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Regulation of endogenous glucose production after a mixed meal in type 2 diabetes

PARAG SINGHAL,[1] ANDREA CAUMO,[2] PETER E. CAREY,[1]
CLAUDIO COBELLI,[2] AND ROY TAYLOR[1]
[1]*Diabetes Research Group, School of Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, United Kingdom; and*
[2]*Dept of Electronics and Informatics, University of Padova, Padua I35131, Italy*

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Singhal, Parag, Andrea Caumo, Peter E. Carey, Claudio Cobelli, and Roy Taylor. Regulation of endogenous glucose production after a mixed meal in type 2 diabetes. *Am J Physiol Endocrinol Metab* 283: E275–E283, 2002. First published April 2, 2002; 10.1152/ajpendo.00424.2001.—The extent and time course of suppression of endogenous glucose production (EGP) in type 2 diabetes after a mixed meal have been determined using a new tracer methodology. Groups of age-, sex-, and weight-matched normal controls ($n = 8$) and diet-controlled type 2 diabetic subjects ($n = 8$) were studied after ingesting a standard mixed meal (550 kcal; 67% carbohydrate, 19% fat, 14% protein). There was an early insulin increment in both groups such that, by 20 min, plasma insulin levels were 266 ± 54 and 190 ± 53 pmol/l, respectively. EGP was similar basally [2.55 ± 0.12 $mg \cdot kg^{-1} \cdot min^{-1}$ in control subjects vs. 2.92 ± 0.16 $mg \cdot kg^{-1} \cdot min^{-1}$ in the patients ($P = 0.09$)]. After glucose ingestion, EGP declined rapidly in both groups to ~50% of basal within 30 min of the meal. Despite the initial rapid decrease, the EGP was significantly greater in the diabetic group at 60 min (1.75 ± 0.12 vs. 1.05 ± 0.14 $mg \cdot kg^{-1} \cdot min^{-1}$; $P < 0.01$) and did not reach nadir until 210 min (0.96 ± 0.17 $mg \cdot kg^{-1} \cdot min^{-1}$). Between 60 and 240 min, EGP was 47% higher in the diabetic group (0.89 ± 0.09 vs. 1.31 ± 0.13 $mg \cdot kg^{-1} \cdot min^{-1}$, $P < 0.02$). These data quantitate the initial rapid suppression of EGP after a mixed meal in type 2 diabetes and the contribution of continuing excess glucose production to subsequent hyperglycemia.

liver; insulin sensitivity

THE LIVER PLAYS A PRIMARY ROLE in maintaining the fasting plasma glucose concentration within a very narrow range in normal subjects, the basal rate of tissue glucose uptake being precisely equaled by the rate of endogenous glucose production (EGP). After the ingestion of a meal, this delicate balance is disrupted, and maintenance of normal glucose homeostasis in the fed state depends on suppression of EGP, augmentation of glucose uptake by splanchnic tissues and muscle, and stimulation of glucose oxidation (19). Because meals tend to be taken every few hours during the day, knowledge about these processes is central to the appreciation of both normal energy metabolism and the pathophysiology of non-insulin-dependent diabetes, a condition characterized by marked postprandial hyperglycemia (10, 21, 30). Hepatic insulin resistance causing faulty control of glucose production is believed to exacerbate the hyperglycemia (20, 27). The practical importance of this has been emphasized by data linking the extent of postprandial hyperglycemia with the vascular complications of diabetes (18, 28).

Information on insulin regulation of glucose control in the fed state has previously been inferred from studies using intravenous glucose under the nonphysiological condition of constant hyperinsulinemia. Many studies have followed the fate of a pure glucose load (24, 33, 37, 38), but the metabolic response to a glucose load differs from that after a mixed meal (23, 35, 44). Furthermore, the studies of endogenous glucose release after oral glucose have utilized a constant infusion of a glucose tracer, such as that used during steady-state glucose infusion experiments to achieve constant specific activity of plasma glucose. However, the key variable in the assessment of endogenous glucose release is the tracer specific activity, referred not to plasma glucose concentration but to endogenous glucose concentration, i.e., that fraction of the plasma glucose originating from endogenous sources. Because the endogenous glucose concentration falls after eating, specific activity varies markedly during a constant glucose tracer infusion protocol, leading to non-steady-state errors in the calculation of EGP (15, 25, 34, 49). To minimize non-steady-state errors, it is necessary to reduce the changes in endogenous glucose specific activity by use of a variable tracer infusion protocol. Ideally, the tracer infusion is varied in such a way that endogenous specific activity remains constant during the meal absorption, and under these conditions the calculation of EGP becomes model independent.

This alternative approach of varying the tracer infusion rate was used as early as 1973 in experimental work in dogs (22) but only recently in humans (65). The technique has not previously been applied to type 2

Address for reprint requests and other correspondence: R. Taylor, School of Clinical Medical Sciences, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK (E-mail: roy.taylor@ncl.ac.uk).

