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**Research Article**

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# Direct Assessment of Liver Glycogen Storage by [13]C Nuclear Magnetic Resonance Spectroscopy and Regulation of Glucose Homeostasis after a Mixed Meal in Normal Subjects

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## Abstract

Despite extensive recent studies, understanding of the normal postprandial processes underlying immediate storage of substrate and maintenance of glucose homeostasis in humans after a mixed meal has been incomplete. The present study applied ^{13}C nuclear magnetic resonance spectroscopy to measure sequential changes in hepatic glycogen concentration, a novel tracer approach to measure postprandial suppression of hepatic glucose output, and acetaminophen to trace the pathways of hepatic glycogen synthesis to elucidate the homeostatic adaptation to the fed state in healthy human subjects. After the liquid mixed meal, liver glycogen concentration rose from $207 \pm 22$ to $316 \pm 19$ mmol/liter at an average rate of 0.34 mmol/liter per min and peaked at $318 \pm 31$ min, falling rapidly thereafter (0.26 mmol/liter per min). The mean increment at peak represented net glycogen synthesis of $28.3 \pm 3.7$ g ($\sim 19\%$ of meal carbohydrate content). The contribution of the direct pathway to overall glycogen synthesis was $46 \pm 5$ and $68 \pm 8\%$ between 2 and 4 and 4 and 6 h, respectively. Hepatic glucose output was completely suppressed within 30 min of the meal. It increased steadily from 60 to 255 min from $0.31 \pm 32$ to $0.49 \pm 18$ mg/kg per min then rapidly returned towards basal levels ($1.90 \pm 0.04$ mg/kg per min). This pattern of change mirrored precisely the plasma glucagon/insulin ratio. These data provide for the first time a comprehensive picture of normal carbohydrate metabolism in humans after ingestion of a mixed meal. (*J. Clin. Invest.* 1996. 97:126–132.) Key words: glycogen • liver • magnetic resonance spectroscopy • hepatic glucose output • mixed meal

## Introduction

Knowledge of the physiological mechanisms involved in substrate storage and maintenance of glucose homeostasis after a normal meal is central to the appreciation of normal energy metabolism in humans. This is especially so as meals tend to be taken every few hours during the day. Such knowledge is also central to the understanding of abnormal metabolic states, particularly non–insulin-dependent diabetes and obesity which are characterized by relative resistance to the major controlling hormone, insulin (1–3). The liver plays a primary role in glucose homeostasis both fasting and postprandially, but detailed information on the extent and time course both of hepatic storage of glucose as glycogen and of suppression of hepatic glucose release after mixed meals is lacking. Present knowledge has been inferred from studies of intravenous glucose administration, often under the unphysiologic condition of constant hyperinsulinemia. Considerable caution has to be exercised in extrapolating from such data as the time course of changes after eating is not reproduced and as available evidence suggests that the relative disposition of glucose in muscle and liver differs considerably after administration by the two routes (4–6). A few studies have followed the fate of a pure glucose load (7–10), but the metabolic response to a glucose load differs from that after a mixed meal (11–13).

A combination of three new methodologies now permits the full description of hepatic glucose metabolism after a mixed meal for the first time. Nuclear magnetic resonance spectroscopy allows the direct, accurate, and repeated measurement of liver glycogen stores (14, 15). A new approach to the design of isotopic studies of hepatic glucose release after a meal, based upon maintaining constant the specific activity of glucose released from the liver, circumvents the limitations of previous approaches to the assessment of hepatic glucose release under such experimental conditions. Finally, the pathway by which glucose is converted into glycogen can be traced by use of acetaminophen as a noninvasive probe of intrahepatic UDP-glucose (16, 17). The postprandial changes in hepatic glycogen concentration, hepatic glucose production, and pathways of glycogen synthesis were assessed in separate studies under identical nutritional conditions.

This paper describes the physiological changes in human hepatic glucose metabolism after a mixed meal.

## Methods

### Subjects

Healthy volunteers, aged 18–40 yr were recruited. Athletes in training were excluded as were any subjects with metabolic disease or with a first degree family history of non–insulin-dependent diabetes. No subject was taking medication which might affect carbohydrate metabolism. The clinical and metabolic characteristics of the subjects participating in each study are shown in Table I.

### Protocol

Over the 3 d before each study, subjects continued their normal activities but ate a weight-maintaining diet (33 kcal/kg per d; 50% carbohydrate, 20% protein, 30% fat). This food was provided from the

*Table I. Clinical Details of Subjects in Each Group*

| Subject | Age | Weight | Height | BMI |
|---|---|---|---|---|
| **Study A—liver MR** | | | | |
| 01 | 21 | 70 | 1.70 | 24.2 |
| 02F | 22 | 57 | 1.65 | 20.9 |
| 03 | 28 | 88 | 1.83 | 26.3 |
| 04 | 19 | 78 | 1.80 | 24.1 |
| 05F | 32 | 63 | 1.75 | 20.6 |
| 06 | 21 | 80 | 1.85 | 23.4 |
| 07 | 24 | 89 | 1.91 | 24.4 |
| 08 | 22 | 80 | 1.94 | 21.3 |
| Mean | 23.6 | 75.6 | 1.80 | 23.1 |
| SE | 1.5 | 4.0 | 0.035 | 0.7 |
| **Study B—HGO** | | | | |
| 06 | 21 | 80 | 1.85 | 23.4 |
| 09F | 19 | 87 | 1.79 | 25.4 |
| 10 | 26 | 83 | 1.84 | 24.5 |
| 11 | 39 | 83 | 1.75 | 27.1 |
| 12 | 29 | 82 | 1.80 | 25.3 |
| 13 | 26 | 69 | 1.70 | 23.9 |
| Mean | 26.6 | 80.7 | 1.79 | 24.9 |
| SE | 2.9 | 2.5 | 0.023 | 0.5 |
| **Study C—pathways** | | | | |
| 14 | 23 | 66 | 1.80 | 20.3 |
| 15 | 28 | 80 | 1.79 | 25.0 |
| 16 | 27 | 68 | 1.78 | 21.5 |
| 17F | 24 | 71 | 1.77 | 22.7 |
| 18 | 23 | 89 | 1.91 | 24.4 |
| 02F | 21 | 57 | 1.65 | 20.9 |
| Mean | 24.3 | 71.8 | 1.78 | 22.5 |
| SE | 2.7 | 11.2 | 0.08 | 0.8 |

Female subjects are indicated by an F beside the subject number. Subjects 02 and 06 participated in more than one study. BMI, body mass index; MR, magnetic resonance.

metabolic kitchen of the Yale/New Haven Hospital General Clinical Research Center (CRC)[1] and comprised items habitually consumed by each subject. No alcohol was permitted over the 3-d run in period. On the day before each study, the subject was admitted to the CRC at 4.30 p.m. A liquid meal consisting of a milk shake (650 kcal; 60% carbohydrate, 20% protein, 20% fat) was consumed at 5:00 p.m. The protocol thereafter varied in each study.

