A non-invasive assessment of hepatic glycogen kinetics and post-absorptive gluconeogenesis in man

L. J. Gay, Ph. Schneiter, Y. Schutz, V. Di Vetta, E. Jéquier, L. Tappy

Institute of Physiology, Faculty of Medicine, Lausanne University, Lausanne, Switzerland

Summary A novel approach to the study of hepatic glycogen kinetics and fractional gluconeogenesis in vivo is described. Ten healthy female subjects were fed an isocaloric diet containing 55 % carbohydrate energy with a ^{13}C abundance of 1.083 atom percent for a 3-day baseline period; then, a diet of similar composition, but providing carbohydrate with a ^{13}C abundance of 1.093 atom percent was started and continued for 5 days. Resting respiratory gas exchanges, urinary nitrogen excretion, breath $^{13}\text{CO}_2$ and plasma ^{13}C glucose were measured every morning in the fasting state. The enrichment in ^{13}C of hepatic glycogen was calculated from these measured data. ^{13}C glycogen enrichment increased after switching to a ^{13}C enriched carbohydrate

diet, and was identical to the ^{13}C enrichment of dietary carbohydrates after 3 days. The time required to renew 50 % of hepatic glycogen, as determined from the kinetics of ^{13}C glycogen enrichment, was 18.9 ± 3.6 h. Fractional gluconeogenesis, as determined from the difference between the enrichments of glucose oxidized originating from hepatic glycogen and plasma glucose ^{13}C was 50.8 ± 5.3 %. This non-invasive method will allow the study of hepatic glycogen metabolism in insulin-resistant patients. [Diabetologia (1994) 37: 517–523]

Key words Glycogen kinetics, gluconeogenesis, glycogenolysis, glucose metabolism in vivo, ^{13}C glucose.

An increased hepatic glucose production and an impaired suppression of hepatic glucose production during hyperinsulinaemia have been observed in insulin-resistant NIDDM patients [1]. The elevated hepatic glucose production has been recently attributed to an increase in hepatic gluconeogenesis, with glycogenolysis being decreased [2]. The relative contributions of gluconeogenesis and glycogenolysis to total glucose production remain however largely unknown, due to the fact that the tracer techniques used for measurements of gluconeogenesis are limited by a number of

methodological considerations. Firstly, the multiplicity of gluconeogenic precursors (glycerol, lactate, amino acids) makes it difficult to obtain an estimate of total gluconeogenesis by administering labelled precursors. Secondly, dilution of labelled precursors due to equilibration of oxaloacetate arising from pyruvate carboxylation with oxaloacetate oxidized in the tricarboxylic acid cycle leads to an underestimation of gluconeogenesis unless correction factors are introduced [3]; such correction factors will depend on the relative rates of the phosphoenolpyruvate and tricarboxylic acid cycles and may vary acutely when experimental conditions are changed. An approach based on the administration of labelled acetate to determine the specific activity of phosphoenolpyruvate, the immediate precursor pool of glucose, has been designed to overcome this problem [4]. This approach has however recently been challenged on theoretical grounds [5] and on the basis of experimental data [6]. Thirdly, hepatic delivery of infused, labelled, gluconeogenic precursors may vary during experiments due to variations in hepatic blood

Received: 1 September 1993
and in revised form: 7 December 1993

Corresponding author: Dr. L. Tappy, Institut de Physiologie, 7 rue du Bugnon, CH-1005 Lausanne, Switzerland

Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; CHO, carbohydrates; CF-IRMS, continuous flow, isotope ratio mass spectrometry

flow [7–9]; this may lead to variations in specific activity or isotopic enrichment of gluconeogenic precursors in hepatic cells.

A substantial part of carbohydrate ingested with a mixed meal is temporarily stored as hepatic glycogen to be subsequently released during interprandial periods [10]. In addition, it has been recently observed using *in vivo* ^{13}C nuclear magnetic resonance spectroscopy that simultaneous glycogen synthesis and breakdown occurs in rat livers [11]. The rate of hepatic glycogen synthesis is likely to play a role in the regulation of post-prandial glycaemia since an inherited defect in hepatic glycogen synthase is associated with fasting hypoglycaemia and impaired glucose tolerance after carbohydrate loading [12, 13]. The effects of alterations of hepatic glycogen metabolism on glucose tolerance have however not been studied in man due to the absence of a method allowing the assessment of hepatic glycogen kinetics *in vivo*.

