Contributions by Kidney and Liver to Glucose Production in the Postabsorptive State and After 60 h of Fasting

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Contributions of renal glucose production to whole-body glucose turnover were determined in healthy individuals by using the arteriovenous balance technique across the kidneys and the splanchnic area combined with intravenous infusion of [U-$^{13}C_6$]glucose, [3-^{3}H]glucose, or [6-^{3}H]glucose. In the postabsorptive state, the rate of glucose appearance was 11.5 ± 0.6 µmol · kg^{-1} · min^{-1}. Hepatic glucose production, calculated as the sum of net glucose output (9.8 ± 0.8 µmol · kg^{-1} · min^{-1}) and splanchnic glucose uptake (2.2 ± 0.3 µmol · kg^{-1} · min^{-1}) accounted for the entire rate of glucose appearance. There was no net exchange of glucose across the kidney and no significant renal extraction of labeled glucose. The renal contribution to total glucose production calculated from the arterial, hepatic, and renal venous ^{13}C-enrichments (glucose M+6) was 5 ± 2%. In the 60-h fasted state, the rate of glucose appearance was 8.2 ± 0.3 µmol · kg^{-1} · min^{-1}. Hepatic glucose production, estimated as net splanchnic output (5.8 ± 0.7 µmol · kg^{-1} · min^{-1}) plus splanchnic uptake (0.6 ± 0.3 µmol · kg^{-1} · min^{-1}) accounted for 79% of the rate of glucose appearance. There was a significant net renal output of glucose (0.9 ± 0.3 µmol · kg^{-1} · min^{-1}), but no significant extraction of labeled glucose across the kidney. The renal contribution to whole-body glucose turnover calculated from the ^{13}C-enrichments was 24 ± 3%. We concluded that *1*) glucose production by the human kidney in the postabsorptive state, in contrast to recent reports, makes at most only a minor contribution (~5%) to blood glucose homeostasis, but that *2*) after 60-h of fasting, renal glucose production may account for 20–25% of whole-body glucose turnover. ***Diabetes*** **48:292–298, 1999**

In the postabsorptive state, glucose production by the kidney has generally been considered of minimal importance for the maintenance of glucose homeostasis. Thus, no significant net output of glucose from the kidney was found using the arteriovenous balance technique in overnight fasted dogs (1) nor in overnight fasted healthy humans (2). In contrast, in healthy subjects fasted for 60 h, net renal glucose output was reported to be 22% of total glucose production (3), and in individuals fasted for 4–6 weeks, it accounted for ~45% (4). Thus, during more prolonged fasting, renal glucose production becomes increasingly important. However, recent reports have suggested that the kidney contributes substantially to whole-body glucose turnover already in the postabsorptive state (5–11). In these studies, the arteriovenous balance technique was combined with measurements of renal uptake of glucose using ^{3}H-glucose. It is well recognized that the arteriovenous balance technique measures net glucose exchange. In the event of a simultaneous uptake and output of glucose, the technique will measure the sum of the two processes. In dogs fasted overnight, no net output of glucose across the kidney was observed, but there was a significant renal uptake of ^{3}H-glucose (5). The combined measurements indicated renal production of glucose corresponding to ~30% of the rate of glucose appearance in this initial report (5), but in more recent reports, that amount was 15–20% (6–8). The human kidney was initially reported to account for ~28% of glucose turnover (9), but more recently ~15% (10,11).

Applying the combined arteriovenous balance and labeled-glucose techniques across the kidney, estimations depend on small differences in arterial and renal venous glucose concentrations and ^{3}H-glucose–specific activities. Relatively high renal blood flow contributes to the difficulties in determining renal glucose exchange and renal uptake of glucose with precision. Simultaneous measurements across the renal and splanchnic vascular beds could therefore be of value to clarify the site(s) of glucose production in the overnight-fasted state in healthy subjects. In the present study, whole-body glucose production was measured simultaneously with renal and splanchnic glucose net exchange and measurements of splanchnic and renal glucose uptake. Specifically, the arteriovenous balance technique was used together with intravenous infusion of [U-$^{13}C_6$]glucose. Considering the methodological problems involved in quantitating the kidney's contribution to glucose homeostasis as the sum of net glucose output and uptake of radioactive-labeled glucose, the use of [U-$^{13}C_6$]glucose could offer a more reliable approach. Determination of the renal contribution can then be based solely on the difference in glucose M+6, i.e., the percentage of glucose molecules having ^{13}C in all of the six carbons, determined in arterial, hepatic, and renal venous blood. For comparison with previous studies, [3-^{3}H]glucose and [6-^{3}H]glucose were also used for measurements across both the splanchnic and the renal tis-

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FE, fractional extraction; HPLC, high-performance liquid chromatography; ICG, indocyanine green dye; PAH, *p*-aminohippuric acid.

sues. Measurements were performed in healthy subjects both in the postabsorptive state and after 60 h of fasting.

