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Effects of lactate infusion on hepatic gluconeogenesis and glycogenolysis

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Summary. Endogenous glucose production rate (EGPR) remains constant when lactate is infused in healthy humans. A decrease of glycogenolysis or of gluconeogenesis from endogenous precursors or a stimulation of glycogen synthesis, may all be involved; This autoregulation does not depend on changes in glucoregulatory hormones. It may be speculated that alterations in basal sympathetic tone may be involved.

To gain insights into the mechanisms responsible for autoregulation of EGPR, glycogenolysis and gluconeogenesis were measured, with a novel method (based on the prelabelling of endogenous glycogen with ^{13}C glucose, and determination of hepatic ^{13}C glycogen enrichment from breath $^{13}CO_2$ and respiratory gas exchanges) in healthy humans infused with lactate or saline. These measurements were performed with or without β-adrenergic receptor blockade (propranolol).

Infusion of lactate increased energy expenditure, but did not increase EGPR; the relative contributions of gluconeogenesis and glycogenolysis to EGPR were also unaltered. This indicates that autoregulation is attained, at least in part, by inhibition of gluconeogenesis from endogenous precursors.

β-adrenergic receptor blockade alone (with propranolol) did not alter EGPR, glycogenolysis or gluconeogenesis. During infusion of lactate, propranolol decreased the thermic effect of lactate but EGPR remained constant. This indicates that alterations of β-adrenergic activity is not required for autoregulation of EGPR.

Key words: hepatic glucose production, carbohydrate oxidation, thermogenesis, sympathetic nervous system, humans.

Introduction

There is evidence in healthy humans that basal hepatic glucose production remains constant when gluconeogenesis is acutely increased by infusion of gluconeogenic precursors (Jahoor *et al.*, 1990; Jenssen *et al.*, 1990; Tounian *et al.*, 1994). A decrease in

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hepatic glycogenolysis, a decrease in gluconeogenesis from gluconeogenic substrates other than the infused precursor, and an increase in hepatic glycogen synthesis may all participate in this autoregulation of hepatic glucose production (HGP).

The signals responsible for the autoregulation of HGP remain unidentified. The glucoregulatory hormones insulin and glucagon are unlikely to be involved because the autoregulation persists when these hormones are clamped by exogenous infusions of somatostatin, insulin and glucagon (Jenssen *et al.*, 1990; Tounian *et al.*, 1994). Changes in the intrahepatic concentrations of (yet undefined) intermediary substrates may be involved. Alternatively, alterations of the autonomic innervation to the liver may participate in this autoregulation by regulating the relative rates of gluconeogenesis, glycogenolysis and glycogen synthesis. In particular, the sympathetic nervous system, when activated, stimulates hepatic glycogenolysis and gluconeogenesis (Cherrington *et al.*, 1984; Webber & MacDonald, 1993). These effects in man are mainly produced by β-adrenergic effects (Deibert & DeFronzo, 1980; Rizza *et al.*, 1980). An inhibition of the basal sympathetic activity triggered by infusion of gluconeogenic precursors might therefore participate in the autoregulation of hepatic glucose production. Little, however, is known on the effect of basal sympathetic nervous system activity in the control of hepatic glucose metabolism.

The aims of the present study were to determine:

(a) whether lactate infusion alters the fractional rates of hepatic gluconeogenesis and glycogenolysis;
(b) whether basal sympathetic nervous system activity regulates intrahepatic glucose metabolism;
(c) whether alteration of SNS activity is involved in the autoregulation of hepatic glucose production during infusion of lactate.

For this purpose, hepatic glycogenolysis and gluconeogenesis during lactate infusion were assessed from indirect calorimetry, breath $^{13}CO_2$ and plasma ^{13}C-glucose according to a method recently developed (Gay *et al.*, 1994b). The effects of β-adrenergic blockade on hepatic glucose production, glycogenolysis and gluconeogenesis were also studied in both the post-absorptive state and during infusion of sodium lactate.

Materials and methods

SUBJECTS

Six healthy male volunteers with no family history of diabetes or related disorders were selected to participate in the study. All subjects were in good physical condition, did not take any medication and had normal glucose tolerance as documented by a standard 75 g oral glucose tolerance test. Their mean (±SD) age was 26.4±2.9 years, their mean

weight was 79.8±12.8 kg (body mass index 23.1±2.2 $kg \cdot m^{-2}$) and their mean percentage fat, determined by skinfold thickness measurement (Durnin & Womersley, 1974) was 15.5±5.0%.

The experimental protocol was approved by the Ethical Committee of Lausanne University and every subject provided an informed written consent.

