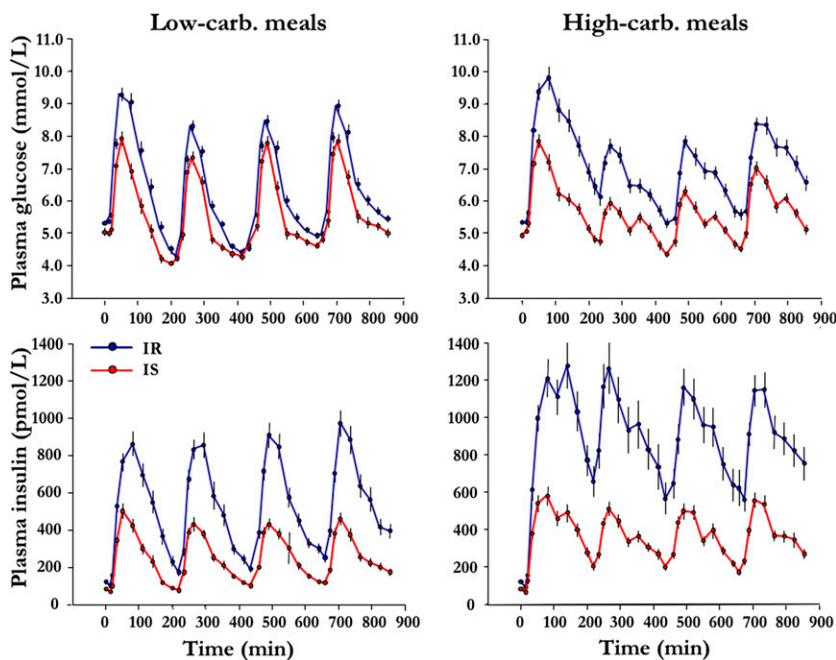


Figure 1—Plasma glucose and insulin concentrations during low and high carbohydrate (carb.) loading in IS and IR subjects. Plasma glucose (top) and insulin concentrations (bottom) in 64 IS and 79 IR subjects in response to four low-carbohydrate (300 g total) and high-carbohydrate (600 g total) meals over 14 h. Plots represent means \pm SEM.

insulin production. Therefore, the daily insulin output on the low-carbohydrate day averaged approximately four times the OGTT-stimulated insulin output. With the high carbohydrate load, however, 14-h insulin output was substantially less than eight times the OGTT-stimulated output; these data suggest that total insulin output would plateau at 250–300 nmol \cdot m² in the IS group and at roughly twice that level in the IR group (Table 3 and Fig. 3). During both the low- and high-carbohydrate day, total insulin output was reciprocally related to insulin sensitivity in the pooled groups, with the high-carbohydrate values lying above the low-carbohydrate values at each level of insulin sensitivity (Supplementary Fig. 3).

β -Cell Function

Both β -cell glucose sensitivity and rate sensitivity were better in IR than IS groups on both carbohydrate load days; notably, glucose sensitivity, but not rate sensitivity, improved with high-carbohydrate loading (Table 3; Fig. 4). Potentiation, however, was impaired in the IR compared with IS group despite the fact that the high carbohydrate load greatly increased potentiation compared with the low carbohydrate load in both groups (Fig. 3). By multivariate analysis, mean plasma glucose concentrations over the two 14-h periods were simultaneously dependent on carbohydrate load (positively, resulting in a plasma glucose difference of 0.45 mmol/L on average) and negatively on insulin sensitivity (-0.55 mmol/L), glucose sensitivity (-0.60 mmol/L), and potentiation (-0.40 mmol/L) (Supplementary Fig. 4). Furthermore, a significant ($P = 0.001$)

interaction term between carbohydrate load and potentiation indicated that the defect in potentiation was amplified by the high carbohydrate load. On balance, β -cell compensation for insulin resistance did not control glucose tolerance in the IR as effectively as in the IS group; thus, in IR subjects the percentage of plasma glucose values ≥ 11.1 mmol/L rose from 2.3 to 6% on doubling the carbohydrate load, whereas it declined from 0.6 to 0.2% in the IS group (Supplementary Fig. 5).

