

# Gluconeogenesis and the Cori cycle in 12-, 20-, and 40-h-fasted humans

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**Katz, Joseph, and John A. Tayek.** Gluconeogenesis and the Cori cycle in 12-, 20-, and 40-h-fasted humans. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E537–E542, 1998.—Six subjects were infused with [U-<sup>13</sup>C]glucose (0.03–0.05 mg·kg<sup>-1</sup>·min<sup>-1</sup>) starting 8–9 h after a meal, and the production of glucose, the recycling of glucose (the Cori cycle), the dilution of glucose by unlabeled carbon into the hepatic lactate-pyruvate pool, and gluconeogenesis were determined in these fasted volunteers by use of mass isotopomer analysis and equations previously described [J. A. Tayek and J. Katz. *Am. J. Physiol.* 272 (*Endocrinol. Metab.* 35): E476–E484, 1997]. A primed continuous 11-h infusion was started at 6:00 AM, and the above parameters were calculated after 3 h (for the 12-h fast) and at the end of the infusion (for the 20-h fast). Another group of five subjects was fasted for 40 h, and the above parameters were calculated as before. At 12, 20, and 40 h of fasting, respectively, blood glucose was 93 ± 2, 83 ± 2, and 71 ± 2 (SE) mg/dl; glucose production was 2.3, 1.8, and 1.77 mg·kg<sup>-1</sup>·min<sup>-1</sup>; the recycling of labeled carbon was 8, 15, and 15%, and that of glucose molecules (Cori cycle) was 18, 35, and 36%; the contribution of gluconeogenesis to glucose production was 41, 71, and 92% or 0.96, 1.29, and 1.64 mg·kg<sup>-1</sup>·min<sup>-1</sup>; and the contribution of other sources to glucose production was 1.37, 0.53, and 0.15 mg·kg<sup>-1</sup>·min<sup>-1</sup>. The recycling of glucose is important in prolonged fasting for the maintenance of plasma glucose concentration. We demonstrate here that gluconeogenesis can be easily measured and that it accounts for ~90% of glucose production after a 40-h fast.

glucose production; glucose recycling; free fatty acids; insulin; glycogenolysis; glycogen

THE AIM of the present study is to examine the effects of prolonged fast on gluconeogenesis (GNG) and the recycling of glucose in humans. We use [U-<sup>13</sup>C]glucose, mass isotopomer analysis, and equations derived by us previously (24, 25) to determine a number of parameters of glucose metabolism such as glucose production, GNG, the recycling of labeled carbon, and the recycling of glucose molecules. We determine the total recycling from all molecules entering in pyruvate. We designate this recycling, as discussed here, as the Cori cycle. We show that, in prolonged fasting, this recycling has an important role in the maintenance of blood glucose concentrations. Our methods are not yet in general use, and thus comparison of our assumptions and results with those obtained by other investigators with stable isotopes and nontracer methods is of interest. We show

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here that the determination of GNG by our method is very similar to that found by other studies.

A major assumption of all methods is that, in the fasted state, there is no flux from glucose to glycogen. This is presently controversial. We discuss the implications of the assumption of the calculations of GNG.

## PROCEDURES AND METHODS

**Clinical characteristics.** Eleven normal volunteers were studied under an approved Institutional Review Board. All volunteers were male and had an average age of 41 ± 2 (SE) yr and weight of 71 ± 2 kg (Table 1). Baseline glucose and hormonal concentrations were normal in all volunteers. None of the volunteers had a family history of diabetes. No one was on medications other than acetaminophen.

**Infusion protocol.** All patients were admitted for 3 days to the Clinical Research Center, where they ingested 2,500 kcal and a minimum of 200 g of carbohydrate per day. On the day before study, the patients were fed until 9:00 PM and were fasted overnight. Six patients were fasted overnight and at 6:00 AM received a primed continuous infusion of [U-<sup>13</sup>C]glucose for 11 h. Blood was taken after 12 and 20 h of fasting. An additional five volunteers fasted for 37 h and then were given a 3-h primed continuous infusion of [U-<sup>13</sup>C]glucose. Fasting volunteers were given water and diet soda ad libitum. Results from the 40-h fast were obtained in this group. The [U-<sup>13</sup>C]glucose was 99% *M*<sub>6</sub> (isotopomer fraction of glucose C-6) and was purchased from Isotec (Miamisburg, OH). The priming dose was 0.05 mg/kg, and the constant infusion varied from 0.031 to 0.050 mg·kg<sup>-1</sup>·min<sup>-1</sup>. Bloods were sampled every 20 min between hours 11 and 12, 19 and 20, and 39 and 40 of the fast to obtain values of [<sup>13</sup>C]glucose and [<sup>13</sup>C]lactate enrichments.