GENERAL PROCEDURES

During the two days preceding each protocol, the subjects were requested not to perform heavy physical exercise and were given instructions to eat a diet containing at least 250 g carbohydrate per day. They ingested an orange juice to which 0.1 g of uniformly-labeled ^{13}C glucose (Isotec Inc., Miamisburg, Ohio, USA) had been added with their dinner, 36 h before and with the three main meals the day before the experiments. This procedure produces substantial ^{13}C enrichment of hepatic glycogen pool, with no change in body protein and lipid ^{13}C content (Gay *et al.*, 1994b). Blood and breath samples had been collected prior to the intake of the first ^{13}C glucose enriched drink to determine basal plasma glucose and breath CO_2 $^{13}C/^{12}C$ isotope ratios. To allow these values to return to basal levels, at least 1 week elapsed between each protocol.

The experiments began between 07.00 hours and 08.00 hours, after a 10–12 h fast, with the subjects lying quietly semi-recumbent throughout the experiment. A venous cannula was inserted into an antecubital vein for blood sampling. A second cannula was inserted into a vein of the contralateral arm for infusion of 6,6-^2H glucose (Tracer Technology, Sommerville, Massachusetts, USA; prime 33.0 $\mu mol \cdot kg^{-1}$ at 0 min, and 0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ throughout the 6-h experiments) and of the test substances (propranolol, sodium lactate).

Blood samples were collected at 0, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min for determination of plasma glucose ^{13}C- and 6,6-^2H enrichments and concentrations of plasma glucose, insulin, glucagon, lactate, urea and free fatty acids. Urine was collected between 0 and 360 min for determination of urinary nitrogen, urea and lactate excretion rates.

The head of the subject was inserted in a ventilated canopy tightened around the neck and respiratory gas exchanges were monitored from time 90 to the end of the experiment as described by Jéquier *et al.* (1987).

Breath collections were obtained at 120, 150, 180, 240, 300, 330 and 360 min for determination of breath $^{13}CO_2$.

EXPERIMENTAL PROTOCOLS

Protocol 1. This protocol aimed to determine the effects of sodium lactate infusion on hepatic glucose production, glycogenolysis, gluconeogenesis and resting energy expen-

diture (REE). A solution of sodium lactate 0.5 M was infused at a rate of 20.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ from time 180 to 360 min.

Protocol 2. This protocol aimed to determine the effects of sodium lactate infusion on hepatic glucose production, glycogenolysis, gluconeogenesis and REE during β-adrenergic blockade.

The experimental protocol was identical to protocol 1. In addition, propranolol (Inderal, ICI, Staines, UK) was infused from time 180 to 360 min (prime 100 $\mu g \cdot kg^{-1}$, continuous 1 $\mu g \cdot kg^{-1}$ min^{-1}). Heart rate and electrocardiogram were monitored throughout the experiment, and arterial blood pressure was measured every 30 min.

Protocol 3. This protocol aimed to determine the effects of an inhibition of the basal β-adrenergic stimulation on hepatic glucose production, glycogenolysis, gluconeogenesis and REE.

The experimental protocol was identical to protocol 2, except no sodium lactate was infused.

Protocol 4. In this control study, basal conditions were maintained from 0 to 360 min with saline infusion only.

ANALYTICAL PROCEDURES

Plasma glucose was determined by the glucose oxidase method using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma free fatty acids (Wako, Freiburg, Germany) were determined by colorimetric assay. Plasma insulin (Herbert *et al.*, 1965) and glucagon (Faloona & Unger, 1974) were measured by radioimmunoassay. Plasma and urinary urea were analysed enzymatically using a Beckman urea analyzer (Beckman Instruments, Fullerton, CA). Plasma and urinary lactate were measured enzymatically (Hohorst, 1965).

For plasma ^{13}C and 6,6-^{2}H glucose measurements, plasma samples were deproteinized with perchloric acid (3% vol/vol), neutralized with 3.2 M K_2CO_3, partially purified by sequential anion-cation exchanger resins (AG 1-X8 and AG 50W-X8, Bio-Rad, Richmond, CA) and the neutral fraction was evaporated to dryness. In the case of plasma 6,6-^{2}H glucose, the glucose dry residue was derivated to pentacetyl glucose and analysed in a gas chromatography-mass spectrometer (Hewlett-Packard Co., Palo Alto, CA) in electron impact mode, with selective monitoring of masses 242.1 and 244.1. In the case of plasma ^{13}C glucose, the glucose dry residue was resuspended with H_2O and glucose was purified by high performance liquid chromatography on an HPX-87-C column (Bio-Rad, Richmond, CA) eluted with H_2O 0.6 ml min^{-1} at 80°C. This procedure allows complete separation of plasma glucose from glycerol (Gay *et al.*, 1994b). Purified glucose was again evaporated to dryness, resuspended with H_2O and the $^{13}C/^{12}C$ ratio was measured by combustion and continuous flow isotope ratio mass spectrometry (IRMS) on a Roboprep CN-Tracermass (Europa Scientific Ltd., Crewe, UK).

