Rate of Glucose Entry Into Hepatic Uridine Diphosphoglucose by the Direct Pathway in Fasted and Fed States in Normal Humans

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We used the glucuronate (GlcUA) probe technique to measure the rate of glucose entry into hepatic uridine diphosphoglucose (UDP-glc) by the direct pathway, to quantify the rate of appearance (R_a) of hepatic UDP-glc, and to calculate hepatic glucose cycling in vivo in normal humans. The direct pathway contribution to UDP-glc as determined by the ratio of [1-d_1]-GlcUA to plasma [LD_1]-glucose enrichments was minor (15% to 20%) in normal men after an overnight fast. After 9 hours of refeeding with intravenous (IV) glucose or an oral liquid formula meal each at a rate of 7 mg carbohydrate/kg/min, the direct pathway increased to 66.3% ± 6.7% and 61.6% ± 6.0% (mean ± SE), respectively. Plasma glucose concentrations remained below 7.8 mmol/L and could not account for most of the variability in direct pathway contribution. The dilution of labeled [L-D_1]-galactose in excreted acetaminophen-GlcUA was used to measure R_a UDP-glc, on the assumption that labeled galactose passes through the liver during its assimilation. R_a UDP-glc was 1.1 ± 0.1 mg/kg/min after an overnight fast and increased to 2.0 ± 0.1 with IV glucose and 2.6 ± 0.2 with the oral liquid mixed meal. By combining the fractional glucose contribution with the R_a of hepatic UDP-glc, the rate of direct glucose entry into hepatic UDP-glc was 0.2 mg/kg/min (fasted) and increased to 1.3 to 1.6 (fed). This represented approximately 18% to 21% of systemic glucose disposal or 19% to 23% of the administered carbohydrate load during IV or oral refeeding. Correction for labeled glucose cycling through hepatic glucose-6-phosphate increased hepatic glucose production (HGP) by 0.55 ± 0.09 mg/kg/min in the fasted state and by 0.34 ± 0.08 in the IV glucose–fed state. The partitioning ratio of hepatic glucose-6-phosphate into UDP-glc versus plasma glucose increased from 0.36 to 3.82 from fasted to IV glucose states, respectively ($P < .01$). We conclude that the contribution from the direct pathway to hepatic UDP-glc increases with progressive carbohydrate repletion in humans, the proportion of hepatic glucose-6-phosphate partitioned into UDP-glc increases with refeeding, the turnover of the intrahepatic metabolite UDP-glc can be measured noninvasively, and the liver takes up approximately 20% of an exogenous glucose load into UDP-glc by the direct pathway.

AN UNDERSTANDING of the role of different tissues in the disposal of a carbohydrate load is central to our understanding of glucose tolerance and the pathophysiology of glucose intolerance; however, quantitative estimates remain controversial. Most estimates in experimental animals and in humans[1-6] have attributed approximately 20% to 35% of the uptake of an oral load to the liver. These estimates have been fairly consistent, although they are based on diverse methods such as splanchnic balances,[1-4] liver glycogen accumulation,[5] radioisotope distribution,[6] and hepatic glycogen mobilization by glucagon.[7] Shulman et al[8] recently used in vivo nuclear magnetic resonance spectroscopy to quantify gastrocnemius muscle glycogen content in normal humans and reported that more than 95% of intravenous (IV) glucose infused during a hyperglycemic, hyperinsulinemic clamp could be accounted for as muscle glycogen. Obviously, this would not allow 20% to 35% hepatic uptake. In contrast, Kelley et al[4] reported that muscle uptake represented only 26% of an oral glucose load, and of that, more than two thirds was oxidized or released as lactate, whereas splanchnic uptake was at least 25% to 30% of the load. Marin et al[9] reported a similar low estimate for the entry of a labeled oral glucose load into muscle glycogen in normal women.

A complicating factor in interpreting the role of the liver in disposal of a glucose load is that glucose may enter into hepatic hexose phosphate pools directly (by phosphorylation) or indirectly (by glycolysis followed by gluconeogenesis). It is apparent that both pathways contribute to glycogen synthesis in fasted-refed rats.[10-13] Portal hyperglycemia may increase the direct pathway contribution,[1,11,12,14,15] consistent with the kinetic properties of hepatic glucokinase, which is a high-K_m/high-Vmax enzyme.[16] However, to what extent this mechanism applies under usual glycemic conditions remains uncertain, since glucose concentrations of 30 to 50 mmol/L are required in vitro for net glucose uptake by hepatocytes,[11,17] and some investigators have reported no difference in pathways in rats at 8 versus 11 mmol/L[18] or until more than 20 mmol/L,[14] whereas others have reported net hepatic glucose uptake at physiologic glucose concentrations.[1] Alternatively, tissue carbohydrate stores may influence the direct pathway contribution. After replenishment of liver glycogen, the direct pathway increases in isolated hepatocytes[19] and intact rats[20] and is higher in fed as compared with overnight-fasted humans at equal serum glucose concentrations[21] during hyperglycemic glucose clamps. The absolute rate of glucose entry into hepatic hexose phosphate pools by the direct and indirect pathways has not yet been measured in humans.

Estimations of the role of the liver in disposal of a

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carbohydrate load and of the contribution from the direct pathway have been limited by the absence of a simple measurement technique. Invasive procedures such as splanchnic catheterization[3] have revealed valuable information but are not easily performed, carry some risk, and are highly dependent on blood flow estimates. Liver biopsy is also invasive, associated with risk, and reveals only net storage. A noninvasive isotopic method for estimating the rate of glucose entry into liver uridine diphosphoglucose (UDP-glc) would be helpful in this regard.

Here we use the glucuronide (GlcUA) probe technique to address these questions in humans. The GlcUA probe is a technique for sampling hepatic UDP-glc noninvasively in living organisms (Fig 1). We[10,22-25] and others[15,21,26,27] have previously used this approach to study hepatic UDP-glc and glycogen metabolism in vivo. We apply here a method, with stable isotopes and mass spectrometry (MS) analysis in lieu of radioisotopes[26] or the less-sensitive nuclear magnetic resonance analysis,[21] for measuring the fraction of hepatic UDP-glc derived directly from plasma glucose. By using this method, a sequential record or moving picture of the direct pathway contribution is generated over time in an individual subject.

We also present a method for measuring the R_a of UDP-glc in the liver of humans in vivo. The method uses the GlcUA probe during infusions of labeled galactose. The rate of glucose entry into the liver by the direct pathway and the partitioning of glucose-6-phosphate between blood glucose and hepatic UDP-glc are determined by combining the two techniques.

SUBJECTS AND METHODS

[1-D_1]-Glucose was purchased from Tracer Technologies (Somerville, MA). MS revealed this to be more than 99% enriched. Enzymes and chemicals were reagent grade. Acetaminophen for IV administration was prepared by Dr Lou Tomimatsu of the University of California at San Francisco School of Pharmacy and mixed by the San Francisco General Hospital Pharmacy. Parenteral acetaminophen was administered under Investigational New Drug No. 29,483 from the Food and Drug Administration (to M.K.H.). Plasma glucose concentrations were determined by the glucose oxidase method. Serum insulin measurements were made by radioimmunoassay (Cambridge Medical Diagnostics, Billerica, MA).

