The Effect of Ingested Glucose Dose on the Suppression of Endogenous Glucose Production in Humans

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Insulin clamp studies have shown that the suppressive actions of insulin on endogenous glucose production (EGP) are markedly more sensitive than for stimulating glucose disposal (R_d). However, clamp conditions do not adequately mimic postprandial physiological responses. Here, using the variable infusion dual-tracer approach, we used a threefold range of ingested glucose doses (25, 50, and 75 g) to investigate how physiological changes in plasma insulin influence EGP in healthy subjects. Remarkably, the glucose responses were similar for all doses tested, yet there was a dose-dependent increase in insulin secretion and plasma insulin levels. Nonetheless, EGP was suppressed with the same rapidity and magnitude (~55%) across all doses. The progressive hyperinsulinemia, however, caused a dose-dependent increase in the estimated rates of R_d, which likely accounts for the lack of a dose effect on plasma glucose excursions. This suggests that after glucose ingestion, the body preferentially permits a transient and optimal degree of postprandial hyperglycemia to efficiently enhance insulin-induced changes in glucose fluxes, thereby minimizing the demand for insulin secretion. This may represent an evolutionarily conserved mechanism that not only reduces the secretory burden on β-cells but also avoids the potential negative consequences of excessive insulin release into the systemic arterial circulation.

Glucose homeostasis is achieved through the integrated regulation of endogenous glucose production (EGP) and glucose disposal (R_d). (1,2). Our understanding about the regulation of EGP and R_d is largely derived from euglycemic–hyperinsulinemic and hyperglycemic clamp studies. These approaches have been useful for determining the dose-response characteristics of insulin and glucose-induced suppression of EGP and stimulation of R_d. These studies revealed that suppression of EGP is more responsive to insulin and hyperglycemia (i.e., is more glucose effective) than is the stimulation of R_d (3–7). Specifically, maximal and nearly complete suppression of EGP can be achieved in healthy individuals with only small increments in systemic insulin and glucose (3–7). However, clamp studies are non-physiological and do not represent the dynamic nature of the postprandial period (1).

Relatively little is known about the regulation of EGP during the postprandial period. This is somewhat surprising given that humans spend most of their waking hours in the postprandial state. Considering the well-established dose-response relationship between plasma insulin levels and EGP suppression under clamp conditions, we thought it pertinent to revisit this phenomenon to investigate how incremental increases in postprandial insulin influence the dynamics of EGP suppression after glucose ingestion. To achieve this, studies were performed across a threefold dose range of orally ingested glucose (25, 50, and 75 g), which was designed to elicit a range of physiological postprandial insulin responses. This was combined with the variable infusion dual-tracer technique to enable the accurate determination of EGP during the postprandial period (8).

RESEARCH DESIGN AND METHODS

Participants

Eight healthy men were studied (26 ± 1.5 years; 1.78 ± 0.01 m; 76.6 ± 4.5 kg). Participants were weight stable (±2 kg) for ≥6 months before the study, none had type 2 diabetes, or were taking medications known to alter glucose metabolism. The Deakin University Human Research Ethics Committee approved the study, which was conducted in accordance with the Declaration of Helsinki. The purpose, nature, and potential risks were explained, and informed written consent was obtained.

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Study Design

Participants completed three trials, separated by ≥4 days, in random order ingesting 25, 50, or 75 g glucose. Participants were provided a standardized diet (8,478 kJ, 18% protein, 35% fat, and 47% carbohydrate) to consume on the day before the study. Trials commenced after an overnight (10-h) fast, with participants instructed to only consume water from 2130 h on the evening before the study. Strenuous exercise was avoided 48 h before the study.

Upon arrival at the laboratory at 0700 h, height and weight were recorded. A 22-gauge catheter was inserted into a forearm vein for blood sampling. Blood (3 mL) was collected in EDTA and lithium-heparin Vacutainers for determination of fasting parameters. A second 22-gauge catheter was inserted into a vein in the contralateral forearm for [6,6-^{2}H]glucose infusion (Cambridge Isotope Laboratories, Inc., Tewksbury, MA). A primed continuous infusion of [6,6-^{2}H]glucose (33 μmol/kg/min prime; 0.33 μmol/kg/min continuous) commenced at 0730 h. After the prime, [6,6-^{2}H]glucose was infused for 120 min (−120 to 0 min), with blood sampled at −30, −20, −10, and 0 min.