In this study, a non-invasive method was developed to obtain estimates of 1) the contribution of gluconeogenesis to total endogenous glucose production and 2) the rate of hepatic glycogen renewal in healthy female subjects during standard living conditions. This method is based on simultaneous monitoring of resting respiratory gas exchanges and determination of breath $^{13}\text{CO}_2$ and plasma ^{13}C glucose under post-absorptive conditions during several days following initiation of a diet containing naturally labelled carbohydrates.

Subjects and methods

Subjects

Ten female volunteers participated in the study. They were all in good physical condition, had no family history of diabetes, and were not taking any medication. Body composition was assessed using skinfold thickness measurement [14]. Their mean age was 24 years (range 21–28), body weight 54.9 kg (49.2–63.9), height 164 cm (153–171), and percent body fat 25.6 (18.2–31.4). The experimental protocol was approved by the Ethical Committee of Lausanne University, School of Medicine, and the subjects gave their informed written consent before entering the study.

Study design

The experimental study was performed for 9 consecutive days. During this period, the subjects were fed an isocaloric diet containing 55% carbohydrate energy, 30% fat, 15% protein as three meals at 08.00, 12.00, 20.00 hours and two snacks at 10.00 and 16.00 hours. Resting metabolic rate was estimated from fat free mass determination using the equation of Weinsier et al. [15]. Total 24-h energy requirement was adjusted to the usual physical activity estimated from an interview.

The experimental study was divided into two periods. During period 1 (day –3 at 07.00 hours to day 0 at 18.00 hours), subjects were fed the major part of their carbohydrate from plants which use the C_3 photosynthetic pathway which leads to a low ^{13}C

Table 1. ^{13}C abundance of food in atom percent

C3 Photosynthetic pathway		C4 Photosynthetic pathway	
	atom percent ^{13}C		atom percent ^{13}C
Wheat flour	1.082 ± 0.001	Millet	1.095 ± 0.001
Rice	1.082 ± 0.001	Corn	1.096 ± 0.001
Beet sugar	1.082 ± 0.001	Cane sugar	1.096 ± 0.001
Apple	1.083 ± 0.001	Pineapple	1.095 ± 0.001

Results are the mean $\pm$ SD of four determinations

abundance (Table 1). During the second period (day 0 at 18.00 hours to day 5 at 07.00 hours), subjects were fed the major part of their carbohydrates from plants which use the C_4 photosynthetic pathway, i.e. naturally ^{13}C enriched carbohydrates (Table 1). Respiratory gas exchanges, urinary nitrogen excretion and breath $^{13}\text{CO}_2$ were measured every morning from day 0 to day 5 in the postabsorptive state under resting conditions after an overnight fast. A plasma sample was taken each morning from day 0 to day 5 for determination of plasma ^{13}C glucose enrichment.

Indirect calorimetry

Respiratory gas exchange monitoring was performed using a canopy ventilated at about 40 l/min [16]. Fractions of CO_2 and O_2 were measured at the inlet and the outlet of the canopy using an infrared paramagnetic carbon dioxide analyser (Uras 2T; Hartmann and Braun, Frankfurt, Germany), and a thermomagnetic oxygen analyser (Magnos T2; Hartmann and Braun). Air flow was measured at the outlet of the canopy by a pneumotachograph (digital pneumotachograph model 47303A; Hewlett-Packard, Palo Alto, Calif., USA). Urine was collected between 06.00–09.00 hours for determination of the urinary nitrogen excretion rate [17]. Substrate oxidation rates were calculated using the equations of Livesey and Elia [18].

Isotope analysis

Breath samples were collected in evacuated, air tight glass tubes (Europa Scientific Inc., Crewe, UK). Breath $^{13}\text{CO}_2$ isotope enrichment was determined by CF-IRMS on a Roboprep G/Tracermass (Europa Scientific Inc.).