Human Subjects and Experimental Design

Protocols were approved by the University of California, San Francisco, Committee on Human Research, and written informed consent was obtained for all procedures. Healthy control subjects were less than 120% of ideal body weight, had no history of diabetes, liver disease, substance abuse, recent weight loss, or systemic illness, and were documented to be human immunodeficiency virus–seronegative. The mean ± SE age was 42 ± 5 years. All subjects were men. Screening laboratory tests (complete blood count, routine chemistry profile, urinalysis) were normal.

The technique for measuring the direct contribution to hepatic UDP-glc synthesis was the same in all subjects. The GlcUA conjugate of administered acetaminophen was isolated from urine during an infusion of [1-d_1]-glucose.[10] Secreted GlcUA reflects the labeling in hepatic UDP-glc (Fig 1) at the time of conjugation.[10] The ratio of GlcUA to plasma glucose enrichments reveals the fraction of hepatic UDP-glc derived directly from plasma glucose.[10,21] We initially used this technique in rats with radioisotopes to study the pathways of UDP-glc synthesis[10] and other metabolic processes relating to hepatic nucleotide sugars.[22,23] A similar method has been used by Magnusson et al[26] in humans with radioisotopes and by Shulman et al[21] with nuclear magnetic resonance analysis.

Two experimental protocols were used (Fig 2):

Group I: Label and Acetaminophen During Overnight Fasting and Daytime Refeeding

Subjects were admitted to the General Clinical Research Center of the San Francisco General Hospital and fed ad libitum until 6 PM of the study day. After their 6-PM supper, subjects were fasted but noncaloric beverages were permitted. At 10 PM an IV line was placed, and at 2 AM a constant infusion of acetaminophen 2 mg/mL in sterile 0.45% saline was begun at 50 mL/h. Mixed with the acetaminophen solution was [1-d_1]-glucose, delivered at 0.04 to 0.06 mg/kg body weight/min. Urine aliquots were collected for the duration of the infusion (2 AM until 6 PM) whenever the subject voided (urine was not pooled). Blood was drawn hourly from a heparin lock placed in the contralateral arm from 8 AM to 5 PM. Subjects were refed from 9 AM until 6 PM with either IV glucose infused at 7 mg/kg/min or an oral liquid mixed-meal formula (Ensure; Ross Laboratories, Columbus, OH) hourly to provide carbohydrate at 7 mg/kg/min. The nutrient composition of the oral

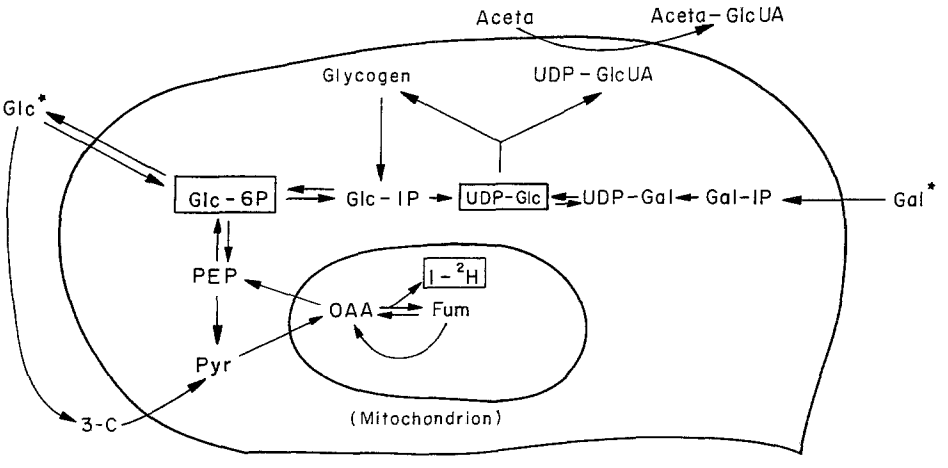

Fig 1. Pathways of hepatic UDP-glc synthesis and their routes of labeling from [1-d_1]-glucose or [1-d_1]-galactose (*). Abbreviations: Glc, glucose; P, phosphate; Pyr, pyruvate; PEP, phosphoenolpyruvate; OAA, oxaloacetate; Fum, fumarate; 3-C, 3-carbon metabolites; Gal, galactose; Aceta, acetaminophen.

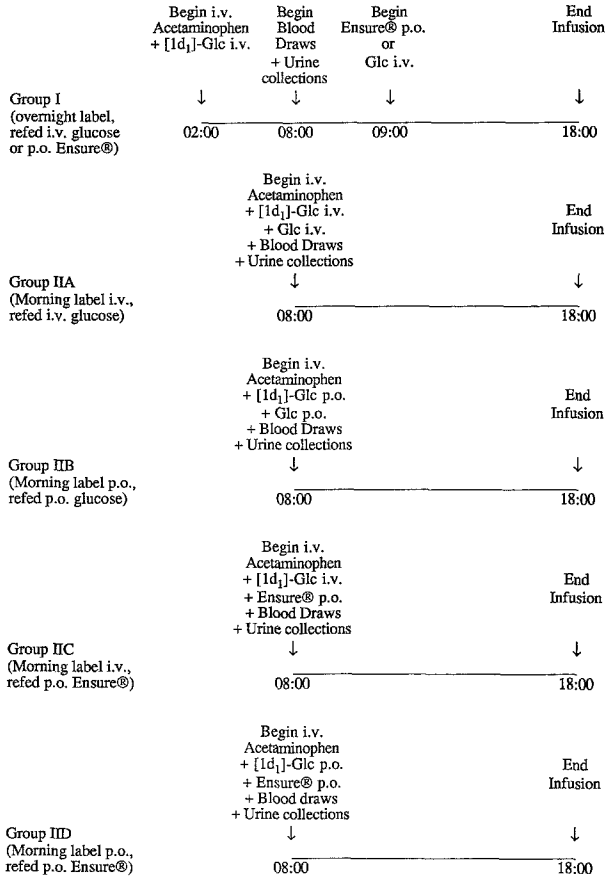

Fig 2. Experimental protocols used in human subjects (groups I and II). p.o., orally.

formula is 15% of calories as protein, 30% as fat, and 55% as carbohydrate (85% of carbohydrate calories as glucose and 15% as fructose). The intent in using this protocol was to evaluate the direct pathway contribution over time during the transition from fasted to fed states and to compare the effects of IV glucose with those of oral mixed nutrients. The identical protocol was used for measurement of R_a UDP-glc (see below). Because [1-d_1]-glucose and [1-d_1]-galactose cannot be administered concurrently (since labeling of GlcUA could be from either), subjects in whom both the direct pathway and R_a UDP-glc were measured were studied on separate occasions 1 week apart under otherwise identical experimental conditions.

Group II: Label and Acetaminophen During Daytime Refeeding Only

For group II subjects, the protocol was the same as for group I except that neither acetaminophen nor [1-d_1]-glucose were administered overnight. For groups IIA and IIC, IV infusions of acetaminophen at 100 mg/h and [1-d_1]-glucose at 0.06 to 0.08 mg/kg/min were begun at 8 AM concurrently with the refeeding regimens described above (IV glucose in group IIA, oral formula in group IIC). Blood and urine collections were as for group I. For groups IIB and IID, the [1-d_1]-glucose was administered as hourly oral boluses to provide an average rate of 0.06 to 0.08 mg/kg/min, mixed with 10% dextrose solution or oral formula, respectively. Acetaminophen was administered IV as for groups IIA and IIC. Blood and urine collections were as described above. The intent of the protocols used in group II subjects was first to test whether pharmacokinetics (delayed clearance of acetaminophen GlcUA synthesized during the fasting period) could explain differences observed during refeeding, and second, whether the route of label administration (oral or IV) was a potentially confounding variable.