At 0930 h (0 min), participants consumed a drink containing 25, 50, or 75 g glucose (Glucodin; iNova Pharmaceuticals, Chatswood, NSW, Australia). The drink was enriched with 3 g [1-^{13}C]glucose (Cambridge Isotope Laboratories) and made to 300 mL with water. A small aliquot was taken for [1-^{13}C]glucose enrichment. After the glucose ingestion, blood was collected at 10, 20, 30, 60, 90, 120, 150, and 180 min. Blood was placed on ice, later spun in a centrifuge (4°C; 4,400 rpm; 10 min), and plasma was stored at −80°C.

Upon glucose ingestion, the [6,6-^{2}H]glucose infusion rate (Table 1) was altered to mimic the fall in endogenous glucose concentrations (8). This variable infusion dual tracer uses the tracer-to-tracee clamp to maintain a nearly constant ratio between [6,6-^{2}H]glucose and endogenous glucose concentrations, thereby minimizing non–steady-state error (8).

Plasma Analysis

Glucose was determined using the glucose oxidase method. Free fatty acids (FFAs) were determined using the NEFA C assay (Wako Chemicals, Richmond, VA). Commercially available ELISA kits were used to determine insulin (ALPCO, Salem, NH), C-peptide (Millipore, Billerica MA), and glucagon (Millipore). Insulin secretion rates were calculated from C-peptide concentrations using ISEC (Insulin SECretion) software. Tracer enrichment was analyzed by methane positive chemical ionization gas chromatography–mass spectrometry using the glucose aldonitrile pentapropionate derivative by monitoring the molecular ions of 384 mass-to-charge ratio (m-z), 385 m-z, and 386 m-z corresponding to the M0 (naturally occurring glucose), M+1 ([1-^{13}C]glucose), and M+2 ([6,6-^{2}H]glucose) isotopomers, respectively.

Table 1—The infusion profiles of [6,6-^{2}H]glucose

	Infusion rate (% of basal)		
Time (min)	25 g glucose	50 g glucose	75 g glucose
−120 to 0	100	100	100
0–10	75	70	70
10–20	65	60	60
20–30	55	50	50
30–90	50	35	35
90–120	55	45	35
120–180	60	55	35

Rates are presented as a percentage of the basal [6,6-^{2}H]glucose infusion rate, which was 0.33 μmol/kg/min.

Calculations and Statistics

Basal EGP was calculated using steady-state equations, and the Steele non–steady-state single-compartment model (8) was used to calculate postprandial EGP. Equations for determination of EGP, R_d, and rate of meal glucose appearance (R_a) have been detailed elsewhere (8). All data are reported as mean ± SEM. Significance was accepted when P was <0.05. The area under the curve (AUC) was calculated using the trapezoidal method. Areas below fasting were subtracted from areas above fasting levels to give a net area. One-way or two-way repeated-measures ANOVA were used where appropriate. Post hoc analysis was conducted using the Tukey multiple comparisons test. The relationship between postprandial EGP calculated using steady-state and non–steady-state equations was determined using linear regression. GraphPad Prism software was used for all analyses.

RESULTS

Plasma Parameters

Fasting glucose, insulin, C-peptide, glucagon, and FFAs were similar for all trials (Fig. 1). Postprandial glycemic responses were similar for all trials (Fig. 1*A* and *B*); however, there was a dose-dependent increase in plasma insulin concentrations such that the integrated response for the 75-g dose was approximately fivefold greater than for 25 g and approximately twofold higher than 50 g, whereas 50 g caused an approximately twofold increase compared with 25 g (Fig. 1*C* and *D*). Dose-dependent increases in C-peptide (Fig. 1*F* and *G*) and insulin secretion rates (Fig. 1*G* and *H*) were also observed. The suppression of plasma glucagon was similar for all doses (Fig. 1*I* and *J*). Plasma FFAs were not different across doses throughout the postprandial period (Fig. 1*K*), but the integrated response revealed modestly greater suppression with the 75- vs. 25-g dose (Fig. 1*L*). There was no difference between 25- vs. 50-g and 50- vs. 75-g doses (Fig. 1*L*).