$^{13}\text{CO}_2$ enrichment from day 1 to 5 was expressed in atom percent excess as:

$^{13}\text{CO}_2$ enrichment (day 1–5) = Breath $^{13}\text{CO}_2$ (day 1–5) – Breath $^{13}\text{CO}_2$ (day 0).

Abundance of ^{13}C in food items was determined by combustion and CF-IRMS on a Roboprep CN/Tracermass (Europa Scientific Inc.). ^{13}C abundance of the carbohydrate containing food items used in this study is shown in Table 1. These food items contain 78 to 100% carbohydrate, and it was assumed that ^{13}C abundance of carbohydrate was identical with ^{13}C abundance of the whole food items.

Dietary ^{13}C carbohydrate enrichment was calculated from the ^{13}C abundance of individual food items, and was expressed in atom percent excess as:

^{13}C CHO diet enrichment = ^{13}C CHO (diet day 1–5) – ^{13}C CHO (diet day –3 to 0).

Diet composition was calculated in order to obtain an overall ^{13}C carbohydrate enrichment of 0.010 atom percent excess.

Fasting plasma ^{13}C glucose enrichment was determined in nine subjects as described [19]. Briefly, plasma samples were deproteinized with perchloric acid (3% final), neutralized with K_2CO_3 and partially purified over sequential cation/anion exchanger resins. The neutral fraction obtained was evaporated to dryness, resuspended in 140 μl H_2O , and plasma glucose was purified by HPLC on an Aminex HPX 87-C column (Bio-Rad, Richmond, Calif., USA), eluted with H_2O at a flow rate of 0.6 ml/min and at a temperature of 80°C [19]. With this procedure, glucose elutes between 12 and 13.5 min, fructose between 14 and 15.5 min, and glycerol between 18 and 19 min. Purified glucose was evaporated to dryness, resuspended with 20 μl H_2O and its ^{13}C abundance was analysed by combustion and CF-IRMS. Plasma ^{13}C glucose enrichment was determined by means of a standard curve of $\text{U-}^{13}\text{C}$ glucose diluted with plasma glucose to correct for minor day-to-day variations of the analytical equipment, and was expressed in atom percent excess.

Plasma ^{13}C protein enrichment was measured by combustion and CF-IRMS of plasma protein precipitated with 90% ammonium sulphate. VLDL were obtained by ultracentrifugation of plasma at 45,000 rev/min for 18 h at 4°C. A 5 μl aliquot of the supernatant VLDL was combusted and its ^{13}C enrichment was measured by CF-IRMS.

Calculations

Measurement of respiratory gas exchanges and urinary nitrogen excretion rate allows the calculation of net substrate oxidation rates (indirect calorimetry) [16, 18] as follows:

Net protein oxidation (mg/min) = $6.25 \cdot \text{N}$ where N is urinary nitrogen excretion in mg/min.

Since oxidation of 6.25 mg protein (corresponding to 1 mg N) requires 6.31 ml O_2 and produces 5.27 ml CO_2 , non-protein oxygen consumption (NPVO_2), non-protein carbon dioxide production (NPVCO_2 (ml/min)) and respiratory quotient (NPRQ) can be calculated as:

$$\begin{aligned}\text{NPVO}_2 &= \text{VO}_2 - 6.31 \times \text{N} \\ \text{NPVCO}_2 &= \text{VCO}_2 - 5.27 \times \text{N} \\ \text{NPRQ} &= \frac{\text{NPVCO}_2}{\text{NPVO}_2}\end{aligned}$$

Net carbohydrate (CHO) oxidation is calculated as follows:

The fraction of NPVO_2 used for oxidation of carbohydrate ($\text{F}_{\text{O}_2(\text{CHO})}$) is calculated as:

$$\text{F}_{\text{O}_2(\text{CHO})} = \frac{\text{NPRQ} - 0.707}{0.293}$$

Since oxidation of 1 mg glycogen requires 0.829 ml O_2 , net CHO oxidation (mg/min) = $\frac{\text{F}_{\text{O}_2(\text{CHO})} \times \text{NPVO}_2}{0.829}$

