Effects of Type 2 Diabetes on Insulin Secretion, Insulin Action, Glucose Effectiveness and Postprandial Glucose Metabolism

Ananda Basu, MD
Chiara Dalla Man, PhD*
Rita Basu, MD
Gianna Toffolo, PhD*
Claudio Cobelli, PhD*
Robert A. Rizza, MD

Division of Endocrinology and Metabolism, Mayo Clinic College of Medicine,
200, First Street SW
Rochester, MN 55905

* Department of Information Engineering, University of Padova, Padova, Italy

Corresponding author and contact for reprints:
Ananda Basu MD, FRCP.
basu.ananda@mayo.edu

Additional information for this article can be found in an online appendix at http://care.diabetesjournals.org.


This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
**Objective**: This study sought to determine whether postprandial insulin secretion, insulin action, glucose effectiveness and glucose turnover were abnormal in type 2 diabetes.

**Research Design**: 14 subjects with type 2 diabetes and 11 non-diabetic subjects matched for age, weight and body mass index underwent a mixed meal test using the triple tracer technique. Indices of insulin secretion, insulin action, and glucose effectiveness were assessed using the oral “minimal” and C-peptide models.

**Results**: Fasting and postprandial glucose concentrations were higher in the diabetic than non-diabetic subjects. Although peak insulin secretion was delayed ($p<0.001$) and lower ($p<0.05$) in type 2 diabetes, integrated total postprandial insulin response did not differ between groups. Insulin action, insulin secretion, disposition indices and glucose effectiveness all were lower ($p<0.05$) in diabetic than non-diabetic subjects. Whereas the rate of meal glucose appearance did not differ between groups, the percent suppression of EGP was slightly delayed and increment in glucose disappearance was substantially lower ($p<0.01$) in diabetic subjects during the first three hours after meal ingestion. Together, these defects resulted in an excessive rise in postprandial glucose concentrations in the diabetic subjects.

**Conclusion**: When measured using methods that avoid non-steady error, the rate of appearance of ingested glucose was normal and suppression of EGP only minimally impaired. However, when considered in light of prevailing glucose concentration, both were abnormal. In contrast, rates of postprandial glucose disappearance were substantially decreased due to defects in insulin secretion, insulin action, and glucose effectiveness.
Glucose concentrations are determined by the balance between the rate of glucose entering and leaving the systemic circulation. Fasting hyperglycemia in type 2 diabetes (DM) occurs when endogenous glucose production (EGP) is inappropriately increased and glucose disappearance (Rd) inappropriately decreased when considered in light of prevailing glucose and insulin concentrations (1-4). The cause of excessive rise in glucose that occurs following mixed meal ingestion is not well established. Whereas postprandial suppression of EGP has consistently been reported to be delayed (5-7), rates of appearance of glucose in the meal (MRa) have been reported to be increased, decreased or no different than those observed in non-diabetic subjects (5; 6; 8). Similarly, postprandial Rd has been reported to be increased or not different when compared to those observed in non-diabetic subjects (5-10).

However, all the above studies have used a dual tracer approach in which one tracer is added to ingested glucose and another infused intravenously. The intravenously infused tracer is used to trace the rate of appearance of both ingested tracer and rate of total glucose appearance (Ra). MRa is calculated by multiplying the rate of appearance of ingested tracer by its enrichment in the meal and EGP by subtracting MRa from Ra. Rd is calculated by subtracting the change in plasma glucose mass from Ra. Unfortunately, the validity of all of these calculations is jeopardized by the marked change in tracer to tracee ratios that occurs following carbohydrate ingestion with the dual tracer approach (11; 12). Perhaps even more problematic, differences in MRa and Ra can lead to differences in tracer to tracee ratios (12). Depending on the magnitude of change in glucose concentration and turnover, non-steady state error could account for the discrepant results amongst previous studies that have used the dual tracer method to compare the pattern of postprandial glucose metabolism in diabetic and non-diabetic subjects (5-10).