Oral Versus Endogenous Glucose Concentrations

There were dose-dependent increases in orally derived glucose (Fig. 2*A* and *B*). Endogenous glucose concentrations were rapidly suppressed and remained below basal levels 3 h after glucose ingestion (Fig. 2*C*). Although the 50- and 75-g doses caused greater reductions in endogenous glucose compared with 25 g, there was no difference between the 50- and 75-g doses (P = 0.09) (Fig. 2*D*). A nearly constant ratio between [6,6-^{2}H]glucose and endogenously

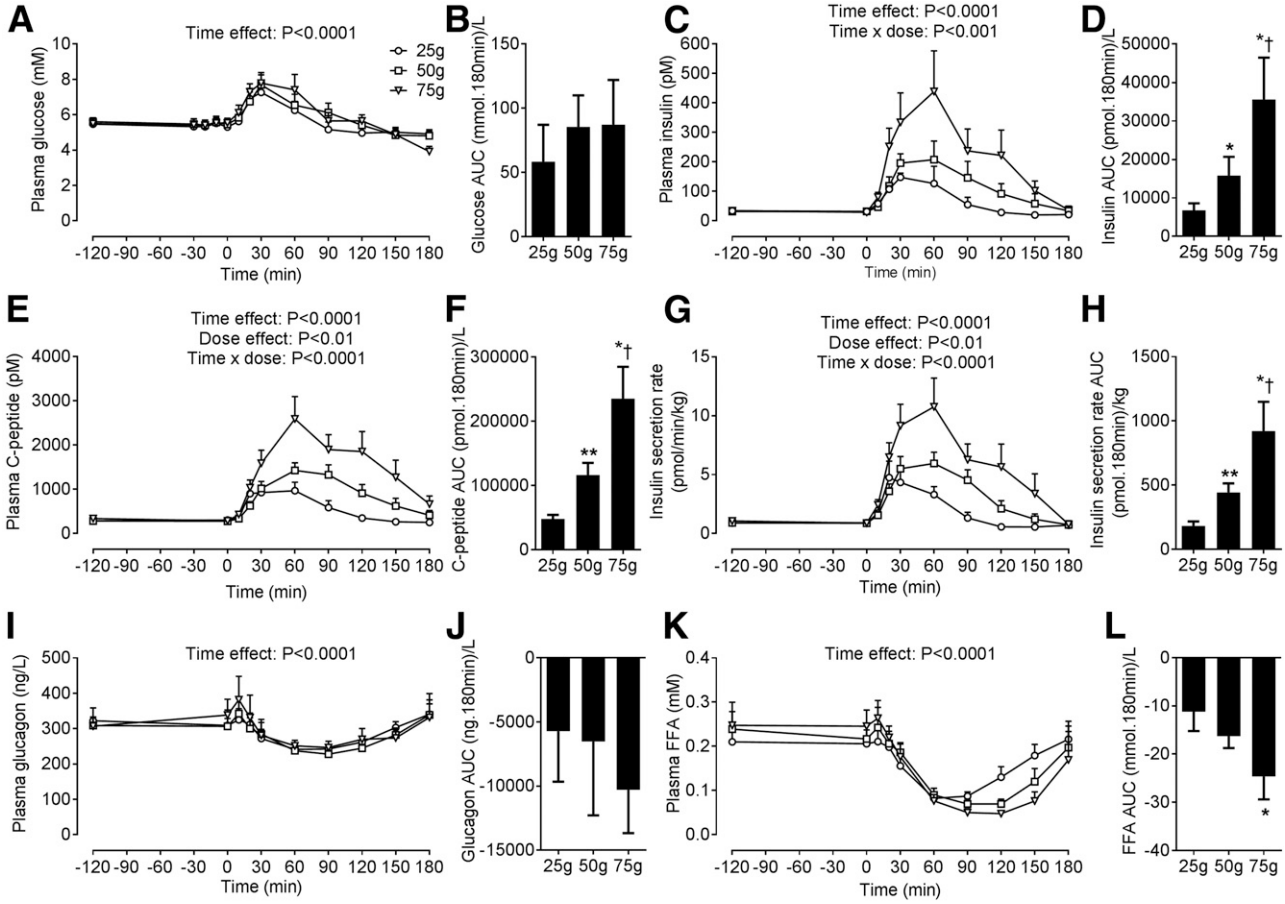

Figure 1—Plasma metabolite and hormone concentrations observed after ingesting 25, 50, or 75 g glucose, which was ingested at time 0 min. *A*: Plasma glucose concentrations. *B*: Net AUC for the glucose response. *C*: Plasma insulin concentrations. *D*: Net AUC for the insulin response. *E*: Plasma C-peptide concentrations. *F*: Net AUC for the C-peptide response. *G*: Insulin secretion rates. *H*: Net AUC the insulin secretion response. *I*: Plasma glucagon concentrations. *J*: Net AUC for the glucagon response. *K*: Plasma FFA concentrations. *L*: Net AUC for the FFA response. Data are mean ± SEM. *$P < 0.05$ vs. 25 g; **$P < 0.01$ vs. 25 g; †$P < 0.05$ vs. 50 g.

derived glucose was achieved (Fig. 2*E*). Consequently, there was strong agreement between EGP determined using steady-state or non–steady-state equations (Fig. 2*F*), demonstrating accuracy and model independence of the tracer clamp approach (8).

Glucose Fluxes