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diabetes. By allowing direct measurement of both EGP and plasma insulin after eating, the hypothesis that insulin resistance operates at the level of the liver could be tested. The absolute contribution of hepatic dysregulation to postprandial hyperglycemia could also be defined. This paper describes the pathophysiological changes in EGP after a mixed meal by applying the new technique to matched groups of normal and type 2 diabetic subjects.

MATERIALS AND METHODS

Subjects. Type 2 diabetic subjects controlled for diet alone ($n = 8$) and age-, sex-, and weight-matched healthy volunteers ($n = 8$) were recruited. Athletes in training were excluded, as were any subjects with metabolic disease or controls with first-degree family history of type 2 diabetes. No subject was taking medication that might affect carbohydrate metabolism. The mean duration of diabetes was 3.9 yr. The clinical and metabolic characteristics of the subjects are shown in Table 1. For 3 days before study, subjects consumed their normal weight-maintaining diet and completed a dietary diary. The diabetic and control groups reported similar calorie intakes (1,811 ± 403 vs. 1,976 ± 581 kcal) and similar food composition (carbohydrate 239 ± 46 vs. 252 ± 77 g, fat 63 ± 15 vs. 78 ± 23 g, and protein 79 ± 16 vs. 83 ± 23 g). The nature, purpose, and potential risks of the study were explained to all the subjects, and their informed, voluntary, written consent was obtained before their participation. The study protocol was reviewed and approved by the Joint Ethics Committee, University of Newcastle upon Tyne.

Experimental protocol. Subjects were studied in the recumbent position after a 12-h overnight fast. At 7 AM, an intravenous cannula for infusion was placed in an antecubital fossa vein, and a second cannula was placed in a distal forearm vein in a retrograde fashion, this hand being placed in a heated box at 50°C to allow sampling of arterialized blood. At time −180 min a primed continuous (0.40 μCi/min) infusion of [3-^{3}H]glucose was started. The prime was 40 μCi in the control group but was adjusted according to fasting plasma glucose in the diabetic group to avoid delay in achieving steady-state plasma glucose specific activity (48). The prime in 10 ml of normal saline was flushed through the cannula with 5 ml of saline. A period of 180 min was allowed for equilibrium of tritiated glucose; the end of this equilibrium period was taken to be *time zero.* A liquid meal (550 kcal; 67.3% carbohydrate, 18.5% fat, 14.2% protein) including 2 g of [2-^{2}H]glucose was consumed within 10 min, starting at *time zero.* After the meal, the rate of infusion of tritiated glucose was adjusted to reproduce the anticipated pattern of endogenous glucose release after the meal. Subjects voided just before ingestion of the glucose load, and urine was collected at 210 min (diabetic group only) and at the end of the experiment for determination of urinary glucose loss. Glucose oxidation rates were calculated from indirect calorimetry data derived by use of a constant-flow hood calorimeter (Delta Trac 17). Measurements were made over 20-min periods in the fasting state and at regular intervals after the meal. Fuel oxidation data were calculated using equations of Lusk (41). Frequent blood samples were taken for the determination of plasma glucose, [2-^{2}H]glucose, [3-^{3}H]glucose, lactate, free fatty acids, insulin, and glucagon.

Variable tracer infusion rate. The [3-^{3}H]glucose infusion rate was varied in a stepwise fashion to anticipate the decrease and subsequent increase in EGP after the meal. The protocol was determined iteratively in preliminary studies. The variable infusion protocol remained the same for both groups to avoid potential complicating factors, as the main aim of the study was to compare the pattern of postprandial EGP between the control and diabetic groups and was as follows: basal period, 100% of basal infusion rate; 0–5 min, 100%; 5–10 min, 90%; 10–15 min, 75%; 15–20 min, 55%; 20–25 min, 40%; 25–30 min, 30%; 30–220 min, 20%; 220–240 min, 30%; 240–260 min, 35%; 260–280 min, 40%; 280–300 min, 45%; 300–400 min, 55%; and 400–480 min, 65%.

Calculations of EGP. The profile of exogenous glucose concentration, i.e., the component of total glucose concentration due to exogenous glucose ingestion, was initially calculated. Because this is proportional to the concentration of [2-^{2}H]glucose, its calculation is straightforward and model independent (16). We then calculated the time course of endogenous glucose concentration, i.e., the component of total glucose concentration due to EGP only, by subtracting the calculated exogenous component from the measured total glucose concentration. The steady-state values of plasma clearance rate (PCR) and basal EGP (basal EGP = PCR × basal glucose concentration) were estimated from the [3-^{3}H]glucose decay curve after the primed continuous infusion of [3-^{3}H]glucose given 3 h before the administration of the meal (14). Subsequently, the time course of EGP was calculated from endogenous glucose concentration and [3-^{3}H]glucose data. Because [3-^{3}H]glucose had been infused to mimic the expected behavior of EGP, the specific activity given by the ratio of [3-^{3}H]glucose to endogenous glucose was steady, thus allowing a more reliable estimation of EGP. EGP was calculated using the two-compartment model of Radziuk et al. (54). The concentration of [3-^{3}H]glucose and the ratio between [3-^{3}H]glucose and endogenous glucose concentration were smoothed using an algorithm based on stochastic nonparametric deconvolution (63).