The present studies were undertaken to reassess the relative contribution of alterations in EGP, MRa and Rd to postprandial hyperglycemia in people with DM using a triple (rather than dual) tracer approach (11) that is designed to minimize postprandial changes in tracer to tracee ratios that are used to measure MRa and EGP. We also used the oral “minimal” and C-peptide models (13; 14) to determine whether changes in pattern of postprandial glucose metabolism are accompanied by alterations in insulin secretion, insulin action and glucose effectiveness.

**RESEARCH DESIGN AND METHODS:**

**Participants:** After approval from Mayo Clinic Institutional Review Board, 14 diabetic and 11 non-diabetic subjects provided written informed consent to participate in the study. Subject characteristics are provided in Table A1 (available in an online appendix at [http://care.diabetesjournals.org](http://care.diabetesjournals.org)).

All participants were in good health and none regularly engaged in vigorous physical exercise. Oral anti-hyperglycemic medications were discontinued three weeks prior to study. Two diabetic and one nondiabetic subjects were on thyroxine replacement therapy but had normal TSH. All participants were instructed to follow a weight maintenance diet for three days prior to study. At screening, body composition and visceral fat were measured using dual-energy x-ray absorptiometry and a single cut CT scan (15).

Subjects were admitted to Mayo Clinical Research Unit at 1700 hours on the evening prior to study. After eating a 10
Prandial glucose turnover in type 2 diabetes

4 kcal/kg meal (55% carbohydrate, 30% fat and 15% protein) subjects received nothing by mouth except water until the following morning. At ~0600 on morning of study, an 18-G cannula was inserted in a hand vein and the hand placed in a heated plexiglass box (~55°C) to obtain arterialized venous blood samples. Another 18-G cannula was inserted into the opposite forearm for tracer infusion. A primed-continuous infusion of [6,6-²H₂] glucose (14.8 mg/kg prime; 0.148 mg/kg continuous; MassTrace, Woburn, MA) was started at 0700 (time –120) and continued until end of study. For diabetic individuals, the prime dose was adjusted upwards depending on the ambient glucose concentration. At 0900 (time 0) a mixed meal (10 kcal/kg, 45% carbohydrate, 40% fat, 15% protein) consisting of scrambled eggs, Canadian bacon, 100 ml water and Jell-O (1.2 g/kg body weight of glucose) containing [1-¹³C] glucose was consumed within 15 minutes. An infusion of [6-³H] glucose also was started at time 0 and the rate varied to mimic the anticipated rate of appearance of [1-¹³C] glucose contained within the meal (11; 14). Simultaneously, the [6,6-²H₂] glucose infusion rate was altered to mimic the anticipated fall in rate of EGP.

**Analytical Techniques:** Arterialized samples were placed on ice, centrifuged at 4°C, separated and stored at –20°C until assay. Plasma glucose concentration was measured using a glucose oxidase method (YSI, Inc., Yellow Springs, OH). Plasma insulin concentration was measured using a chemiluminescence assay (Access Assay, Beckman Coulter, Inc., Chaska, MN) and C-Peptide using radioimmunoassay (Linco Research, St. Louis, MO). Plasma [6-³H] glucose specific activity was measured by liquid scintillation counting as previously described (11). Plasma enrichment of [1-¹³C] glucose and [6,6-²H₂] glucose were measured using GCMS (Thermoquest, San Jose, CA) to simultaneously monitor the C₁₂ and C₃-₆ fragments (16).

**Calculations:** Fasting and postprandial rates of glucose turnover were calculated as detailed elsewhere (11). Briefly, the systemically infused [6-³H] glucose was used to trace the systemic rate of appearance of [1-¹³C] glucose that was contained in the meal whereas [6,6-²H₂] glucose was used to trace the rate of appearance of endogenously produced glucose. The ratio of plasma concentration of [6-³H] glucose to [1-¹³C] glucose was used to calculate the rate of appearance of ingested [1-¹³C] glucose and the ratio of plasma concentration of [6,6-²H₂] glucose to plasma concentration of endogenously produced glucose was used to calculate EGP. The plasma concentration of endogenously produced glucose was calculated by subtracting the concentration of exogenously derived (ingested) glucose (i.e. plasma [1-¹³C] glucose concentration times meal [1-¹³C] glucose enrichment) from total plasma glucose concentration (11).