FFA

Plasma FFAs were suppressed in-phase with the plasma insulin surges after each meal on the low-carbohydrate day but remained higher in IR (FFA area under the curve [AUC_{FFA}] = 41.7 ± 1.4 mmol \cdot L⁻¹ \cdot h) than IS (36.1 ± 1.6 mmol \cdot L⁻¹ \cdot h, $P < 0.02$) subjects. On the high-carbohydrate day, AUC_{FFA} (31.6 ± 1.1 mmol \cdot L⁻¹ \cdot h) were more markedly suppressed than with the low carbohydrate load (46.3 ± 1.1 mmol \cdot L⁻¹ \cdot h, $P < 0.02$).

By cross-correlation, plasma FFAs were significantly related to potentiation in a reciprocal fashion ($r = -0.32$, $P < 0.0001$) with an optimal time shift of 67 min (Fig. 5). The cross-correlation maintained statistical significance ($r = -0.30$, $P < 0.0001$) after adjusting for concomitant plasma glucose values. Similar results were obtained when cross-correlating data from the high-carbohydrate day (not shown).

FMD

FMD measurements ($n = 1,722$ in total) were obtained in 82 subjects (39 from the IS and 43 from the IR group) at

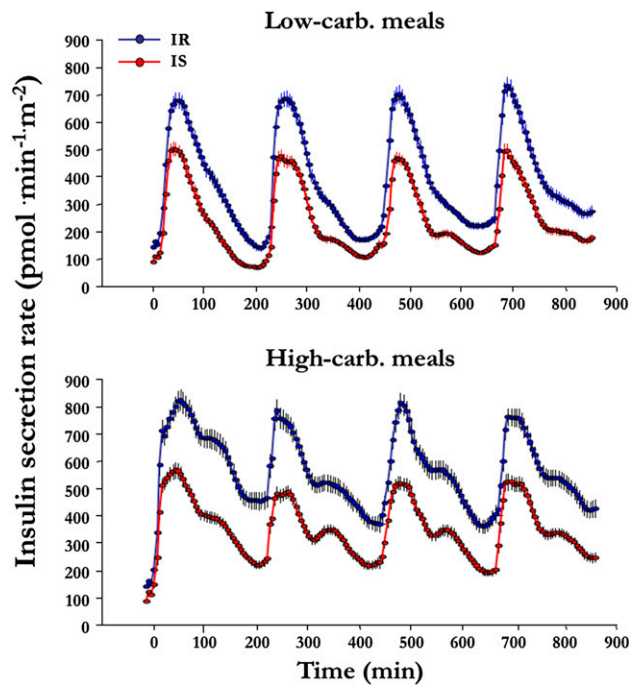


Figure 2—Insulin secretion rates during low and high carbohydrate loading in IS and IR subjects. Insulin secretion rate in 64 IS and 79 IR subjects in response to four low-carbohydrate (top) and high-carbohydrate (bottom) meals over 14 h. Plots represent mean \pm SEM.

multiple times during the OGTT and the meal days. The clinical characteristics of these subjects were not different from those of the entire cohort (data not shown). Fasting FMD values (before oral glucose and meals) were lower in IR ($4.8 \pm 0.7\%$) than IS ($6.7 \pm 0.5\%$, $P < 0.05$) subjects. Meals induced an attenuation of FMD (1-h postmeal relative to the premeal values), which was significantly stronger with high-carbohydrate than low-carbohydrate

meals similarly in IS and IR groups ($-8.7 \pm 2.3\%$ vs. $-10.3 \pm 2.5\%$ for low vs. high carbohydrate, respectively; $P < 0.02$ for the interaction term). Comparable postmeal FMD attenuation was observed in IS and IR groups (Supplementary Fig. 6).

By cross-correlation analysis, plasma FFA levels and FMD values from the low-carbohydrate day were reciprocally related to one another ($r = -0.49$, $P < 0.001$), with a mean time shift of 111 min (Fig. 5). The cross-correlation maintained statistical significance ($r = -0.38$, $P < 0.0001$) after adjustment for concomitant plasma glucose values. Similar results were obtained when cross-correlating data from the high-carbohydrate day (not shown).

