Insulin Administration and Rate of Glucose Appearance in People With Type 1 Diabetes

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OBJECTIVE — To assess whether prandial insulin, in addition to basal insulin, has an effect on the rate of glucose appearance from a meal in people with type 1 diabetes.

RESEARCH DESIGN AND METHODS — The rate of glucose appearance from a mixed meal (Ra_{meal}) was investigated in six adult (aged 24 ± 2 years), lean (BMI 23.6 ± 1.5 kg/m^2) subjects with well-controlled type 1 diabetes (duration 7.9 ± 6.9 years, A1C 7.6 ± 0.9%) with/without prandial insulin. Actrapid was infused to maintain euglycemia before meals were consumed. Subjects consumed two identical meals on separate occasions, and Ra_{meal} was measured using a dual isotope method. [6,6-^{2}H$_2$]glucose was incorporated into the meal (0.081 g/kg body wt), and a primed constant/variable rate infusion of [1,2,3,4,5,6,6-^{2}H$_2$]glucose was administered. In the tests with prandial insulin, an additional bolus dose of Actrapid was given 20 min before the meal at 0.1 units/kg body wt.

RESULTS — Insulin concentration with prandial insulin was significantly higher than during basal insulin studies (119 ± 16 vs. 66 ± 15 pmol/l, $P = 0.03$ by paired t test). Despite differences in insulin concentration, there were no differences in total glucose appearance (3,398 ± 197 vs. 3,307 ± 343 μmol/kg) or time taken for 25% (33.1 ± 3.3 vs. 31.7 ± 3.5 min), 50% (54.6 ± 3.5 vs. 54.1 ± 4.7 min), and 75% (82.9 ± 7.1 vs. 82.8 ± 5.8 min) of total glucose appearance. The fraction of the glucose dose appearing in the circulation was the same for basal (73 ± 8%) and prandial (75 ± 4%) study days.

CONCLUSIONS — These results suggest that meal glucose appearance is independent of prandial insulin concentration in people with type 1 diabetes.

P lasma glucose concentration is determined by several factors: the production of glucose by the body, the uptake of glucose by splanchnic and peripheral tissue, and the appearance of exogenous glucose from meals (1). Plasma insulin regulates the production and uptake of glucose (2,3), but its role in regulating rates of glucose appearance from meals (Ra_{meal}) is uncertain.

Ra_{meal} is determined by the rate at which glucose is emptied from the stomach and absorbed across the intestinal membrane, and by the extent of extraction during first pass of the liver and other splanchnic tissues, before reaching the general circulation. The modification by insulin of any of these processes would act to regulate postprandial glucose levels and would be an important consideration for people with diabetes. In particular, for people with type 1 diabetes, the effect of insulin on Ra_{meal} has important implications for the timings of prandial insulin

injections and the appropriateness of pre-/post-meal insulin dosing.

Research in this area is limited and, although there is some in vitro and in vivo evidence that insulin plays a role in regulating glucose appearance in rats (4–6), these findings have yet to be reproduced in human studies. In people with poorly controlled type 1 diabetes, Ra_{meal} was normal and unchanged with intensive insulin therapy (7). However, these studies were designed to investigate the effects of longer-term hyperglycemia/insulin deficiency, and the effect of acute insulin deficiency on Ra_{meal} was not independently investigated.

No study to date has examined the immediate independent effect of bolus exogenous insulin administration on Ra_{meal} in people with type 1 diabetes. This study therefore aimed to compare Ra_{meal} in the presence of prandial insulin with that measured at basal insulin concentrations in people with type 1 diabetes.

RESEARCH DESIGN AND METHODS — This study was approved by the Norfolk local research ethics committee, and written informed consent was obtained from each subject before participation. Volunteers with type 1 diabetes were invited to the Medical Research Council (MRC) Human Nutrition Research (HNR) where their blood pressure and hemoglobin were determined. A1C levels were measured and subjects excluded if they had elevated blood pressure, anemia, or A1C >11%. Six lean (BMI 23.6 ± 1.5 kg/m^2) adult (24 ± 2 years) subjects with well-controlled type 1 diabetes (duration 7.9 ± 6.9 years, A1C 7.6 ± 0.9%) treated with multiple daily injection (MDI) regimens were recruited.