Fasting EGP was similar for all trials (Fig. 3*A*). Regardless of the ingested glucose dose, EGP was rapidly suppressed and remained below basal 3 h after glucose ingestion (Fig. 3*A*). The pattern of EGP suppression was similar for all doses (Fig. 3*A*), with the only significant differences at 150 and 180 min, where EGP was higher in the 25- and 50-g doses vs. the 75-g dose. Nevertheless, the percentage suppression of EGP (~55%) was similar for all doses (Fig. 3*B*). In contrast, there was a dose-dependent increase in the R_a of meal-derived glucose (Fig. 3*C* and *D*) and R_d (Fig. 3*E* and *F*).

DISCUSSION

In healthy young adults, ingestion of increasing amounts of glucose did not cause a dose-dependent increase in plasma glucose. In contrast, dose-dependent increases in postprandial insulin secretion rates and insulin levels were observed. Nonetheless, postprandial EGP was suppressed with the same rapidity and magnitude with all glucose doses. The progressive hyperinsulinemia, however, caused a dose-dependent increase in R_d that acted to maintain identical glycemic responses regardless of the amount of glucose ingested. Thus, after carbohydrate ingestion, the body appears to preferentially permit an optimal degree of hyperglycemia to efficiently coordinate glucose fluxes to appropriately deal with the amount of carbohydrate ingested, rather than secreting large amounts of insulin to prevent an increase in blood glucose. This reliance on glucose effectiveness (i.e., the ability of glucose itself to regulate glucose fluxes) could be advantageous because it minimizes the secretory burden on β-cells while avoiding potential negative consequences associated with excessive insulin release into the systemic arterial circulation.