DISCUSSION

The main findings of this study are that, in nondiabetic subjects under conditions simulating free living, the secretory adaptation to high-carbohydrate loading 1) is less than proportional to the carbohydrate load, i.e., it tends to level off; 2) involves an enhancement of both β -cell glucose sensitivity and potentiation; 3) is critically dependent on insulin sensitivity; and 4) is associated with changes in endothelial-dependent vasodilatory function.

With regard to 1, we found that doubling daily carbohydrate intake (from half to two-thirds of total calorie intake partitioned over four meals) increased absolute insulin output by substantially less than 100%, suggesting that secretion may level off at 70–85 units/14 h in IS individuals. In IR subjects, this dose response was similar in shape but shifted upwards, suggesting a plateau of 140–170 units/14 h (Fig. 3). Between subjects, total insulin output was strongly related to insulin sensitivity in a reciprocal fashion, with the values from high carbohydrate loading lying consistently above those of low carbohydrate loading (Supplementary Fig. 3). This result confirms previous findings (3,30,31) of an inverse association of absolute insulin secretion and insulin sensitivity

Table 3—Glucose, insulin, and FFA response to the meal challenges (300 vs. 600 carbohydrate kcal/meal) and β -cell function parameters for the IS and IR groups

	IS (n = 64)		IR (n = 79)		P§
	300 kcal	600 kcal	300 kcal	600 kcal	
AUC _G (mol · L ⁻¹ · h)	0.66 \pm 0.07	0.69 \pm 0.12	1.02 \pm 0.06	1.59 \pm 0.10	*
AUC _I (mol · L ⁻¹ · h)	155 \pm 24	255 \pm 51	371 \pm 20	659 \pm 46	*
AUC _{FFA} (mol · L ⁻¹ · h)	-69.2 \pm 11.9	-140.2 \pm 12.8	-100.6 \pm 10.7	-173.2 \pm 11.5	†
ISR _{5mM} (pmol · min ⁻¹ · m ⁻²)	97 (49)	90 (33)	118 (54)	116 (52)	*
Total IS (nmol · m ⁻²)	206 (75)	283 (90)	312 (113)	448 (165)	†
β -GS (pmol · min ⁻¹ · m ⁻² · [mmol/L] ⁻¹)	90 (44)	113 (55)	108 (50)	122 (67)	‡
Rate sensitivity (nmol · m ⁻² · [mmol/L] ⁻¹)	1.11 (0.67)	1.11 (0.90)	1.41 (0.83)	1.33 (1.15)	*
Potentiation (ratio)	1.47 (0.71)	2.22 (1.05)	1.42 (0.54)	1.79 (1.08)	‡

Data are means \pm SE or median (interquartile range). Potentiation (ratio) = (1–14 h)/(0–20 min). AUC_G, glucose AUC; AUC_I, insulin AUC; β -GS, β -cell glucose sensitivity; ISR_{5mM}, insulin secretion at 5 mmol/L glucose; total IS, total insulin secretion. §ANCOVA adjusted for sequence of carbohydrate loading days (i.e., 300 day \rightarrow 600 day vs. 600 day \rightarrow 300 day). *Group = IR > IS ($P < 0.0001$). †Group \times load = $P < 0.0001$. ‡Group = IR < or > IS ($P < 0.01$); load = 600 > 300 ($P < 0.0001$).

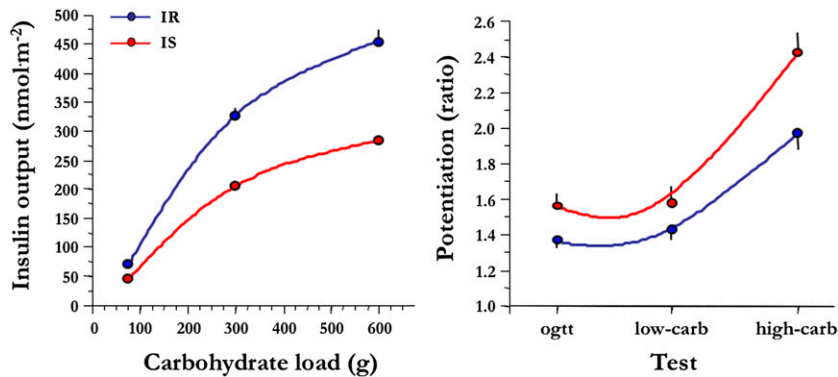


Figure 3—Parameters of β -cell function as a function of carbohydrate (carb) load. Total insulin output and potentiation in response to a 75-g OGTT, a 300-g carbohydrate meal sequence, and a 600-g carbohydrate meal sequence in 64 IS and 79 IR subjects. Plots represent means \pm SEM.