RESEARCH DESIGN AND METHODS

Subjects. We studied 29 healthy men aged 27 ± 1 years with no family history of diabetes and with body weights of 77 ± 1 kg, and BMI of 22.9 ± 0.3 kg/m^2; seven of the subjects were studied twice. The subjects were informed of the nature, purpose, and possible risks of the study before consenting to participate. The experimental protocol was approved by the Institutional Human Ethics Committee and the Isotope Radiation Committee.

Protocol. In seven subjects fasted overnight, an antecubital vein was catheterized and a primed continuous infusion of [U-$^{13}C_6$]glucose was given at a rate of 0.4–0.5 µmol · kg^{-1} · min^{-1} for 5 h (prime dosage 25–30 µmol/kg). In 16 additional subjects also fasted overnight, a primed continuous infusion of [6-^{3}H]glucose (n = 10) or [3-^{3}H]glucose (n = 6) was given for 3 h (20 µCi prime and 10 µCi/h). In 13 subjects fasted for 60 h, [U-$^{13}C_6$]glucose (n = 3), [3-^{3}H]glucose (n = 3), or [6-^{3}H]glucose (n = 7) was infused, as described above. Glucose production in the subjects fasted 60 h and given [U-$^{13}C_6$]glucose has been reported previously (12). In all subjects, catheters were inserted into a brachial artery and a left-sided renal vein. In addition, a right-sided hepatic vein catheter was introduced in all subjects given [U-$^{13}C_6$]glucose and in the subjects given [6-^{3}H]glucose (four fasted overnight and six fasted for 60 h). The renal and hepatic catheters were positioned under fluoroscopic control, and the position of the renal catheter was also confirmed by demonstrating a blood oxygen saturation level >88%. The tip of the renal vein catheter was positioned as close to the organ as possible to avoid sampling of blood from the left spermatic vein. Hepatic blood flow was determined by the constant infusion technique, using indocyanine green dye (ICG) (13). Renal blood flow was estimated using p-aminohippuric acid (PAH) (14). In the subjects given [U-$^{13}C_6$]glucose, blood samples were drawn simultaneously from the arterial, hepatic vein, and renal vein catheters at five time points during the last hour of infusion for determination of glucose, ICG, and PAH concentrations and glucose M+6. In a similar manner, in the subjects given ^{3}H-glucose, samples were drawn for determination of glucose, ICG, PAH, and ^{3}H-labeled glucose at nine time points between 115 and 185 min of infusion. In these subjects, samples were also drawn for determination of lactate, glycerol, β-hydroxybutyrate, alanine, insulin, and glucagon. [U-$^{13}C_6$]Glucose was purchased from Isotec (Miamisburg, OH) and determined by gas chromatography/mass spectrometry (GC/MS) to be 94% of the mass M+6. [3-^{3}H]Glucose and [6-^{3}H]glucose were purchased from Du Pont-NEN (Du Medical Scandinavia AB, Sollentuna, Sweden); ICG, from ICG Pulsion (Pulsion Medical System, Munich, Germany); and PAH from Merck (West Point, PA). The labeled glucoses were dissolved in saline and the solutions shown to be sterile and pyrogen-free.

Analyses. Glucose concentrations in whole blood were determined enzymatically using glucose dehydrogenase (15) and in plasma using glucose oxidase (Beckman Glucose Analyzer; Beckman, Fullerton, CA). Glucose M+6 in plasma was determined using GC/MS under ammonia chemical ionization conditions, as previously described (12). The whole ion from the aldonitrile penta-acetate derivative of glucose was monitored at m/z 411. ^{3}H activity in plasma was determined after deproteinization of 1 ml of plasma diluted with 5 ml of water by the addition of 2 ml of 0.3 N $ZnSO_4$ and 2 ml of 0.3 N $Ba(OH)_2$, as previously described (16). After centrifugation, the supernatant was deionized by passage through anion and cation exchange resins (AG2-X8 and AG50-X8) (Bio-Rad, Hercules, CA). An evaporated aliquot of the eluate was counted in a β-scintillation counter. Deproteinized and deionized plasma samples from nine of the subjects fasted overnight were also subjected to high-performance liquid chromatography (HPLC), and ^{3}H-specific activity in glucose was determined. Analyses of glucose concentration and isotopic content in samples from each subject were carried out in one run and in the same sequence as the sequence of sampling. Concentrations of lactate, glycerol, and β-hydroxybutyrate were analyzed enzymatically (17); insulin and glucagon by radioimmunoassay (18,19); and alanine by HPLC (20).