High-Performance Liquid Chromatography/MS

We use a VG30-250 quadruple mass spectrometer (VG Mass Laboratory, Altrincham, Cheshire, UK) linked to a Waters 600 Multisolvent Delivery System (Waters/Millipore, Millford, MA) via a VG instruments plasmaspray-thermospray LC/MS interface.[27] The samples are chromatographed on a 155 × 4–mm C-18 5-μm cartridge column (Econosphere, Alltech Associates, Deerfield, IL) with a solvent system of methanol:0.2-mol/L ammonium acetate (9:1 vol/vol) at a flow rate of 1.0 mL/min. The mass spectrometer is tuned and calibrated daily before analyzing samples. The source remains at 245°C. The repeller electrode voltage and capillary temperature vary somewhat around the settings shown. The abundance of the molecular anion [M $^-$H] negative and an isotope [M + [M + 1 $^-$H] negative are monitored in the selected ion recording mode. Techniques for maximizing analytic precision and reproducibility of the LC/MS analyses are described in detail elsewhere.[27] For both glucose and acetaminophen GlcUA, replicate analyses were performed on each set of samples until the standard error of each measurement did not exceed 0.05 molar percent excess (MPE). Generally, quadruplicate or quintuplicate analyses were performed. Sequential time points were then averaged within each 3-hour block (fasted, early refed, mid-refed, and late refed), further reducing the error. Two to three samples were analyzed for each time period, resulting in an analytic precision of ± less than 0.03 MPE (see below). Measured M_1 isotope enrichments were converted to true [1-d_1]-enrichments by correction for the natural abundance mass isotopomer distribution of the remainder of the molecule,[24] for both plasma glucose and acetaminophen GlcUA. The calculated correction for isotopomeric distribution in glucose was confirmed by comparison with standard curves made by mixing known proportions of [1-d_1]-glucose and natural-abundance glucose.

Sample Preparation

Glucose was isolated from plasma before HPLC-MS analysis.[27] The purity of isolated glucose was tested using a Bio-Rad (Hercules, CA) HPX-87H carbohydrate analysis column and detection with a Waters Model 410 Differential Refractometer (Milford, MA). The pump was a Beckman (Palo Alto, CA) Model 110B. The elution buffer was 0.01N H_2SO_4 at 65°C using a column heater (Model 1061, Rainin, Woburn, MA). The remainder of the sample was analyzed by HPLC-MS. Acetaminophen GlcUA was isolated from urine as described previously.[10,22] Peak identification of acetaminophen GlcUA was confirmed by comparison with an authentic acetaminophen GlcUA standard (McNeil Pharmaceuticals, Fort Washington, PA). Aliquots of infusates were taken before and after infusions, and infusate glucose concentrations and isotope enrichments were determined to calculate actual [1-d_1]-glucose infusion rates.

Isotopic Model

The isotopic dilution principle is commonly used to estimate the R_a or turnover of metabolites located in the plasma compartment—for example, glucose, amino acids, glycerol, or palmitate.[28,29] We reasoned that the R_a of an intracellular metabolite such as hepatic UDP-glc might also be determined using this principle. The prerequisites for using the isotopic dilution principle are (1) that the label can be injected into the metabolite pool of interest at a

known and preferably constant rate; (2) that the metabolite pool of interest or a product derived exclusively from it can be sampled in vivo and that its isotopic content (specific activity or enrichment) can be determined; and (3) that serial measurements are possible so that the attainment of an isotopic steady state can be documented. If these three conditions apply, the standard steady-state dilution equation can be used[28]:

$$R_a(\text{mg/kg/min}) = [\text{label infusion rate (mg/kg/min)/plateau} \cdot \text{enrichment (molar excess)}] - [\text{label infusion rate}]. \quad \text{Eq 1}$$

Condition 1 above is met when [1-d_1]-galactose is infused IV at a known rate in rats or humans, since more than 90% of galactose is metabolized by the liver in mammals[30] and passes through UDP-glc in an obligate manner (Fig 1). Condition 2 is met by using the GlcUA probe as an end-product indicator of hepatic UDP-glc labeling (Fig 1), and condition 3 is readily achieved due to the noninvasive nature of the secreted-probe technique, which permits serial monitoring of UDP-glc. Accordingly, the R_a of UDP-glc can be calculated as follows:

$$R_a\text{UDP-glc(mg/kg/min)} = \frac{I}{\text{plateau molar excess GlcUA} \times \text{body weight (kg)}} - I, \quad \text{Eq 2}$$

where I is the infusion rate of [^{2}H]-galactose.

Results are expressed for R_a UDP-glc per kilogram body weight rather than liver weight, for comparison to systemic glucose disposal.

Calculations

The direct pathway contribution to UDP-glc was calculated as described previously[10]:

$$\text{Direct pathway contribution (\%)} = \frac{\text{GlcUA enrichment} \cdot [m/z\ 327/(m/z\ 326 + m/z\ 327)]}{\text{glucose enrichment} \cdot [m/z\ 180/(m/z\ 179 + m/z\ 180)]} \times 100. \quad \text{Eq 3}$$

Time-weighted GlcUA and glucose values over the preceding 3-hour period were used.

$$\text{Weighted interval MPEs} = \sum_0^n \frac{t_x(\text{MPE}_x)}{t},$$

where t_x is the interval time period and MPE_x is the MPE of GlcUA during this time period.

The absolute rate of direct glucose entry into hepatic UDP-glc was calculated by combining the UDP-glc flux rate with the fractional contribution from plasma glucose:

$$\text{direct glucose entry into UDP-glc (mg/kg/min)} = R_a\ \text{UDP-glc} \times \text{direct pathway contribution}. \quad \text{Eq 4}$$

R_a glucose was calculated in the standard fashion,[28] from steady-state glucose enrichments (fasted and refed):

$$R_a\ \text{glucose (mg/kg/min)} = \frac{I\ (\text{mg/kg/min})}{\text{glucose enrichment}\ [m/z\ 180/(m/z\ 179 + m/z\ 180)]} - I,$$

where I is the infusion rate of [1-d_1]-glucose.

Hepatic glucose cycling was calculated according to the method recently described by Rossetti et al.[31] The direct pathway is a measure of the steady-state contribution from circulating glucose to flux into the glucose-6-phosphate pool. Total flux in the reverse direction, from hepatic glucose-6-phosphate to plasma glucose, will be underestimated to this same extent, since secretion of labeled glucose-6-phosphate (ie, that portion of glucose-6-phosphate with the same enrichment as plasma glucose) will not dilute plasma glucose. Thus, hepatic glucose production (HGP) measured by dilution of infused [1-d_1]-glucose can be corrected by use of the UDP-glc to glucose labeling ratio to calculate labeled glucose cycling, which is defined here as direct glucose uptake via phosphorylation followed by release by dephosphorylation without loss of label in the liver.