Indices of net insulin action (Si), glucose effectiveness (Sg) and of the ability of insulin (Si*) and glucose (Sg*) to stimulate glucose disposal were calculated using the oral “minimal” model as previously described (13; 14; 17). Indices of insulin secretion including Φ_total (total response to the glycemic stimuli) Φ_dynamic (response to a change in glucose concentration), and Φ_static (response to a given glucose concentration) were calculated using the oral C-peptide secretion model as previously described (14). Disposition indices (DI) were calculated by multiplying indices of insulin secretion times indices of insulin action in order to determine whether insulin secretion was appropriate for the degree of insulin resistance.

**Statistics:** All results are expressed as mean ± sem. Students two-tailed unpaired t tests were performed to compare normally distributed glucose turnover data while non-parametric Mann-Whitney tests were performed to
RESULTS:

**Plasma glucose, insulin, C-peptide and glucagon concentrations (Figure 1):** Plasma glucose concentrations were higher (p<0.001) in diabetic than non-diabetic individuals before the meal (9.1 ± 0.7 vs. 5.2 ± 0.1 mmol/L) and increased to a higher peak (p<0.001) after the meal (18.1 ± 0.9 vs. 10.8 ± 0.7 mmol/L) resulting in a greater (p<0.0001) postprandial integrated response above basal in diabetic than non-diabetic subjects (1671 ± 125 vs. 557 ± 92 mmol/L over 6 hours).

Fasting insulin concentrations (59 ± 9 vs. 37 ± 6 pmol/L) did not differ between diabetic and non-diabetic individuals. However, peak postprandial plasma insulin concentrations were lower (382 ± 54 vs. 673 ± 126 pmol/L; p<0.02) and occurred later (156 ± 16 vs. 62 ± 7 minutes; p<0.001) in diabetic than non-diabetic subjects. This led to a smaller (p<0.001) increase in insulin above basal (21 ± 4 vs. 52 ± 8 nmol/L over 2 hours) in diabetic subjects during the first two hours after the meal but no difference in the integrated insulin response above basal for the entire six hours of study (79 ± 14 vs. 80 ± 13 nmol/L over 6 hours).

C-peptide concentrations did not differ between diabetic and non-diabetic individuals before meal ingestion but increased more (p<0.05) in diabetic than non-diabetic subjects during the first two hours after meal ingestion (5.1 ± 0.9 vs. 2.1 ± 1.0 ng/ml/2 hours). Glucagon concentrations did not differ in the two groups from two hours onward resulting in a comparable integrated response over six hours of study (13.2 ± 1.8 vs. 11.3 ± 2.1 ng/ml over 6 hours).

**Indices of insulin action (figure 2a):** Net insulin action (Si: 4.6 ± 0.8 vs. 10.4 ± 2.9 10^-4 min^-1 per µU/ml) and net glucose effectiveness (Sg: 0.019±0.002 vs. 0.034±0.006 min^-1) were lower (p<0.03) in diabetic than non-diabetic subjects following meal ingestion. Likewise, the effect of insulin on glucose disposal (S_i*:1.25 ± 0.25 vs. 4.78 ± 1.53 10^-4 min^-1 per µU/ml) and the effect of glucose on glucose disposal (S_g*:0.0016 ± 0.0006 vs. 0.172 ± 0.001 min^-1) were lower (p<0.02) in diabetic than non-diabetic subjects (data not shown).

**Indices of insulin secretion (figure 2b):** Insulin secretion in response to the total glycemic stimulus (Φ_total) was lower (p<0.01) in diabetic than non-diabetic subjects (20.7 ± 3.0 vs. 52.6 ± 4.1 10^-9/min). This was due to a decrease (p<0.0001) in both the dynamic response to a change in glucose (Φ_dynamic: 269.5 ± 44.0 vs. 638.1 ± 69.1 10^-9), and a decrease (p<0.001) in the response to a given glucose concentration (Φ_static:19.3 ± 2.9 vs. 46.6 ± 3.8 10^-9/min).