Calculations of net glucose balance. Net splanchnic glucose exchange was calculated as the product of the arterial-hepatic venous blood glucose concentration difference and hepatic blood flow. Net renal glucose exchange was calculated as the product of the arterial-renal venous blood glucose concentration difference and renal blood flow.

Calculations, ^{13}C-labeled glucose. The fractional extraction (FE) of glucose across the splanchnic area and the kidneys was calculated as $[(IE_a \times glu_a) - (IE_v \times glu_v)] / (IE_a \times glu_a)$, where IE_a is the arterial glucose M+6, glu_a is the arterial concentration of glucose, and IE_v and glu_v are the corresponding parameters for hepatic or renal vein samples.

The uptake of glucose by the splanchnic bed and kidneys was calculated as regional FE $\times$ $glu_a \times (1 - IE_a) \times$ regional blood flow. The rate of glucose appearance was calculated as $[(IE_{infusate} / IE_a) - 1] \times R$, where $E_{infusate}$ is the glucose M+6 in the infusate and R is the rate of infused ^{13}C-labeled glucose in µmol · kg^{-1} · min^{-1}.

The renal contribution to total glucose production was calculated by dividing kidney production of glucose by the sum of kidney and splanchnic production of glucose. The production of glucose from the splanchnic bed and kidney were determined as $([IE_a (1 - FE) / IE_v] - 1 + FE)\ glu_a \times$ regional blood flow. Even if fractional extractions across the splanchnic and renal tissues had reached 3%, as has previously been reported (10,21), their contribution to the calculation would still be negligible. Consequently, FE can be disregarded and the above expression for calculation of glucose molecules produced within the regions can be simplified to $[(IE_a / IE_v) - 1]\ glu_a \times$ regional blood flow.

Calculations: ^{3}H-labeled glucose. The fractional extraction of glucose across the splanchnic area and the kidneys was calculated as (^{3}H activity$_a$ – ^{3}H activity$_v$) / ^{3}H activity$_a$, where ^{3}H activity$_a$ is the ^{3}H activity (DPM/ml plasma) in arterial plasma and ^{3}H activity$_v$ is the ^{3}H activity in hepatic or renal venous plasma. In the samples from deproteinized, deionized plasma subjected to HPLC, the fractional extraction of glucose was calculated as $[(SA_a \times glu_a) - (SA_{rv} \times glu_{rv})] / (SA_a \times glu_a)$, where SA_a is the arterial specific activity and glu_a is the arterial plasma concentration of glucose, and SA_{rv} and glu_{rv} are the corresponding parameters for the renal venous plasma.

The uptake of glucose by the splanchnic bed and kidneys was then calculated by multiplying the regional FE by the arterial concentration and the regional flow.

The rate of glucose appearance was calculated by dividing the rate of infusion of ^{3}H-labeled glucose by the specific activity of glucose in arterial plasma.

Statistical analyses. For all results, the mean of the five measurements for each subject during the 5th hour of infusion of ^{13}C-glucose was calculated. Similarly, during the ^{3}H-glucose infusions, the mean of nine determinations between 115 and 185 min was calculated for each subject. The mean ± SE for the individual means is presented for each study group. Differences between groups were assessed using Student's t test.

RESULTS

Glucose M+6 in arterial, hepatic, and renal venous plasma reached a relative steady state during the 5th h of [U-$^{13}C_6$]glucose infusion, with a coefficient of variation between 1 and 4% (Table 1). Likewise, the activities of ^{3}H-glucose in arterial plasma and in hepatic and renal venous plasma were stable during the latter part of the 3-h infusion period. The coefficient of variation for glucose ^{3}H activity was ~2%, calculated for the nine values obtained during the sampling period for each subject individually and for the three sampling sites (Table 1). The means of the individual arterial-renal venous differences of ^{3}H activities and glucose M+6 are presented in Fig. 1.

Postabsorptive state. Results obtained for rate of glucose appearance, FE of labeled glucose, and uptake of glucose across the splanchnic bed and the kidney did not differ significantly among the groups given [U-$^{13}C_6$]glucose, [6-^{3}H]glucose, or [3-^{3}H]glucose (Table 2). Likewise, the splanchnic and renal net exchanges of glucose were similar in these three groups (Table 2). Therefore, the results for the three groups have been combined. The arterial concentration of glucose was 4.81 ± 0.07 mmol/l, the arterial-hepatic venous concentration difference was –0.58 ± 0.04 mmol/l, and the net splanchnic glucose output amounted to 9.8 ± 0.8 µmol · kg^{-1} · min^{-1}. The arterial-renal venous concentration difference was 0.01 ± 0.02 mmol/l; individual data are presented in Fig. 2. Thus there was no significant net output of glucose across the kidney. The whole-body rate of appearance of glucose was 11.5 ± 0.6 µmol · kg^{-1} · min^{-1}. There was significant fractional extraction of labeled glucose across the splanchnic area (2.7 ± 0.4%; P < 0.001), corresponding to an estimated glucose uptake of 2.2 ± 0.3 µmol · kg^{-1} · min^{-1} (P < 0.001). In contrast to the splanchnic fractional extraction, there was no statistically significant renal fractional extraction (0.4 ± 0.3%). In the samples from deproteinized, deionized plasma subjected to HPLC, all the ^{3}H activity was found in glucose. Also for this group of measurements, no statistically significant fractional extraction across the kidney was detected (–0.1 ± 0.6%). Using the glucose M+6 in arterial, hepatic, and renal venous