The partitioning fraction of hepatic glucose-6-phosphate into blood glucose versus hepatic UDP-glc can then be estimated by comparing glucose cycling with the rate of direct glucose entry into hepatic UDP-glc, since these represent alternative fates of hepatic glucose-6-phosphate:

$$\text{labeled glucose cycling (mg/kg/min)} = \frac{\text{HGP}}{(1 - \text{fractional direct pathway contribution})} - \text{HGP}, \quad \text{Eq 5A}$$

$$\text{where HGP equals } (R_a\ \text{glucose} - \text{glucose infusion rate}). \quad \text{Eq 5B}$$

$$\text{Partitioning of hepatic glucose-6-phosphate} = \frac{\text{direct glucose entry into UDP-glc}}{\text{labeled glucose cycling}}. \quad \text{Eq 6}$$

It is important to note that this measure of glucose cycling does not include labeled glucose lost within the liver (eg, [2-^{2}H]glucose lost in the glucose phosphate isomerase reaction), but represents only the labeled glucose retained in glucose-6-phosphate (see below).

Statistical Analyses

Repeated-measures ANOVA was used to obtain estimates of variability during sequential fasting-refeeding protocols. Follow-up evaluation was performed using one-tailed Dunnett's multiple comparison procedure for comparing treatment values with baseline values at a 5% procedurewise use error rate. Comparisons among treatment groups were by ANOVA.

RESULTS

Pathway of Hepatic UDP-glc Synthesis From Glucose After Overnight Fasting and After Refeeding

After overnight fasting, the direct pathway contribution to UDP-glc synthesis was only 16% to 20% (Tables 1 and 2). IV glucose administration at 7 mg/kg/min increased the direct pathway contribution over time from 19.5% ± 2.7% to 66.3% ± 6.7% ($P < .05$; Table 1). The direct pathway also became increasingly important as oral refeeding with a liquid formula meal progressed (16.6% ± 3.1% to 66.1% ± 6.0%; Table 2). Plateau values for the direct pathway were not significantly different after 9 hours from the IV glucose–refeeding route than the oral formula–refeeding route in these subjects ($P = .22$). Urinary acetaminophen GlcUA concentrations reached a plateau after 4 to 8 hours of acetaminophen infusion, consistent with established kinetics of free acetaminophen in plasma and acetaminophen glucuronidation.[32] Since urinary acetaminophen GlcUA enrichments reached new plateau values within 4 or 5 hours after a metabolic perturbation in humans (see below), delayed excretion of previously synthesized acetaminophen GlcUA cannot explain the stable or increasing GlcUA isotope enrichments during refeeding in protocol I subjects (Tables 1 and 2). These presumably reflect the combination of two processes: decreasing plasma glucose enrichments with an increasing fractional direct contribution from glucose.

Table 1. Enrichments and Direct Pathway Contribution (mean ± SE) in IV Glucose–Refed Subjects (group I)

Subject No./ Time	Glucose Enrichment (MPE)	GlcUA Enrichment (MPE)	Direct Pathway Contribution (%)
1			
Fasted	0.91 ± .03	0.22 ± .02	24.2
Early	0.60 ± .02	0.36 ± .04	60.0
Middle	0.41 ± .02	0.38 ± .02	92.7
Late	0.41 ± .03	0.35 ± .03	85.4
2			
Fasted	2.52 ± .04	0.56 ± .04	22.2
Early	1.26 ± .02	0.48 ± .03	38.7
Middle	0.52 ± .03	0.45 ± .02	86.5
Late	0.44 ± .03	0.43 ± .03	97.7
3			
Fasted	1.27 ± .03	0.19 ± .01	15.0
Early	0.52 ± .03	0.21 ± .03	40.4
Middle	0.43 ± .04	0.27 ± .03	62.8
Late	0.48 ± .03	0.31 ± .02	64.6
5			
Fasted	1.44 ± .03	0.23 ± .03	16.0
Early	0.85 ± .04	0.21 ± .02	24.7
Middle	0.51 ± .03	0.30 ± .03	58.8
Late	0.40 ± .01	0.21 ± .01	52.5
6			
Fasted	1.28 ± .02	0.38 ± .02	29.7
Early	0.99 ± .03	0.34 ± .03	34.3
Middle	0.99 ± .04	0.59 ± .03	59.6
Late	0.72 ± .02	0.52 ± .02	72.2
9			
Fasted	2.20 ± .03	0.36 ± .02	16.4
Early	0.59 ± .03	0.38 ± .03	64.4
Middle	0.52 ± .02	0.31 ± .03	59.6
Late	0.72 ± .03	0.31 ± .02	43.1
10			
Fasted	2.19 ± .04	0.14 ± .03	6.4
Early	1.00 ± .03	0.20 ± .02	20.0
Middle	0.79 ± .01	0.42 ± .04	53.2
Late	0.81 ± .03	0.38 ± .01	46.9
11			
Fasted	0.76 ± .02	0.20 ± .03	26.3
Early	0.40 ± .02	0.20 ± .02	50.2
Middle	0.38 ± .03	0.30 ± .01	78.9
Late	0.43 ± .03	0.29 ± .02	67.4
Mean ± SE			
Fasted			19.5 ± 2.7
Early			41.5 ± 5.6*
Middle			69.0 ± 5.2†
Late			66.3 ± 6.7†

NOTE. Direct pathway contribution was calculated as described in the text. Subjects were fasted overnight until 9 AM and then infused with IV glucose at 7 mg/kg/min. [1-d_1]-glucose was infused IV from 2 AM until 6 PM. Fasted, 8 to 9 AM to noon; middle, noon to 3 PM; late, 3 to 6 PM.

*$P < .05$ *v* fasted.

†$P < .05$ *v* fasted and early refed.

Direct Pathway Contribution in Protocol II Subjects

To confirm that the early time points during refeeding reflected a lower direct pathway contribution than did late time points, subjects were studied without prior administration of labeled glucose or acetaminophen during the overnight fast (protocol II). In these subjects, who received IV labeled and unlabeled glucose concurrently in the morning, enrichments of both serum glucose and urinary acetaminophen GlcUA increased over time (Fig 3). The direct pathway contribution tended to increase during IV glucose refeeding, from 30.6% ± 6.5% to 49.2% ± 6.7% to 51.5% ± 6.7% ($P < .01$) in early, middle, and late refed stages, respectively, for group IIA (Fig 3), and from

Table 2. Enrichments and Direct Pathway Contribution (mean ± SE) in Orally Refed Subjects (group I)

Subject No./ Time	Glucose Enrichment (MPE)	GlcUA Enrichment (MPE)	Direct Pathway Contribution (%)
1			
Fasted	0.88 ± .03	0.28 ± .02	31.8
Early	0.49 ± .02	0.24 ± .02	49.0
Middle	0.42 ± .02	0.23 ± .03	54.8
Late	0.46 ± .02	0.24 ± .02	52.2
2			
Fasted	2.52 ± .03	0.19 ± .02	7.5
Early	0.69 ± .03	0.26 ± .03	37.7
Middle	0.64 ± .02	0.30 ± .02	46.9
Late	0.52 ± .03	0.27 ± .02	51.9
3			
Fasted	1.86 ± .02	0.07 ± .02	3.8
Early	0.43 ± .01	0.17 ± .02	39.5
Middle	0.41 ± .02	0.19 ± .03	46.3
Late	0.35 ± .02	0.21 ± .01	60.2
4			
Fasted	1.22 ± .03	0.29 ± .03	23.8
Early	0.73 ± .04	0.33 ± .02	45.2
Middle	0.56 ± .02	0.40 ± .02	71.4
Late	0.53 ± .02	0.51 ± .03	96.2
5			
Fasted	1.77 ± .02	0.28 ± .03	15.8
Early	0.89 ± .03	0.23 ± .02	25.8
Middle	0.43 ± .01	0.22 ± .03	51.2
Late	0.31 ± .02	0.22 ± .02	71.0
6			
Fasted	2.38 ± .03	0.44 ± .02	18.5
Early	1.71 ± .03	0.47 ± .03	27.5
Middle	1.45 ± .03	0.57 ± .02	39.3
Late	0.93 ± .02	0.64 ± .03	68.8
7			
Fasted	1.58 ± .03	0.21 ± .03	13.3
Early	0.69 ± .03	0.23 ± .02	33.3
Middle	0.58 ± .02	0.27 ± .03	46.6
Late	0.57 ± .02	0.25 ± .02	42.1
8			
Fasted	2.03 ± .03	0.36 ± .03	17.7
Early	0.82 ± .02	0.37 ± .03	45.1
Middle	0.59 ± .02	0.28 ± .02	47.5
Late	0.62 ± .03	0.31 ± .02	50.0
Mean ± SE			
Fasted			16.5 ± 3.1
Early			37.9 ± 3.0*
Middle			50.5 ± 3.4*
Late			61.6 ± 6.0†