Our finding that increasing the amount of ingested glucose caused a dose-dependent increase in plasma insulin, yet did not exacerbate glycemic responses, may seem

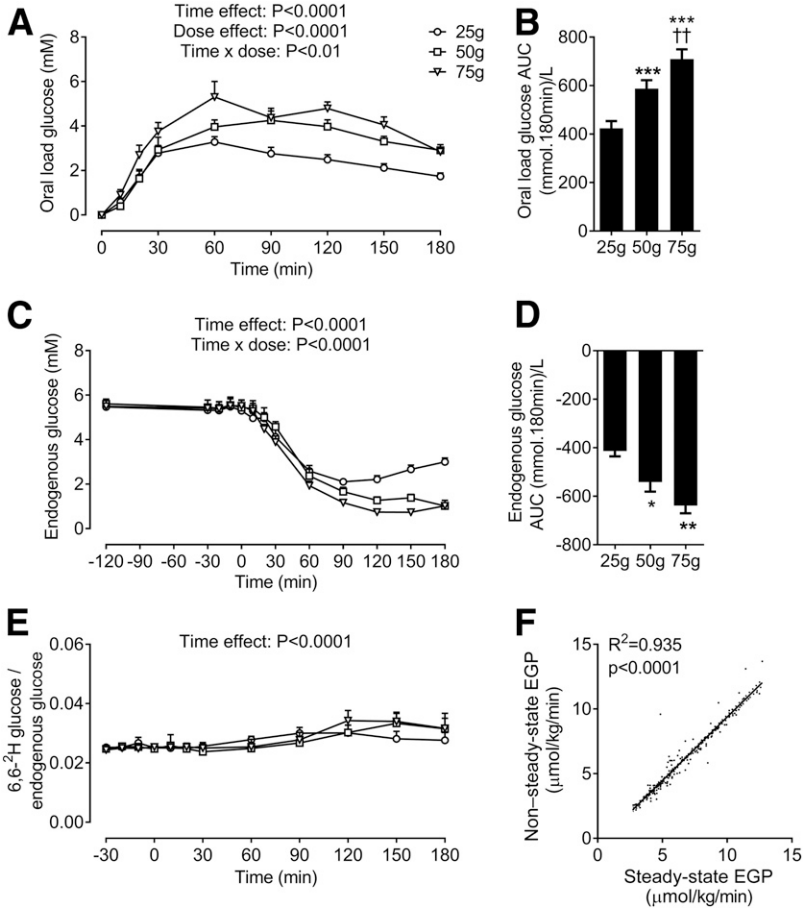

Figure 2—The concentrations of orally derived glucose, endogenous glucose, and the tracer-to-tracee ratios observed after ingesting 25, 50, or 75 g glucose, which was ingested at time 0 min. *A*: Oral load plasma glucose concentrations. *B*: Net AUC for the oral load glucose response. *C*: Endogenous plasma glucose concentrations. *D*: Net AUC for the endogenous plasma glucose response. *E*: The ratio between [6,6-^{2}H]glucose and endogenously derived glucose. *F*: Relationship between EGP calculated with steady-state and non–steady-state equations. Data are mean ± SEM. **P* < 0.05 vs. 25 g; ***P* < 0.01 vs. 25 g; ****P* < 0.001 vs. 25 g; ††*P* < 0.01 vs. 50 g.

surprising but is not without precedent. In fact, similar findings have been documented in healthy adults (9–11), suggesting that healthy humans have the capacity to maintain nearly identical postprandial glucose responses after the ingestion of vastly differing amounts of carbohydrate.

The pattern of EGP suppression was remarkably similar for all doses. The only difference occurred late in the postprandial period where EGP remained maximally suppressed with 75 g, while it began to rise with the 25- and 50-g doses. Considering the addition of other macronutrients to carbohydrate meals, particularly protein, generally potentiates the insulin response (12), future studies comparing postprandial glucose fluxes in response to varying doses of glucose alone versus mixed meals would be of value as would comparing liquid versus solid meals. Of note, unlike what is commonly observed in clamp experiments, we found a lack of complete EGP suppression (~55%), consistent with the fact that physiological hyperinsulinemia inhibits hepatic glycogenolysis but not gluconeogenesis (1,13). Nearly complete EGP suppression requires persistent inhibition of gluconeogenesis, which can only be achieved by prolonged supraphysiological hyperinsulinemia (1). Thus, the residual nonsuppressed EGP is almost certainly of gluconeogenic origin.