TABLE 1
^{3}H activity and glucose M+6 in arterial, hepatic, and renal venous plasma

	Fast	Arterial plasma		Hepatic venous plasma		Renal venous plasma	
		Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
[6-^{3}H]	12-h	1,749	2.3 ± 0.5	1,688	1.8 ± 0.4	1,759	1.8 ± 0.4
	60-h	2,555	1.9 ± 0.3	2,625	2.0 ± 0.4	2,562	2.4 ± 0.3
[3-^{3}H]	12-h	2,242	1.4 ± 0.7	—	—	2,220	1.8 ± 0.2
	60-h	2,267	1.5 ± 0.4	—	—	2,239	1.8 ± 0.3
[U-^{13}C]	12-h	3.018	2.3 ± 0.3	2.599	3.6 ± 1.0	2.995	2.0 ± 0.3
	60-h	3.894	1.1 ± 0.2	3.567	1.6 ± 0.4	3.781	1.0 ± 0.0

Data for means are expressed as DPM/ml for [6-^{3}H] and [3-^{3}H], and as % for [U-^{3}C]; data are means ± SE for coefficient of variation (CV).

plasma and allowing for blood flow, the renal contribution to total glucose production was calculated to be 5 ± 2% (P < 0.05).

60-h fasted state. In 60-h fasted subjects, no statistically significant differences in regional net glucose exchange, rate of glucose appearance, FE of glucose, and glucose uptake were found among the subjects given [U-$^{13}C_6$]glucose, [6-^{3}H]glucose, or [3-^{3}H]glucose, respectively (Table 3). These data for the three groups have been combined. The average arterial glucose concentration was 3.48 ± 0.12 mmol/l. The arterial-hepatic venous concentration difference was –0.38 ± 0.07 mmol/l, and the net splanchnic glucose output averaged 5.8 ± 0.7 μmol · kg^{-1} · min^{-1}. The arterial-renal venous concentration difference was –0.05 ± 0.02 mmol/l (P < 0.05); individual data are given in Fig. 2. There was a small but significant net output of glucose from the kidney (–0.9 ± 0.3 μmol · kg^{-1} · min^{-1}; P < 0.025). The whole-body rate of glucose appearance was 8.2 ± 0.3 μmol · kg^{-1} · min^{-1}. Fractional extraction of labeled glucose across the splanchnic area was 1.3 ± 0.3% (P < 0.01), indicating a glucose uptake by the splanchnic bed of 0.6 ± 0.3 μmol · kg^{-1} · min^{-1}. No significant fractional extraction of glucose across the kidney was found (0.4 ± 0.3%). Using glucose M+6 in arterial, hepatic, and renal venous plasma and allowing for blood flow, the renal contribution to total glucose production was estimated at 24 ± 3% (P < 0.05).

Regional blood flows and blood glucose concentrations were stable during the sampling periods (Tables 2 and 3). Blood and plasma concentrations of lactate, glycerol, β-hydroxybutyrate, alanine, insulin, and glucagon, measured after 12 and 60 h, respectively, are presented in Table 4. As expected, the insulin concentration was lower and the glucagon and β-hydroxybutyrate concentrations were higher in the 60-h fasted subjects than in those fasted overnight. In addition, an increased uptake of lactate and glycerol by the splanchnic bed and the kidney was observed in the 60-h fasted individuals, as expected.

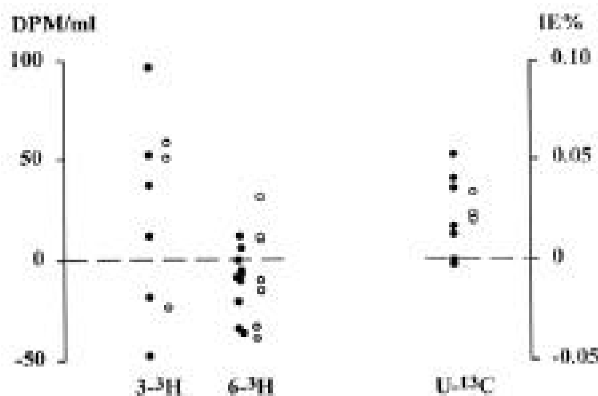

FIG. 1. Individual mean values for arterial-renal venous differences in ^{3}H-activity (DPM/ml) and isotopic enrichment (IE%) of glucose M+6 in the postabsorptive (●) and 60-h (○) fasted state.

