The effect of ingested glucose dose on the suppression of endogenous glucose production in humans.

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Abstract

Insulin clamp studies have shown that insulin's suppressive actions on endogenous glucose production (EGP) are markedly more sensitive than that for stimulating glucose disposal (Rd). However, clamp conditions do not adequately mimic postprandial physiological responses. Here, in healthy subjects, using the variable infusion dual-tracer approach, we used a 3-fold range of ingested glucose doses (25, 50 and 75 g) to investigate how physiologic changes in plasma insulin influence EGP. 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 Rd which likely accounts for the lack of a dose-effect on plasma glucose excursions. This suggests that following glucose ingestion, the body preferentially permits a transient and optimal degree of postprandial hyperglycemia so as 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 (Rd) (1; 2). Our understanding about the regulation of EGP and Rd 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 Rd. These studies revealed that suppression of EGP is more responsive to insulin and hyperglycemia (ie. is more glucose effective) than is the stimulation of Rd (3-7) . Specifically, maximal and near 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-physiologic 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 the majority of 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 following glucose ingestion. To achieve this, studies were performed across a three-fold dose range of orally ingested glucose (25, 50 and 75 g) which were 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 males were studied (26±1.5 yr; 1.78±0.01 m; 76.6±4.5 kg). Participants were weight-stable (±2 kg) for ≥6 months prior to study, none had type 2

diabetes, nor were taking medications known to alter glucose metabolism. 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 obtained.

Study design: Participants completed three trials, separated by ≥4 days, in random order ingesting either 25, 50 or 75 g of glucose. Participants were provided with a standardised diet (8,478 kJ, 18% protein, 35% fat and 47% carbohydrate) to consume on the day prior to study. Trials commenced following an overnight (10 h) fast, with participants instructed to only consume water from 21:30 h on the evening prior to study. Strenuous exercise was avoided 48 h prior to study. Upon arrival at the laboratory at 07:00 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, MA, USA). A primed continuous infusion of [6,6-^{2}H]glucose (33 μmol/kg/min prime; 0.33 μmol/kg/min continuous) commenced at 07:30 h. Following 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 09:30 h (0 min), participants consumed a drink containing 25, 50 or 75g of glucose (Glucodin, iNova Pharmaceuticals, NSW, Australia). The drink was enriched with 3 g of [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. Following 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 near 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. FFAs were determined using the NEFA C assay (Wako Chemicals, VA, USA). Insulin (ALPCO, NH, USA), C-peptide (Millipore, MA, USA) and glucagon (Millipore) were determined using commercially available ELISA kits. Insulin secretion rates were calculated from C-peptide concentrations using ISEC software. Tracer enrichment was analysed by GC-MS using the glucose aldonitrile pentapropionate derivative by monitoring the molecular ions of 384mz, 385mz, and 386mz corresponding to the M0 (naturally occurring glucose), M+1 ([1-^{13}C]glucose) and M+2 ([6,6-^{2}H]glucose) isotopomers respectively.

Calculations and statistics: Basal EGP was calculated using steady-state equations while Steele's non-steady-state single compartment model (8) was used to calculate postprandial EGP. Equations for determination of EGP, Rd and meal rate of appearance have been detailed elsewhere (8). All data are reported as mean±SEM. Significance was accepted when $P<0.05$. 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 Tukey's multiple comparisons test. The relationship between postprandial EGP calculated using steady-state and non-steady state equations was determined using linear regression. GraphPad Prism was used for all analyses.

Results

Plasma parameters: Fasting glucose, insulin, C-peptide, glucagon and FFAs were similar for all trials (Figure 1). While postprandial glycemic responses were similar for all trials (Figure 1A&B), there was a dose-dependent increase in plasma insulin concentrations such that the integrated response for the 75 g dose was ~5-fold greater than for 25 g and ~2-fold higher than 50 g, while 50 g caused a ~2-fold increase when compared with 25 g (Figure 1C&D). Dose-dependent increases in C-peptide (Figure 1 F&G) and insulin secretion rates (Figure 1 G&H) were also observed. The suppression of plasma glucagon was similar for all doses (Figure I&J). While plasma FFAs were not different across doses throughout the postprandial period (Figure 1K), the integrated response revealed modestly greater suppression with the 75 vs. 25 g dose (Figure 1L). There was no difference between 25 vs. 50 g and 50 vs. 75 g doses (Figure 1L).