Breath $^{13}CO_2$ isotopic enrichment was measured by continuous flow IRMS on a Roboprep G-Tracermass (Europa Scientific Ltd., Crewe, UK).

CALCULATIONS

Endogenous glucose production, hepatic glycogenolysis, gluconeogenesis, energy expenditure and net substrate oxidation rates were calculated for periods of steady state of infused substances (6,6-^{2}H glucose, sodium lactate and propranolol) only, i.e. from 120 to 180 min and from 300 to 360 min.

Endogenous glucose production rate (EGPR in $\mu mol \cdot min^{-1}$), i.e. total rate of glucose appearance, was calculated from plasma 6,6-^{2}H glucose isotopic enrichment using Steele's equation for steady state (Steele, 1959):

$$EGPR = \frac{F}{\text{plasma } 6,6\text{-}^2H\text{-glucose}} - F$$

with plasma 6,6-^{2}H glucose expressed in mole % excess. F is the rate of 6,6-^{2}H glucose infusion in $\mu mol \cdot min^{-1}$.

The rate of hepatic glycogenolysis was calculated from indirect calorimetry, breath $^{13}CO_2$ and plasma ^{13}C glucose. The principle of this calculation has been described in detail (Gay *et al.*, 1994b). Briefly, feeding labelled carbohydrate during the 36 h prior to the experiment results in a labelling of the endogenous glycogen pool, with no detectable increase in the ^{13}C content of plasma lipids or protein. The hepatic ^{13}C glycogen enrichment can be calculated from indirect calorimetry and breath $^{13}CO_2$ in resting, post-absorptive conditions, knowing that (a) oxidation of glucose produced from glycogen hydrolysis is the sole source of excess $^{13}CO_2$; and (b) net carbohydrate oxidation reflects, essentially, oxidation of glucose originating from hepatic glycogen. Therefore, hepatic glycogen ^{13}C enrichment, expressed in atom % excess, was estimated during the basal period of each test (120 to 180 min) as:

$$\text{Hepatic } ^{13}C \text{ glycogen} = {}^{13}CO_2 \cdot \frac{\dot{V}CO_2}{\dot{V}CO_2(CHO)} \cdot \frac{1}{0.8}$$

where $^{13}CO_2$ is the isotopic enrichment of breath CO_2 expressed in atom % excess, $\dot{V}CO_2$ is total breath CO_2 elimination ($l \cdot min^{-1}$), $\dot{V}CO_2$ (CHO) represents the breath CO_2 elimination due to carbohydrate oxidation as calculated from $\dot{V}CO_2$ and respiratory quotient (Livesey & Elia, 1988), and 0.8 is $^{13}CO_2$ recovery in breath as determined previously (Robert *et al.*, 1987).

In these experimental conditions, plasma glucose originates from hydrolysis of ^{13}C labelled hepatic glycogen, and from conversion of unlabelled gluconeogenic precursors (i.e. glycerol, amino acids; lactate may be partially labelled: see Discussion). The ratio of plasma ^{13}C glucose to hepatic ^{13}C glycogen enrichments allows calculation of the fractional rate of glycogenolysis. Thus, hepatic glycogenolysis is obtained as follows:

$$\text{Hepatic glycogenolysis} = \frac{\text{plasma } ^{13}C \text{ Glucose}}{\text{hepatic } ^{13}C \text{ glycogen}} \cdot EGPR$$

where plasma ^{13}C glucose is the isotopic enrichment expressed in atom % excess of

plasma glucose. Protocol 4 (control) showed that ^{13}C glycogen enrichment calculated, as described above, remained constant during the measurements (data not shown). Therefore, hepatic glycogenolysis of both periods (120 to 180 min and 300 to 360 min) was calculated using ^{13}C glycogen enrichment obtained during baseline in each protocol.

Energy expenditure was calculated from respiratory O_2 and CO_2 exchanges and from urinary nitrogen excretion corrected for the change in urea nitrogen pool size as described previously (Livesey & Elia, 1988; Tappy *et al.*, 1988). During infusion of sodium lactate, a correction was introduced for extrapulmonary CO_2 losses as described (Burnier *et al.*, 1992; Chioléro *et al.*, 1993).