in nondiabetic subjects and extends them to free-living conditions over a 20-fold range of insulin sensitivity. Thus, in nondiabetic subjects the degree of insulin sensitivity determines the individual set point of absolute β -cell secretory activity, which the amount of carbohydrate modulates acutely. The current quantitative estimates of insulin release highlight the secretory burden that a high carbohydrate intake poses to the β -cell in the IR individual. With long-standing obesity/insulin resistance, islets are known to hypertrophy (10,11), and their structure may develop diabetogenic changes (12). In fact, relative to the IS group a high carbohydrate intake in the IR group was associated with the emergence of an excess of plasma glucose values ≥ 11.1 mmol/L (Supplementary Fig. 5), a signal of incipient “diagnostic” glucose intolerance (see plasma glucose levels on the OGTT [Table 2]). In other words, in the presence of insulin resistance, adaptation of insulin release to high carbohydrate intake is actually maladaptive.

With regard to the underlying mechanisms, the augmented insulin secretory response to the high carbohydrate load was mediated by an increase in β -cell glucose sensitivity and, to an even greater extent, potentiation. Notably, that glucose sensing (i.e., augmentation of insulin release for any glycemic increment) can be acutely (1 day) upregulated (by $\sim 30\%$) upon doubling the carbohydrate content of a mixed meal is a novel finding. At the physiological level, this upregulation can be explained by the concomitant increase in potentiation, which includes both glucose-induced (i.e., persistence of raised glycemia or glucose memory) and non-glucose-induced (i.e., incretin effects) mechanisms (32). Further insight into the interplay of glucose sensitivity and potentiation is provided by the differences observed between IS and IR individuals. In the latter group, β -cell glucose sensitivity was not only preserved but actually better than in the IS group on both carbohydrate loads, possibly as a result of chronic carbohydrate excess (33,34); in contrast, potentiation was

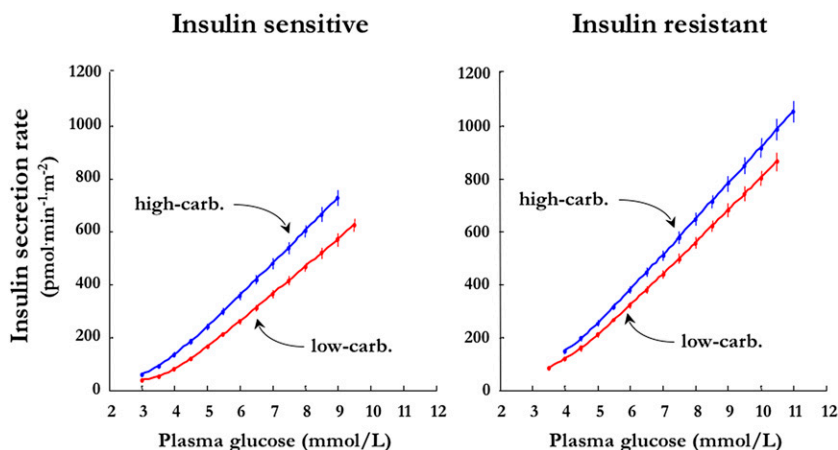


Figure 4—Insulin secretion dose-response function. Insulin secretion rate as a function of concomitant plasma glucose concentrations throughout 14 h of low or high carbohydrate (carb.) loading in IS and IR nondiabetic subjects. The mean slope of these functions represents glucose sensitivity. Plots (mean \pm SEM) encompass the actual glucose concentration range observed in each subgroup of subjects.