All subjects participated in 2 study days where their response to glucose incorporated into a solid meal (sweet pancakes) was measured. Studies were conducted at Addenbrooke's Hospital Wellcome Trust Clinical Research facility (WTCRF). Subjects were admitted to the WTCRF volunteer suite at 1800 h on the evening before each study day, and two cannulas were inserted into antecubital veins: one for frequent blood sampling and one for intravenous infusions. Subjects

were given an evening meal of their choice, identical on each study visit, and took their normal short-acting prandial insulin dose. Subjects omitted their usual long-acting evening insulin dose, and plasma glucose levels were stabilized overnight using a variable-rate intravenous insulin infusion (Actrapid) based on the recommendation of a model-predictive controller (8).

At 0600 h the next day, a primed (1 mg/kg) intravenous infusion of $[1,2,3,4,5,6,6-^2H_2]$glucose (0.1 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) was begun and continued for the duration of studies. As implemented by other investigators (9), in order to minimize non–steady state errors in calculating the endogenous glucose production (EGP), changes in glucose specific activity were reduced by maintaining the ratio of tracer ($[1,2,3,4,5,6,6-^2H_2]$glucose) to tracee (endogenous glucose component) as constant as possible. During studies at basal insulin, where no change in EGP were anticipated, the infusion rate of $[1,2,3,4,5,6,6-^2H_2]$glucose was maintained constant. In studies when an additional bolus dose of insulin was administered, EGP was anticipated to vary and infusion rates were reduced accordingly: 0–10 min, 100%; 10–20 min, 95%; 20–30 min, 80%; 30–40 min, 70%; 40–50 min, 45%; 50–60 min, 40%; 60–110 min, 35%; 110–140 min, 40%; 140–180 min, 45%; 180–240 min, 55%; 240–270 min, 65%; and 270–300 min, 70%. At 0700 h, the intravenous insulin infusion was fixed at the average rate required to maintain euglycemia for the previous hour and maintained constant for the remainder of the study day.

At ~0800 h on both study days, subjects were given a meal (sweet pancakes) with energy derived as 45% from carbohydrate, 40% from fat, and 15% from protein sources and contained 0.9 g glucose/kg body wt. Meal size was adjusted for body weight (8 kcal/kg body wt), and $[6,6^2H_2]$glucose was incorporated to replace 9% of meal glucose. Two baseline blood samples were taken to establish fasting glucose, insulin, and isotope concentrations, and subjects were then given the meal. A further 22 blood samples (5 ml) were taken at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 165, 180, 195, 210, 240, 270, and 300 min. On basal insulin study days, no additional insulin was given, but on prandial insulin study days, a dose of Actrapid
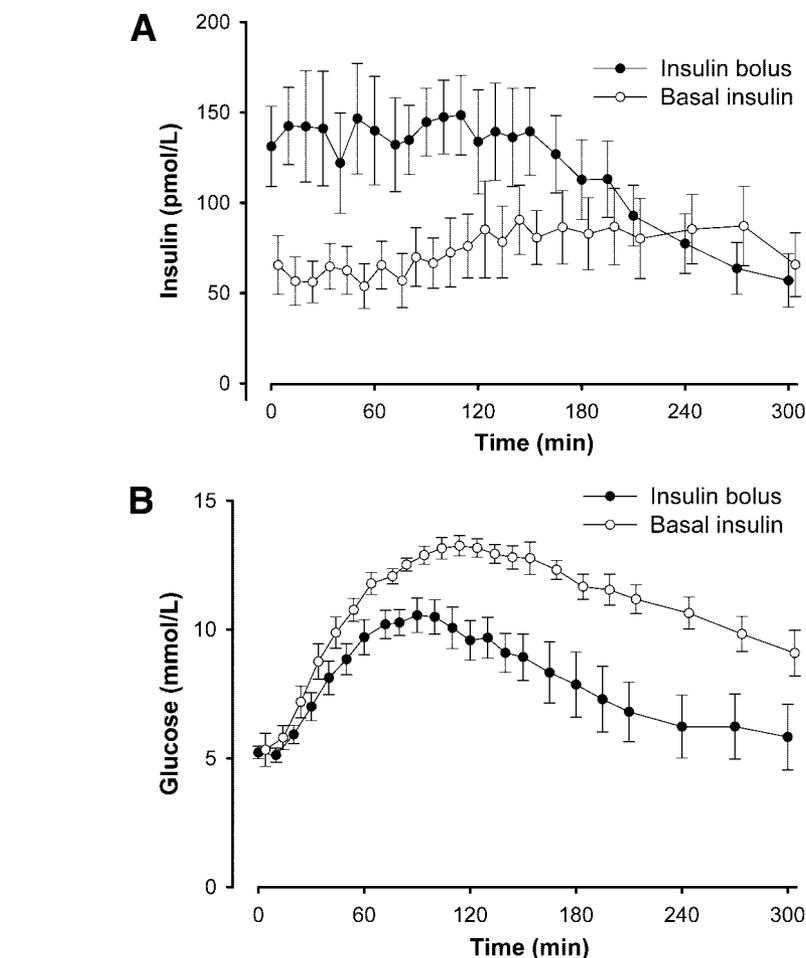
was given 20 min before the meal (0.1 unit/kg body wt).