Disposition indices, calculated in order to adjust insulin secretion for the prevailing level of insulin action, all were lower (p<0.002) in diabetic than non-diabetic subjects including DI_total (227 ± 51 vs. 1104 ± 276 10^-14 dl/kg/min per pmol/L), DI_dynamic (2897 ± 748 vs. 11403.4 ± 1700 10^-14 dl/kg/min^2 per pmol/L), and DI_static (209 ± 47 vs. 964 ± 228 10^-14 dl/kg/min per pmol/L).

**Tracer to tracee ratios (Figure 3a):** The plasma ratio of [6-^3H] glucose to [1-^13C] glucose...
Prandial glucose turnover in type 2 diabetes

glucose (used to calculate MRa) increased during the first ten minutes after start of the meal in both groups then changed minimally thereafter. The plasma ratio of \([6,6-2H_2]\) glucose to plasma endogenous glucose concentration (used to calculate EGP) decreased slightly but equally in both groups during the first hour after meal ingestion. With the exception of a marked increase that occurred in one non-diabetic subject, this ratio then gradually rose from sixty minutes onward in both groups.

**MRa, EGP and Rd (Figure 3b):** MRa increased rapidly reaching a peak in both groups between twenty to thirty minutes postprandially then returned towards baseline over the next six hours. Although peak MRa (74.9 ± 5.4 vs. 93.6 ± 7.0 µmol/kg/min) was slightly lower (p<0.05) in diabetic than non-diabetic subjects MRa during the first two hours (6.7 ± 0.5 vs. 7.3 ± 0.5 mmol/kg over 2 hours) or over the six hours of study (12.8 ± 0.7 vs. 13.4 ± 0.8 mmol/kg over 6 hours) did not differ between groups. Hence, splanchnic extraction of ingested glucose also did not differ between the diabetic and non-diabetic subjects (8.8±0.4 vs. 8.2±0.5 mmol/kg over 6 hours).

EGP did not differ in diabetic and non-diabetic subjects prior to meal ingestion (19.4 ± 0.9 vs. 18.8 ± 0.7 µmol/kg/min) and decreased in both groups after meal ingestion. This resulted in no difference in suppression below basal over the entire six hours of study (−4.0 ± 0.3 vs. −3.6 ± 0.2 mmol/kg over 6 hours) but a lower (p<0.05) percent suppression from baseline in diabetic than non-diabetic subjects during the first three hours (45.9 ± 1.8 vs. 53.0 ± 3.8%).

Rd did not differ in diabetic and non-diabetic subjects before meal ingestion (20.2 ± 0.85 vs. 19.6 ± 0.74 µmol/kg/min). While the increment above basal did not differ in diabetic and non-diabetic individuals over the entire six hours of study (8.7 ± 0.7 vs. 9.9 ± 0.7 mmol/kg over 6 hours), it was lower (p<0.01) in diabetic subjects during the first three hours after meal ingestion (4.8 ± 0.5 vs. 7.9 ± 0.8 mmol/kg over 3 hours).

**DISCUSSION**

The present data indicate that a low Rd is the primary cause of excessive postprandial rise in glucose concentration in DM. Defects in insulin secretion, insulin action and glucose effectiveness likely contribute to the low rates of postprandial Rd. While postprandial suppression of EGP is modestly delayed and not appropriate for the prevailing glucose concentration, the absolute rate of postprandial EGP was only minimally greater in diabetic than non-diabetic subjects. Therefore, while lack of appropriate suppression of EGP may have exacerbated the postprandial rise in glucose, it did not cause it. MRa did not differ between groups.