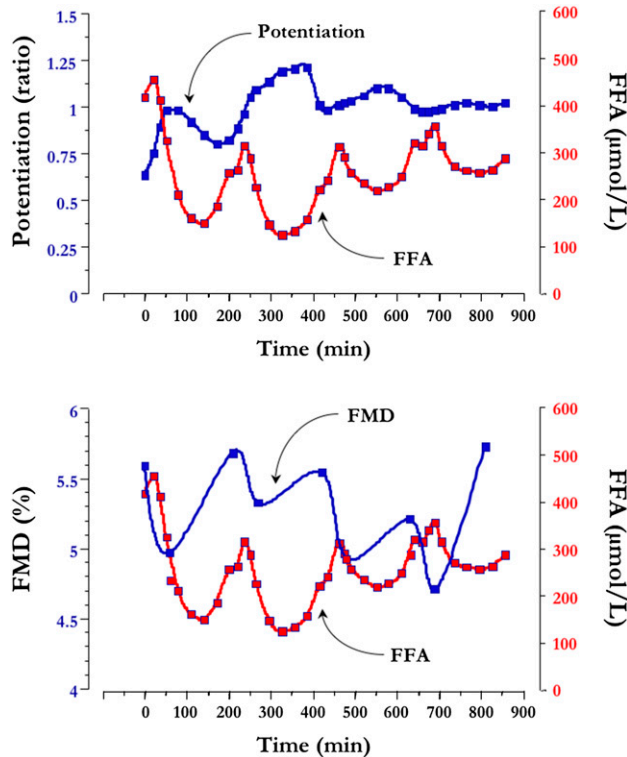


Figure 5—Cross-correlation of plasma FFA with potentiation and FMD. The time courses of potentiation and plasma FFA (top panel) and FMD and plasma FFA (bottom panel) are juxtaposed to highlight their respective phase shifts. Plots are mean data for the whole group of participants during the low-carbohydrate-loading day.

significantly impaired across all tests (Fig. 3). This result is compatible with the notion that in nondiabetic IR subjects, slightly raised glucose levels (Table 2) potentiate insulin release, but overall potentiation is impaired owing to deficient incretin effects. In fact, in previous studies using isoglycemic glucose infusion protocols, incretin effects were shown to be decreased not only as a function of hyperglycemia (i.e., in IGT and type 2 diabetic individuals) but also as an independent function of insulin resistance (35). In subsequent analyses of potentiation mechanisms (36), it was confirmed that in hyperglycemic subjects glucose-induced potentiation is enhanced, whereas incretin-induced potentiation is markedly depressed. Although incretin hormones (GLP-1 and glucose-dependent insulinotropic peptide) were not measured in the current studies, a recent literature survey (37) has documented a poor correlation between the size of incretin effects and the plasma excursions of incretin hormones.

With regard to the overall impact of IR, the nondiabetic IR subjects in this study displayed virtually all the anthropometric and metabolic features that cluster with IR (overweight/obesity, visceral fat accumulation, dyslipidemia, and markers of subclinical inflammation, Table 1). The interaction of IR with β -cell function manifested itself as absolute insulin hypersecretion, enhanced glucose and rate sensitivity, and reduced potentiation, resulting

in sporadic mild hyperglycemia. By quantitative analysis (Supplementary Fig. 4), a difference of 1 SD of insulin-mediated whole-body glucose uptake ($-3.18 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) translated into a rise of 0.55 mmol/L in mean 14-h glucose concentration independently of glucose sensitivity and potentiation; this effect was magnified by the high carbohydrate load. Thus, even in the presence of normal glucose tolerance by conventional diagnostic criteria, insulin resistance is associated with a relative intolerance to carbohydrate excess. The impairment in insulin secretory potentiation is compatible with the finding that the incretin defect of patients with overt type 2 diabetes is not reversed by chronic antihyperglycemic treatment with sitagliptin (a DPP-IV inhibitor) or metformin (38). Persistence of incretin defects has therefore been ascribed to non-glucose-mediated factors (35,38), which the current analysis imputes, at least in part, to endogenous FFA. In fact, the reciprocal association of plasma FFA with potentiation (both in absolute terms and in time course) (Fig. 5) confirms the previously mentioned reciprocal relation of the incretin effect to IR (35) as well as providing an explanation for recent studies showing that pharmacological lowering of plasma FFA restores the incretin effect through GLP-1 signaling in mice (39).