**Substrate analysis.** Insulin, cortisol, and growth hormone (GH) were assayed by RIA, as previously described (23). Glucagon was assayed by RIA kit (Linco, St. Charles, MO). Free fatty acid (FFA) concentration was measured by a calorimetric method (12).

**Gas chromatography-mass spectroscopy analysis.** The isolation of glucose and lactate and the measurement of molar enrichments and mass isotopomers have been previously described (24). The molar enrichments are the weighted sums of the isotopomer fractions designated as  $\Sigma_1^6 M_n$  for glucose and  $\Sigma_1^3 m_n$  for lactate (24).

**Calculations.** The terminology and equations for the parameters measured in the present study are shown below. We list here the equations for glucose production (GP), the recycling of carbon, the recycling of glucose molecules (Cori cycle), and the fraction of GNG (25). *M* refers to the isotopomer fractions of glucose and *m* to those of lactate. GP is determined by the infused dose of glucose divided by the enrichment of uniformly labeled glucose in plasma (Eq. 1). Recycling of carbon is the enrichment  $\Sigma_1^3 M_n$  in the three carbons of glucose (*M*<sub>1</sub> to *M*<sub>3</sub>) divided by the enrichment  $\Sigma_1^6 M_n$  of glucose (*M*<sub>1</sub> to *M*<sub>6</sub>; Eq. 2). *M*<sub>4</sub> and *M*<sub>5</sub> are negligible. The recycling of glucose molecules is the number of recycled molecules (*M*<sub>1</sub> to *M*<sub>3</sub>) divided by the total number of molecules of glucose (*M*<sub>1</sub> to *M*<sub>6</sub>; Eq. 3). The dilution of hepatic lactate is the enrichment of plasma

Table 1. Patient characteristics and fasting blood parameters

Subject No.	<i>n</i>	Age, yr	Wt, kg	Fast Duration, h	Glucose, mg/dl	Insulin, $\mu\text{U/ml}$	Cortisol, $\mu\text{g/dl}$	Glucagon, ng/ml	GH, ng/ml	FFA, meq/ml
1-6	6	41 $\pm$ 2	70 $\pm$ 2	12	93 $\pm$ 3	9.4 $\pm$ 3.3	8.5 $\pm$ 1.0	55 $\pm$ 11	1.0 $\pm$ 0.3	580 $\pm$ 20
1-6	6	41 $\pm$ 2	70 $\pm$ 2	20	83 $\pm$ 2*	3.9 $\pm$ 0.7	6.6 $\pm$ 0.8	54 $\pm$ 10	2.0 $\pm$ 0.9	910 $\pm$ 30*
7-11	5	42 $\pm$ 4	71 $\pm$ 2	40	71 $\pm$ 2*	5.1 $\pm$ 0.5	8.1 $\pm$ 1.8	64 $\pm$ 4	4.6 $\pm$ 1.4*	890 $\pm$ 68*

Values are means  $\pm$  SE; *n*, no. of subjects. GH, growth hormone; FFA, free fatty acid concn. \*  $P < 0.05$  vs. 12-h fast.

glucose divided by two times the enrichment in plasma lactate (Eq. 4). The fraction of GNG is the product of the Cori cycle and the dilution of hepatic lactate (Eq. 5).

$$\text{GP (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \text{infused dose}/M_6 \quad (1)$$

$$\text{Recycling of carbon (\%)} = \frac{\sum_1^3 M_n}{\sum_1^6 M_n} \quad (2)$$

$$\text{Cori cycle (\%)} = \frac{\sum_1^3 M}{\sum_1^6 M} \quad (3)$$

$$\text{Dilution of hepatic lactate (in multiples of increase)} = \frac{\sum_1^6 M_n/2}{\sum_1^3 m_n} \quad (4)$$