Net lipid oxidation is calculated, knowing that oxidation of 1 mg lipids requires 2.02 ml O_2 , as:

$$\text{Net lipid oxidation (mg/min)} = \frac{1 - \text{F}_{\text{O}_2(\text{CHO})} \times \text{NPVO}_2}{2.02}$$

When endogenous glycogen is labelled with ^{13}C but not endogenous lipids or protein, the enrichment in ^{13}C glycogen can be calculated from post-absorptive respiratory gas exchanges, urinary nitrogen excretion, and breath $^{13}\text{CO}_2$ enrichment according to the following principles.

In basal, post-absorptive conditions, oxidation of ^{13}C enriched, endogenous glycogen is the sole source for $^{13}\text{CO}_2$ enrich-

ment. Moreover, lipogenesis and amino acid synthesis are insignificant, and net CHO oxidation corresponds to oxidation by peripheral tissues of glucose issued from glycogen hydrolysis.

Net lipid oxidation yields unlabelled $^{12}\text{CO}_2$ and corresponds to the following metabolic pathways:

Gluconeogenesis from glycerol produces unlabelled glucose and oxidation of the neoformed glucose is included in net lipid oxidation.

Net protein oxidation yields unlabelled $^{12}\text{CO}_2$ and corresponds to:

Gluconeogenesis from unlabelled protein produces unlabelled glucose and oxidation of the neoformed glucose is included in net protein oxidation. De novo lipogenesis from amino acid is insignificant in postabsorptive conditions.

CO_2 produced from carbohydrate oxidation has an enrichment in ^{13}C identical with that of glycogen. This labelled $^{13}\text{CO}_2$ is diluted with unlabelled $^{12}\text{CO}_2$ produced from lipid and protein oxidation;

$$\begin{aligned}\text{breath } ^{13}\text{CO}_2 \text{ (atom percent excess)} &= \\ \frac{^{13}\text{C glycogen enrichment} \cdot \text{VCO}_{2(\text{CHO})}}{\text{total VCO}_2} \cdot 0.8\end{aligned}$$

where $\text{VCO}_{2(\text{CHO})}$ is the fraction of total VCO_2 used for oxidation of carbohydrates. Since the RQ for carbohydrates is 1,

$$\text{VCO}_{2(\text{CHO})} = \text{VO}_{2(\text{CHO})} = \frac{\text{NPRQ} - 0.707}{0.293} \cdot \text{NPVO}_2; \text{ the term } 0.8$$

is the recovery factor of $^{13}\text{CO}_2$ in breath

$$\text{and } ^{13}\text{C glycogen enrichment} = \frac{\text{breath } ^{13}\text{CO}_2 \times \text{VCO}_2}{\text{VCO}_{2(\text{CHO})} \cdot 0.8}$$

Example of calculation

^{13}C glycogen enrichment can be calculated from the following set of data measured in the resting state:

$$\begin{array}{ll}\text{VO}_2 & 190 \text{ ml/min} \\ \text{VCO}_2 & 150 \text{ ml/min} \\ \text{N} & 5 \text{ mg/min}\end{array}$$

Breath $^{13}\text{CO}_2 = 0.003$ atom percent excess.

$\text{VCO}_2(\text{CHO})$ can be calculated as:

$$\text{VCO}_2(\text{CHO}) = \text{VO}_2(\text{CHO}) = \text{NPVO}_2 \cdot \frac{\text{NPRQ} - 0.707}{0.293}$$

where $\text{NPVO}_2 = 190 - (5 \times 6.31) = 158.45$

$\text{NPVCO}_2 = 150 - (5 \times 5.27) = 123.65$

$$\text{NPRQ} = \frac{123.65}{158.45} = 0.780$$

and

$$VCO_2(\text{CHO}) = 158.45 \times \frac{0.780 - 0.707}{1 - 0.293} = 39.5 \text{ ml/min.}$$

^{13}C glycogen enrichment can then be calculated as:

^{13}C glycogen (atom percent excess) =

$$\frac{\text{Breath } ^{13}\text{CO}_2 (\text{atom percent excess}) \cdot VCO_2}{VCO_{2(\text{CHO})} \cdot 0.8} =$$

$$0.003 \cdot \frac{150}{31.56} = 0.0143$$

The enrichment of glycogen oxidized at rest was assumed to be equal to the enrichment of hepatic glycogen (see discussion), and was further normalized for dietary ^{13}C CHO enrichment.