DISCUSSION

In the postabsorptive state, the average rate of glucose appearance for all individuals (n = 20) was 11.5 μmol · kg^{-1} · min^{-1} (Table 2). Net glucose output from the splanchnic area (9.8 μmol · kg^{-1} · min^{-1}) accounted for 85% of the total rate of glucose appearance. The fractional extraction of glucose across the splanchnic bed indicated a simultaneous uptake of glucose. Consequently, the hepatic glucose production, calculated as the sum of the net output and the splanchnic uptake, as measured by the isotope technique, accounted for the entire rate of glucose appearance. When the average value of hepatic glucose production was calculated for each of the individuals in whom both splanchnic and whole-body measurements were obtained (n = 11), the hepatic glucose production covered 96 ± 8% of the rate of glucose appearance (Fig. 3). In agreement with these data, neither net output of glucose nor extraction of isotopically labeled glucose across the kidney were found in the postabsorptive state. Based on glucose M+6 in arterial, renal, and hepatic venous plasma, the renal contribution to total glucose production amounted to 5 ± 2%. Thus we concluded that the liver was the predominant site of glucose production in the overnight fasted state and that the renal contribution at most must be quite small.

The present results for the postabsorptive state were in accordance with previous measurements of net glucose exchange and uptake of ^{3}H from [3-^{3}H]glucose across the splanchnic bed (21,22), in which hepatic glucose production after 10–12 h of fasting accounted for 94 and 99% of glucose turnover, respectively. In contrast, using the same techniques across the kidney, but infusing [6-^{3}H]glucose, Meyer and colleagues (10,11) recently reported that net renal glucose output and renal glucose production accounted for 15% of whole-body glucose production in overnight-fasted subjects. In a previous study involving healthy subjects, Stumvoll et al. (9) reported renal production of glucose accounting for ~28% of the rate of glucose appearance in the postabsorptive state. In their study, the fractional extraction of ^{3}H-glucose across the kidney was 2.9 ± 0.3%, measured from the product of the specific activity and the concentrations of glucose entering

TABLE 2
Blood flow, glucose concentrations, net glucose balance, fractional extraction of glucose and uptake of glucose across the splanchnic and renal tissues in the 12-h fasted state

	[6-^{3}H]	[3-^{3}H]	[U-^{13}C]	Combined groups
n	10 (4 splanchnic)	6	7	20 (11 splanchnic)
Hepatic blood flow (l/min)	1.37 ± 0.05	—	1.26 ± 0.05	1.30 ± 0.04
Renal blood flow (l/min)	1.50 ± 0.06	1.59 ± 0.05	1.46 ± 0.08	1.51 ± 0.04
Arterial glucose concentration (mmol/l)	4.90 ± 0.13	4.69 ± 0.11	4.77 ± 0.13	4.81 ± 0.07
Hepatic vein glucose concentration (mmol/l)	5.32 ± 0.22	—	5.37 ± 0.14	5.35 ± 0.11
Renal vein glucose concentration (mmol/l)	4.91 ± 0.13	4.65 ± 0.04	4.76 ± 0.12	4.80 ± 0.07
Splanchnic net glucose exchange (μmol · kg^{-1} · min^{-1})	−9.60 ± 1.40	—	−9.92 ± 0.98	−9.80 ± 0.76
Splanchnic fractional extraction (%)	2.32 ± 0.31	—	2.96 ± 0.60	2.73 ± 0.40
Splanchnic glucose uptake (μmol · kg^{-1} · min^{-1})	1.90 ± 0.20	—	2.31 ± 0.50	2.16 ± 0.32
Renal net glucose exchange (μmol · kg^{-1} · min^{-1})	−0.29 ± 0.39	0.99 ± 1.77	0.16 ± 0.18	0.18 ± 0.48
Renal fractional extraction (%)	−0.59 ± 0.30	0.87 ± 0.84	0.99 ± 0.46	0.40 ± 0.34
Renal glucose uptake (μmol · kg^{-1} · min^{-1})	−0.54 ± 0.31	I .00 ± 0.90	0.83 ± 0.36	0.40 ± 0.33
Rate of glucose appearance (μmol · kg^{-1} · min^{-1})	11.40 ± 0.74	9.27 ± 0.90	13.60 ± 0.65	11.54 ± 0.58

Data are means ± SE.

and leaving the kidney. In the present study, we measured renal fractional extraction by exactly the same procedure as Stumvoll et al. (9) and found it to be −0.1 ± 0.6%.