Oral vs. endogenous glucose concentrations: There were dose-dependent increases in orally-derived glucose (Figure 2A&B). Endogenous glucose concentrations were rapidly suppressed and remained below basal levels 3 h after glucose ingestion (Figure 2C). While 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; Figure 2D). A near constant ratio between [6,6-^{2}H]glucose and endogenously-derived glucose was achieved (Figure 2E). Consequently, there was strong agreement between EGP determined using either steady-state or non-steady-state equations (Figure 2F), demonstrating accuracy and model independence of the tracer clamp approach (8).

Glucose fluxes: Fasting EGP was similar for all trials (Figure 3A). Regardless of ingested glucose dose, EGP was rapidly suppressed and remained below basal 3 h after glucose ingestion (Figure 3A). The pattern of EGP suppression was similar for all doses (Figure 3A), with the only significant differences at 150 and 180 min where EGP was higher in both the 25 and 50 g vs. the 75 g dose. Nevertheless, the percent suppression of EGP (~55%) was

similar for all doses (Figure 3B). In contrast, there was a dose-dependent increase in both the rate of appearance of meal-derived glucose (Figure 3C&D) and Rd (Figure 3E&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 Rd which acted to maintain identical glycemic responses regardless of the amount of glucose ingested. Thus, following 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, ie. the ability of glucose itself to regulate glucose fluxes, could be advantageous as it minimizes the secretory burden on β-cells, whilst 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 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 near identical postprandial glucose responses following 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 vs. mixed meals would be of value as would comparing liquid vs. 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, not gluconeogenesis (1; 13). Near complete EGP suppression requires persistent inhibition of gluconeogenesis which can only be achieved by prolonged supraphysiological hyperinsulinemia (1). Thus, the residual non-suppressed EGP is almost certainly of gluconeogenic origin.

As 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 Rd 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 Rd, thereby maintaining similar glycemic excursions for all doses. As 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 meal glucose appearance and Rd are estimates and likely suffer from non-steady-state error. However, our peak rates of meal glucose appearance and disposal following 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, 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 Rd, 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 (GIP and GLP-1) were not assayed as blood was not collected in tubes containing protease inhibitor, a necessary step for their reliable measurement due to their short half-lives (15). It would be informative to measure these peptides, particularly their active forms, in the future.

Secondly, following ingestion of small glucose loads, why does the system avoid secreting more insulin to stimulate Rd to prevent or minimize the rise in blood glucose? Mammals appear to be evolutionarily geared to minimise 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 ~2-3-fold higher than systemic levels (16-19). The portal to systemic insulin gradient is also maintained by high rates of hepatic insulin clearance, with 40-80% of insulin being cleared prior to entering the systemic circulation (16). Mammals therefore have a conserved mechanism that minimises arterial insulin concentrations. This may be advantageous as 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).

Additionally, the requirement for arterial insulin is minimised by preferentially utilising the liver, the most anatomically and physiologically efficient glucoregulatory system. Following glucose ingestion, the liver is directly and preferentially exposed to rising glucose and insulin levels due to 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 Rd

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 minimising spill over of excessive insulin into the systemic circulation. It is important 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 glucose disposal following oral glucose ingestion (2). Consistent with the notion of minimising systemic insulin spill over, insulin's other major actions, 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 was similar across doses, indicating the postprandial hyperinsulinemia, and perhaps glycemia, achieved with 25 g of 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 as these require minimal insulin demand. However, with increasing doses of carbohydrate, these systems become overwhelmed, and the naturally insulin resistant muscle Rd system is engaged. It is clear that cumulatively, muscles have a substantial capacity to dispose of glucose, however activation of this process is sluggish due to 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 Rd system can be viewed as a balancing act between the need to attain higher Rd 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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Tables

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 - 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 percent of the basal [6,6-^{2}H]glucose infusion rate which was 0.33 μmol/kg/min.