Fraction of GNG (%)

$$= \text{Eq. 3} \times \text{Eq. 4} = \frac{\sum_1^3 M \times \sum_1^6 M_n}{\sum_1^6 M \times 2 \times \sum_1^3 m_n} \quad (5)$$

**Data analysis.** All data are represented as means  $\pm$  SE. Data were compared by paired *t*-test between 12 and 20 h of fasting and also by ANOVA between groups for hour 20 and hour 40 of fasting. Confidence intervals were determined for the glucose and lactate enrichments in the volunteers after a 12-, 20-, and 40-h fast (as shown in Table 2). Simple linear regression analysis was by least squares approach. Significance was defined as  $P < 0.05$ .

## RESULTS

Table 1 describes the population and their fasting hormonal profile. As expected, both insulin and glucose concentrations were reduced with fasting. The concentration of plasma glucose decreased from 93 mg/dl after an overnight fast to 83 mg/dl 20 h after a meal and decreased to 71 mg/dl at 40 h. Mean glucagon concentrations for 12-, 20-, and 40-h-fasted volunteers were

similar. GH increased at 40 h, and FFA concentration increased after 20- and 40-h fasting ( $P < 0.05$ ).

Table 2 illustrates typical isotopomer patterns obtained with 12-, 20-, and 40-h-fasted subjects. There was an increase with time in the fraction of labeled glucose, indicating the increase in the contribution of GNG to GP. Uniformly labeled glucose ( $M_6$ ) constituted 80% of the total labeled species at 12 h and 93% of the total  $^{13}\text{C}$  content. The value of  $M_1$ , 0.05  $\pm$  0.016% after a 12-h fast in volunteer 6 (Table 2), is at the limit of resolution of the isotopomer assay, and the value of  $M_2$ , 0.12  $\pm$  0.03%, was of low precision. This does not affect greatly the total  $^{13}\text{C}$  fraction ( $M_1 + M_2 + M_3 + M_6 = \sum_1^6 M$ ) or the enrichment ( $1 \times M_1 + 2 \times M_2 + 3 \times M_3 + 6 \times M_6 = \sum_1^6 M_n$ ), but the sum of the  $M_1 + M_2 + M_3$  is a critical parameter in the calculation of the glucose recycling, the Cori cycle, and GNG. However, in summation there is partial cancellation of random errors in the individual values, and, as can be shown by numerical examples, the mean error of the sum is of the order of  $\pm 10\%$ . As shown below, the results at the 12-h fast are highly reproducible even at an infusion as low as 0.03  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . The yield and the randomization of  $^{13}\text{C}$  increase markedly with prolonged fasting. At 20 and 40 h,  $M_6$  constitutes 65% of the total  $^{13}\text{C}$  fraction. There was a large increase in the  $M_1$  fraction. These changes indicate the increased contribution of GNG to GP and an increase in the flux in the tricarboxylic acid cycle in prolonged fasting, as will be presented elsewhere.

Table 3 presents the mass isotopomer values for all of our subjects. One group was infused for 11 h, beginning 8-9 h after a meal, and blood was sampled after 4 h and at the termination of the infusion at 20 h after a meal. For a second group, infusion was started 36 h after a meal, and blood was sampled at the end of infusions or at 40 h. This infused dose ranged from 0.03 to 0.05  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Table 4 presents the calculated values for the recycling of labeled carbon, the Cori cycle, the

Table 2. Typical isotopomer yields

Subject No.	Fast Duration, h	Infusion Duration, h	$M_1$ , %	$M_2$ , %	$M_3$ , %	$\sum_1^3 M$ , %	$M_6$ , %	$\sum_1^6 M$ , %
6	12	4	0.05 $\pm$ 0.016	0.12 $\pm$ 0.03	0.19 $\pm$ 0.05	0.36 $\pm$ 0.04	1.80 $\pm$ 0.03	2.16 $\pm$ 0.04
6	20	11	0.50 $\pm$ 0.001	0.38 $\pm$ 0.008	0.51 $\pm$ 0.02	1.39 $\pm$ 0.005	2.24 $\pm$ 0.001	3.62 $\pm$ 0.03
10	40	4	0.44 $\pm$ 0.03	0.71 $\pm$ 0.06	0.33 $\pm$ 0.10	1.48 $\pm$ 0.05	2.92 $\pm$ 0.017	4.40 $\pm$ 0.02
95% Confidence interval								
	Hour 12		(0.02-0.08)	(0.06-0.18)	(0.10-0.29)	(0.28-0.44)	(1.74-1.86)	(2.08-2.24)
	Hour 20		(0.49-0.51)	(0.36-0.40)	(0.37-0.55)	(1.38-1.40)	(2.23-2.25)	(3.56-3.68)
	Hour 40		(0.38-0.50)	(0.59-0.83)	(0.13-0.53)	(1.38-1.58)	(2.89-2.95)	(4.36-4.44)