No immediate explanation for the different results is available. The subjects were similarly prepared for the study, and it is unlikely that dietary or ethnic differences contributed to the variations. Results similar to those of Stumvoll et al. (9) and Meyer and colleagues (10,11) were obtained in dogs using the same technique, but with [3-^{3}H]glucose as the tracer (5,6,8). The differences in the reported renal contributions to glucose production may reflect compounded experimental errors from relying on such small concentration differences, as is the case when measurements are performed across the kidney, with its relatively high blood flow. In Fig. 1, where the individual mean values of nine determinations of arterial-renal venous differences in ^{3}H activities are presented, the variation between subjects and the occurrence of negative numbers is apparent. Obviously, the negative values cannot reflect production of labeled glucose by the kidney, but rather are the consequence of experimental errors. Therefore, in the present study, the negative arteriovenous differences of radioactivity that were encountered were all included in the calculations to avoid introducing a bias. Differences in the treatment of negative uptakes of radioactivity across the kidney could contribute to the differences between our estimates and those of Cersosimo and associates (5,6,8), Stumvoll et al. (9), and Meyer and associates (10,11). Mistakenly setting negative values to zero or repeating the assays giving negative values until zero or positive values are obtained has two consequences: actual glucose production will be overestimated and the variation in the measurements (e.g., expressed as SE) will be erroneously decreased. The larger the experimental error in the measurements, the more such treatment of data will overestimate production by the kidney and its statistical significance.

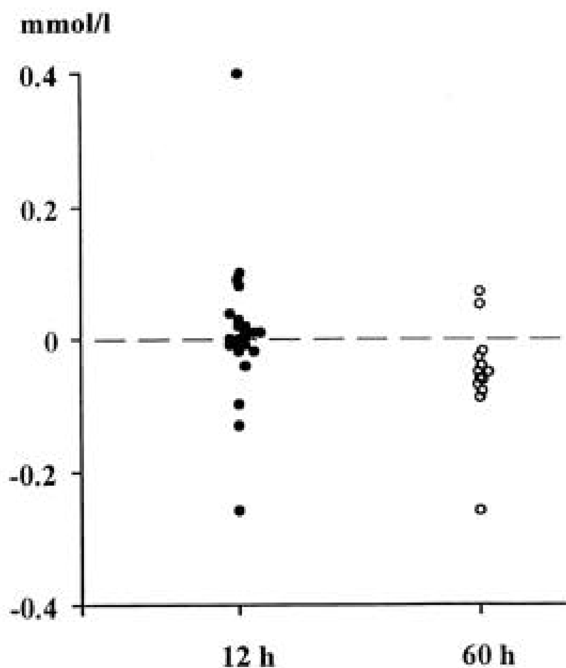

FIG. 2. Individual mean values for arterial-renal venous concentration differences for glucose in the postabsorptive (●) and 60-h (○) fasted state.

By using [U-$^{13}C_6$]glucose, we determined the contribution of renal glucose production in a new way. Measuring glucose M+6 across the kidney and the splanchnic bed offered a more direct approach; the extent of dilution of glucose M+6 in the renal vein was the measure of renal glucose production. Estimation was based solely on glucose M+6 in arterial, hepatic, and renal venous plasma. Consequently, the number of analyses required for estimation was reduced to a minimum. Applying this technique in the 12-h fasted state, the kidney was found to account for 5 ± 2% of the total glucose production.

In the present study, estimates of glucose uptake across the splanchnic bed and the kidneys were obtained using three different tracers. The tritiated glucose studies were performed for comparison with previous studies. Metabolism of the tracers could differ between splanchnic tissue and kidney. Estimation of glucose production using ^{3}H- or ^{13}C-labeled glucose is based on the dilution of the label by endogenously produced glucose. To the extent that the ^{3}H or ^{13}C is recycled

TABLE 3
Blood flow, glucose concentrations, net glucose balance, fractional extraction of glucose, and uptake of glucose across the splanchnic and renal tissues in the 60-h fasted state