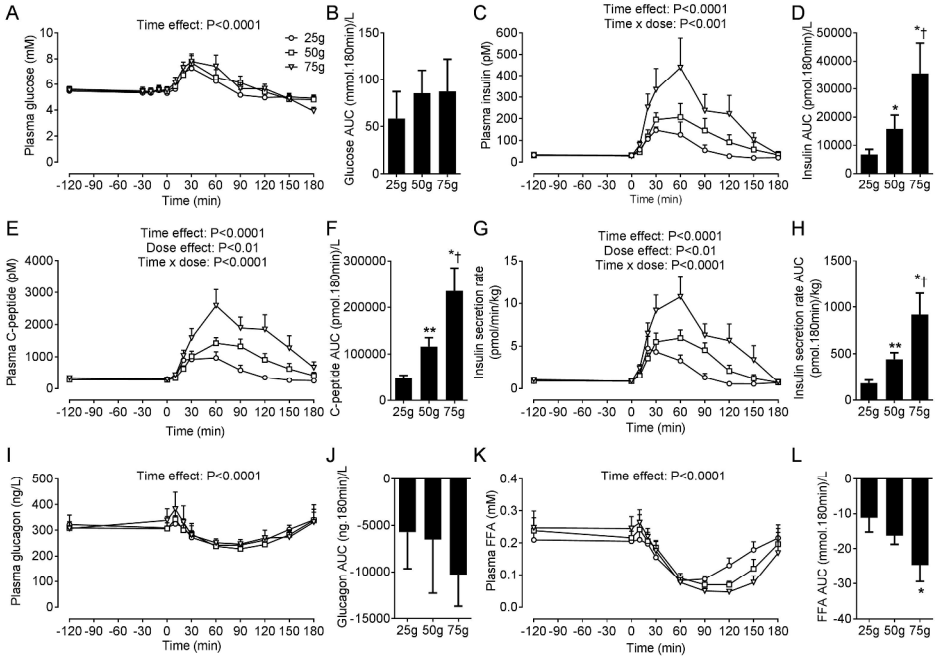
Figure legends

Figure 1. Plasma metabolite and hormone and hormone concentrations observed after ingesting either 25, 50 or 75 g of glucose which was ingested at time 0 min. A: Plasma glucose concentrations. B: Net area for the glucose response. C: Plasma insulin concentrations. D: Net area for the insulin response. E: Plasma C-peptide concentrations. F: Net area for the C-peptide response. G: Insulin secretion rates. H: Net area for the insulin secretion response. I: Plasma glucagon concentrations. J: Net area for the glucagon response. K: Plasma free fatty acid (FFA) concentrations. L: Net area 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.

Figure 2. The concentrations of orally-derived glucose, endogenous glucose and the tracer to tracee ratios observed after ingesting either 25, 50 or 75 g of glucose which was ingested at time 0 min. A: Oral load plasma glucose concentrations. B: Net area for the oral load glucose response. C: Endogenous plasma glucose concentrations. D: Net area for the endogenous plasma glucose response. E: The ratio between [6,6-^{2}H]glucose and endogenously-derived

glucose. F: Relationship between endogenous glucose production (EGP) calculated with steady-state and non-steady 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.

Figure 3. Whole-body glucose fluxes determined after ingesting either 25, 50 or 75 g of glucose which was ingested at time 0 min. A: Rates of endogenous glucose production (EGP). B: Percent suppression of EGP. C: Rates of meal glucose appearance (Ra). D: Net area for meal glucose appearance. E: Rates of glucose disposal (Rd). F: Net area for glucose disposal. Data are mean ± SEM. ***P<0.001 vs. 25 g; ††P<0.01 vs. 50 g.



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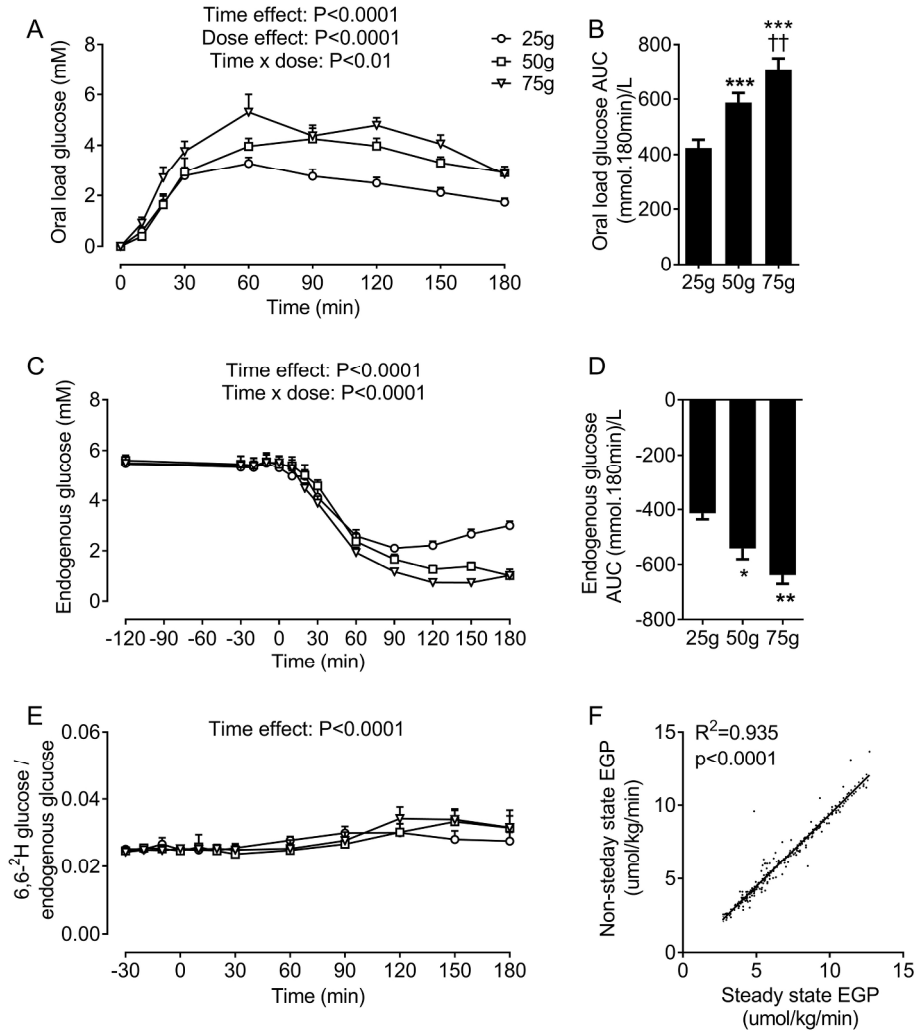