Insulin sensitivity. The homeostatic model assessment (HOMA) index of insulin sensitivity was calculated using fasting insulin and glucose concentrations (43).

Analytical procedures. Plasma glucose concentration was measured by the glucose oxidase method on a Beckman glucose analyzer. Plasma insulin concentration was measured using an enzyme-linked immunosorbant assay kit (Dako) and plasma glucagon by radioimmunoassay (50). Plasma lactate was measured on perchloric acid extracts on a Cobas Bio centrifugal analyzer (Roche, Welwyn Garden City, UK) (29). Plasma FFA were measured by centrifugal enzymatic analysis (39). Atom percent enrichment of plasma [2-^{2}H]glucose was determined by gas chromatography-mass spectrometry (8). Plasma [3-^{3}H]glucose radioactivity was determined after deproteinization of plasma with $ZnS0_4$ and $Ba(OH)_2$, as described (56).

Statistical methods. Data are stated as means ± SE. Student's two-tailed *t*-test and Pearson's correlation were employed as appropriate with the use of the Minitab statistical program (Minitab, State College, PA).

Table 1. *Clinical characteristics of the study subjects*

Characteristic	Normal Subjects	Diabetic Subjects
Age, yr	50.8 ± 2.9	52.4 ± 2.9
Sex, M/F	5/3	5/3
Body mass index	29.6 ± 1.3	30.1 ± 1.2
Fasting glucose, mmol/l	5.0 ± 0.1	7.7 ± 0.5
Hb A_{1C}, %	5.5 ± 0.1	7.2 ± 0.4

Values are means ± SE.

RESULTS

Plasma glucose. After glucose ingestion, plasma glucose in the control group increased from a basal level of 4.8 ± 0.1 mmol/l to peak concentration of 7.8 ± 0.4 mmol/l at 60 min and returned to basal values between 180 and 240 min (Fig. 1). In the diabetic subjects, plasma glucose increased from a basal level of 6.8 ± 1.2 mmol/l to a peak concentration of 14.2 ± 2.2 mmol/l at 150 min (both $P < 0.001$ compared with controls) and did not return to basal levels until 330 min. The mean plasma glucose concentration in the diabetic subjects during the 8-h experimental period was 9.5 mmol/l compared with 5.7 mmol/l in the control group ($P < 0.001$). The rise in enrichment of [2-^{2}H]glucose in plasma was very similar in the two groups, diverging modestly only after 80 min (Fig. 2).

Plasma metabolites and hormones. There was no significant difference in the fasting insulin concentration in the two groups (50 ± 10 pmol/l in controls vs. 67 ± 12 pmol/l in patients, $P = 0.23$). After glucose ingestion, there was an early, brisk increment in both groups such that, by 20 min, plasma insulin levels were 266 ± 54 and 190 ± 53 pmol/l in the control and diabetic groups, respectively. Plasma insulin increased to a peak concentration at 60 min in the control group (614 ± 179 pmol/l) and more slowly to peak concentration at 150 min in the diabetic group (449 ± 123 pmol/l; Fig. 1). In the control group, plasma insulin levels returned to basal values between 240 and 270 min, but in the diabetic group basal levels were not reached until 400 min after the meal. Immediately before the meal, plasma glucagon concentrations were similar in the two groups (53.3 ± 6.0 ng/l in control vs. 59.7 ± 10.4 ng/l in patients, $P = 0.3$). After glucose ingestion, plasma glucagon rose briskly in both groups to peak at 20 min (69.6 ± vs. 94.2 ± 15.1 ng/l, respectively, $P = 0.17$; Fig. 1). Plasma glucagon decreased in the control group to 36.6 ± 2.3 ng/l at 150 min and returned to basal values between 300 and 360 min. In the diabetic subjects, the plasma glucagon at 150 min was still elevated at 74.0 ± 12.9 ng/l ($P = 0.03$) and did not reach lowest concentrations until 270 min. Plasma lactate levels were similar in control and diabetic subjects both fasting (0.66 ± 0.14 vs. 0.58 ± 0.07 mM) and at peak (80 min: 0.95 ± 0.11 vs. 1.02 ± 0.14 mM). The subsequent fall in plasma lactate was slightly but not significantly delayed in the diabetic subjects (150 min: 0.65 ± 0.04 vs. 0.99 ± 0.18 mM, respectively).

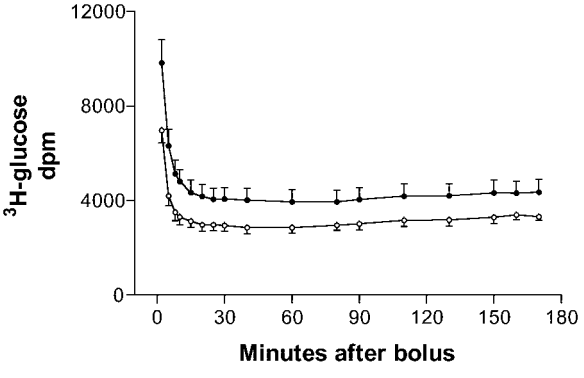

Fig. 1. Plasma specific activity during the primed continuous infusion of [^{3}H]glucose during the basal period before the test meal.