Values are means  $\pm$  SE. See data for all subjects in Table 3. For subjects 6 and 10, infused dose was 0.45  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . Confidence intervals are shown within parentheses for each isotopomer fraction of glucose ( $M_1 \dots$ ).

Table 3. Mass isotopomer distribution in subjects fasted for 12, 20, and 40 h

Subject No.	Dose, mg·kg <sup>-1</sup> ·min <sup>-1</sup>	Hour of Fast	M <sub>0</sub>	M <sub>6</sub>	Σ <sub>3</sub> <sup>1</sup> M	Σ <sub>6</sub> <sup>1</sup> M	Σ <sub>3</sub> <sup>1</sup> M <sub>n</sub>	Σ <sub>6</sub> <sup>1</sup> M <sub>n</sub>	m <sub>0</sub>	Σ <sub>3</sub> <sup>1</sup> m	Σ <sub>3</sub> <sup>1</sup> m <sub>n</sub>
1	0.031	12	98.2	1.41	0.33	1.74	0.67	9.13	99.2	0.79	1.98
		20	96.6	1.72	1.51	3.23	2.78	13.2	98.4	1.69	3.90
2	0.036	12	98.2	1.35	0.37	1.72	0.86	8.96	98.9	1.06	2.37
		20	96.4	2.44	1.10	3.54	3.48	16.9	98.5	1.45	3.40
3	0.047	12	97.6	2.05	0.43	2.53	1.05	13.4	98.9	1.03	2.54
		20	98.2	2.20	0.60	2.80	1.42	14.6	99.1	1.00	2.42
4	0.044	12	97.6	1.80	0.39	2.19	1.02	11.8	98.9	1.16	2.75
		20	96.0	2.19	1.65	3.84	3.27	16.4	98.1	1.82	4.25
5	0.043	12	97.1	2.23	0.43	2.66	0.99	14.4	98.6	1.35	3.4
		20	95.6	2.97	1.27	4.24	2.35	20.2	97.7	2.32	5.6
6	0.045	12	97.4	1.80	0.36	2.16	0.86	11.7	98.9	0.93	2.34
		20	96.3	2.24	1.39	3.62	2.78	16.2	98.3	1.74	4.53
7	0.050	40	96.2	2.64	1.14	3.74	2.6	18.4	97.6	1.59	3.47
8	0.047	40	95.5	2.68	1.69	4.37	3.3	19.4	98.3	1.68	3.58
9	0.034	40	97.3	1.72	1.13	2.85	2.2	12.6	98.8	1.13	2.44
10	0.045	40	95.4	2.92	1.48	4.40	2.85	20.4	97.9	1.86	4.12
11	0.041	40	96.5	1.97	1.12	3.10	1.9	13.8	98.9	1.04	2.61
Mean ± SE		12	97.7 ± 0.2	1.77 ± 0.14	0.39 ± 0.02	2.14 ± 0.16	0.91 ± 0.06	11.6 ± 0.90	98.9 ± 0.1	1.05 ± 0.08	2.56 ± 0.19
Mean ± SE		20	96.2 ± 0.2	2.29 ± 0.17	1.36 ± 0.09	3.68 ± 0.17	2.88 ± 0.21	16.5 ± 1.11	98.3 ± 0.2	1.67 ± 0.18	4.02 ± 0.44
Mean ± SE		40	96.2 ± 0.35	2.38 ± 0.23	1.31 ± 0.12	3.69 ± 0.31	2.57 ± 0.24	16.9 ± 1.56	98.3 ± 0.26	1.46 ± 0.16	3.24 ± 0.31

Values are individual or mean ± SE isotopomer distribution values for molar fractions of glucose (M<sub>n</sub>) and lactate (m<sub>n</sub>).