## Study A

*Measurement of liver glycogen concentration.* Liver volume was determined by magnetic resonance imaging $\sim$ 2 h after the evening meal using a 1.5 T magnet (Signa; General Electric Co. Milwaukee, WI) as previously described (14). 4 h after the meal, liver glycogen concentration was measured as described below. Subjects slept overnight in the CRC. At 6:00 a.m., fasting liver glycogen concentration was measured. Subjects returned to the CRC and the liquid test meal (824 kcal; 67.3% carbohydrate as glucose, 18.5% fat, 14.2% protein) was consumed over a 10-min period. Time 0 was set at the commencement of the test meal. Throughout the day subjects remained sedentary apart from moving between the CRC and the magnetic resonance center.

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1. *Abbreviations used in this paper:* CRC, clinical research center; HGO, hepatic glucose output; HGR, hepatic glucose release; NMR, nuclear magnetic resonance.

## Study B

*Measurement of hepatic glucose output.* At 6:00 a.m. on the morning of study an intravenous cannula for infusion was placed in an antecubital fossa vein, and a cannula was placed in a distal forearm vein in a retrograde fashion; this hand was placed in a heated box at 50°C to allow sampling of arterialized blood. At time $-120$ min a prime-continuous (0.40 $\mu$Ci/min) infusion of [3-$^3$H]glucose was started. The 40 $\mu$Ci prime in 10 ml normal saline was flushed through the cannula with 5 ml saline. The composition of the test meal was identical to that used in study A except that 3 g of [2-$^2$H]glucose replaced 3 g of unlabeled glucose to trace absorption of glucose in the mixed meal. Indirect calorimetry was performed using an indirect calorimeter (Delta Trac, Anaheim, CA) as previously described (18). Measurements were made over 20-min periods in the fasting state and at intervals after the meal. Fuel oxidation data were calculated using the equations of Lusk (19). Hepatic glucose release (HGR) was calculated using the strategy detailed below. Frequent blood samples were taken for the determination of plasma glucose, [2-$^2$H]glucose, [3-$^3$H]glucose, lactate, FFA, insulin, glucagon, epinephrine, and NE.

*Endogenous glucose concentration.* Glucose concentration is the sum of two components. One is exogenous glucose concentration, i.e., the fraction due to glucose ingestion, while the other is endogenous glucose concentration which is due to hepatic glucose production. Endogenous glucose concentration was calculated from total glucose concentration and [2-$^2$H]glucose measurements using the stable isotope equivalent of specific activity, i.e., the tracer-to-tracee ratio (20, 21). Exogenous glucose concentration was then calculated by subtracting endogenous glucose concentration from total glucose concentration. The knowledge of endogenous glucose concentration allows the straightforward estimation of hepatic glucose production. In fact, instead of being calculated as the difference between the estimates of total and exogenous appearance rate of glucose, hepatic glucose production is estimated directly from endogenous glucose concentration and the known rate of appearance and plasma concentrations of [3-$^3$H]glucose.

*Variable tracer infusion rate.* To minimize non–steady state error, it is necessary to reduce the changes in glucose specific activity during the experiment (22–25). As the glucose whose specific activity must be clamped is endogenously released, and as endogenous glucose concentration varies markedly during the study, [3-$^3$H]glucose had to be infused in such a way as to mimic such variations. Based on pilot experiments, the rate of basal [3-$^3$H]glucose infusion was adjusted in the attempt to reproduce the anticipated pattern of hepatic glucose release after the meal. A similar approach has recently been used in studies using meal-like intravenous glucose administration (26). The rate of [3-$^3$H]glucose infusion was refined until the following schedule was developed: basal period: 100% of basal infusion rate; 0–10 min: 50%; 10–80 min: 20%; 80–220 min: 15%; 220–240 min: 30%; 240–260 min: 50%; 260–280 min: 75%; 280–300 min: 100%; 300–400 min: 120%; 400–500 min: 100%; 500–600 min: 80%. In this way, the resulting [3-$^3$H]glucose concentration mimicked the time course of endogenous glucose concentration and changes in endogenous glucose specific activity were thus reduced.

*Calculation of hepatic glucose output.* Hepatic glucose release was calculated with the two-compartment model of Radziuk et al. (27) from [3-$^3$H]glucose and endogenous glucose concentration. The steady state values of model parameters were estimated from the [3-$^3$H]glucose decay curve after the primed, continuous infusion of [3-$^3$H]glucose given 2 h before the administration of the meal (28). The concentration of [3-$^3$H]glucose and the ratio between endogenous glucose and [3-$^3$H]glucose concentration during the mixed meal were subjected to smoothing using the optimal segments technique (29).

## Study C

*Pathways of glycogen synthesis.* At 5:30 a.m. on the morning of study a distal forearm cannula was inserted to allow sampling of arterialized blood. Acetaminophen (1.5 g) was given orally at 8:30 a.m., and the test meal was taken at 9:00 a.m. The test meal was identical to that

used in study A except that 50 μCi [1-14C]glucose was added to allow determination of 14C specific activity of urinary acetaminophen glucuronide and hence quantitation of the percent contribution of the direct and indirect pathways of glycogen synthesis (17). Urine samples were collected every 2 h for measurement of 14C-acetaminophen-glucuronide specific activity, and frequent blood samples were taken for measurement of glucose, [14C]glucose, and plasma insulin.