Analytical techniques

All blood samples were kept on ice before plasma was separated by centrifugation and subsequently kept at $-80°C$ until analysis was performed. Plasma insulin was measured by a 1235 AutoDELFIA automatic immunoassay system using a two-step time-resolved fluorometric assay (kit no. B080-101; DAKO, Ely, Cambridgeshire, U.K.) (coefficient of variation 2%) using the method previously described (10). Plasma glucose concentration was determined from whole blood samples immediately after each blood sample was taken using a Yellow Springs Instrument (Lynchford House, Farnborough, Hants, U.K.), and $[6,6-^2H_2]$glucose and $[1,2,3,4,5,6,6-^2H_2]$glucose isotopic enrichment was measured in duplicate by gas chromatography–mass spectrometry using an Agilent 5973N (Agilent Technologies, Workingham, U.K.).

Mathematical methods and calculations

The method utilizing a two compartment glucose model and the maximum likelihood approach combined with a regularization method was used for calculation of Ra$_{meal}$ and EGP from the measured concentrations of glucose, $[6,6-^2H_2]$glucose and $[1,2,3,4,5,6,6-^2H_2]$glucose (11).

The method, originally designed for the triple tracer meal study design, was adapted for the dual tracer study design. Briefly, the method assumes smoothness of *1*) the fractional glucose clearance, *2*) EGP, and *3*) Ra$_{meal}$, whereas the traditional approaches assume smoothness of the measured concentrations of native glucose and tracer-to-tracee ratios (12). The extent of smoothness is determined by the SD of the measurement error for native and tracer glucose. A two compartmental model of glucose kinetics was assumed with population values for model parameters identical across all glucose species with $k_{21} = 0.05$/min,

Figure 1—*Plasma insulin (A) and glucose (B) concentrations following a meal under conditions of basal insulin and with an additional insulin bolus at −20 min.*

$k_{12} = 0.07/min$, and $V_1 = 160$ ml/kg body wt (13), where k_{21} and k_{12} represent rate constants of glucose transfer between plasma and interstitial glucose compartments and V_1 represents the glucose volume of distribution.

Total glucose appearance was calculated from the area under the curve (AUC) of the glucose appearance/time profiles. Time taken for 20, 50, and 75% of total glucose appearance was calculated by assuming a linear profile between appearance rates at each time point. The fraction of ingested glucose appearing in the circulation was calculated as the ratio of total glucose appearance to the dose size administered.

The total amount of glucose appearing in the circulation on each study day was compared using paired *t* tests, as was the time taken for 25, 50, and 75% of total glucose appearance to take place. Fractional glucose appearance on prandial and basal insulin study days were also compared by paired *t* test. Results are presented as means ± SEM (± SD for baseline measurements). Statistical significance was declared at $P < 0.05$.

RESULTS

Baseline data

Before the meal was given, basal glucose levels were not different on basal (I_B) and prandial (I_P) study days (I_P 5.5 ± 0.5 vs. I_B 5.3 ± 0.2 mmol/l, P = NS). As insulin was administered 20 min before the meal on prandial insulin study days, basal insulin levels at time 0 were significantly elevated on prandial study days (I_P 119 ± 16 vs. I_B 66 ± 15 pmol/l, P = 0.03).