NOTE. Subjects were fasted overnight until 9 AM and then administered an hourly oral bolus of a liquid formula diet to provide carbohydrate at 7 mg/kg/min, as described in the text. Statistical symbols as in Table 1.

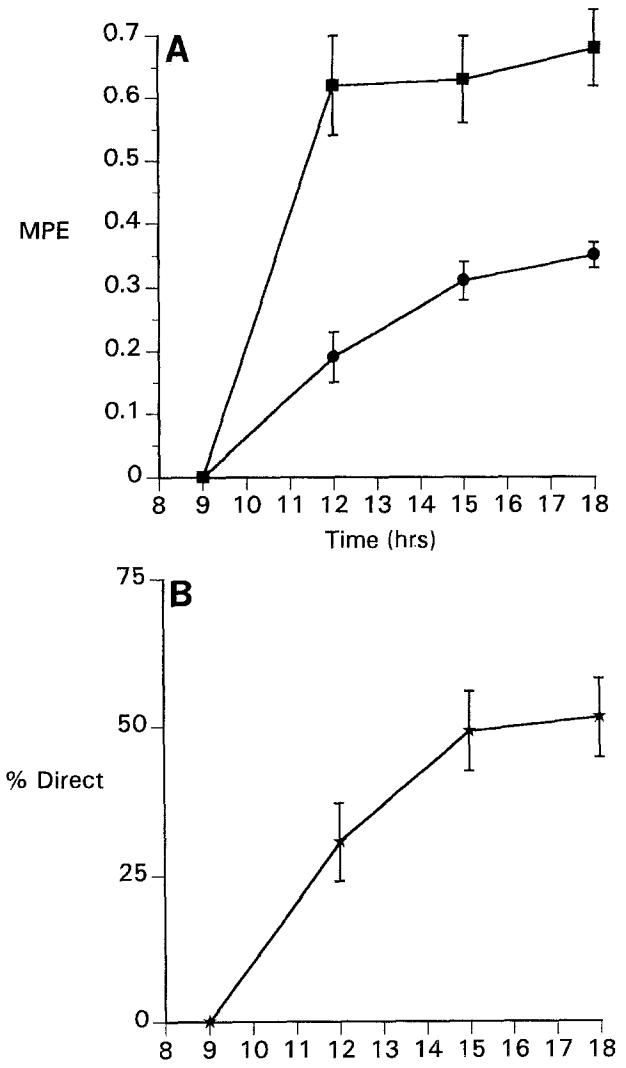

Fig 3. Time course of plasma glucose (■) and urinary acetaminophen GlcUA (●) enrichments (A) and direct pathway contribution (B) in subjects on protocol IIA (morning start of label IV, refed with glucose IV).

32.5% ± 7.0% to 43.5% ± 9.8% from middle to late stages for group IIC.

Effect of Route of Label Administration

We considered the possibility that IV administration of labeled glucose could artifactually influence the effect of oral carbohydrate refeeding, since tracer and tracee were entering the liver by different routes (systemic *v* portal circulation). As a test of the importance of the route of label administration or refeeding, we compared oral versus IV routes. These subjects did not receive labeled glucose until 8 AM, to simplify the kinetics and comparisons between IV and oral routes. When both labeled and unlabeled glucose routes were IV (group IIA), the plateau for the direct pathway was 51.5% ± 6.7% (mean ± SE, n = 6). When the labeled glucose was mixed with 10% glucose solution orally (group IIB), the plateau direct contribution was 57.7% ± 8.1% (n = 3). When label was administered IV but the refeeding route was the oral liquid formula (group IIC), the plateau direct contribution was 43.5% ± 9.8% (n = 3). When label was mixed with the oral formula (group IID), the plateau for the direct pathway was 44.5% ± 8.2% (n = 6). These values do not differ significantly, although the sample size is too small to exclude differences definitively.

Plasma Glucose R_a and Endogenous Glucose Production

HGP was suppressed by IV glucose administration. The R_a glucose (fasted) was 2.5 ± 0.2 mg/kg/min (mean ± SE). During IV glucose at 7 mg/kg/min, HGP was 0.2 mg/kg/min. During oral liquid-formula diet ingestion, the apparent R_a glucose was 7.6 ± 1.0 mg/kg/min. Since complete absorption was not documented and a true isotopic steady state is unlikely to have been present with this protocol, endogenous glucose production cannot be calculated accurately. It is of interest that the apparent plateau R_a glucose for group IID (oral label mixed with the liquid formula) was higher than for group IIC (IV label, oral formula) at 8.9 ± 0.7 and 7.7 ± 0.2 mg/kg/min ($P < .05$), respectively. The systemic appearance of enterally administered [1-d_1]-glucose was therefore incomplete, representing approximately 87% of the tracer glucose administered. This is consistent with previous non–steady-state studies of [1-^{14}C]glucose R_a systemically[4,33,34] and could in theory be due to either direct splanchnic uptake of tracer with storage as glycogen or de-deuteration (glycolysis or pentose phosphate pathway) in splanchnic tissues. The apparent R_a glucose for group IIB (oral label mixed with oral glucose) was 9.0 ± 0.1 mg/kg/min, as compared with 8.0 ± 0.5 ($P < .05$) for group IIA (IV label, IV glucose). This difference might be explained by loss of deuterium label during splanchnic glucose metabolism with recycling of carbon skeletons back to glucose (ie, dissociation between tracer and tracee), resulting in greater dilution of blood glucose.