The thermic effect of infused lactate (TEL) was calculated by dividing the increase in resting energy expenditure (REE in kJ) by the energy content (1363 kJ per mol lactate) of lactate infused at a rate of 20.0 $\mu mol \cdot kg \cdot min^{-1}$ during the last hour of the experiment (60 min) (steady state):

$$TEL = \frac{REE(300-360) - REE(120-180)}{20 \cdot BW \cdot 60 \cdot 0.001363} \cdot 100$$

where BW is body weight in kg. TEL is expressed in % of energy content of the infused lactate.

STATISTICAL ANALYSES

All data are presented as means ± SEM. Differences between 300 to 360 min means and basal values within each protocol were tested using Student's paired *t*-test. Changes in measured or calculated parameters were compared between protocols by repeated-measures analysis of variance and paired *t*-test with Bonferroni's correction. Thermic effect of lactate was compared between protocol 1 and 2 using Student's paired *t*-test. Significance was determined at $P<0.05$ (two-tailed).

Results

BLOOD PARAMETERS

Plasma glucose, glucagon and free fatty acids remained nearly constant in all four protocols (Table 1). Plasma insulin decreased slightly with time in all 4 protocols, but the decrease reached statistical significance in protocol 3 (propranolol) only. Plasma lactate concentrations had reached a steady state during the third hour of lactate infusion. The plateau lactate concentration was similar with or without propranolol. During the experiments without lactate infusion, plasma lactate decreased, slightly with time by 25% ($P<0.01$) with propranolol infusion and by 10% ($P<0.05$) with saline only.

Table 1. Blood parameters

	Protocol 1		Protocol 2		Protocol 3		Protocol 4	
	Basal	Lactate	Basal	Lactate+ propranolol	Basal	Propranolol	Basal	Saline
Glucose ($mmol \cdot l^{-1}$)	5.0±0.1	4.9±0.1	4.9±0.1	4.9±0.2	4.9±0.2	4.9±0.2	4.8±0.1	4.9±0.1
Insulin ($pmol \cdot l^{-1}$)	53±13	47±10	55±12	41±7	51±9	36±7*	44±5	37±5†
Glucagon ($ng \cdot l^{-1}$)	73±7	71±5	70±9	65±9	61±5	56±7	56±4	5964
FFA ($mmol \cdot l^{-1}$)	359±73	363±72	404±65	407±83	456±125	377±88	446±84	598±113
Lactate ($mmol \cdot l^{-1}$)	1.0±0.1	2.9±0.1†‡	1.1±0.1	2.7±0.2†‡	1.2±0.1	0.9±0.1†	1.0±0.1	0.9±0.1*

Data represent mean±SEM. FFA = free fatty acids.
*$P<0.05$ *vs.* basal; †$P<0.01$ *vs.* basal; ‡$P<0.01$ *vs.* protocol 4.

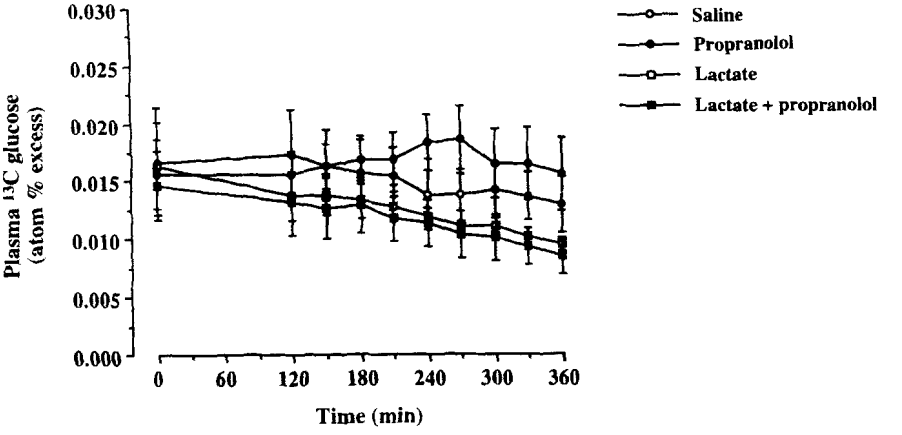

Fig. 1. Breath $^{13}CO_2$ enrichment in atom percent excess (APE) without (m, p) and with (l, p) propranolol; without (l, m) and with (n, p) lactate. No statistical difference was observed between the four different protocols.