The third conclusion concerns the relationship between IR status and vascular endothelial dysfunction. The current study used stringent exclusion criteria (e.g., current smoking, hypertension, etc.) to limit confounding of the study outcomes. As in previous studies (22,23), the meal challenges induced a significant postprandial FMD attenuation, which, however, did not differ between IS and IR subjects. This suggests that the meal-related effect on endothelial function is not directly mediated by mechanisms linked with insulin resistance per se. Of note, the postprandial FMD attenuation was only modestly, though significantly, worsened by doubling the carbohydrate load. This finding likely reflects the opposing influences on FMD of elevations in glycemia and insulinemia. In fact, in normal subjects insulin at physiological doses is a weak vasodilator (40) and potentiates acetylcholine-induced (endothelial-dependent) vasodilation (41).

A novel finding was the difference in the baseline value of FMD between IR and IS subjects and the inverse association of plasma FFA with FMD. In fact, postprandial FFA levels were higher in IR than IS on both carbohydrate loads and were reciprocally related to FMD. Furthermore, by cross-correlation analysis the time course of FFA was tightly related to that of FMD with an anticipation of ~ 2 h and independently of plasma glucose levels (Fig. 5). This pattern of association strongly suggests that higher FFA levels are causally related to endothelial-dependent vascular function, thereby extending to endogenous FFA the results seen with exogenous FFA infusions (25,26). The proposed mechanisms for the role of postprandial lipids in vascular endothelial dysfunction include the production of highly reactive oxygen species and other oxidants

(hydrogen peroxide and peroxyxynitrite species) and the reduction of NO availability (42).

In summary, insulin resistance can be mechanistically linked with insulin hypersecretion, defective incretin potentiation, and endothelial dysfunction via the raised FFA concentrations (generated by adipose tissue) and the raised glucose concentrations (resulting from impaired insulin-mediated glucose uptake). Of note, this construct emerged as a coherent interpretation of data in nondiabetic subjects once the impact of insulin resistance was amplified by carbohydrate overloading and by studying a large group of individuals covering a wide range of insulin sensitivity.