dilution of pyruvate by unlabeled carbon, and the contribution of GNG to GP. We present also the results with overnight-fasted volunteers in two previous experiments. The means for the three studies, with a total of 21 subjects and performed over a 4-yr period, are highly reproducible. They are 2.3, 2.3, and 2.0 mg·kg<sup>-1</sup>·min<sup>-1</sup>

for GP; 8, 8, and 8% for the recycling of labeled carbon; 19, 19, and 20% for the Cori cycle; 41, 35, and 40% for the contribution of GNG; and 2.3-, 1.9-, and 2.0-fold for the dilution of pyruvate by unlabeled carbon. The confidence interval has been determined for the isotopomer enrichments (See Table 2). Thus, despite the low

Table 4. Gluconeogenesis and other parameters in 12-, 20-, and 40-h-fasted humans

Subject No.	Hour of Fast	Glucose Production, mg·kg <sup>-1</sup> ·min <sup>-1</sup>	Recycling Carbon, %	Cori Cycle, %	Dilution of Pyruvate, multiples of increase	Gluconeogenesis		Other Sources, mg·kg <sup>-1</sup> ·min <sup>-1</sup>
						%	mg·kg <sup>-1</sup> ·min <sup>-1</sup>	
1	12	2.20	7	19	2.3	44	0.97	1.23
	20	1.80	19	47	1.7	78	1.41	0.39
2	12	2.67	9	21	1.9	40	1.07	1.60
	20	1.48	13	31	2.5	77	1.14	0.34
3	12	2.29	8	17	2.6	44	1.01	1.28
	20	2.14	10	21	3.0	64	1.38	0.76
4	12	2.44	9	18	2.2	40	0.98	1.46
	20	2.01	20	43	1.9	81	1.63	0.38
5	12	1.93	7	16	2.1	37	0.71	1.22
	20	1.45	12	31	1.8	56	0.81	0.64
6	12	2.48	7	17	2.5	42	1.03	1.45
	20	2.01	17	38	1.8	68	1.38	0.64
7	40	1.76	14	29	2.65	77	1.36	0.40
8	40	1.77	17	39	2.70	105	1.86	0
9	40	1.93	19	42	2.58	101	1.96	0
10	40	1.53	14	34	2.47	83	1.27	0.26
11	40	1.85	14	36	2.83	95	1.76	0.09
Mean ± SE	12	2.33 ± 0.11	8 ± 0.6 (6.8–9.2)	18 ± 0.8 (16–20)	2.3 ± 0.10 (2.1–2.5)	41 ± 1 (39–43)	0.96 ± 0.05 (0.86–1.06)	1.37 ± 0.07 (1.23–1.51)
Mean ± SE	20	1.81 ± 0.12*	15 ± 2* (11–19)	35 ± 4* (27–43)	2.1 ± 0.22 (1.7–2.5)	71 ± 4* (63–78)	1.29 ± 0.12* (1.05–1.53)	0.53 ± 0.07* (0.39–0.67)
Mean ± SE	40	1.77 ± 0.07*	15 ± 1* (13–17)	36 ± 2* (32–40)	2.6 ± 0.07 (2.4–2.8)	92 ± 5† (82–102)	1.64 ± 0.13† (1.38–1.90)	0.15 ± 0.08† (–0.01–0.31)
Ref. 24	12	1.99	8	20	2.0	40	0.80	1.19
Ref. 25	12	2.35	8	19	1.9	35	0.83	1.52

Values are individual data, means ± SE, 95% confidence intervals (in parentheses), or comparative data from Refs. 24 and 25. \*P < 0.01 vs. 12-h fasted; †P < 0.01 vs. 20-h fasted.

isotopomer yields, results are reproducible, and the overall error is of the order of 10%.

After a 20-h fast, GP decreased from 2.3 to 1.8  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and it remained unchanged at 40 h. The recycling of labeled carbon doubled, and glucose recycling increased to 35% at 20 h and 36% at 40 h. However, the contribution of the unlabeled carbon to GNG was greater, with 2.6/3.6 or 72% of the pyruvate derived from unlabeled carbon stores. This compares with ~50% contribution at the overnight fast.