Postprandial glucose, insulin, Ra_{meal}, and EGP

Postprandial glucose, insulin, Ra_{meal}, and EGP profiles are presented in Figs. 1 and 2. On prandial compared with basal insulin study days, there were significantly higher AUC insulin and average insulin concentrations, whereas AUC total glucose concentrations were significantly lower (Table 1). There was no difference in total glucose appearance (Table 1), Ra_{meal} at any time point (Fig. 2A), or time taken for 25, 50, and 75% of total glucose appearance to take place (Table 1). The fraction of the glucose dose appearing in the circulation was the same under both conditions and was, on average, 75 ± 4% and 73 ± 8% (P = NS) under prandial and basal conditions, respectively. There was no significant difference in total AUC
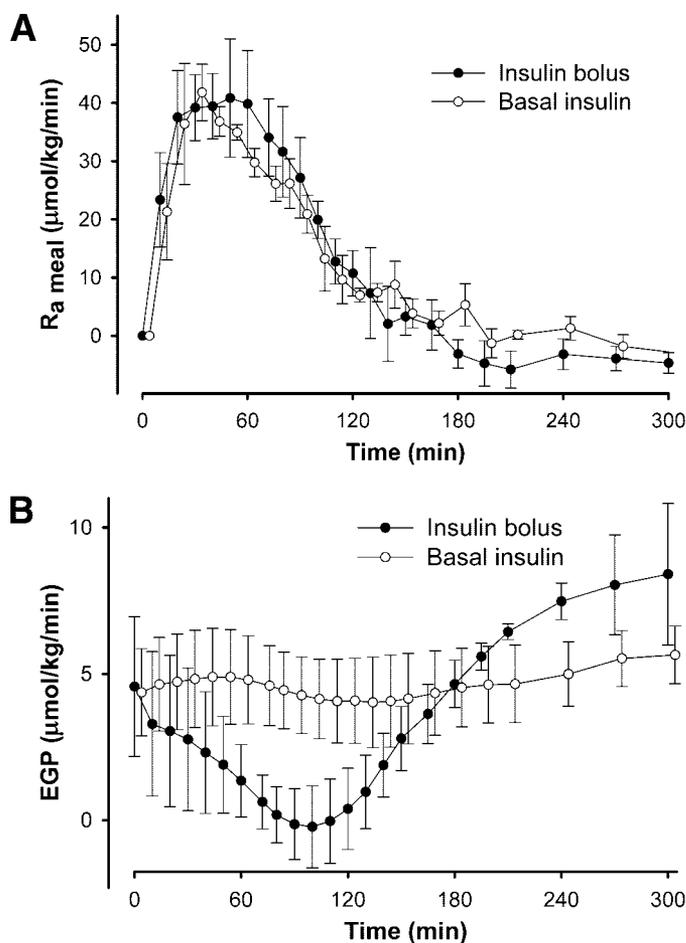
Figure 2—*Rates of postprandial meal–derived glucose appearance (Ra_{meal}) (A) and EGP (B) following a meal under conditions of basal insulin and with an additional insulin bolus at −20 min.*

EGP on different study days, but different patterns were evident (Fig. 2B).

CONCLUSIONS — The current work shows that an exogenous dose of insulin, given before a meal, had no significant effect on rates of glucose appearance or total fractional glucose appearance in people with type 1 diabetes.

Insulin and Ra_{meal}

Rat studies suggest that insulin may have important effects on intestinal glucose absorption (4–6). Conversely, a study of dogs showed no effect of insulin on glucose absorption (14), although direct application of animal work to humans may be unsuitable due to differences in the physiology of glucose metabolism.

Table 1—*AUC and mean plasma insulin, AUC and mean plasma glucose, AUC Ra_{meal}, fractional glucose appearance, and time taken for 25, 50, and 75% appearance on basal insulin (I_B) and prandial insulin (I_P) study days*

	I_B	I_P	P
AUC insulin (nmol/l per 300 min)	18.6 ± 5.1	34.1 ± 3.8	0.030
Mean insulin (pmol/l)	66 ± 15	119 ± 16	0.030
AUC glucose (mmol/l per 300 min)	3,351 ± 104	2,625 ± 124	0.004
Mean glucose (mmol/l)	10.9 ± 0.3	8.2 ± 0.6	0.004
AUC Ra_{meal} (μmol/kg)	3,307 ± 343	3,398 ± 197	NS
Fractional appearance (%)	73 ± 8	75 ± 4	NS
Time taken for glucose appearance (min)			
25% dose	31.7 ± 3.5	33.1 ± 3.3	NS
50% dose	54.1 ± 4.7	54.6 ± 3.5	NS
75% dose	82.8 ± 5.8	82.9 ± 7.1	NS

A study in humans, investigating the effect of hyperglycemia on meal glucose appearance rate, showed that at plasma glucose concentrations of 6 or 10 mmol/l, Ra_{meal} was equivalent (15). Pehling et al. (7) investigated rates of glucose appearance in healthy subjects and those with poorly controlled type 1 diabetes and subsequently restudied those with type 1 diabetes during intensive insulin therapy. Despite insulin deficiency and fasting and postprandial hyperglycemia, people with untreated diabetes showed no difference in Ra_{meal} compared with healthy individuals (7), and following intensive insulin therapy, there was similarly no difference in rates of meal glucose appearance. These studies are in agreement with the current work, where insulin and glucose levels did not appear to affect rates of meal glucose appearance.