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References

- Fabbrini E, Higgins PB, Magkos F, et al. Metabolic response to high-carbohydrate and low-carbohydrate meals in a nonhuman primate model. *Am J Physiol Endocrinol Metab* 2013;304:E444–E451
- Høst U, Kelbaek H, Rasmussen H, et al. Haemodynamic effects of eating: the role of meal composition. *Clin Sci (Lond)* 1996;90:269–276
- Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G; European Group for the Study of Insulin Resistance (EGIR). Insulin resistance and hypersecretion in obesity. *J Clin Invest* 1997;100:1166–1173
- Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. *Diabetes* 2001;50:1–11
- Steil GM, Trivedi N, Jonas JC, et al. Adaptation of beta-cell mass to substrate oversupply: enhanced function with normal gene expression. *Am J Physiol Endocrinol Metab* 2001;280:E788–E796
- Vernier S, Chiu A, Schober J, et al. β -cell metabolic alterations under chronic nutrient overload in rat and human islets. *Islets* 2012;4:379–392
- Drucker DJ. The biology of incretin hormones. *Cell Metab* 2006;3:153–165
- Marchetti P, Lupi R, Bugliani M, et al. A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia* 2012;55:3262–3272
- Ferrannini E, Mari A. β cell function and its relation to insulin action in humans: a critical appraisal. *Diabetologia* 2004;47:943–956
- Saisho Y, Butler AE, Manesso E, Elashoff D, Rizza RA, Butler PC. β -cell mass and turnover in humans: effects of obesity and aging. *Diabetes Care* 2013;36:111–117
- Linnemann AK, Baan M, Davis DB. Pancreatic β -cell proliferation in obesity. *Adv Nutr* 2014;5:278–288
- Mezza T, Muscogiuri G, Sorice GP, et al. Insulin resistance alters islet morphology in nondiabetic humans. *Diabetes* 2014;63:994–1007
- Hopkins PN. Molecular biology of atherosclerosis. *Physiol Rev* 2013;93:1317–1542
- Halcox JP, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002;106:653–658
- McLenachan JM, Vita J, Fish DR, et al. Early evidence of endothelial vasodilator dysfunction at coronary branch points. *Circulation* 1990;82:1169–1173
- Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging* 2010;26:631–640
- Manrique C, Lastra G, Sowers JR. New insights into insulin action and resistance in the vasculature. *Ann N Y Acad Sci* 2014;1311:138–150
- Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 1996;97:2601–2610
- Mather KJ, Steinberg HO, Baron AD. Insulin resistance in the vasculature. *J Clin Invest* 2013;123:1003–1004
- Natali A, Toschi E, Baldeweg S, et al. Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. *Diabetes* 2006;55:1133–1140
- Natali A, Bonadonna R, Santoro D, et al. Insulin resistance and vasodilation in essential hypertension. Studies with adenosine. *J Clin Invest* 1994;94:1570–1576
- Kawano H, Motoyama T, Hirashima O, et al. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. *J Am Coll Cardiol* 1999;34:146–154
- Title LM, Cummings PM, Giddens K, Nassar BA. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J Am Coll Cardiol* 2000;36:2185–2191
- Zhu W, Zhong C, Yu Y, Li K. Acute effects of hyperglycaemia with and without exercise on endothelial function in healthy young men. *Eur J Appl Physiol* 2007;99:585–591
- Steinberg HO, Tarshoby M, Monestel R, et al. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest* 1997;100:1230–1239
- Steinberg HO, Paradisi G, Hook G, Crowder K, Cronin J, Baron AD. Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. *Diabetes* 2000;49:1231–1238
- Wascher TC, Schmoelzer I, Wiegatz A, et al. Reduction of postchallenge hyperglycaemia prevents acute endothelial dysfunction in subjects with impaired glucose tolerance. *Eur J Clin Invest* 2005;35:551–557
- Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of beta-cell function: modeling analysis in normal subjects. *Am J Physiol Endocrinol Metab* 2002;283:E1159–E1166
- Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 1992;41:368–377
- Polonsky KS, Given BD, Hirsch LJ, et al. Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 1988;318:1231–1239
- Jones CN, Abbasi F, Carantoni M, Polonsky KS, Reaven GM. Roles of insulin resistance and obesity in regulation of plasma insulin concentrations. *Am J Physiol Endocrinol Metab* 2000;278:E501–E508
- Holst JJ, Vilsbøll T, Deacon CF. The incretin system and its role in type 2 diabetes mellitus. *Mol Cell Endocrinol* 2009;297:127–136
- Garg A, Grundy SM, Koffler M. Effect of high carbohydrate intake on hyperglycemia, islet function, and plasma lipoproteins in NIDDM. *Diabetes Care* 1992;15:1572–1580

34. Garg A, Grundy SM, Unger RH. Comparison of effects of high and low carbohydrate diets on plasma lipoproteins and insulin sensitivity in patients with mild NIDDM. *Diabetes* 1992;41:1278–1285
35. Muscelli E, Mari A, Casolaro A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes* 2008;57:1340–1348
36. Tura A, Muscelli E, Gastaldelli A, Ferrannini E, Mari A. Altered pattern of the incretin effect as assessed by modelling in individuals with glucose tolerance ranging from normal to diabetic. *Diabetologia* 2014;57:1199–1203
37. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 2011;54:10–18
38. Holst JJ, Knop FK, Vilsbøll T, Krarup T, Madsbad S. Loss of incretin effect is a specific, important, and early characteristic of type 2 diabetes. *Diabetes Care* 2011;34(Suppl. 2):S251–S257
39. Kang ZF, Deng Y, Zhou Y, et al. Pharmacological reduction of NEFA restores the efficacy of incretin-based therapies through GLP-1 receptor signalling in the beta cell in mouse models of diabetes. *Diabetologia* 2013;56:423–433
40. Natali A, Buzzigoli G, Taddei S, et al. Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes* 1990;39:490–500
41. Taddei S, Virdis A, Mattei P, Natali A, Ferrannini E, Salvetti A. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. *Circulation* 1995;92:2911–2918
42. Botham KM, Wheeler-Jones CP. Postprandial lipoproteins and the molecular regulation of vascular homeostasis. *Prog Lipid Res* 2013;52:446–464