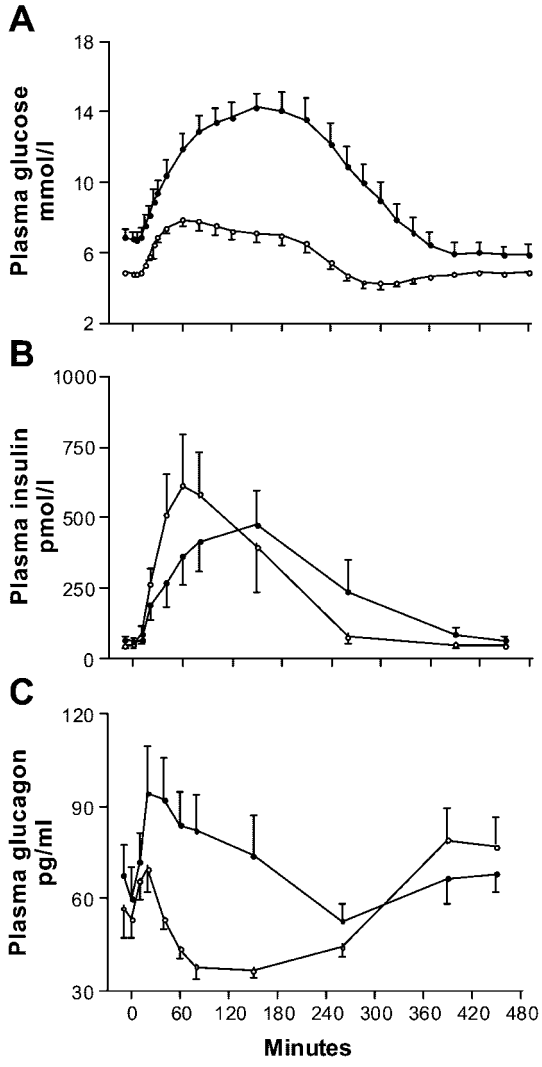

Fig. 2. Change in plasma glucose (*A*), insulin (*B*), and glucagon concentrations (*C*) after the test meal in the type 2 diabetic group (●) and the matched normal control group (○). Data are shown as means ± SE.

Endogenous glucose output. Steady-state plasma levels of [3-^{3}H]glucose were achieved in the basal period (Fig. 3).

Fasting EGP was 2.55 ± 0.12 mg·kg^{-1}·min^{-1} in control subjects vs. 2.92 ± 0.16 mg·kg^{-1}·min^{-1} in the patients ($P = 0.09$). After glucose ingestion, EGP declined rapidly in both groups to ~50% of basal within 30 min of the meal (Fig. 4). The parallel rates of decline in EGP over this 30-min period are striking. In control subjects EGP decreased further and remained less than 1 mg·kg^{-1}·min^{-1} until 240 min but recovered steeply thereafter. The greatest suppression was observed at 120 min (0.75 ± 0.16 mg·kg^{-1}·min^{-1}). Despite the initial rapid decrease in EGP in the diabetic group, the EGP was significantly greater than in con-

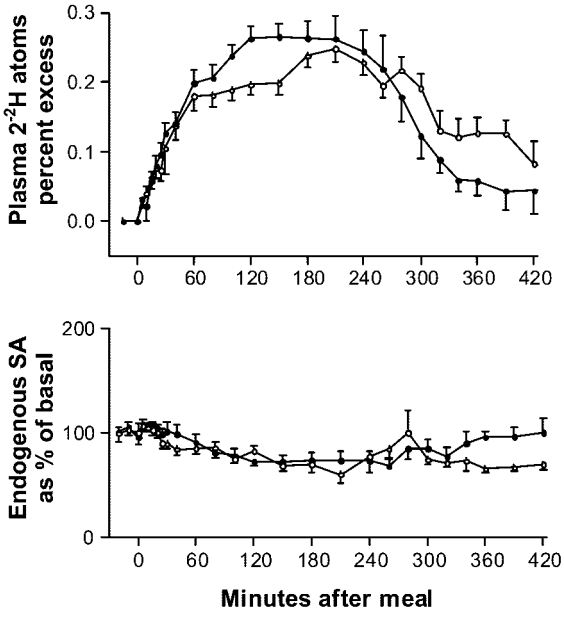

Fig. 3. *Top*: rate of absorption of meal glucose shown as atom percent excess of 2-D-glucose in plasma in the type 2 diabetic group (●) and the matched normal control group (○). *Bottom*: specific activity (SA) of endogenously derived glucose in plasma expressed as percentage of basal plasma glucose specific activity.

trols at 60 min (1.75 ± 0.12 vs. 1.05 ± 0.14 $mg \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$) and did not reach nadir until 210 min (0.96 ± 0.17 $mg \cdot kg^{-1} \cdot min^{-1}$). Between 60 and 240 min, EGP was 47% higher in the diabetic group (0.89 ± 0.09 vs. 1.31 ± 0.13 $mg \cdot kg^{-1} \cdot min^{-1}$, $P < 0.02$). EGP recovered rapidly in nondiabetic subjects between 240 and 360 min, plateauing thereafter. In the diabetic subjects the rise was less rapid (0.006 vs. 0.011 $mg \cdot kg^{-1} \cdot min^{-1}$; Fig. 5).