Relationship Between Plasma Glucose Concentration and the Direct Pathway Contribution to UDP-glc

In intact rats,[11,14,15] perfused liver,[17] and isolated hepatocytes,[19] ambient glucose concentrations in the hyperglycemic range (30 to 50 mmol/L) have been reported to increase the direct pathway contribution. In our subjects, plasma glucose concentrations were not in the hyperglycemic range (Fig 4A), yet the direct pathway contribution increased fourfold to fivefold from postabsorptive to fed states. Moreover, there was only a relatively weak relationship between systemic glucose concentrations and the direct pathway contribution in IV glucose–refed subjects ($R^2 = .34$; Fig 4B) and almost no correlation in liquid formula–refed subjects ($R^2 = .05$; Fig 4C) or in both groups combined ($R^2 = .10$). The portal glucose level was not measured in the orally refed subjects, which makes the result difficult to interpret in that group, but portal glucose concentrations should not be different than systemic concentrations during IV glucose refeeding. Thus, hyperglycemia may stimulate the direct pathway, but under nonhyperglycemic refeeding conditions in normal humans, factors other

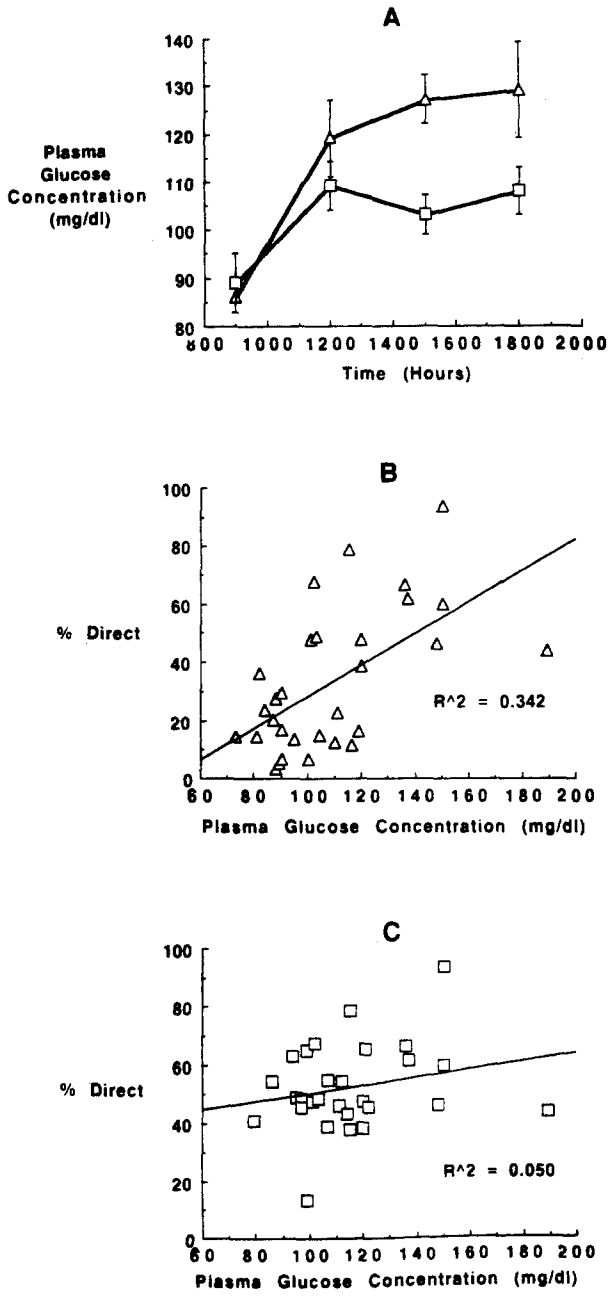

Fig 4. (A) Plasma glucose concentrations during fasting and refeeding with IV glucose (△) and oral Ensure (□). (B) Relationship between weighted interval glycemia and direct pathway in subjects fasted and then refed with glucose (IV or oral routes). (C) Relationship between weighted interval glycemia and direct pathway in subjects fasted and then refed with Ensure orally.

than glycemia need to be invoked to explain the marked change in hepatic UDP-glc synthetic pathways from the overnight-fasted condition.

Relationship Between Serum Insulin Concentrations and the Direct Pathway

Fasting serum insulin concentrations were 11.0 ± 1.3 μU/mL (n = 23). During the late refeeding phase with IV glucose and the liquid formula diet, insulin concentrations increased to 50.4 ± 12.7 (n = 13) and 57.5 ± 18 (n = 10) μU/mL, respectively. There were nonsignificant correlations between serum insulin and the percent direct pathway contribution (R^2 = .06, fasted/IV glucose–refed group; R^2 = .28, fasted/liquid formula–refed group).

R_a UDP-glc

The enrichment of urinary acetaminophen GlcUA reached stable values during constant IV infusions of [1-d_1]-galactose under fasting conditions and decreased to new plateau values within 4 to 5 hours after refeeding was begun (Fig 5). The time course of human GlcUA labeling in subjects refed with IV glucose or liquid formula diet after an overnight fast during a constant infusion of [1-d_1]-galactose is shown. Plateau enrichments were 2.71 ± 0.19 MPE during the overnight fast and decreased to 1.55 ± .05 MPE during IV glucose refeeding and to 1.15 ± 0.09 MPE during liquid formula refeeding. The R_a UDP-glc was 1.2 ± 0.1 mg/kg/min in fasted subjects, 2.0 ± 0.1 in IV glucose–refed subjects, and 2.6 ± 0.2 in oral formula–refed subjects (Table 3). Fed values were significantly higher ($P < .01$) as compared with fasted values by repeated-measures ANOVA. There were no significant differences between IV glucose and liquid formula diet values.

Estimation of Direct Glucose Entry Into UDP-glc

The R_a UDP-glc values were combined with values of the direct glucose contribution to UDP-glc, measured in the same subjects under identical conditions 1 week apart, to estimate net glucose entry into liver UDP-glc (Table 3). Direct entry of glucose into UDP-glc was 0.2 ± 0.03

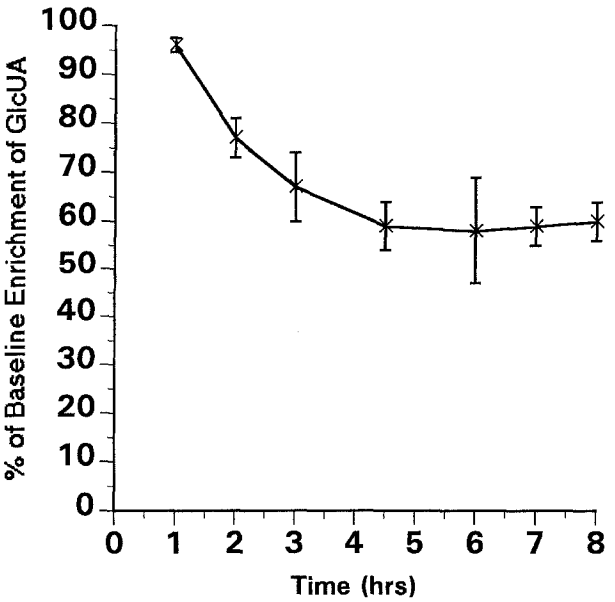

Fig 5. Time course of change in enrichments of urinary acetaminophen GlcUA during constant IV infusions of [1-d_1]-galactose in subjects refed at t = 0 after an overnight fast. Results from both IV glucose and liquid formula diets are shown, since the time course of change did not differ between the two feeding regimens.