Because there was no dose-dependent effect on glucose excursions or EGP suppression, other glucose fluxes must have been altered to maintain glycemia. Indeed, rates of systemic glucose appearance and R_d increased with each increment in ingested glucose dose. Thus with escalating glucose dose, the progressive increase in gut-derived glucose appearance was matched by enhanced rates of R_d, thereby maintaining similar glycemic excursions for all doses. Because our major outcome measure was EGP, we used the less complex dual-tracer method, as opposed to the triple-tracer approach, and thus acknowledge that R_a and R_d are estimates and likely suffer from non–steady-state error. However, our peak rates of R_a and R_d after the 75-g dose are similar to those reported in healthy young adults using the triple-tracer technique with similarly sized glucose-containing mixed meals (14), suggesting our approach has some degree of accuracy. Overall,

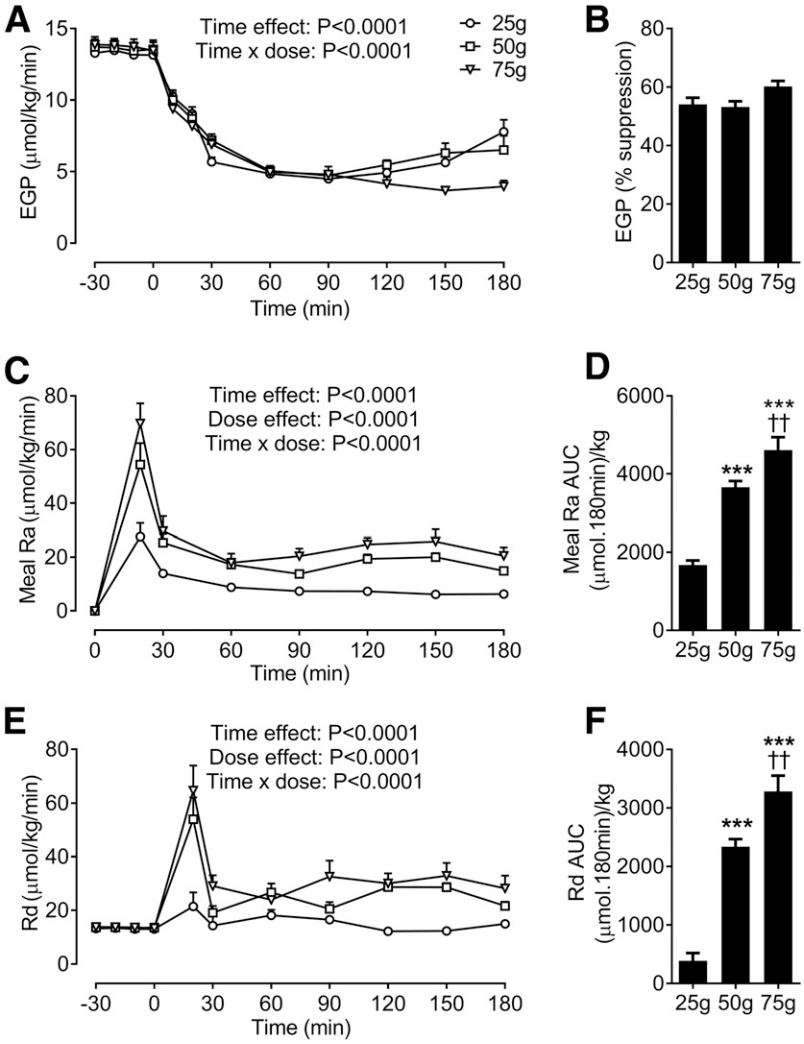

Figure 3—Whole-body glucose fluxes determined after ingesting 25, 50, or 75 g glucose, which was ingested at time 0 min. *A*: Rates of EGP. *B*: Percentage suppression of EGP. *C*: R_a. D: Net R_a. E: R_d. *F*: Net AUC R_d. Data are mean ± SEM. ***$P < 0.001$ vs. 25 g; ††$P < 0.01$ vs. 50 g.

our data support findings from clamp studies showing that EGP is rapidly and maximally, although incompletely, suppressed at relatively low insulin concentrations, while stimulation of R_d, particularly by muscle, requires higher levels of systemic hyperinsulinemia (3–7) and thereby imposes a greater insulin secretory burden on β-cells.