At 20 h GNG contributed 71% and at 40 h over 90% of GP. The contribution of recycling to GP was 18% at the end of the overnight fast and 36% at 40 h. Our results demonstrate the importance of recycling in maintaining blood glucose concentration in the fasted human. The role of glycogen declines markedly in fasting. It contributed 1.4  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  after an overnight fast, 0.5  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at 20 h, and 0.2  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  at 40 h. However, this includes any contribution from substrates entering at the triose phosphate stage, such as glycerol and serine. The contribution of these substrates is probably very low (see DISCUSSION).

## DISCUSSION

*Comparison with other methods.* Earlier studies of GNG employed  $^{14}\text{C}$ -labeled precursors. The methods are cumbersome, and the interpretation is subject to question. Recently more direct methods using stable isotopes have been developed and used in humans. Our method has not yet been widely used, and comparison with other studies is in order. We examine here the assumptions and results obtained by us and others, and we compare the estimates of GNG with those by nontracer methods.

*Mass isotopomer distribution analysis.* This elegant and simple method was developed and named by Hellerstein (Neese et al., Ref. 13). After the administration of a precursor singly labeled with  $^{13}\text{C}$ , the enrichment of the precursor in blood and the mass isotopomer pattern of blood glucose are determined and the contribution of GNG is calculated. Hellerstein et al. (6) used [ $^{13}\text{C}$ ]glycerol, but in principle other  $^{13}\text{C}$ -labeled precursors may be used. They found the contribution of GNG after an overnight fast to average 38% (range 27–54%), nearly the same as our overall value of 40%. Lee et al. (10) used [ $1\text{-}^{13}\text{C}$ ]lactate and reported %GNG to range from 39 to 50% in three subjects fasted overnight and 70% for one subject fasted for 24 h. Sunehag et al. (22) studied premature infants receiving parenteral nutrition. These investigators determined GNG by mass isotopomer distribution analysis (MIDA) with [ $2\text{-}^{13}\text{C}$ ]glycerol and by our method. GNG by MIDA was at 4, 6, 8, and 10 h  $25 \pm 5$ ,  $25 \pm 4$ ,  $30 \pm 3$ , and  $32 \pm 6\%$ , respectively. Values were similar to those obtained by our method, which were  $24 \pm 7$ ,  $27 \pm 6$ ,  $27 \pm 7$ , and  $26 \pm 8\%$ , respectively. It is of interest that the infants received a large infusion of glucose ( $3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). It appears that our method developed for endogenous metabolism is also valid in the presence of large glucose loads.

*Deuterium.* Landau et al. (9) determined the contribution of GNG from the ratio of enrichment of the deuterium on C-5 and C-2 of glucose (or in plasma water) after the administration of  $^3\text{H}_2\text{O}$  (9). GNG averaged 48% (33–65%) after a 14-h fast. In a recent study these investigators (Chandramouli et al., Ref. 2) reported a GNG of 54% for 18 h and of 93% after 42 h. It appears that the contribution of GNG increases by 2–3% per hour in the interval from 12 to 20 h after a meal. Our results in the present study at 12, 20, and 40 h of 41, 71, and 92% are very similar to those obtained by the  $^3\text{H}_2\text{O}$  method.

Our method measures the contribution of GNG of compounds entering at the pyruvate stage, whereas MIDA and the deuterium methods include any direct input occurring at the triose phosphate stage, such as glycerol and serine. However, it is likely that these compounds formed in the periphery are to a large extent oxidized to pyruvate. The contribution of glycerol to GP is very low. Thus there is no significant difference among the three methods. It should be noted that MIDA and the deuterium method measure only the fractional contribution of GNG, and another tracer is required to measure GP. Isotopomer analysis provides with a single tracer an estimate of numerous parameters.

All three methods assume no incorporation of glucose into hepatic glycogen in the fasted state. This would cause a cycling of glucose via UDP glucose and glycogen and an underestimate of GNG by the three methods. The operation of such a cycle is controversial, as discussed subsequently.