At 60 min after the meal, the degree of suppression of EGP correlated both with plasma insulin ($r = -0.60$, $P < 0.02$) and with the insulin/glucagon ratio ($r = -0.63$, $P < 0.01$; Fig. 6). There was an inverse correlation between the HOMA index of insulin sensitivity and EGP 60 min after the meal in both the control ($r = 0.81$, $P < 0.02$) and diabetic groups ($r = 0.82$, $P < 0.02$).

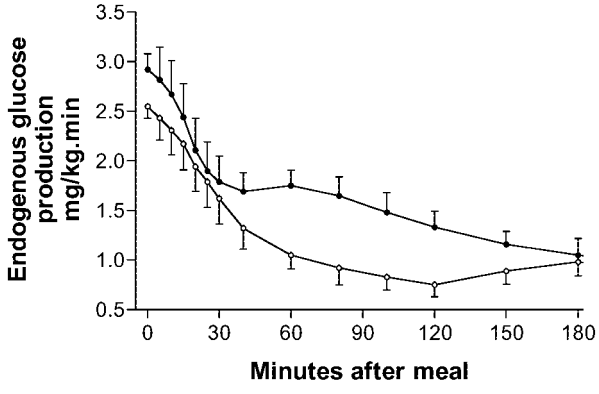

Fig. 4. Change in endogenous glucose production (EGP) during the early postmeal phase in the type 2 diabetic group (●) and the matched normal control group (○). Data are shown as means ± SE.

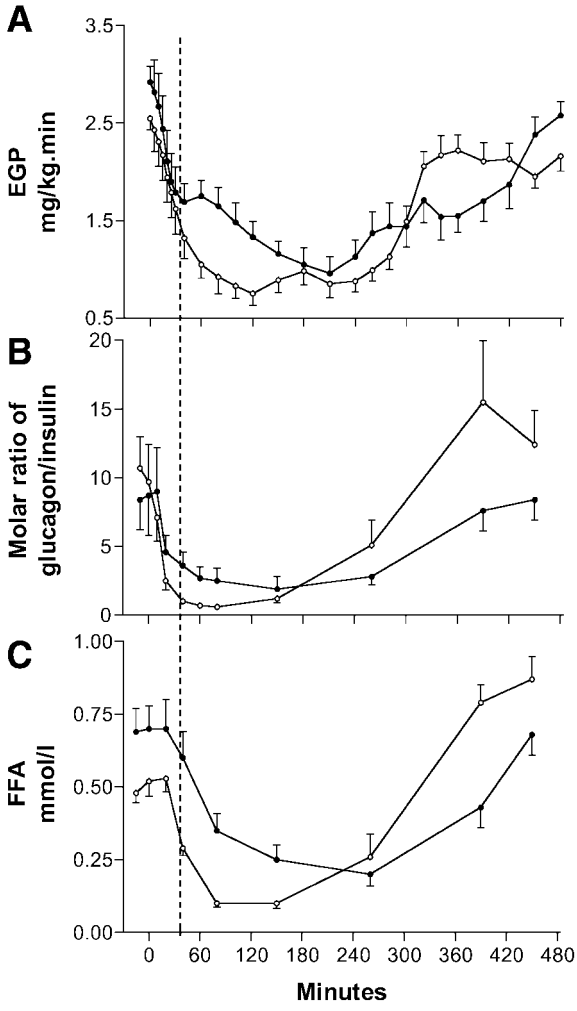

Fig. 5. *A*: time course of EGP during the entire study period after the test meal. *B*: molar ratio of plasma glucagon to insulin. *C*: change in plasma free fatty acid (FFA) concentration after the test meal in the type 2 diabetic group (●) and the matched normal control group (○). The vertical dashed line at 40 min is included to assist comparison between the relative time courses. Data are shown as means ± SE.

Plasma FFA. Fasting FFA concentration was higher in the diabetic group (0.48 ± 0.03 vs. 0.69 ± 0.08 mmol/l, $P < 0.05$). Plasma FFA concentration fell to 0.09 ± 0.05 mmol/l at 80 min in the control group (Fig. 5). After remaining suppressed for over 3 h, plasma FFA concentration rebounded, reaching 0.87 ± 0.06 mmol/l at 460 min. In the diabetic group, FFA concentration had fallen by 80 min, but it remained elevated compared with controls (0.35 ± 0.01 mmol/l, $P < 0.005$). The lowest concentration was not reached until 270 min (0.20 ± 0.10 mmol/l, $P < 0.05$ compared with controls at 80 min).