Table 3. Direct Glucose Entry Rate Into Hepatic UDP-glc in Humans

Group (n)	R_a UDP-Glc (mg/kg/min)	Contribution From Direct Pathway (%)	Direct Glucose Entry Rate Into UDP-Glc (mg/kg/min)	Steady-State Glucose Disposal (mg/kg/min)	Glucose Disposal Into Direct Pathway (%)	Labeled Glucose Cycling (mg/kg/min)	Partitioning Ratio of Glucose-6-Phosphate
Fasted (7)	1.2 ± 0.1	18.0 ± 3	0.2 ± 0.3	2.5 ± 0.2	8 ± 1	0.55 ± 0.09	0.36
Refed IV glucose at 7 mg/kg/min × 9 h (4)	2.0 ± 0.1*	63 ± 7*	1.3 ± 0.2*	7.2 ± 0.2*	18 ± 4*	0.34 ± 0.08*	3.82
Refed oral liquid formula at 7 mg CHO/kg/min × 9 h (4)	2.6 ± 0.2*	62 ± 6*	1.6 ± 0.2*	7.6 ± 1.0*	21 ± 3*	—	—

NOTE. R_a UDP-glc values are calculated as described in the text. Direct pathway fractional contributions of glucose to hepatic UDP-glc are in the same subjects studied on a separate occasion (see text). Glucose disposal is taken to equal the steady-state R_a glucose. The direct glucose entry rate into hepatic UDP-glc (mg/kg/min) is calculated as the product of R_a UDP-glc times percent direct pathway contribution to UDP-glc. The percent glucose disposal into the direct pathway is the direct glucose entry rate divided by steady-state glucose disposal. Labeled glucose cycling and partitioning ratio of glucose-6-phosphate into UDP-glc to plasma glucose are described in the text.

Abbreviation: CHO, carbohydrate.

*$P < .01$ *v* fasted state by repeated-measures ANOVA.

mg/kg/min during a fast, representing less than 10% of fasting glucose disposal (2.5 ± 0.2 mg/kg/min). In subjects refed IV glucose at 7 mg/kg/min for 9 hours, direct glucose entry in hepatic UDP-glc was 1.3 ± 0.2 mg/kg/min, representing 18% ± 4% of glucose disposal or 19% ± 4% of exogenous glucose delivered. With the liquid formula diet, refeeding at 7 mg carbohydrate/kg/min (85% glucose, 15% fructose) increased direct glucose entry to 1.6 ± 0.2 mg/kg/min, which was 21% ± 3% of apparent glucose disposal or 23% ± 3% of exogenous carbohydrate administered. Fed values were significantly higher than fasted values ($P < .01$) for both refeeding routes, but there were no significant differences between refeeding routes. Thus, direct glucose entry into hepatic UDP-glc in humans was approximately 19% to 23% of an exogenous load and 18% to 21% of glucose disposal under these oral and IV refeeding regimens.

Hepatic Glucose Cycling and Partitioning of Hepatic Glucose-6-Phosphate

The cycling of labeled glucose across hepatic glucokinase/glucose-6-phosphatase was calculated from HGP, corrected for the percent direct pathway contribution (equation 5A above). Labeled glucose cycling was 0.55 ± 0.09 mg/kg/min in fasted and 0.34 ± 0.08 mg/kg/min in IV glucose–refed subjects (NS by ANOVA). The partitioning ratio of glucose-6-phosphate between blood glucose and hepatic UDP-glc was calculated on the assumption that the fate of glucose-6-phosphate derived from the direct pathway was not different from the fate of glucose-6-phosphate derived from any other source. Accordingly, the relative rates of labeled glucose cycling (labeled glucose-6-phosphate flux back to plasma glucose) and absolute entry of plasma glucose into UDP-glc (labeled glucose-6-phosphate flux to UDP-glc) represents the partitioning of glucose-6-phosphate flux. The ratio of direct glucose entry into UDP-glc to glucose cycling in the fasted state was 0.36, whereas in the fed state it was 3.82 (Table 3). Thus, 27% of glucose-6-phosphate flux entered UDP-glc in the fasted state and 78% entered UDP-glc in the fed state ($P < .01$ for effect of feeding state on partitioning fraction).

DISCUSSION

The intent of these studies was to measure the flux or turnover of an intracellular intermediary metabolite, hepatic UDP-glc, and then to use this to calculate the rate of direct glucose entry into UDP-glc during fasting and carbohydrate administration. The technique for measuring the R_a UDP-glc can be used during periods of either net glycogen breakdown or net deposition, and allows serial monitoring in an individual subject. We observed R_a UDP-glc to be stable and reproducible during a fast in humans. R_a UDP-glc increased markedly during IV glucose refeeding (Table 3). In rats, R_a UDP-glc is significantly correlated with the rate of net glycogen deposition during IV glucose refeeding (Hellerstein MK, Kaempfer S, Wu K, unpublished observations, 1993). Glycogen deposition was not directly measured here in humans.

Our results suggest that roughly 20% of an exogenous glucose load directly passes through the hepatic UDP-glc pool during refeeding after a fast. This is at odds with the recent nuclear magnetic resonance study noted above.[8] There are several possible explanations for the discrepancy. First, Shulman et al[8] estimated muscle mass to be 26% in their normal subjects, which is less than the consistent 36% to 45% observed in mammalian species ranging from rodent to elephant, including humans.[32,35] Using the standard 36% or 40% estimate would have resulted in too-high estimates of muscle glycogen accumulation—ie, more than 150% of the infused glucose load[8]—calling into question the validity of extrapolating values using spectroscopy of a single muscle to the whole body. Second, their experimental design involved 2 hours of constant hyperglycemia (180 mg/dL) and hyperinsulinemia (400 pmol/L) and glucose was administered IV, thus bypassing the usual absorptive route. Our findings are more consistent with previous estimates of liver glucose uptake,[1-7] and reaffirm the role of the liver in glucose disposal and in the suppression of glucose production in response to a glucose load.[3,4]

It should be noted that the GlcUA-probe technique allows the pathways of hepatic UDP-glc synthesis to be studied when there is net glycogen breakdown (fasting) or no net change in glycogen concentrations (previously fed

state). The reliance on hepatic glycogen as an analyte had previously restricted studies of hepatic hexose phosphate synthesis to states of glycogen accumulation, in addition to precluding human studies. Moreover, the transition from the postabsorptive state through early refeeding and into late refeeding was able to be characterized because each subject was his own control. Sequential studies were possible because of the lack of radiation exposure, the need for only microgram quantities of GlcUA for HPLC-MS analysis, and the high degree of sensitivity and accuracy of the MS analyses (SE ± <0.03 MPE), which permitted prolonged isotope infusions at low cost and no risk. The major assumptions of the R_a UDP-glc measurement are that all infused [1-d_1]-galactose enters hepatic UDP-glc pools and that the GlcUA probe samples a hepatic UDP-glc pool derived from the general hexose phosphate pool in liver. The first assumption is reasonable[31] but difficult to confirm experimentally, and R_a UDP-glc will be systematically overestimated to the extent that tracer galactose is not metabolized by the liver. With regard to the second assumption, we have recently observed identical labeling patterns in acetaminophen GlcUA and blood glucose in rats infused with [2-^{13}C]glycerol[36] under gluconeogenic conditions (fasting and glycerol or fructose infusions) both for their enrichments and the precursor pool from which they were synthesized, indicating that acetaminophen GlcUA is derived from the same hepatic hexose phosphate pool as glucose that is secreted into the blood. These results and reports from other laboratories[26] suggest that our initial observation[10] that GlcUA and hepatic glycogen labeling diverged in rats may have been due to non–steady-state kinetics of the two products under the conditions studied. In any event, most experimental evidence now supports the conclusion that GlcUA reflects UDP-glc derived from the general glucose-6-phosphate pool in liver.[15,26,36]

The results demonstrate (Tables 1 and 2) that the direct pathway makes only a minor contribution to UDP-glc in postabsorptive humans. Although not surprising, this need not have been the case and had not previously been documented in humans. If glucose derived from gluconeogenic precursors (amino acids, glycerol, lactate) or glycogen was secreted from one population of hepatocytes and mixed with circulating glucose before re-entering hepatic UDP-glc pools, as might have been predicted from the two-cell population model of liver metabolism,[33] the enrichment of UDP-glc relative to plasma glucose would remain high and the UDP-glc synthesis pathway would appear to be mainly direct even during times of active hepatic gluconeogenesis. Losses of [1-^{2}H] label by the pentose pathway are unlikely to alter the interpretation of our results, since this pathway is minor[34] and can be seen as representing flow through an "indirect" pathway in any case.