Despite a greater glycemic excursion, MRa was slightly lower in diabetic than non-diabetic subjects during the first hour after meal ingestion. Therefore increased meal appearance was not the cause of excessive postprandial hyperglycemia. This is consistent with results from most, but not all of previous experiments that have measured meal appearance using the dual tracer method (5-10). Since the ratio of ingested tracer to the infused tracer markedly increases as ingested glucose enters the circulation the resultant rapid change in tracer to tracee ratio introduces error into the calculation of MRa (12; 19). This does not happen when the plasma ratio of the ingested and infused tracers is kept constant as was done in the present experiments since measurement of meal appearance becomes essentially model independent (13; 14). When the rate of intravenously infused tracer is kept constant, the plasma ratio of the ingested to infused tracer is solely determined by the rate of appearance of ingested tracer since both the tracer and tracee are cleared in parallel. However, it is noteworthy that since
Prandial glucose turnover in type 2 diabetes

hyperglycemia is a potent stimulus of hepatic glucose uptake (18), the fact that MRa was comparable in diabetic and non-diabetic subjects despite far higher glucose concentrations in the former is consistent with impaired hepatic glucose uptake (18; 20).

Rate of suppression of EGP following meal ingestion was slightly slower in diabetic than non-diabetic subjects. This is similar to the report by Taylor et al (21) when postprandial suppression of EGP was measured using a variable intravenous glucose tracer infusion analogous to that employed in the present experiments. Delayed suppression of EGP also has been reported in studies using the dual tracer approach (5; 6). EGP measured with this approach is calculated by subtracting MRa from Ra. Since Ra also is measured with the constant intravenous tracer, non-steady state error caused by the rapid fall of plasma tracer to tracee ratio that occurs immediately after eating results in an underestimation of Ra. This is followed by a rise in tracer to tracee ratio that results in an overestimation of Ra. Therefore, while the absolute rates may be wrong, if the size of the error in both MRa and Ra are the same in both groups, then conclusions regarding the temporal pattern of suppression of EGP of one group relative to the other could be correct. This possibly accounts for the fact that previous studies using the dual tracer method (5-10) and the present studies as well as those of Taylor et al (21) have concluded that postprandial suppression of EGP is delayed in DM.

EGP did not differ in diabetic and non-diabetic subjects before meal ingestion. However, plasma glucose concentrations were substantially higher in diabetic than non-diabetic subjects. Since hyperglycemia suppresses EGP, this indicates that rates were not appropriate for the prevailing glucose concentration (22). We and others have reported that absolute rate of EGP is increased in people with severe diabetes as indicated by marked fasting hyperglycemia (2; 3; 23; 24). In addition, diabetic subjects in the present study had a HbA1c of 6.8% at time of screening indicating excellent glycemic control. Therefore, it is probable that abnormalities in regulation of EGP would be even more marked in individuals with poor glycemic control. Therefore, excessive hepatic glucose release likely contributes to postprandial hyperglycemia in people with substantially elevated preprandial glucose concentrations. Delayed insulin secretion, insulin resistance, and increase in glucagon concentrations all could have contributed to impaired postprandial suppression of EGP. Furthermore, EGP was lower in diabetic than non-diabetic subjects during 3-6 hours postprandially likely due to higher insulin and glucose concentrations and slightly lower glucagon concentrations in diabetic subjects. The present experimental design can not distinguish between these possibilities.