Glucose oxidation. Net glucose oxidation rate in the basal state was higher in the control than the diabetic group (1.69 ± 0.14 vs. 1.14 ± 0.10 $mg \cdot kg^{-1} \cdot min^{-1}$, $P = 0.01$). The rate of rise was similar in both groups such that, at peak net glucose oxidation rate in the control group (180 min), there was a significant difference between the groups (2.35 ± 0.22 vs. 1.69 ± 0.25 $mg \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$; Fig. 7). In the diabetic group,

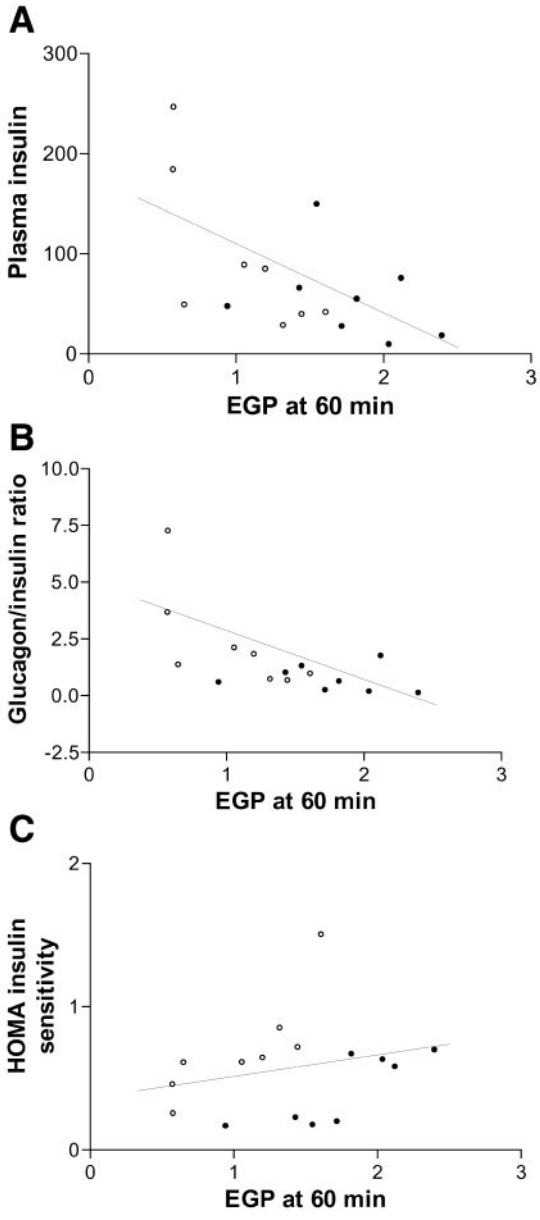

Fig. 6. Relationships between EGP 60 min after the meal and plasma insulin at 60 min (*A*), plasma glucagon/insulin ratio (*B*), and homeostatic model assessment (HOMA) index of insulin sensitivity (*C*).

peak rates were achieved at 240 min (1.82 ± 0.26 $mg \cdot kg^{-1} \cdot min^{-1}$). In both groups, carbohydrate oxidation rates declined thereafter.

Urinary glucose excretion. During the early postprandial period (0–210 min), urinary glucose excretion rate was 0.29 ± 0.22 $mg \cdot kg^{-1} \cdot min^{-1}$, and between 210 and 480 min it was 1.9 ± 0.9 $mg \cdot kg^{-1} \cdot min^{-1}$ in the diabetic group. There was no quantifiable loss of glucose in urine in the control group.

DISCUSSION

This study demonstrates the time course and extent of suppression of EGP after a mixed meal in subjects with type 2 diabetes. The initial rate of suppression was similar in the diabetic and matched control groups but was not sustained in the diabetic group, such that EGP was 47% higher between 60 and 240 min after eating. This is important, as meals are normally taken every 4–5 h during waking hours. The nadir of EGP was delayed by 120 min, and, unlike in the control group, this was not sustained. In both groups, plasma insulin increased briskly during the initial 20 min. Subsequently, plasma insulin in the type 2 diabetic group rose more slowly to a subnormal peak. Conversely, plasma glucagon concentrations were higher in the diabetic group throughout the postprandial period. One hour after the meal, the degree of suppression of EGP correlated with plasma insulin and with the insulin/glucagon ratio.

Hepatic insulin resistance is widely believed to play an important role in the pathophysiology of type 2 diabetes (5, 20, 27). The observed modest elevation of fasting plasma insulin in the type 2 diabetic subjects, together with the slightly elevated EGP and the subnormal suppression of EGP between 60 and 240 min, are consistent with this. However, the present data indicate that the response to a similar acute increment in hepatic sinusoidal insulin concentration produces the same initial rate of suppression of EGP in type 2 diabetic subjects and matched controls (Fig. 4). These observations were made under the normal day-to-day circumstances of elevated plasma glucose and glucagon in type 2 diabetes, and direct inferences about hepatic insulin sensitivity cannot be made. Glucose effectiveness in suppression of EGP is similar in normal and diabetic subjects (4); hence, the elevated plasma glucose will exert an additional suppressive effect in the diabetic group. However, the apparently normal acute hepatic insulin responsiveness under everyday conditions is of interest. In contrast to the universal observation of insulin resistance of muscle in type 2 diabetes, normal insulin sensitivity of the liver has previously been reported by use of exogenous insulin infusion to mimic postprandial profiles (36). The same group demonstrated a normal rate of onset of hepatic insulin action (68). In obese type 2 diabetic subjects, elevation of plasma insulin to a mean of 30 mU/l was observed to be associated with fall in EGP of 51 vs. 54