^{13}C hepatic glycogen enrichment (%) =

$$\frac{^{13}\text{C Glycogen enrichment (atom percent excess)}}{^{13}\text{C CHO diet enrichment (atom percent excess)}} \cdot 100$$

The time required to renew 50% of hepatic glycogen was estimated from regression analysis of log ^{13}C glycogen enrichment (plateau day 4–5) – ^{13}C glycogen enrichment (day 1, 2, 3) vs time.

Fractional gluconeogenesis {GNG(F)} was calculated as:

GNG(F) =

$$\frac{^{13}\text{C glycogen enrichment} - ^{13}\text{C plasma glucose enrichment}}{^{13}\text{C glycogen enrichment}}$$

Statistical analysis

All results in the test, figures and tables are expressed as mean $\pm$ SEM.

Results

Resting VCO_2 , VO_2 and urinary nitrogen excretion averaged 0.147 ± 0.004 l/min, 0.178 ± 0.005 l/min and 5.3 ± 0.2 mg/min, respectively throughout the study period and did not change during the 5 days after the diet had been switched to naturally enriched carbohydrates. Carbohydrate oxidation represented 33%, fat 49% and protein 17% of calories expended at rest.

Hepatic glycogen turnover

Resting, post-absorptive breath $^{13}\text{CO}_2$ increased rapidly when the ^{13}C naturally enriched diet was started, and reached a plateau after 3 days. The ^{13}C enrichment of hepatic glycogen (i.e. of glucose oxidized at rest, assumed to be essentially of hepatic origin) was $103 \pm 16\%$ of the ^{13}C enrichment of dietary carbohydrates after 3 days (Fig. 1). The time required to renew 50% of hepatic glycogen was calculated to be 18.9 ± 3.6 h (Table 2).

Fig. 1. ^{13}C enrichment of hepatic glycogen oxidized at rest (calculated from breath $^{13}\text{CO}_2$ and respiratory gas exchanges) (top panel) and ^{13}C plasma glucose (bottom panel) during 5 consecutive days after initiation of a diet naturally labelled with ^{13}C carbohydrates. Results (mean $\pm$ SEM) are expressed as percent enrichment of dietary carbohydrates (0.01 atom percent excess)

Table 2. Hepatic glycogen kinetics and resting fractional gluconeogenesis

Subjects	$\frac{T}{2}$ glycogen ^a (h)	Fractional gluconeogenesis (%)
1	14.6	45.9
2	24.4	44.6
3	22.7	29.2
4	40.3	–
5	3.7	47.9
6	16.3	62.0
7	31.8	37.3
8	19.0	57.1
9	7.6	48.1
10	8.2	84.3
Mean	18.9	50.8
SEM	3.6	5.3

^a $\frac{T}{2}$ glycogen refers to the time required to renew 50% of hepatic glycogen

Fractional gluconeogenesis

Plasma ^{13}C glucose enrichment increased rapidly when the ^{13}C naturally enriched diet was started and reached a plateau after 2 days (Fig. 1). Plasma ^{13}C glucose enrichment at plateau represented $54.6 \pm 5.5\%$ of the ^{13}C

carbohydrate enrichment of the diet and $49.2 \pm 5.5\%$ of the enrichment of hepatic glycogen. This corresponds to a fractional gluconeogenesis of $50.8 \pm 5.3\%$.