### Nuclear magnetic resonance (NMR) spectroscopy

The method for measuring glycogen concentration by 13C NMR spectroscopy has been described previously (14). A 9-cm observation coil and a 12 × 14–cm coplanar butterfly 1H decoupler coil were placed over the antero-lateral aspect of the liver and held firmly in place by means of a frame attached to the subject support. A 6-mm thick lucite plate was placed between the coil and the chest. The subject was then placed within an NMR spectrometer (1-m bore, 2.1 T, BioSpec; Bruker Instruments Inc., Billerica, MA) with the liver within the homogenous volume of the magnet. 12,800 acquisitions were observed in each 30-min period of observation as previously described (14).

### Analyses

Plasma glucose concentration was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments Inc., Palo Alto, CA). Atom percent enrichment of plasma [2-2H]glucose was determined by gas chromatography–mass spectrometry, and the 14C specific activity of plasma glucose and urine acetaminophen-glucuronide was determined by liquid scintillation analysis. Plasma (0.5 ml) was deproteinized with $ZnSO_4$ and $Ba(OH)_2$ and applied to a mixed-bed anion–cation exchange chromatography (1:1:1, AG1-X8, AG5OW-X8; Bio-Rad Laboratories, Richmond, CA), and glucose was eluted with 2 ml water. Acetaminophen glucuronide was purified by anion exchange chromatography (AG1X8, 100–200 mesh, acetate form) and eluted with increasing concentration of acetic acid.

*Gas chromatography–mass spectroscopy.* Gas chromatography–mass spectroscopy analysis was performed with a gas chromatograph (5890, HP-1 capillary column, 12 m × 0.2 mm × 0.33–μm film thickness; Hewlett-Packard Co., Palo Alto, CA) interfaced to a mass selec-tive detector (5971 A; Hewlett-Packard Co.) operating in the positive chemical ionization mode with methane as reagent gas. GC temperature was isothermal at 200°C. Selected ion monitoring (mass/charge 331, 332, and 333) was used to determine deuterium enrichment in glucose C1 → C6.

*HPLC.* HPLC analysis of acetaminophen-glucuronide was performed with a liquid chromatograph (1092, HP ODS Hypersil column, 5.0 μm, 100 × 4.6 mm, 45°C; Hewlett-Packard Co.) equipped with a filter photometric detector (254 nm). A binary solvent system (flow rate 0.75 ml/min) of sodium acetate (0.45 M in water, pH adjusted to 4.15 with phosphoric acid) and acetonitrile (isocratic for 3 min, 92% aqueous, 8% acetonitrile, followed by a linear increase to 20% acetonitrile in 2 min, with this concentration held constant for 3 min) was used to achieve resolution of acetaminophen glucuronide ($R_t$ 1.6 min). Purified acetaminophen glucuronide used as a standard was the gift of McNeil Pharmaceuticals (Fort Washington, PA).

## Results

*Plasma metabolites and hormones.* Plasma glucose rose from 5.0±0.1 to peak at 8.6±0.7 mmol/liter at 60 min. It had returned to basal levels (5.0±0.3 mmol/liter) by 360 min (Fig. 1). Plasma insulin rose from 4.1±0.5 to peak level of 73±13 mU/liter at 30 min and declined to reach basal levels (5.0±1.0) by 360 min (Fig. 2). Plasma glucagon levels also rose sharply to peak at 30 min (109±16–315±69 pg/ml) but fell much more gradually. At 360 min, plasma glucagon was still 62% above basal levels (177±24 pg/ml; $P < 0.05$ compared with basal), and by the end of the study it was still elevated in six of the eight subjects (130±21 pg/ml). The glucagon/insulin ratio, calculated as (pg/ml) per (mU/liter), fell as plasma insulin concentration increased but subsequently rose sharply from 240 min because of the much slower fall in plasma concentration of
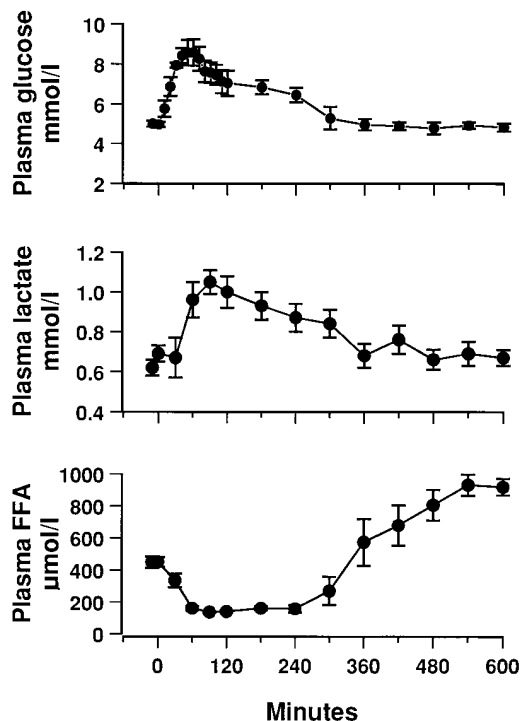