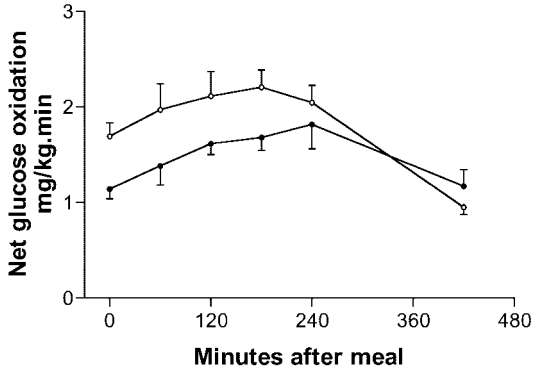

Fig. 7. Change in glucose oxidation rates after the meal in the type 2 diabetic group (●) and the matched normal control group (○). Data are shown as means ± SE.

$mg \cdot m^{-2} \cdot min^{-1}$ in control and diabetic groups, respectively (64). The present data confirm the everyday physiological relevance of acute insulin responsiveness of the liver in type 2 diabetes, although between 60 and 240 min after eating, higher ambient insulin levels do not achieve normal suppression of EGP. Because glucose transport is the major insulin-insensitive step in muscle in type 2 diabetes (57), and because glucose transport is not rate limiting for hepatic glucose metabolism, it would be expected that liver and muscle differ in expression of insulin resistance. It is noteworthy that postprandial EGP correlated with HOMA, an index based on the fasting state. The data on EGP reported in the present study includes a renal component, and the latter has been reported to rise after eating (45).

The control subjects demonstrated a prompt decrease in EGP, although this was less rapid than previously observed in young normal subjects (66), and possible reasons for this must be considered. The meals in the two studies were made up of identical proportions of carbohydrate, protein, and fat, although the total energy content was modestly less in the present study (550 vs. 650 kcal). It is unlikely that this had a major impact on initial rates of EGP suppression. Age per se is unlikely to be responsible, as neither peripheral nor hepatic insulin sensitivity changes with age, provided physical activity and obesity are matched (11). The considerable difference in body mass index (BMI; 30 vs. 21 kg/m^2) between the previously studied subjects and the control subjects in the present study may explain the observations. Obesity itself could be exerting an effect by changing the availability of intrahepatic fatty acids in the hepatic sinusoids (7). In the present study, the two groups were well matched for BMI. The diabetic and control groups demonstrated an identical rate of suppression of EGP over the first 30 min after commencing the meal. However, suppression of EGP was much slower in the diabetic group thereafter. This pattern of abnormality raises the question of precisely which factors regulate EGP during the postprandial period. Both the major regulatory hormones (31, 40, 42) and change in plasma FFA (6) have to be considered.

The rate of rise in plasma insulin concentration in the first 20 min (6.4 $pmol \cdot l^{-1} \cdot min^{-1}$) in this group of diabetic subjects was striking and at 20 min represented 40.1% of the subsequent peak concentration compared with 43.3% in the control subjects. Although the initial insulin response to eating may appear unexpectedly great for subjects with type 2 diabetes, previous studies of such subjects after a mixed meal rather than pure glucose have reported a similar change in plasma insulin (59). The ability of intravenous arginine to elict normal early insulin-secretory responses in type 2 diabetes has been recognized for many years (51). The mixed meal administered in the present study would bring about a stimulus to the pancreas by the increment in plasma amino acids and glucose as happens in everyday life. The subjects were at a relatively early stage of the disease and were reasonably well controlled on diet alone. The actual change in hepatic sinusoidal insulin concentration will be underestimated by measurements on peripheral plasma samples. Portal vein insulin concentrations are ~2.4-fold greater than peripheral plasma concentrations during steady-state conditions (12), and during the initial phase of increasing insulin concentration the degree of increase over basal will be considerably greater. Hence, in the 20 min after eating, the liver would be exposed to an increase in insulin concentration from ~160 pmol/l (2.4 times peripheral levels) to well over 460 pmol/l in the diabetic group. This considerable change could be supramaximal with respect to inducing initial change in EGP, the increase to >630 pmol/l in the controls achieving only a similar rate of suppression of EGP. The subsequent pattern of insulin secretion differs markedly between the groups (Fig. 1), and the sustained difference is consistent with the slower subsequent suppression of EGP in the diabetic group between 60 and 240 min. This analysis of change in the concentrations of one hormone does not by itself explain the pattern of the EGP response to insulin, and the effect of simultaneous change in glucagon concentration must be considered.