An alternative approach to study the contribution of GNG is to study glycogenolysis. The most extensive examination of liver glycogen in humans is a biopsy study with 58 subjects by Nilsson et al. (16). After an overnight fast, liver glycogen content ranged for the great majority of subjects from 3 to 6% of liver weight, with a mean of 4.3%. Liver accounts for ~2% of body weight in humans (14), and thus the overnight mean glycogen content is ~850 mg/kg body weight. Nilsson et al. (16) found the decrease in glycogen content in the next 4 h to be ~50  $\text{mg} \cdot \text{kg} \text{ body weight}^{-1} \cdot \text{h}^{-1}$ . Thus, at 20 h after a meal, glycogen content would be ~400 mg/kg body weight. The rate of nongluconeogenic glucose release in our study was ~80  $\text{mg} \cdot \text{kg} \text{ body weight}^{-1} \cdot \text{h}^{-1}$  at 12 h, 30  $\text{mg} \cdot \text{kg} \text{ body weight}^{-1} \cdot \text{h}^{-1}$  at 20 h, and 10  $\text{mg} \cdot \text{kg} \text{ body weight}^{-1} \cdot \text{h}^{-1}$  at 40 h of fasting. The glycogen content after a 2-day fast was reported (16) to be ~80 mg/kg body weight and showed little change up to 10 days of fasting.

Nilsson and Hultman (15) measured GNG and GP by sampling portal arterial and hepatic venous blood and the change in liver glycogen by biopsy. They estimated GNG in overnight-fasted subjects (by splanchnic balance) to be 35% and hepatic glycogen (by biopsy) to be 67%, in close agreement with values obtained by the three tracer methods.

An indirect estimate of GNG from a noninvasive determination of the content of hepatic glycogen with NMR was developed by Cline et al. (3). They estimated

liver volume by imaging NMR and assayed hepatic glycogen by NMR from its natural  $^{13}\text{C}$  abundance (11). Petersen et al. (19) estimated from the decline in hepatic glycogen content between 6 and 12 h after a meal for GNG to account for an average of 55% of GP. The decrease in liver glycogen ranged from 1 to 10  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and GNG (by difference) ranged from 24 to 80%. In metabolic studies with humans, as in clinical practice, the range is the primary physiological criterion rather than the mean. Averaging of such widely differing data should be taken with caution.

*Recycling of glucose: the Cori cycle.* The concept of the reincorporation and resynthesis of glucose from lactate, "the glucose-lactate cycle," was formulated by Cori before the advent of tracers. Tracer studies have established that pyruvate, lactate, and alanine are extensively equilibrated. Thus, in a review in 1981 (4), Cori extended the concept of the glucose-lactate cycle as a "pyruvate cycle." Thus we retain the term Cori cycle for the recycling via pyruvate. We actually sample blood lactate, but this reflects the specific activity, the enrichment, and the isotopomer pattern in liver pyruvate. The pyruvate cycle encompasses also the glucose-alanine cycle, the glucose-glutamine cycle, or any other possible input in the dicarboxylic or tricarboxylic acid cycle, because all pathways lead to pyruvate and phosphoenolpyruvate. The determination of the Cori cycle by isotopomer analysis with  $[\text{U-}^{13}\text{C}]\text{glucose}$  was first derived by Kalderon et al. (8). Their equation, with a different notation, is identical to ours.

The recycling of glucose does not, of course, serve as a net source of glucose. All body carbohydrates are derived from dietary sources, and in fasting they are derived from tissue proteins. There have been numerous studies to determine what fraction of lactate, alanine, or glutamine is due to recycling and what fraction is derived from tissue protein breakdown, providing synthesis *de novo* (see citations discussed by Periello et al. in Ref. 17). The Cori cycle as defined here is the sum total of all recycling via pyruvate. It measures the contribution of recycling to GP, as measured by Eq. 3 of this paper or by balance studies across liver and kidney.

Periello et al. (17) estimated in overnight-fasted men what fraction of the flux from lactate, alanine, and glutamine represents recycled glucose and what fraction is *de novo* synthesis from muscle proteins. They estimate that the greater part of the flux from lactate and alanine is from recycling but that part of the flux from glutamine is mainly from protein. Our calculation that about one-half of the gluconeogenic flux is from recycling is in general agreement with these estimates. In prolonged fasting, the role of the Cori cycle for maintaining blood glucose concentrations increases, contributing to  $\sim 36\%$  of GP (Table 4). The rest is derived from protein breakdown and from glycolysis of unlabeled muscle glycogen.