*Figure 1.* Plasma glucose, lactate, and FFA concentrations before and after the standard mixed meal.
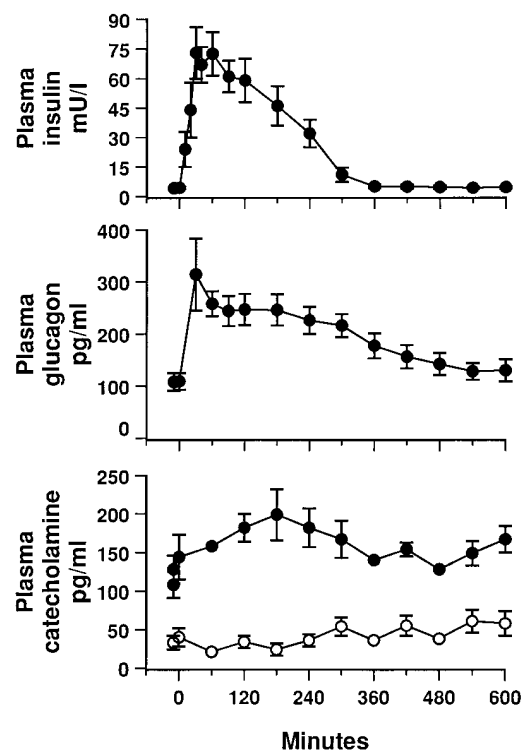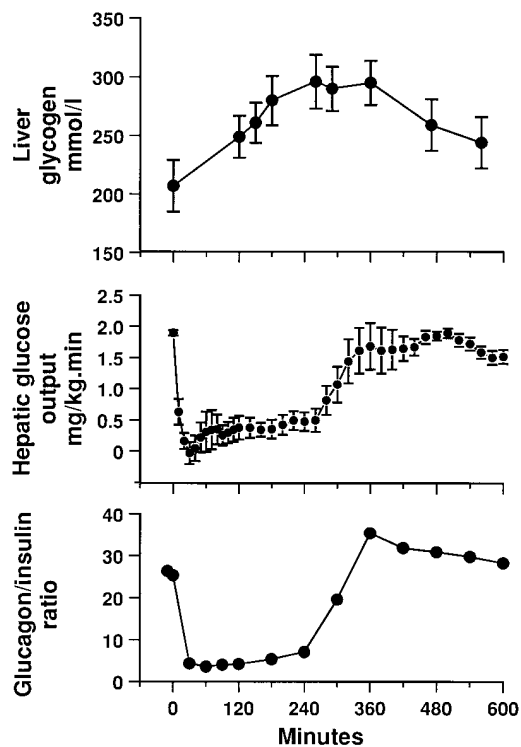


*Figure 2.* Plasma insulin, glucagon, NE (*bottom*, *solid symbols*) and epinephrine (*bottom*, *open symbols*). Catecholamine data were obtained from only four subjects.

glucagon compared with insulin (Fig. 3). Plasma FFA were suppressed rapidly, reaching a nadir at 90 min (basal 449±36; 90 min 137±5 μmol/liter; $P < 0.0001$). After remaining suppressed for over 3 h, plasma FFA levels rebounded, reaching 932±66 μmol/liter at 540 min ($P < 0.0001$ compared with basal). The rise and fall in plasma lactate levels mirrored those of plasma insulin (Fig. 1).

Plasma catecholamines were measured in four subjects. Epinephrine levels did not change during the study, but NE levels rose steadily to peak at 180 min (Fig. 2).

Full hormonal and metabolic data were obtained only during study B (measurement of hepatic glucose output). Plasma glucose levels were measured for 360 min in study C (pathways of glycogen synthesis) and were very similar to study B (basal, peak, and 360 min: 5.2±0.1 vs. 5.0±0.2; 8.6±0.4 vs. 8.6±0.6; 5.3±0.3 vs. 5.0±0.3mmol/liter).
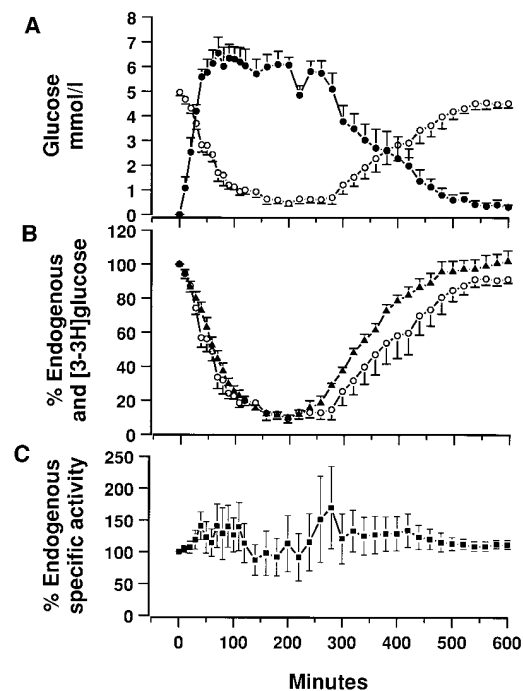
*Liver glycogen concentration.* Liver glycogen concentration fell overnight from 350±18 mmol/liter 4 h after the evening meal to 207±22 mmol/liter in the fasting state ($P < 0.0001$). After the test meal, mean net hepatic glycogen concentration rose at an average rate of 0.34 mol/liter per min for 260 min (Fig. 3). The mean of the individual peak concentrations was 316±19 mmol/liter and the mean time to peak was 318±31 min. This rise was statistically significant by 120 min; the first time point measured ($P < 0.001$). After peaking in individual subjects, mean liver glycogen concentration decreased in approximately a linear fashion ($P < 0.01$) at a rate of 0.26 mmol/liter per min.

Over the 260 min after the test meal the mean of the individual increments in glycogen concentration was 79.2±16.2 mmol/liter. As individual subjects did not all reach maximum liver glycogen concentrations at the same time, the mean increment in liver glycogen concentration was greater (109.8± 13.0 mmol/liter) than the group mean data at peak. Multiplying the individual increase in liver glycogen concentration by liver volume yielded a net hepatic glycogen synthesis of 28.3± 3.7 g. This was equivalent to ~ 19% of the meal carbohydrate content. Mean liver volume was 1.44±0.06 liters. There was no inverse correlation between liver volume and absolute increment in glycogen stored at peak concentration ($r = 0.35$, $P = 0.35$).