^{13}C abundance of plasma proteins and VLDL remained constant throughout the 5-day period (^{13}C proteins: 1.0840 ± 0.0008 atom percent, day 0 vs 1.0843 ± 0.0008 day 5, NS, and ^{13}C VLDL: 1.0827 ± 0.0009 , day 0 vs 1.0826 ± 0.0010 day 5), indicating that intake of ^{13}C enriched carbohydrate did not label the endogenous pools of protein and lipid (Table 2).

Discussion

Indirect calorimetry provides estimates of net substrate oxidation rates. Glucose oxidation determined in the resting, post-absorptive state, is therefore exclusively accounted for by oxidation of endogenous glycogen, since oxidation of glucose formed from glycerol or amino acids gluconeogenesis will be computed as fat and protein oxidation, respectively [21]. This allows the ^{13}C enrichment of previously labelled glycogen to be specifically determined using indirect calorimetry and breath $^{13}\text{CO}_2$ measurement. In this study, the measured ^{13}C enrichment of glycogen at rest observed to be equivalent to the ^{13}C enrichment of dietary carbohydrates 2 days after starting a diet which provided naturally enriched ^{13}C carbohydrates. Glucose oxidized at rest was considered to be essentially of hepatic origin. The observation that muscle metabolism accounts for approximately 20% of the resting metabolic rate [22], and resting muscle oxidizes a mixture of fat and glycogen supports this assumption [23]. Thus, in the postabsorptive state, the contribution of muscle glycogen to carbohydrate oxidation can be estimated to be insignificant.

The calculation used to determine the ^{13}C enrichment of hepatic glycogen assumes that protein and fat oxidized at rest have a ^{13}C abundance identical to breath CO_2 before administration of naturally ^{13}C enriched carbohydrates. The ^{13}C content of endogenous lipids and proteins was assessed from analysis of plasma proteins and VLDL, and was found to be similar to the basal breath CO_2 . The administration of naturally enriched ^{13}C carbohydrate such as corn and millet was accompanied by the absorption of a certain amount of corn and millet proteins, which were also ^{13}C enriched. The amount of ^{13}C enriched proteins administered with the diet was however very small (less than 7% of total dietary proteins). In addition, due to the short duration of this protocol, amino acids from ^{13}C enriched proteins can be assumed to be diluted in a very large pool of endogenous amino acids and proteins, and were therefore not included in our analysis. In addition, the observation that plasma proteins and VLDL were not enriched with ^{13}C during the 5-day study corroborates that neither exogenous labelled proteins nor labelled lipids interfered with the $^{13}\text{CO}_2$ enrichment.

The ^{13}C enrichment of glycogen oxidized at rest was found to be identical to the ^{13}C enrichment of dietary carbohydrate after a 3-day diet which provided carbohydrate naturally enriched in ^{13}C , indicating that hepatic glycogen had been almost completely renewed during this time period.

The kinetics of enrichment of hepatic glycogen over time indicated that 50% of the hepatic glycogen pool had been replaced after approximately 18 h. A relatively large range of individual results is observed. This may be due to the low enrichment in ^{13}C of naturally enriched foods, giving a relatively large noise-to-response ratio in the determination of breath $^{13}\text{CO}_2$. Obviously this may be improved by using artificially enriched carbohydrates. Using magnetic resonance spectroscopy *in vivo*, hepatic glycogen content of normal post-absorptive humans has been reported to be approximately 70 g [2]. The turnover of hepatic glycogen in these healthy, moderately active non-obese women can thus be estimated to be about 47 g per day since hepatic glycogen content remains constant from day-to-day when the diet composition is stable. With an average carbohydrate intake of 250 g, hepatic glycogen synthesis represented about 20% of exogenous carbohydrate disposal.

Glycogen storage in skeletal muscle has been reported to account for nearly 100% of non-oxidative glucose disposal during intravenous administration of glucose and insulin in humans [24]. The situation is different after oral feeding when higher portal glucose/insulin concentrations may favour hepatic glycogen deposition. Using a dual glucose tracer method, hepatic glycogen synthesis was found to account for 25–30% of the disposal of an oral glucose load while glucose oxidation was the major metabolic pathway of ingested glucose in skeletal muscle [10]. The degree of physical activity, the type of diet and the feeding schedule may all affect hepatic glycogen kinetics.