	[6-^{3}H]	[3-^{3}H]	[U-^{13}C]	Combined groups
n	7 (6 splanchnic)	3	3	13 (9 splanchnic)
Hepatic blood flow (l/min)	1.25 ± 0.16	—	1.54 ± 0.10	1.35 ± 0.12
Renal blood flow (l/min)	1.38 ± 0.08	1.49 ± 0.12	1.48 ± 0.10	1.43 ± 0.06
Arterial glucose concentration (mmol/l)	3.59 ± 0.15	3.55 ± 0.25	3.14 ± 0.30	3.48 ± 0.12
Hepatic vein glucose concentration (mmol/l)	4.00 ± 0.19	—	3.36 ± 0.31	3.79 ± 0.18
Renal vein glucose concentration (mmol/l)	3.64 ± 0.13	3.61 ± 0.25	3.19 ± 0.32	3.53 ± 0.12
Splanchnic net glucose exchange (μmol · kg^{-1} · min^{-1})	–6.68 ± 0.83	—	–4.17 ± 0.12	–5.84 ± 0.68
Splanchnic fractional extraction (%)	1.16 ± 0.38	—	1.71 ± 0.68	1.35 ± 0.33
Splanchnic glucose uptake (μmol · kg^{-1} · min^{-1})	0.34 ± 0.41	—	1.12 ± 0.48	0.60 ± 0.33
Renal net glucose exchange (μmol · kg^{-1} · min^{-1})	–0.79 ± 0.63	–1.01 ± 0.25	–0.95 ± 0.33	–0.88 ± 0.34
Renal fractional extraction (%)	–0.27 ± 0.18	1.07 ± 1.35	1.14 ± 0.22	0.36 ± 0.35
Renal glucose uptake (μmol · kg^{-1} · min^{-1})	–0.07 ± 0.16	0.60 ± 0.90	0.67 ± 0.08	0.26 ± 0.22
Rate of glucose appearance (μmol · kg^{-1} · min^{-1})	8.05 ± 0.24	7.15 ± 0.39	9.42 ± 0.38	8.16 ± 0.28

Data are means ± SE.

via Cori cycling, endogenous glucose production will be underestimated. Alternatively, if ^{3}H is detritiated in triose-P cycling (i.e., glucose triose-P glucose), it will be overestimated. The latter occurs to a greater extent using [3-^{3}H]glucose than [6-^{3}H]glucose. Recycling of [3-^{3}H]glucose cannot occur, since lactate formed from [3-^{3}H]glucose will not contain ^{3}H. Recycling of [6-^{3}H]glucose via the formation of [3-^{3}H]lactate has been estimated to be at most 8% in overnight-fasted humans (23). Detritiation of the [3-^{3}H]lactate occurs in the transamination of pyruvate with alanine (24). However, no net renal uptake of alanine was observed in the present study, in agreement with previous findings (3). Detritiation also occurs in oxaloacetate's equilibration in the dicarboxylic acid shuttle before its conversion to glucose. There is evidence for an active dicarboxylic acid shuttle during the renal conversion of pyruvate to glucose (25). If the loss of ^{3}H from [6-^{3}H]glucose is incomplete, renal glucose uptake is underestimated. Although the number of observations in each group was small, there was no significant difference in the results obtained with [U-$^{13}C_6$]glucose, [3-^{3}H]glucose, or [6-^{3}H]glucose. Thus the differences between [6-^{3}H]glucose and [3-^{3}H]glucose metabolism appears to have no effect on our conclusions. Using [U-$^{13}C_6$]glucose, the amount of glucose M+6 formed by kidney and liver due to recycling of lactate of mass m+3 (i.e., with three ^{13}C) should be negligible. In another study (12), when [U-$^{13}C_6$]glucose was administered in the same amount as in the present study, lactate m+3

TABLE 4
Arterial concentrations and splanchnic and renal exchanges of lactate, glycerol, β-hydroxybutyrate, alanine, insulin, and glucagon after a 12- and 60-h fast

	12-h fast	60-h fast
Lactate		
Arterial concentration (μmol/l)	503 ± 40	528 ± 35
Splanchnic net exchange (μmol/min)	130 ± 50	220 ± 40
Renal net exchange (μmol/min)	24 ± 20	89 ± 14
Glycerol		
Arterial concentration (μmol/l)	37 ± 4	89 ± 9
Splanchnic net exchange (μmol/min)	44 ± 7	99 ± 12
Renal net exchange (μmol/min)	22 ± 4	62 ± 6
β-Hydroxybutyrate		
Arterial concentration (μmol/l)	99 ± 25	1,730 ± 306
Splanchnic net exchange (μmol/min)	–51 ± 44	–340 ± 108
Renal net exchange (μmol/min)	20 ± 6	–25 ± 90
Alanine		
Arterial concentration (μmol/l)	246 ± 20	157 ± 10
Splanchnic net exchange (μmol/min)	102 ± 7	177 ± 42
Renal net exchange (μmol/min)	–11 ± 4	–3 ± 3
Insulin		
Arterial concentration (mU/l)	6.2 ± 0.4	3.3 ± 0.5
Glucagon		
Arterial concentration (ng/l)	86 ± 4	131 ± 23

Data are means ± SE.