*Hepatic glucose output.* The time courses of the endogenous and exogenous components of total glucose concentration are shown in Fig. 4 *A*. As described in Methods, to reduce variations in endogenous glucose specific activity, the infusion rate of [3-³H]glucose was adjusted during the meal in such a way that the anticipated changes in endogenous glucose concentration were mirrored by similar changes in [3-³H]glucose concentration. The time courses of endogenous glucose and [3-³H]glucose concentration are shown in Fig. 4 *B*. For the sake of comparison, endogenous glucose and [3-³H]glucose concentration values were expressed in percentage of the respective basal levels. The patterns of [3-³H]glucose and endogenous glucose concentrations were almost superimposable during the first 4 h of the study. The approximation was less good from 240 min on, probably due to the high intrasubject variability in the rate of resumption of hepatic glucose release. However, since the two profiles remained parallel until the end of the study, the overall degree of correlation between them was re-



*Figure 3.* Concurrent changes in liver glycogen concentration (*top*), hepatic glucose output (*middle*), and the plasma glucagon/insulin ratio after the standard mixed meal. The ¹³C-NMR spectroscopy data on liver glycogen were obtained from study A, and the data on hepatic glucose output and plasma hormone levels were obtained under identical conditions from study B.



*Figure 4.* (*A*) Time course of change in the endogenous (*open circle*) and exogenous (*solid circle*) components of total plasma glucose concentration. (*B*) Time course of endogenous glucose (*open circle*) and [3-³H] glucose radiotracer (*triangle*) concentration expressed as a percentage of the respective basal levels. (*C*) Endogenous glucose specific activity expressed as a percentage of the basal level.

markable ($r = 0.98$, $P < 0.01$). Fig. 4 *C* shows the time course of endogenous glucose specific activity, which was calculated as the ratio of the [3-³H]glucose and endogenous glucose data shown above. The endogenous glucose specific activity remained within narrow limits during the first 200 min of the study. Between 200 and 300 min, when both tracer and endogenous glucose concentrations were very low and HGR began its resumption, endogenous specific activity was less stable.

Fasting hepatic glucose output (HGO) was $1.90\pm0.04$ mg/kg per min. Within 10 min of commencing the test meal HGO had decreased by 67% and reached a nadir at 30 min (Fig. 3). Between 60 and 255 min, HGO increased slightly but steadily from $0.31\pm0.32$ to $0.49\pm0.18$ mg/kg per min. Thereafter HGO recovered steeply. In individual subjects the fasting level of HGO was regained between 300 and 460 min after the meal (mean $380\pm28$ min).

*Indirect calorimetry.* By 120 min after eating both $CO_2$ uptake and $O_2$ uptake were significantly elevated ($213\pm12$–$250\pm11$ and $273\pm17$–$307\pm14$; $P < 0.0001$ and $P < 0.005$, respectively; Fig. 5). Respiratory quotient rose from $0.78\pm0.02$ to $0.89\pm0.05$ ($P < 0.005$) at peak at 220 min, and carbohydrate oxidation rose from $0.91\pm0.26$ to $2.51\pm0.41$ mg/kg per min ($P < 0.01$) at the same time. Carbohydrate oxidation rates fell rapidly and were not significantly different from basal by 460 min ($1.39\pm0.29$ mg/kg per min) and below basal by 600 min ($0.72\pm.026$ mg/kg per min; Fig. 5).

*Pathways of glycogen synthesis.* Plasma glucose ¹⁴C specific activity rapidly increased over the 30 min after the test meal to $87\pm7$ dpm/μmol then gradually increased to peak at $134\pm8$ at 160 min. The highest [¹⁴C]glucose specific activity observed in plasma was very similar in each subject to the specific activity

in that individual's test meal (166 and 166; 175 and 168; 151 and 177; 112 and 139; 116 and 158; 130 and 162 dpm/μmol). Urinary acetaminophen glucuronide ¹⁴C specific activity was $46.8\pm5.6$ dpm/μmol between 2 and 4 h after eating and $67.0\pm5.8$ dpm/μmol between 4 and 6 h after eating. The calculated contribution of the direct pathway to overall glycogen synthesis was $46\pm5$ and $68\pm8$% for each time period, respectively.

## Discussion

This study has demonstrated for the first time the postprandial time course of hepatic glycogen storage in normal humans in relation to the time course for suppression of hepatic glucose release. These new data allow a more complete picture to be built up of the processes of carbohydrate economy after ingestion of a mixed meal.

Previous work examining glucose storage after oral glucose has used either isotopic methods (9, 10), hepatic venous cannulation (7), or both techniques combined (8). Estimates of splanchnic glucose uptake after oral glucose have varied from $< 25$ to 60% of the administered load (7, 8). However, such studies were unable to distinguish between liver glycogen storage and splanchnic glucose metabolism. In the present study hepatic glycogen concentration increased by a mean of 42 mmol/liter within 120 min of commencing the meal. The mean rate of net hepatic glycogen synthesis continued, peaking at a mean of 318 min. It should be noted that most previous studies have been terminated at or before 240 min (7, 8, 12), a time at which absorption of either glucose or a mixed meal may be incomplete (7, 9, 30, 31) and at which liver glycogen storage is submaximal. Also, in the present study, a mixed test meal rather than glucose was used. This test meal was chosen to allow reproducible and traceable glucose absorption while mimicking the normal hormonal response to glucose-protein-lipid intake. The carbohydrate content of the meal was entirely glucose, and it must be considered that a normal solid meal containing complex carbohydrates may result in somewhat different rates of glucose absorption with consequent effects upon postprandial glucose metabolism.

The mean fasting hepatic glycogen concentration was slightly lower than that reported in a previous study (32), and this is accounted for by differences in the nutritional preparation. During the 3 d before the study, carbohydrate intake was lower in the present study (33 kcal/kg per d with 50% carbohydrate compared with 35 kcal/kg per d with 60% carbohydrate). The mean rise in hepatic glycogen concentration was 110 mmol/liter. As liver volume was measured in each subject, it was possible to calculate that $29\pm4$ g of glucose, $\sim 19$% of the ingested glucose load, had been stored as glycogen in the liver. This represents net glycogen synthesis and takes no account of glycogen turnover which has been shown to be active under hyperglycemic, hyperinsulinemic conditions (33). Hence, to the degree that hepatic glycogen turnover is occurring during absorption of the meal, the proportion of the meal-derived glucose which becomes at least temporarily incorporated into liver glycogen will be greater than the above net estimates. Although liver size changes with feeding and fasting, this would not be expected to exert a major influence upon the above estimations. During a 72-h fast, liver size decreases by 23%, but the changes after an overnight fast and single meal would not substantially alter the conclusions.