The ^{13}C enrichment of plasma glucose was found to be $49.2 \pm 5.5\%$ of the enrichment of hepatic glycogen at rest. Since ^{13}C plasma glucose enrichment differed significantly from both hepatic glycogen enrichment and dietary ^{13}C carbohydrate enrichment, a substantial portion of plasma glucose carbons did not originate from dietary carbohydrates or hepatic glycogenolysis. The difference observed between enrichment of hepatic glycogen and of plasma glucose suggests that approximately 50% of the glucose endogenously released into the systemic circulation originates from gluconeogenesis from unlabelled amino acids or glycerol. Part of gluconeogenesis corresponds to the Cori and glucose-alanine cycles, in which plasma glucose is taken up by peripheral tissues to be released as lactate or alanine which have the same enrichment as plasma glucose. Subsequent conversion of these labelled compounds into glucose will therefore not be accounted for by this calculation. Such a cycling between plasma glucose and three carbon compounds has been reported to repre-

sent 12–20 % of total glucose production in healthy humans [25] but may be increased two-fold in NIDDM patients [26]. Plasma lactate may also originate from hydrolysis of muscle glycogen, and its conversion into glucose will be measured as glycogenolysis with the present method if muscle and hepatic glycogen enrichment are identical. The muscle glycogen pool is however larger and its turnover may be slower than hepatic glycogen, and further studies are required to assess the kinetics of glycogen in muscle under similar conditions.

This estimate of fractional gluconeogenesis of 50 % in healthy subjects is substantially higher than those reported during infusion of labelled acetate or bicarbonate [27, 28]. This may be due to the fact that the latter technique only reflects gluconeogenesis from lactate/pyruvate, or phosphoenolpyruvate substrates, but fails to detect gluconeogenesis originating from glycerol or some amino acid substrates. The percentage is consistent with a recent report [29] which showed that up to 70 % of hepatic glucose production could be attributed to hepatic gluconeogenesis during a 24-h fast in healthy human subjects.

In conclusion, we report a simple method with which 1) hepatic glycogen kinetics and 2) fractional gluconeogenesis can be determined non-invasively in humans. The results obtained in a group of 10 healthy lean women indicate that the time required to renew 50 % of hepatic glycogen is approximately 18 h; consequently, it can be estimated that 20 % of dietary carbohydrate may be temporarily stored as hepatic glycogen during a 24-h period under normal living conditions. Thus, hepatic glycogen kinetics may play an important role in determining insulin sensitivity and glucose tolerance in humans. Fractional gluconeogenesis was found to be close to 50 % indicating that half of the glucose released into the circulation originates from gluconeogenesis. This estimate does not include however the cycling between plasma glucose and three carbon compounds.

Acknowledgements. The authors wish to thank Mrs. M. Emch for editorial assistance. This work was supported by grants from the Swiss National Foundation for Scientific Research (No. 32-37247-93), from the foundation of Prof. Dr. Max Cloëtta and from the foundation of Mr. Raymond Berger.