The direct pathway contribution remained low during the first few hours of refeeding in humans (Tables 1 and 2, Fig 3), but became increasingly important after prolonged glucose input, as in the rat.[10] In 1944, Boxer and Stetten wrote,[20] "... a greater proportion of the hydrogen atoms of glycogen had been derived from the body water in the previously fasted animal than in the well-nourished animal. The most plausible explanation for this finding is that the previous period of fasting results in a preference of glycogenesis from fragments smaller than hexose rather than from dietary glucose directly." Our results support this formulation, although the non–steady-state values, particularly zero to 3-hour values, in subjects on protocol I must be interpreted cautiously, since these may in part reflect different rates of change in glucose and GlcUA enrichments (eg, delayed acetaminophen GlcUA excretion). On the other hand, the early time points from protocol II subjects, in whom label was begun concurrently with acetaminophen and refeeding, are free of this potential problem of delayed GlcUA excretion and confirm the relatively small direct pathway contribution observed early in refeeding (Fig 3). Although the direct pathway contribution during the early period of transition from fasted to fed states remains uncertain, it is clear that the steady-state fed values are markedly different from fasted values for both fractional and absolute direct glucose contributions. A pharmacokinetic explanation for the increase in the direct pathway contributions from fasted to fed steady states is unlikely for several reasons. Urinary acetaminophen GlcUA enrichments showed large changes within 4 to 5 hours during [1-d_1]-galactose infusions (Fig 5) and decreased rapidly after tracer was discontinued (unpublished observations, 1993). Moreover, the circulating half-life of acetaminophen GlcUA is brief, with an excretion rate greater than the glomerular filtration rate and without known storage pools for this water-soluble conjugate or known delays in hepatic secretion after glucuronidation.[37] Finally, the group II experiments (no prelabeling) were performed to address this question, and increases in GlcUA enrichments were observed over the 8- to 9-hour period (Fig 3), as would be expected under conditions of stable or increasing glucose enrichments with increasing direct pathway contribution. Thus, delayed excretion of previously formed GlcUA is not significant enough to prevent appropriate changes in GlcUA enrichment from being observed. The relatively stable GlcUA enrichments in protocol I subjects must therefore reflect the experimental conditions (decreasing enrichment of the precursor glucose combined with increasing contribution from glucose). Recycling of labeled glucosyl units from glycogen into UDP-glc is another process that deserves consideration, but this also is unlikely to explain the increased direct pathway in going from fasted to fed states. Plasma glucose units during the overnight fast have the highest enrichment and glycogenolysis would also be expected to be maximal during a fast, so any effect of recycled labeled glucose to increase labeling of UDP-glc would be greatest during a fast and least after refeeding. Yet we observed the lowest direct pathway after a fast. Any error from this source would therefore work against our central conclusion. In addition, previous studies of fasted rats revealed very similar results,[10] yet these rats were nearly completely depleted of liver glycogen, precluding an effect of labeled glycogen recycling. Finally, group II (no prelabeling) showed essentially the same change in pathways, yet had no prelabeled glycogen to recycle. Thus, neither pharmacokinetic nor isotopic factors explain the depen-

dence of the direct pathway contribution on the feeding state. Our findings are consistent with those of Shulman et al,[21] who performed glucose clamps in fasted versus fed states in humans and reported a higher percent direct pathway contribution in the fed state.

Cycling of labeled glucosyl units from glycogen to glucose-1-phosphate and then UDP-glc will not be measured as UDP-glc flux. As with any isotope dilution method, recycling of labeled units into the pool will not be measured. However, it can be shown mathematically that cycling of labeled units in an end-product pool has no effect on the calculated incorporation rate from a labeled precursor. Decreases in the estimate of R_a UDP-glc are exactly balanced by increased estimates of the glucose fractional contribution, so the calculated rate of glucose entry is unchanged.* Thus, even if labeled glucosyl units are mobilized and cycled, there will be no effect on our estimates of the absolute rate of direct glucose entry into UDP-glc or the partitioning of glucose-6-phosphate.

The use of hepatic UDP-glc labeling to calculate labeled glucose cycling was recently proposed by Rossetti et al[31] in rats. We applied a modification of this approach in humans by using the GlcUA probe instead of isolating hepatic UDP-glc and by using [1-d_1]-glucose instead of [3-^{3}H]glucose. This approach is based on the opposite strategy to that used in the classic method for measuring hepatic glucose cycling. The classic method is to compare losses of labeled hydrogen from glucose on different positions. In the method used here, we correct for glucose-6-phosphate that has *not* lost label. Retention of label in the ostensibly diluting source (hepatic glucose-6-phosphate) is sought and corrected for. This measurement is essentially a correction factor for the cycling of "labeled" glucose-6-phosphate (glucose units with the same enrichment as the plasma glucose from which they came and to which they are returning). This approach also allowed calculation of a partitioning coefficient for hepatic glucose-6-phosphate, based on the assumption that labeled glucose-6-phosphate derived directly from plasma glucose has the same fate as glucose-6-phosphate from any other source. The marked shift in relative fluxes from glucose-6-phosphate into plasma glucose versus UDP-glc is consistent with studies in animals suggesting a regulation of glucose-6-phosphatase activity by nutritional state.[11,12,38] It should be noted that the partitioning calculation does not include glycolytic/lipogenic flux in the liver, but only compares two fates of labeled glucose-6-phosphate (UDP-glc *v* plasma glucose). Other fates are not measured and are not relevant here, since the labeled-galactose technique for measuring R_a UDP-glc does not measure the entry rate into hepatic glucose-6-phosphate, but instead the entry rate into hepatic UDP-glc. Further studies are necessary to characterize more fully the control over disposal of flux into the hepatic glucose-6-phosphate pool.

In conclusion, we have presented a method for measuring the R_a (flux, turnover) of an intracellular metabolite, hepatic UDP-glc, in normal humans. There appears to be turnover of UDP-glc in the fasted state and a marked increase with IV glucose or liquid formula diet refeeding. Feeding also increases the fractional and absolute contribution of glucose to hepatic UDP-glc and alters the partitioning of glucose-6-phosphate flux, markedly increasing the proportion entering UDP-glc. By using this method, approximately 20% of a glucose load was estimated to be taken up directly into the hepatic UDP-glc pool. The ability to estimate R_a UDP-glc may permit the calculation of rates of UDP-glc synthesis by various biosynthetic pathways where previously only fractional contributions could be calculated.

*A numerical example may make this clear: If direct entry of glucose into UDP-glc is 0.4 mg/kg/min and from other routes into UDP-glc is 0.6 mg/kg/min and there is no cycling, percent direct = 0.4/1.0 = 40% and R_a UDP-glc = 1.0 mg/kg/min. If the technique missed 20% contribution from labeled glycogen cycling, true percent direct = 0.4/1.2 = 33% and true R_a UDP-glc = 1.2 mg/kg/min, so the calculated direct entry rate remains 0.4 mg/kg/min.

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