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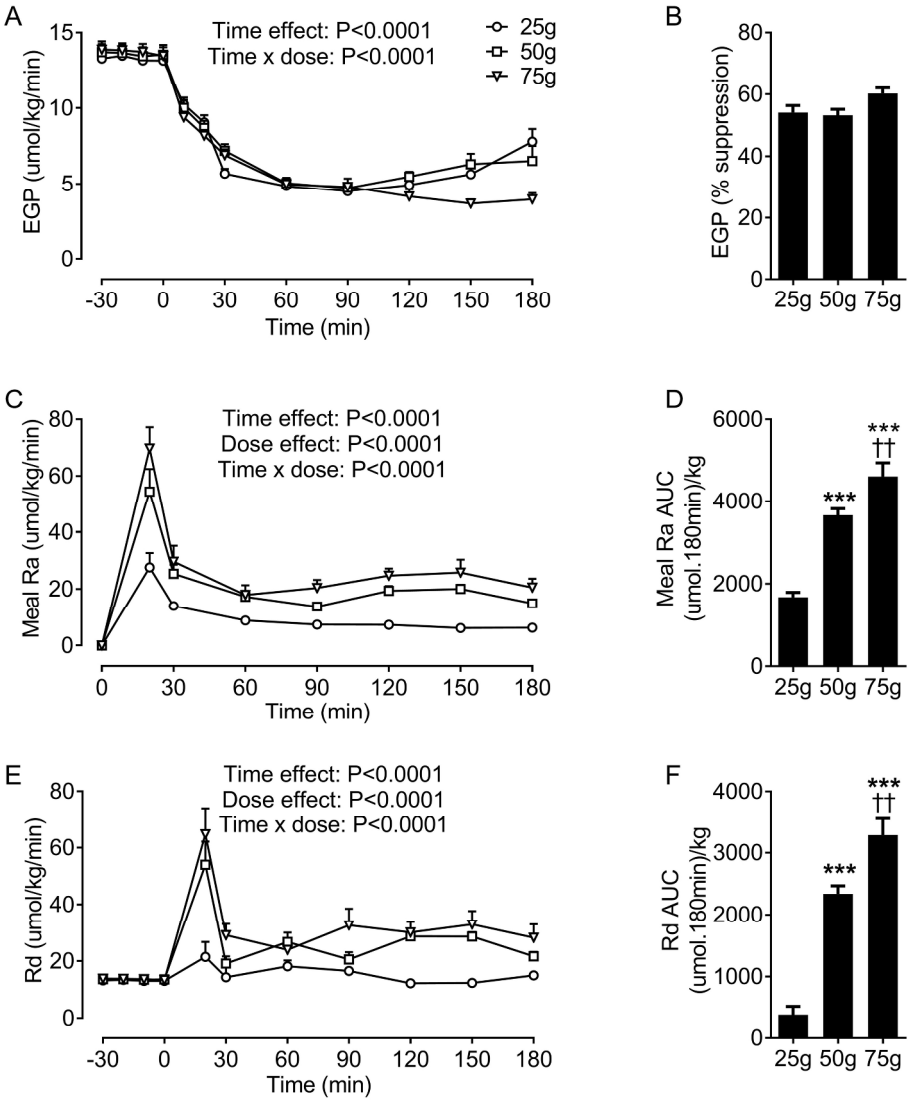

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