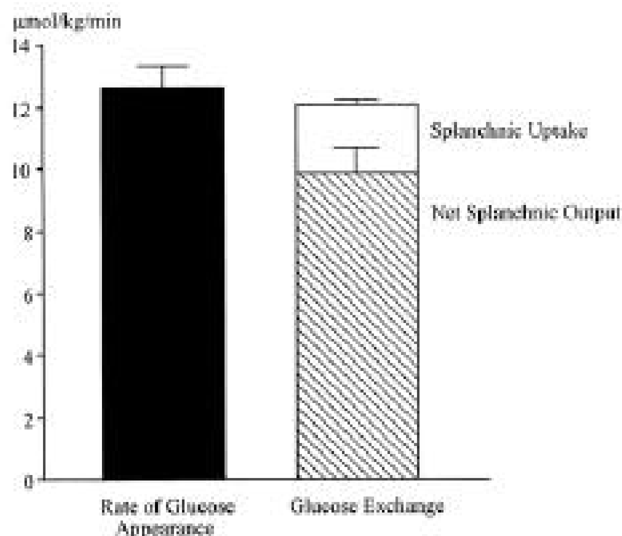

FIG. 3. Rate of glucose appearance, glucose exchange measured as net splanchnic output, and splanchnic uptake of glucose after 12 h of fasting in 11 subjects in whom all measurements were obtained simultaneously. No net renal exchange and no renal uptake of labeled glucose were detected.

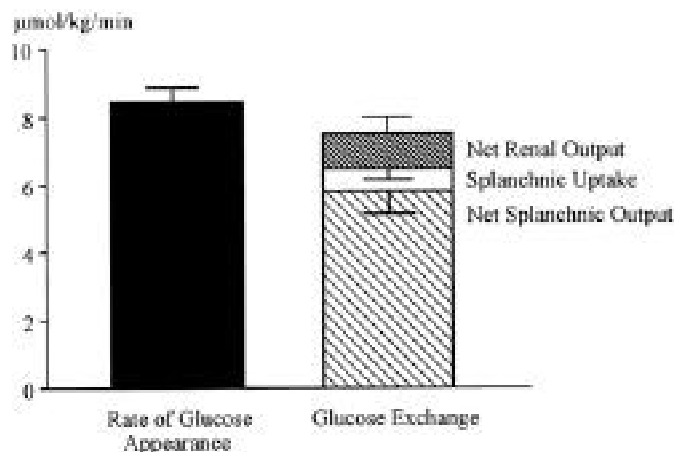

FIG. 4. Rate of glucose appearance, glucose exchange measured as net splanchnic output, splanchnic uptake, and net renal output of glucose after 60 h of fasting in nine subjects in whom all measurements were obtained simultaneously. No renal uptake of labeled glucose was detected.

reached about 2% in the blood. Theoretically, that would result in the formation of only 1 in every 2,500 molecules of glucose with mass M+6.

The situation after 60 h of fasting was different from that after an overnight fast. The arterial glucose concentration was lower and whole-body glucose turnover was reduced by ~30% (Tables 2 and 3). In agreement with previous observations (3), a small net arteriovenous difference for glucose across the kidney was detectable (–0.05 ± 0.02 mmol/l; $P < 0.05$), corresponding to a renal glucose output of 0.9 ± 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ or 11% of the whole-body glucose turnover. As in the overnight-fasted state, no significant extraction of labeled glucose was found across the kidney for any of the tracers or the combined data (Table 3). Net splanchnic glucose output was ~40% lower than in the overnight fasted state (Tables 2 and 3), in keeping with previous observations (3). In addition, splanchnic fractional extraction of glucose had fallen significantly, to 1.4 ± 0.3%, indicating a markedly diminished splanchnic glucose uptake in the 60-h fasted state (–70%) (Tables 2 and 3). Thus splanchnic glucose uptake after 60 h of fasting was reduced both in absolute terms and in proportion to the whole-body glucose turnover (17 [overnight fasted] vs. 10% [60-h fasted]; $P < 0.01$).

The combined data indicate that after 60 h of fasting, hepatic glucose production (net splanchnic output plus splanchnic uptake) may have accounted for 79% of whole-body glucose turnover (Table 2). The corresponding calculation made for the individuals in whom simultaneous splanchnic and renal measurements were performed ($n = 9$) indicated that hepatic glucose production may have accounted for 76% of glucose turnover (Fig. 4). In these subjects, the renal component may have contributed an additional 15%. The failure to account for ~10% of glucose appearance may relate to problems in precisely determining arteriovenous concentration differences that are no more than 1–2% of the arterial concentration and to the small number of subjects studied. The data of glucose M+6 in the three subjects given [U-$^{13}C_6$]glucose indicated that the renal contribution to total glucose production was 24 ± 3%, suggesting that the proportion of total glucose production not accounted for in the above calculations may represent renal glucose production.

In conclusion, the findings demonstrated that the liver is the predominant site of glucose production in both the postabsorptive and the 60-h fasted state. The kidney makes at most only a minor contribution in the overnight-fasted state. After 60 h of fasting, the renal contribution may represent 20–25% of whole-body glucose turnover.

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