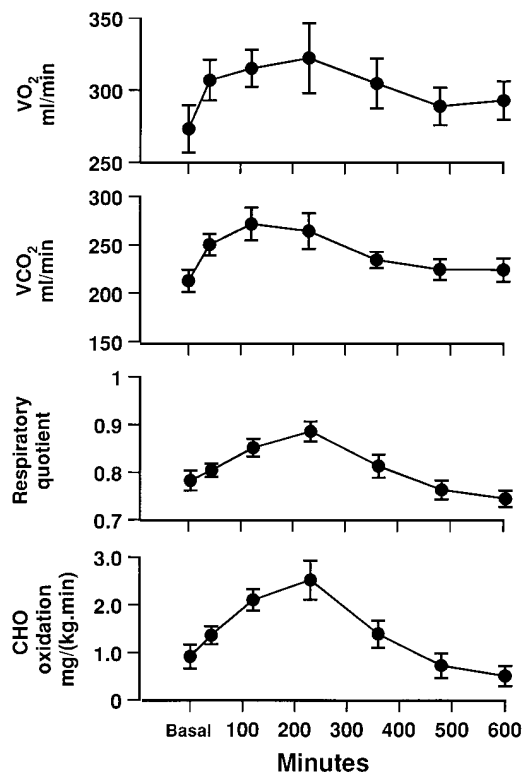Previous studies of hepatic glucose release after eating



*Figure 5.* Changes in fuel oxidation measured by indirect calorimetry after the mixed meal. $VO_2$, oxygen uptake; $VCO_2$, $CO_2$ uptake; CHO, carbohydrate.

have used a constant infusion of a glucose tracer. As the glucose whose specific activity must be clamped is endogenously released, and as endogenous glucose concentration varies markedly during the study, endogenous glucose specific activity (that is the key variable for the calculation of HGR) was far from constant. As shown by non–steady state theory, this introduces errors when HGR is calculated from such data (22–25). Previous estimates of the amount of suppression of hepatic glucose production after an oral glucose load include a mean of 56% between 0 and 270 min (9), a mean of 30% between 45 and 135 min, and a mean of 36% at 210 min (8). In the present study, the basal [3-³H]glucose infusion was adjusted during the test meal to allow minimization of the changes in the specific activity of endogenous glucose concentration (Fig. 4). In addition, Radziuk's two-compartment model, which has been proven to be a better representation of non–steady state glucose kinetics than Steele's model (27), has been used to derive HGR. A rapid and almost complete suppression of HGR was seen, and between 60 and 270 min HGR increased very slowly, averaging 21% of basal rate over this period. In the period when HGR was inhibited (0–200 min), the clamp of endogenous glucose specific activity was reasonably good. This is, by itself, model-independent evidence that our estimate of HGR in that period is substantially correct. In fact, the small deviations of specific activity indicate that the shape of HGR must be very close to that of the variable tracer infusion. Since the latter was rapidly decreased to 20% of the basal level and maintained between 15 and 30% until 240 min, our results showing a rapid and profound inhibition of HGR are likely to be close to the truth.

Clamping specific activity between 200 and 320 min was difficult because the time at which HGR began its resumption to the basal level varied among subjects. In addition, when both tracer and endogenous glucose concentration are very low (like when HGR began its resumption, Figs. 3 and 4), specific activity is very sensitive to changes in either tracer or glucose concentration. Thus, even a small increase in tracer concentration not accompanied by a concomitant increase in endogenous glucose concentration induces a noticeable increase in specific activity. The question arises to what extent this relatively unfavorable pattern of specific activity introduced error in our estimate of HGR between 200 and 320 min. To assess the size of this error we calculated HGR with Steele's model and compared its estimate with that of Radziuk's model reported in Fig. 4. We reasoned that since the error affecting HGR estimation depends on both the model and the rate of change of specific activity, the difference between the predictions of Radziuk's and Steele's model (i.e., between a good and a poor model of glucose kinetics) should be an index of how much the actual specific activity changes influence the estimate of HGR. The graphs of HGR so derived were almost superimposable (data not shown), and hence it may be concluded that despite a nonideal clamp of specific activity, the HGR estimate between 200 and 320 min is sufficiently reliable and better than that obtainable with the usual strategy of tracer infusion.

Since in previous studies pure glucose was administered, while in the present study a mixed meal was used, the differences in observed suppression of HGR could possibly reflect, at least in part, the different substrate and hormonal milieu. However, most previous studies have used a constant tracer infusion, which can lead to an increase in endogenous glucose specific activity during the time when hepatic glucose production is suppressed. Since Steele's model has been shown to overestimate HGR when specific activity is increasing (23), we suggest that in previous studies hepatic glucose release might have been overestimated mainly for this reason.

Early work in animals suggested that the majority of liver glycogen was synthesized not from glucose but from 3-carbon atom precursors (34, 35). More recently, the direct pathway (glucose → glucose-6-phosphate → glucose-1-phosphate → UDP-glucose → glycogen) has been shown to account for 49% of hepatic glycogen synthesis fasting and 69% after breakfast (16, 17). The present study has extended the latter observation in that estimations were made over two time periods postprandially and also in that a mixed meal was used, reflecting more precisely everyday physiology. The measurements during the first period were made during net hepatic glycogen synthesis and during the second period were made during little net synthesis but continued turnover. The observed increase in direct pathway contribution to hepatic glycogen synthesis from 46% between 2 and 4 h postprandially to 68% between 4 and 6 h postprandially is consistent with the previous observations that direct pathway activity is greater after the second meal of the day (16, 36). It is also consistent with the observation that glycogen synthesis directly from glucose occurs at a higher rate in primary cultures of hepatocytes isolated from fed rather than fasted animals (37–39). However, it must be emphasized that 4–6 h after the test meal in the present study rates of net liver glycogen synthesis are low. Hence, < 10% of the carbohydrate component of the meal was stored as liver glycogen via the direct pathway between 4 and 6 h postprandially.