Our findings raise important questions about the regulation of postprandial glycemia. Firstly, considering each glucose dose elicited the same glycemic excursion, why was there a dose effect on insulin secretion? We speculate that the differing insulin secretory responses were mediated, or at least influenced, by incretin hormones. Unfortunately, incretin hormones (gastric inhibitory polypeptide and glucagon-like peptide 1) were not assayed because blood was not collected in tubes containing protease inhibitor, a necessary step for their reliable measurement, owing to their short half-lives (15). Measuring these peptides, particularly their active forms, in the future would be informative.

Secondly, after ingestion of small glucose loads, why does the system avoid secreting more insulin to stimulate R_d to prevent or minimize the rise in blood glucose? Mammals appear to be evolutionarily geared to minimize insulin release into the systemic arterial circulation. Studies in humans, dogs, pigs, and rodents show that under physiological conditions where insulin is secreted into the hepatic portal vein, portal insulin is approximately two- to threefold higher than systemic levels (16–19). The portal-to-systemic insulin gradient is also maintained by high rates of hepatic insulin clearance, with 40% to 80% of insulin being cleared before entering the systemic circulation (16). Mammals therefore have a conserved mechanism that minimizes arterial insulin concentrations. This may be advantageous because high arterial insulin has been linked to adverse effects, including insulin-induced insulin resistance, coagulation abnormalities, weight gain, dyslipidemia, hypoglycemia, atherosclerosis, hypertension, and heart disease (20–22).

In addition, the requirement for arterial insulin is minimized by preferentially utilizing the liver, the most

anatomically and physiologically efficient glucoregulatory system. After glucose ingestion, the liver is directly and preferentially exposed to rising glucose and insulin levels as a result of their appearance in the portal vein (2). Accordingly, the combination of portal hyperinsulinemia and hyperglycemia rapidly suppress EGP and stimulate hepatic glucose uptake, such that the liver disposes of 30–40% of ingested glucose (2). The muscle R_d system, however, likely represents a backup mechanism reliant on high insulin and is engaged when the glucoregulatory actions of the liver reach capacity. By allowing an optimal transient postprandial hyperglycemia that is observed even after the ingestion of small glucose doses, EGP is efficiently suppressed and hepatic glucose uptake stimulated, while minimizing spillover of excessive insulin into the systemic circulation. It is important to note, however, that in contrast to peripheral vein insulin and glucose infusion, as during a clamp, where muscle is responsible for 80–90% of glucose uptake, muscle accounts for ~30–40% of whole-body R_d after oral glucose ingestion (2). Consistent with the notion of minimizing systemic insulin spillover, the other major actions of insulin, including suppression of lipolysis and glucagon secretion, are highly responsive to small increments in systemic insulin. Indeed, the patterns of suppression of FFA and glucagon were similar across doses, indicating the postprandial hyperinsulinemia, and perhaps glycemia, achieved with 25 g glucose had maximal inhibitory effects. The ease at which FFAs were suppressed is consistent with the fact that lipolysis appears to be the most sensitive of the key insulin-regulated processes (23,24). Thus, it seems that the body preferentially relies on liver and adipose tissue for the disposition of small glucose loads because these require minimal insulin stimulation. However, these systems become overwhelmed with increasing doses of carbohydrate, and the naturally insulin-resistant muscle R_d system is engaged. Cumulatively, muscles clearly have a substantial capacity to dispose of glucose; however, activation of this process is sluggish as a result of the requisite transendothelial insulin transport from plasma to muscle interstitium (25), which requires high and sustained systemic insulin levels (3–7). Therefore, engagement of the muscle R_d system can be viewed as a balancing act between the need to attain higher R_d rates at the cost of increasing the insulin secretory burden on the β-cells and the release of relatively high amounts of insulin into the systemic arterial circulation.

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