References

- DeFronzo A, Ferrannini E, Simonson DC (1989) Fasting hyperglycemia in non-insulin dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism* 38: 387–395
- Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI (1991) Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR. *Science* 254: 573–576
- Hetenyi GJ (1982) Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis in vivo. *Fed Proc* 41: 104–109
- Katz J (1985) Determination of gluconeogenesis in vivo with ^{14}C -labeled substrates. *Am J Physiol* 248: R391–R399
- Landau BR (1991) Correction of tricarboxylic acid cycle exchange in gluconeogenesis: why the y's are wrong. *Am J Physiol* 261: E673–E675
- Consoli A, Nurjhan N, Capani F, Pangburn T, Lapenna D, Gerich J (1993) Limitations in the use of $[2-^{14}\text{C}]$ acetate for measuring gluconeogenesis in vivo. *Diabetes* 42: 732–737
- McKaigney JP, Carmichael FJ, Saldivia V, Israel Y, Orrego H (1986) Role of ethanol metabolism in the ethanol-induced increase in splanchnic circulation. *Am J Physiol* 250: G518–G523
- Chou CC (1983) Splanchnic and overall cardiovascular hemodynamics during eating and digestion. *Fed Proc* 42: 1658–1661
- Kock NG, Roding B, Hahnloser P, Tibblin S, Schenk WG (1970) The effect of glucagon on hepatic blood flow. *Arch Surg* 100: 147–149
- Kelley D, Mitrakou A, Marsh H et al. (1988) Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *J Clin Invest* 81: 1563–1571
- David M, Petit WA, Laughlin RG, Shulman RG, King JE, Barrett EJ (1990) Simultaneous synthesis and degradation of rat liver glycogen. An in vivo nuclear magnetic resonance spectroscopic study. *J Clin Invest* 86: 612–617
- Aynsley-Green A, Williamson DH, Gitzelmann R (1977) Hepatic glycogen synthetase deficiency. *Arch Dis Child* 52: 573–579
- Spencer-Peet J (1964) Erythrocyte synthetase in glycogen storage deficiency resulting from the absence of this enzyme from liver. *Clin Chim Acta* 10: 481–483
- Durnin JVG, Womersley J (1974) Body fat assessment for total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr* 32: 77–97
- Weinsier R, Schutz Y, Bracco D (1992) Reexamination of the relationship of resting metabolic rate to fat-free mass and to the metabolically active components of fat-free mass in humans. *Am J Clin Nutr* 55: 790–794
- Jéquier E, Felber J (1987) Indirect calorimetry. *Baillieres Clin Endocrinol Metab* 1: 911–935
- Hawk P (1947) The Kjeldahl method. In: Hawk PB, Oser BL, Simonson WH (eds) *Practical physiological chemistry*. Blackiston, Toronto, pp 814–822
- Livesey G, Elia M (1988) Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry evaluation of errors with special reference to the detailed composition of foods. *Am J Clin Nutr* 47: 608–628
- Vollenweider P, Tappy L, Randin D et al. (1993) Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J Clin Invest* 92: 147–154
- Robert JJ, Koziat J, Chauvet D, Darmaun D, Desjeux JF, Young VR (1987) Use of ^{13}C -labeled glucose for estimating glucose oxidation: some design considerations. *J Appl Physiol* 63: 1725–1732
- Ferrannini E (1988) The theoretical basis of indirect calorimetry. A review. *Metabolism* 37: 287–301
- Zurlo F, Larson K, Bogardus C, Ravussin E (1990) Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J Clin Invest* 86: 1423–1427
- Kelley DE, Mokan M, Simoneau JA, Mandarino LJ (1993) Interaction between glucose and free fatty acid metabolism in human skeletal muscle. *J Clin Invest* 92: 91–98
- Shulman G, Rothman D, Jue T, Stein P, DeFronzo R, Shulman R (1990) Quantitation of muscle glycogen synthesis in

- normal subjects and in subjects with non-insulin dependent diabetes by ^{13}C nuclear magnetic resonance spectroscopy. *N Engl J Med* 322: 223–228
25. Reichard GAJ, Moury NF, Hochella NJ, Patterson AL, Weinhouse S (1963) Quantitative estimation of the Cori cycle in the human. *J Biol Chem* 238: 495–501
26. Tappy L, Acheson K, Curchod B et al. (1994) Overnight glucose metabolism in obese non insulin dependent diabetic patients and in healthy lean individuals. *Clin Physiol*, in press
27. McMahon M, Marsh M, Rizza R (1989) Effects of basal insulin supplementation on disposition of mixed meals in obese patients with NIDDM. *Diabetes* 38: 291–303
28. Tappy L, Acheson K, Normand S et al. (1992) Effects of infused amino acids on glucose oxidation, endogenous glucose production and ^{13}C glucose synthesis from ^{13}C bicarbonate in healthy human subjects. *Am J Physiol* 262: E826–E833
29. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI (1992) Increased rate of gluconeogenesis in type II diabetes mellitus. A ^{13}C nuclear magnetic resonance study. *J Clin Invest* 90: 1323–1327