The present study is unique in that detailed observation of postprandial hepatic metabolism was continued for > 5 h. The factors controlling the processes underlying hepatic glucose production need to be considered. At the time when the glucagon/insulin ratio starts to rise rapidly (at 240 min), HGR rises in step (Fig. 3). The increase in total liver glycogen concentration levels out, and as the glucagon/insulin ratio rises above basal values, liver glycogen concentration falls (Fig. 3).

Direct NMR observation of muscle glycogen content in humans has previously demonstrated that the rise in muscle glycogen concentration after a mixed meal is not maintained, but that from a mean of ∼ 300 min muscle glycogen breakdown supervenes (40). We previously hypothesized that glucose carbon from glycogen would be returned to the liver in the form of lactate, drawing support from observation of increasing lactate flux from muscle starting between 120 and 180 min postprandially (10, 41–43). Splanchnic balance studies have observed uptake of lactate commencing at about the same time (44), which would also support this concept.

In summary, this study has elucidated the changes in hepatic glucose metabolism after a mixed meal. A new approach to measuring hepatic glucose production demonstrated complete suppression within 30 min, and it averaged only 20% of basal HGR over the subsequent 4 h. Meal-derived glucose accounted for almost all the plasma glucose between 3 and 4 h after the meal. A rapid change in the glucagon/insulin ratio thereafter was paralleled by a rapid increase in hepatic glucose production towards basal levels. Net hepatic glycogen synthesis, measured directly by ¹³C-NMR spectroscopy, continued for 4 h after the meal, and net hepatic glycogenolysis was observed after 6 h. Glycogen synthesis via the direct pathway increased from 46% between 2 and 4 h to account for 68% of

overall hepatic glycogen synthesis between 4 and 6 h after the meal. These data provide for the first time a comprehensive picture of the metabolic changes after ingestion of a mixed meal in humans and lay the foundation for similar investigations in subjects with non–insulin-dependent diabetes mellitus.

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## References

1. Bogardus, C., S. Lillioja, K. Stone, and D. Mott. 1984. Correlation between muscle glycogen synthase activity and in vivo insulin action in man. *J. Clin. Invest.* 73:1185–1190.

2. Hollenbeck, C. B., Y.-D. I. Chen, and G. M. Reaven. 1984. A comparison of the relative effects of obesity and non-insulin dependent diabetes mellitus on in vivo insulin stimulated glucose utilization. *Diabetes.* 33:622–626.

3. DeFronzo, R. A., D. Simonson, and E. Ferrannini. 1982. Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin dependent) and type 1 (insulin dependent) diabetes mellitus. *Diabetologia.* 23:313–319.

4. Ishida, T., Z. Chap, J. Chou, R. Lewis, C. Hartley, M. Entman, and J. B. Field. 1983. Differential effects of oral, peripheral intravenous, and intraportal glucose on hepatic glucose uptake and insulin and glucagon extraction in conscious dogs. *J. Clin. Invest.* 72:590–601.

5. Adkins, B. A., S. R. Myers, G. K. Hendrick, P. E. Williams, and A. D. Cherrington. 1987. Importance of the route of intravenous glucose delivery on hepatic glucose balance in the conscious dog. *J. Clin. Invest.* 79:557–565.

6. Myers, S. R., D. W. Biggers, D. W. Neal, and A. D. Cherrington. 1991. Intraportal glucose delivery enhances the effects of hepatic glucose load on net hepatic glucose uptake in vivo. *J. Clin. Invest.* 88:158–167.

7. Katz, L. D., M. G. Glickman, S. Rapoport, E. Ferrannini, and R. A. DeFronzo. 1983. Splanchnic and peripheral disposal of oral glucose in man. *Diabetes.* 32:675–679.

8. Ferrannini, E., O. Bjorkman, G. A. Reichard, A. Pilo, M. Olsson, and J. Wahren. 1985. The disposal of an oral glucose load in healthy subjects: a quantitative study. *Diabetes.* 34:580–588.

9. Jackson, R. A., R. D. Roshania, M. I. Hawa, B. M. Sim, and L. DiSivio. 1986. Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: an analysis based on simultaneous use of the forearm and double isotope techniques. *J. Clin. Endocrinol. & Metab.* 63:541–549.

10. Kelley, D., A. Mitrakou, H. Marsh, F. Schwenk, J. Benn, G. Sonnenberg, M. Arcangell, T. Aoki, J. Sorensen, M. Berger, et al. 1988. Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *J. Clin. Invest.* 81:1563–1571.

11. McMahon, M., H. Marsh, and R. A. Rizza. 1989. Comparison of the pattern of postprandial carbohydrate metabolism after ingestion of a glucose drink or a mixed meal. *J. Clin. Endocrinol. & Metab.* 68:647–653.

12. Elia, M., P. Folmer, A. Schlatmann, A. Goren, and S. Austin. 1988. Carbohydrate, fat and protein metabolism in muscle and in the whole body after mixed meal ingestion. *Metab. Clin. Exp.* 37:542–551.

13. Jones, I. R., D. R. Owens, S. Luzio, S. Williams, and T. M. Hayes. 1989. The gastrointestinal inhibitory peptide response to oral glucose and mixed meals is increased in patients with type 2 (NIDDM) diabetes mellitus. *Diabetologia.* 32:668–677.

14. Rothman, D. L., I. Magnusson, L. D. Katz, R. G. Shulman, and G. I. Shulman. 1991. Quantitation of hepatic glycogenolysis and glucsneogenesis in fasting humans with $^{13}$C NMR. *Science (Wash. DC).* 254:573–576.

15. Gruetter, R., I. Magnussen, D. L. Rothman, M. J. Avison, R. G. Shulman, and G. I. Shulman. 1994. Validation of $^{13}$C NMR measurements of liver glycogen in vivo. *Mag. Reson. Med.* 4:583–588.

16. Magnusson, I., V. Chandramouli, W. C. Schumann, K. Kumaran, J. Wahren, and B. R. Landau. 1989. Pathways of hepatic glycogen formation in humans following ingestion of a glucose load in the fed state. *Metab. Clin. Exp.* 38:583–585.

17. Shulman, G. I., G. Cline, W. C. Schumann, V. Chandramouli, K. Kumaran, and B. R. Landau. 1990. Quantitative comparison of pathways of hepatic glycogen repletion in fed and fasted humans. *Am. J. Physiol.* 260:E335–E341.