Glucagon exerts a powerful effect on EGP (31). Plasma glucagon levels increased more markedly in the diabetic group (Fig. 1), and, during the important phase from 60 to 240 min after eating, plasma glucagon levels were elevated approximately twofold in the diabetic group compared with the controls. Similar data in type 2 diabetes have previously been reported (2, 62). This is generally consistent with an effect on the observed pattern of EGP but, as with the pattern of change of insulin, it does not match the time course of change in EGP in the two groups. However, if the hormones are regarded as part of a coordinated response to eating and the molar ratio of glucagon to insulin is calculated, a different picture emerges. It can be seen from Fig. 5 that the glucagon/insulin ratio changes in a manner highly suggestive of this being the major regulatory factor. A similar close relationship was previously observed in the study of young healthy subjects (66). Although the concept of dual regulation of EGP by glucagon and insulin is not new (69), it has not previously been linked so closely to physiological regulation in vivo.

Several previous studies have indicated a role for FFA in controlling EGP (9, 13, 26, 52, 55, 60). The "single gateway hypothesis" was originally put forward to integrate observations on change in plasma hormones and FFA, suggesting that peripheral action of hormones exerted effects on the liver primarily by modulating plasma FFA concentrations (6). However, some studies have not observed any effect of FFA on EGP (53, 58), and evidence exists to suggest that insulin acts directly to inhibit EGP (42). In the present study, FFA concentration did not change in control and diabetic groups, respectively, in the 20 min after eating, whereas EGP fell by 25 and 35%, respectively, over the same time. In the 40 min after eating, FFA concentration fell by 42 and 14% in control and diabetic

groups, and EGP fell by 59 and 57%, respectively, over the same time. The relatively slow and small change in plasma FFA has to be compared with the major rapid changes in plasma insulin and glucagon. The comparative time courses are shown in Fig. 6. There were, however, weak correlations between basal FFA and the extent of suppression of EGP ($r = 0.54$, $P < 0.05$) and between the decrease of FFA concentrations at 40 min and the time to maximum suppression of EGP ($r = -0.58$, $P < 0.05$). The observations do not support the concept of an important acute role for plasma FFA in regulating EGP but do not exclude the possibility of regulation over longer periods. It has recently been demonstrated that 8 h of lipid infusion blunted suppression of EGP during a hyperinsulinemic clamp (61).

Non-steady-state errors in the estimation of EGP can be avoided if the tracer specific activity of endogenous glucose is maintained constant during meal absorption. If a continuous infusion of tracer is used throughout the postprandial period, the specific activity will rise sharply as EGP is suppressed. By steady-state theory, this will cause EGP to be overestimated after a meal and, hence, create the appearance of slow suppression (15, 25, 34, 49). Indeed, previous studies using a constant tracer infusion after an oral glucose load have reported slow suppression in both normal and diabetic subjects to 20–50% of basal EGP (24, 33, 46). A similar problem of overestimation has been identified if tracer specific activity is increasing during estimation of fasting EGP (48). In the present study, this difficulty was avoided in the basal state by adequate priming of the glucose pool and in the postprandial state by adjusting the rate of tracer infusion in a stepwise manner to match the expected decrease in EGP. Our previous work (66) in young healthy subjects demonstrated that constant specific activity of endogenous glucose could be achieved using this technique. Previous work on human subjects has demonstrated normal rates of glucose absorption in type 2 diabetes (3).

Knowledge of the dynamics of change in EGP after eating, coupled with observations on whole body glucose oxidation rates, allows an analysis of the factors underlying postprandial hyperglycemia in type 2 diabetes. During the 30 min after eating, the suppression of EGP did not account for any major difference between control and diabetic groups. However, by 60 min after eating, plasma glucose had reached maximum in the controls, but in the diabetic group it was still rising at 2.1 $mg \cdot kg^{-1} \cdot min^{-1}$ (Fig. 1). At 60 min, the difference in EGP between the groups was 0.70 $mg \cdot kg^{-1} \cdot min^{-1}$ (Fig. 4), this representing 33% of the excess accumulation of glucose in extracellular fluid. The difference in glucose oxidation rates was 0.63 $mg \cdot kg^{-1} \cdot min^{-1}$, or 30% of the excess accumulation of glucose. The remainder must be accounted for by inadequate rates of glucose disposal. It can thus be seen that inadequate suppression of EGP, subnormal glucose oxidation rate, and slow tissue uptake of glucose are each an important factor in the genesis of postprandial hyperglycemia in type 2 diabetes. Previous studies using magnetic resonance spectroscopy have demonstrated that, at 5 h after eating in nondiabetic subjects, ~20% of the absorbed carbohydrate is stored in liver and 30% in muscle (65, 67). This is very different from the pattern during hyperinsulinemic clamp studies. Previous evidence suggests that the relative disposition of glucose in muscle and liver differs considerably after oral and intravenous routes (1, 32, 47). In conclusion, direct study of the pathophysiological changes after eating has allowed quantitation of the major factors underlying postprandial hyperglycemia in type 2 diabetes.

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