Rd was substantially lower in diabetic subjects during the first several hours after meal ingestion when the excessive rise in glucose occurred. Since splanchnic glucose clearance and therefore by implication hepatic glucose uptake, did not differ between groups, this indicates that decreased Rd was the primary cause of hyperglycemia in diabetic subjects. The overall pattern of postprandial glucose metabolism, namely marked decrease in Rd immediately after meal ingestion when glucose concentrations are rising in the presence of normal MRa and minimal changes in postprandial suppression of EGP, strongly resemble that which we have recently reported in people with impaired fasting glucose (25). The pattern differs from previous studies (5-10) using the dual tracer approach where postprandial Rd has been reported to be unchanged or increased relative to non-diabetic subjects. In retrospect, these discrepancies are readily explainable since Rd is calculated by subtracting the change in glucose mass from Ra. Therefore, errors in
Multiple factors likely contributed to lower postprandial Rd. The difference in Rd between diabetic and non-diabetic subjects closely paralleled the difference in the pattern in insulin concentrations with both being lower in diabetic subjects during the initial several hours after meal ingestion followed by concentrations that were higher than those observed in non-diabetic subjects from three hours onward. In addition, postprandial insulin action and glucose effectiveness were impaired in diabetic subjects. These abnormalities all likely contributed to the lower Rd during the initial two hours after meal ingestion. Factors that contribute to postprandial glucose turnover include meal content, meal size, duration and severity of diabetes amongst others. Our experimental design is unable to determine the impact of these factors. Furthermore, although disposition indices were lower in diabetic subjects, their interpretation needs to be considered in the context of a mixed meal (14).

Although the total 6 hour postprandial insulin concentrations were similar between groups, the insulin secretory response in the diabetic subjects was sluggish for the first 2 hours likely due to reduced sensitivity of the beta cells to the glucose challenge. Furthermore, our observations are relevant only to those subjects who are relatively early in the natural history of type 2 diabetes and are well controlled on oral antidiabetic agents and therefore cannot be extrapolated to those with grossly impaired beta cell reserves after a longer duration of the disease.

In conclusion, when measured using methods that avoid non-steady error, MRa of ingested glucose was normal and suppression of EGP only slightly impaired. However, when considered in the light of prevailing glucose concentration, both are abnormal. In contrast, Rd was lower in diabetic subjects for several hours after meal ingestion due to a combination of defects in insulin secretion, action and glucose effectiveness. Therefore, agents that correct only one of these abnormalities are unlikely to fully restore postprandial metabolism to normal in type 2 diabetes.

ACKNOWLEDGEMENTS
This study was supported by U.S. Public Health Service (DK29953, R-00585, U 54RR 24150-1) and a Takeda research grant. We wish to thank Cathy Dvorak RN, Barb Norby RN and Jean Feehan RN for execution of the studies; Betty Dicke, Robert Rood and staff of the Immunochemical Core Laboratory and the Mass Spectroscopy Core for technical assistance; the staff of the Mayo Clinical Research Unit and Center for Clinical and Translation Science Activities for assistance with the studies.
REFERENCES

Legends:

Figure 1
Glucose, insulin, C-peptide and glucagon concentrations observed in diabetic (closed squares) and non-diabetic (open circles) subjects before and after ingestion of a mixed meal at time zero.

Figure 2
a., Indices of net insulin action (Si) and net glucose effectiveness (Sg) observed in diabetic (shaded bars) and non-diabetic (open bars) subjects after ingestion of a mixed meal. * denotes p<0.05 vs. non-diabetic.

b., Indices of insulin secretion (Φ_{total}, Φ_{dynamic}, Φ_{static}) and disposition Indices (DI_{total}, DI_{dynamic} and DI_{static}) observed in diabetic (shaded bars) and non-diabetic (open bars) subjects after ingestion of a mixed meal. * denotes p<0.05 vs. non-diabetic.

Figure 3
a., Pattern of change in the plasma [6-\textsuperscript{3}H] glucose to [1-\textsuperscript{13}C] glucose ratio (used to calculate the rate of meal appearance) and the [6,6-\textsuperscript{2}H\textsubscript{2}] glucose to endogenous glucose ratio (used to calculate endogenous glucose production) observed in diabetic (closed squares) and non-diabetic (open circles) subjects before and after ingestion of a mixed meal at time zero.

b., Pattern of change of the rate of appearance of meal glucose, endogenous glucose production, and the rate of glucose disappearance observed in diabetic (closed squares) and non-diabetic (open circles) before and after ingestion of a mixed meal at time zero.
Prandial glucose turnover in type 2 diabetes

Figure 1

Figure 2a
Figure 3b

**Meal Glucose Appearance**

**Endogenous Glucose Production**

**Glucose Disappearance**

- ○ Non-diabetic
- ■ Diabetic