18. Cooper, B. G., J. A. McClean, and R. Taylor. 1991. Evaluation of the Deltatrac indirect calorimeter by gravimetric gas injection and alcohol burning. *Clin. Physics Physiol. Meas.* 12:333–341.

19. Lusk, G. 1924. Analysis of the oxidation of mixtures of carbohydrate and fat. *J. Biol. Chem.* 59:41–42.

20. Cobelli, C., and G. Toffolo. 1990. Constant specific activity input allows reconstruction of endogenous glucose concentration in non-steady state. *Am. J. Physiol.* 258:E1037–E1040.

21. Cobelli, C., G. Toffolo, and D. M. Foster. 1992. Tracer-to-tracee ratio for the analysis of stable isotope tracer data: the link with the radioactive kinetic formalism. *Am. J. Physiol.* 262:E968–E975.

22. Norwich, K. N. 1977. Molecular dynamics in Biosystems. The Kinetics of Tracers in the Intact Organism. Pergamon Press Ltd., Oxford.

23. Cobelli, C., A. Mari, and E. Ferrannini. 1987. Non-steady state: error analysis of Steel's model and developments for glucose kinetics. *Am. J. Physiol.* 252:E679–E689.

24. Finegood, D. T., R. N. Bergman, and M. Vranic. 1987. Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes.* 36:914–924.

25. Jacquez, J. A. 1992. Theory of production rate calculations in steady and non-steady states and its application to glucose metabolism. *Am. J. Physiol.* 262:E779–E790.

26. Alzaid, A. A., S. F. Dinneen, D. J. Turk, A. Caumo, C. Cobelli, and R. A. Rizza. 1978. Assessment of insulin action and glucose effectiveness in diabetic and nondiabetic humans. *J. Clin. Invest.* 94:2341–2348.

27. Radziuk, J., K. H. Norwich, and M. Vranic. 1978. Experimental validation of measurements of glucose turnover in nonsteady state. *Am. J. Physiol.* 234:E84–E93.

28. Carson, E. R., C. Cobelli, and L. Finkelstein. 1983. The Mathematical Modeling of Metabolic and Endocrine Systems. John Wiley & Sons, Inc. New York.

29. Finegood, D. T., and R. N. Bergman. 1983. Optimal segments: a method for smoothing tracer data to calculate metabolic fluxes. *Am. J. Physiol.* 244:E472–E479.

30. Price, T. B., R. Taylor, G. F. Mason, D. L. Rothman, G. I. Shulman, and R. G. Shulman. 1994. Turnover of human muscle glycogen with low-intensity exercise. *Med. Sci. Sports Exercise.* 26:983–991.

31. Collins, P. J., M. Horowitz, and B. E. Chatterton. 1988. Proximal, distal and total stomach emptying of a digestible solid meal in normal subjects. *Br. J. Radiol.* 61:12–18.

32. Hwang, J.-H., G. Perseghin, D. L. Rothman, G. W. Cline, I. Magnusson, K. Falk-Petersen, and G. I. Shulman. 1995. Impaired net hepatic glycogen synthesis in insulin-dependent diabetic subjects during mixed meal ingestion. *J. Clin. Invest.* 95:783–787.

33. Magnusson, I., D. L. Rothman, B. Jucker, G. W. Cline, R. G. Shulman, and G. I. Shulman. 1994. Liver glycogen turnover in fed and fasted humans. *Am. J. Physiol.* 266:E796–E803.

34. Katz, J., S. Golden, and P. Wals. 1979. Glycogen synthesis by rat hepatocytes. *Biochem. J.* 180:389–402.

35. Katz, J., and J. D. McGarry. 1984. The glucose paradox. Is glucose a substrate for liver metabolism? *J. Clin. Invest.* 74:1901–1909.

36. Friedman, J. E., J. F. Caro, W. J. Pories, J. L. Azevedo, Jr., and G. L. Dohm. 1994. Glucose metabolism in incubated human muscle: effect of obesity and non-insulin-dependent diabetes mellitus. *Metab. Clin. Exp.* 43:1047–1054.

37. Agius, L., M. Peak, and K. G. M. M. Alberti. 1990. Regulation of glycogen synthesis from glucose and gluconeogenic precursors by insulin in periportal and perivenous rat hepatocytes. *Biochem. J.* 266:91–102.

38. Agius, L., D. Tosh, and M. Peak. 1993. The contribution of pyruvate cycling to loss of [6-$^{3}$H]glucose during conversion of glucose to glycogen in hepatocytes: effects of insulin, glucose and acinar origin of hepatocytes. *Biochem. J.* 289:255–262.

39. Tosh, D., G. Beresford, and L. Agius. 1994. Glycogen synthesis from glucose by direct and indirect pathways in hepatocyte cultures from different nutritional states. *Biochim. Biophys. Acta.* 1224:205–212.

40. Taylor, R., T. B. Price, L. D. Katz, R. G. Shulman, and G. I. Shulman. 1993. Direct measurement of change in muscle glycogen concentration after a mixed meal in normal subjects. *Am. J. Physiol.* 265:E224–E229.

41. Jackson, R. A., J. B. Hamling, B. M. Sim, M. I. Hawa, P. M. Blix, and J. D. N. Nabarro. 1987. Peripheral lactate and oxygen metabolism in man: the influence of oral glucose loading. *Metab. Clin. Exp.* 36:144–150.

42. Mitrakou, A., D. Kelley, T. Veneman, T. Jenssen, T. Pangburn, J. Reilly, and J. Gerich. 1990. Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. *Diabetes.* 39:1381–1390.

43. Radziuk, J., and R. Inculet. 1983. The effects of ingested and intravenous glucose and glucogenic substrate in normal man. *Diabetes.* 32:977–981.

44. Waldhausl, W. K., S. Gasic, P. Bratusch-Marrain, and P. Nowotny. 1983. The 75-g oral glucose tolerance test: effect on splanchnic metabolism of substrates and pancreatic hormone release in healthy man. *Diabetologia.* 25:489–495.