If Ra_{meal} is considered to represent the sum of processes of gastric emptying, glucose absorption, and first pass splanchnic extraction, then although counterbalancing changes in these processes cannot be ruled out, it seems likely that all three were not affected by plasma insulin concentration. The lack of effect on first pass splanchnic extraction may be surprising. Insulin exerts large effects on peripheral glucose uptake into muscle and adipose tissue (16), and a similar function in splanchnic tissues might be expected. The mode of glucose transport, predominately via GLUT4 into peripheral tissue and GLUT2 into splanchnic tissue, can explain this difference. GLUT4 is insulin regulated, while GLUT2 is almost entirely regulated by, and directly proportional to, plasma glucose concentration (3); the absence of an effect by insulin may therefore be expected.

Fractional systemic glucose appearance

The fractional appearance of meal-derived glucose can be determined by the percentage of ingested glucose that appears in the circulation. In the current work, fractional glucose appearance was, on average, 74 ± 4% (I_B 73 ± 8 vs. I_P 75 ± 4%, P = NS). The difference between glucose ingested and that appearing in the systemic circulation may be accounted for by retention in the small intestine, fermentation to lactate, and splanchnic tissue and liver extraction on first pass into the general circulation. The contribution of glucose fermentation to lactate is thought to be small (17), but direct arterio-hepatic-venous difference experiments have shown first pass hepatic glucose extraction to be important and to be responsible for extracting ~5–8% of the ingested dose (18).

Despite this, far lower values and large variations in fractional glucose appearance have been observed by investigators working in different laboratories. The review by Livesey et al. (19) suggests that fractional appearance of glucose following oral glucose administration ranges from 65 to 104% in healthy adults and people with type 1 or type 2 diabetes. There appears to be systematic differences in measurement, since errors reported in individual studies are reasonably small compared with the range of mean values obtained.

Although some investigators suggest that differences can be accounted for by retention in the intestinal lumen and first pass hepatic extraction (15), others argue that discrepancies are due to errors in modeling methods used. In the current work, a two-compartmental model is applied to the dual isotope approach with assumed values for model kinetic parameters. A two-compartmental model has been shown to improve estimates of Ra_{meal} (19) compared with Steele's one-compartmental model (20) and, assuming population values for rate constants, simplifies further calculation of Ra_{meal}. More recently, however, investigators argue that the dual isotope method is inadequate and that three tracers are needed for accurate estimation of Ra_{meal} (21). During these comparative studies, although the dual-tracer method appeared to underestimate overall systemic glucose appearance (16% lower), profiles for dual- and triple-tracer techniques were very similar (21), and it may be considered that a two-compartmental dual isotope approach is acceptable.

Incomplete absorption could also explain the lower rates of fractional appearance. Nearly complete absorption has been estimated over 3.5 h following an oral glucose load (19), but following glucose given as part of a mixed meal, >5 h was necessary for complete glucose absorption (7). In the current work, measurements were made for only 5 h, but due to the composition of the meal (carbohydrate in the form of pure glucose), glucose was likely to have been absorbed more rapidly. Indeed, from the average Ra_{meal} profiles (Fig. 2A) it appears that absorption was finished, and incomplete absorption seems unlikely to have been an issue.

From studies reviewed by Livesey et al. (19), there is no difference between average values for fractional appearance rates in healthy people (80.6 ± 3.1%) and those with impaired glucose tolerance/type 2 diabetes (85 ± 2.3%, P = 0.31). Only one study investigated total appearance in subjects with type 1 diabetes, and the estimate of 93% (7) is well within the range of that observed in healthy individuals. There appears to be no evidence that the fraction of glucose appearing in the systemic circulation is affected in type 1 diabetes, and this is supported by the current work, where typical values were obtained.

Exogenous insulin affects not only plasma insulin concentrations but also plasma glucose levels. Since insulin and glucose levels changed simultaneously in opposite directions in the current work (increased insulin but reduced glucose concentrations), it may be speculated that they acted in opposite directions and, by counterbalancing one another, Ra_{meal} remained unchanged. Despite this possibility, the current work still gives evidence for the overall impact of a bolus dose of insulin: Ra_{meal} and total fractional appearance are independent of exogenous insulin administration in people with type 1 diabetes.

In conclusion, the present study suggests that, in people with type 1 diabetes, exogenous insulin administration does not affect postprandial Ra_{meal} or the fraction of glucose appearing in the circulation. The injection of an insulin dose before or after a meal, in contrast to its affect on glucose utilization, would not affect the rate of glucose appearance. These findings provide important information for people with type 1 diabetes when considering the size and timing of meal-time insulin doses.

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