*Recycling of labeled carbon.* A number of studies equaled the reincorporation of labeled glucose with the Cori cycle. Streja et al. (21) were the first to stress the difference between the recycling of tracer and the recycling of glucose molecules. The reincorporation of

labeled carbon into glucose depends largely on the dilution of pyruvate by unlabeled carbon and to a lesser extent on the exchange of labeled carbon with  $^{12}\text{C}$  in the tricarboxylic acid cycle. The recycling of tracer is conventionally determined by the difference in GP determined with  $[\text{}^3\text{H}]\text{-}$  and  $[\text{}^{14}\text{C}]\text{glucose}$ , or with  $[\text{U-}^{13}\text{C}]\text{glucose}$  by isotopomer analysis with Eq. 2. In fasted humans, the recycling of labeled carbon averaged 8 and 19% in the 12- and 40-h fasts (Table 4). This is a tracer parameter rather than a physiological rate.

*Glycogen cycle.* Several studies have demonstrated the simultaneous synthesis and breakdown of hepatic glycogen in rats (5), dogs (1), and humans (11). The simultaneous activities of glycogen synthase and phosphorylase would operate as a cycle (glucose-6 phosphate-glucose-1 phosphate, UDP-glucose, glycogen, and glucose-1 phosphate). This contributes a bypass for gluconeogenic flux to glucose. It may trap  $^{13}\text{C}$  in glycogen, and the flux from glycogen to glucose would also become labeled. If such a cycle operates in the fasting state, it will cause an underestimate of GNG by our method as well as by the MIDA and  $^3\text{H}_2\text{O}$  methods.

Hellerstein et al. (7) presented evidence for the operation of such a cycle in men even after a 60-h fast. According to these investigators, after an 11-h fast, total gluconeogenic flux into glucose-6 phosphate accounts for 54% of GP. Only 39% proceeds directly into glucose, and 15% is recycled via glycogen. Thus the flux from glycogen to glucose would be greater than the net decrease of the glycogen stores. According to their Fig. 1 (7), at 60 h, 75–80% of GP was via direct gluconeogenic flux and 20–25% was produced directly via glycogen. According to Hellerstein et al., GNG is from both pathways and accounts for 100% of GP without a significant change in hepatic glycogen content. However, we find GNG at 40 h to be  $>90\%$ , and it is unlikely that any cycling via glycogen is significant at that stage of fasting.

The calculations of Hellerstein et al. (7) assume a random incorporation of label in the terminal glycosyl of glycogen and a random phosphorylation. They would also depend on the duration of the infusion, because the enrichment in glycogen will increase with time. When the enrichment of the glycosyl glucose approaches that of gluconeogenic flux, the effect of the bypass will vanish.

The operation of synthase and glycogen cycling in the fasted state is a controversial subject. Thus Magnusson et al. (11) infused glucose at a rate of  $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and found cycling to be  $\sim 30\%$ . On the other hand, Wajngot et al. (26) infused glucose at  $4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and estimated glycogen cycling at a maximum of 2–12% of GP. Using NMR, Roden et al. (20) showed that glycogen cycling depends greatly on the glucose load. At hyperglycemia and hyperinsulinemia, recycling is extensive, but it is very low or absent at low plasma glucose concentrations and at basal insulin. Recently, Petersen et al. (18) reported no glycogen synthase flux in men at 5 mM glucose and 40 pM of insulin, and negligible flux at 10 mM glucose and basal insulin. Further studies are required to resolve the controversy.

## NOTE ADDED IN PROOF

Landau et al. [Landau, B. R., J. Wahren, K. Ekberg, S. F. Previs, D. Yang, and H. Brunengraber. *Am. J. Physiol.* 274 (*Endocrinol. Metab.* 35): E954–E961, 1998] claim that our equation of gluconeogenesis is in error. Landau's estimates for Cori cycle and gluconeogenesis are about one-half of our values, and one-half of values in other published methods (7, 9). We believe that Landau's estimates are incorrect. This issue will be discussed in detail in an upcoming Letter to the Editor (in press).

This grant was supported by National Institute of Diabetes and Digestive and Kidney Diseases Clinical Investigator Award KO8-DK-02083 (to J. A. Tayek) and Harbor-University of California Los Angeles Medical Center General Clinical Research Center Grant MO1RR00425.

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Received 14 January 1998; accepted in final form 28 May 1998.

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