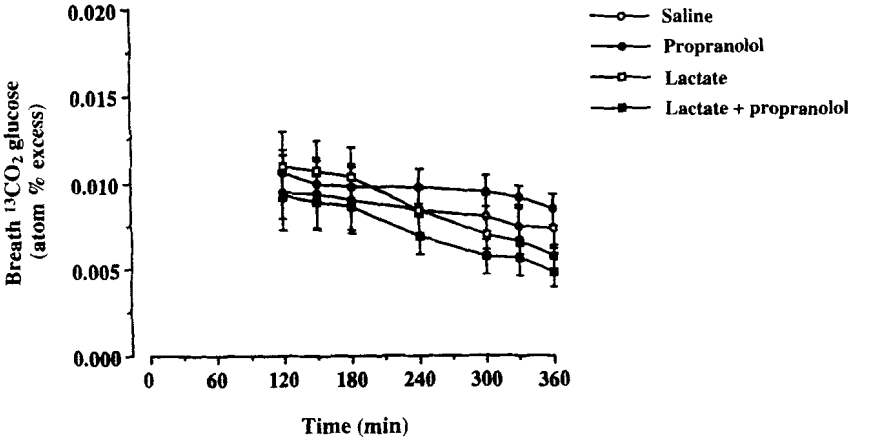

Fig. 2. Plasma ^{13}C glucose enrichment in atom percent excess (APE) without (m, p) and with (l, p) propranolol; without (l, m) and with (n, p) lactate. No statistical difference was observed between the four different protocols.

GLUCOSE METABOLISM

Infusion of lactate failed to increase EGPR both without and without propranolol (protocol 1 and 2). EGPR decreased slightly throughout the experiments during all protocols.

^{13}C plasma glucose and breath $^{13}CO_2$ enrichments are shown in Figs 1 and 2. Basal ^{13}C hepatic glycogen enrichments were 0.027 ± 0.006; 0.026 ± 0.004, 0.029 ± 0.005; and 0.025 ± 0.006 at % excess in protocol 1, 2, 3 and 4 respectively. In protocols 3 and 4, when no lactate was infused, it was observed that the calculated enrichment of hepatic glycogen remained constant over the 6 h study period. This indicates homogenous enrichment of hepatic glycogen and supports the use of the ^{13}C glycogen enrichment

Table 2. Hepatic glucose production (HGP), hepatic gluconeogenesis and glycogenolysis

	Protocol 1		Protocol 2		Protocol 3		Protocol 4	
	Basal	Lactate	Basal	Lactate+ propranolol	Basal	Propranolol	Basal	Saline
Hepatic glucose production ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	10.9±0.7	10.1±0.5	10.9±0.9	9.9±0.7	10.9±0.6	10.0±0.5	10.5±0.6	9.7±0.5
Glycogenolysis ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	5.5±0.8	4.7±1.5	5.9±1.1	4.1±0.9*	7.4±1.0	7.0±1.7	6.4±0.7	5.3±0.9
Gluconeogenesis ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	5.5±0.7	5.4±1.0	5.0±0.5	5.8±0.2*	3.5±1.0	3.0±1.6	4.0±0.7	4.460.7

Data represent mean±SEM.
*$P<0.05$ *vs.* basal.

Table 3. Resting energy expenditure (REE) and thermic effect of lactate (TEL)

	Protocol 1		Protocol 2	
	Basal	Lactate	Basal	Lactate+ propranolol
REE ($kJ \cdot min^{-1}$)	5.0±0.2	5.5±0.3*	5.1±0.2	5.4±0.2†
TEL (%)	—	24±4	—	14±4‡

Data represent mean±SEM.
*$P<0.01$ *vs.* basal; †$P<0.05$ *vs.* basal; ‡$P<0.01$ *vs.* protocol 1.

obtained at baseline for the subsequent calculation of fractional glycogenolysis. Hepatic glycogenolysis decreased slightly in all four protocols, although significance was reached only during infusion of both lactate and propranolol (Table 2). Infusion of lactate however, did not suppress glycogenolysis compared to infusion of saline Gluconeogenesis did not increase significantly during infusion of lactate or during infusion of saline with or without propranolol. A modest 16% rise ($P<0.05$) was observed only during infusion of lactate+propranolol.

ENERGY EXPENDITURE, THERMIC EFFECT OF LACTATE AND SUBSTRATE OXIDATION RATES

Lactate infusion increased REE by 10% ($P<0.01$) without propranolol and by 6% ($P<0.01$) with propranolol (Table 3). The thermic effect of lactate (TEL) represented 24±4% of the energy content of exogenous lactate when infused alone, and was decreased to 14±4% when infused with propranolol ($P<0.01$).

HEART RATE

Infusion of propranolol alone decreased heart rate from 67±3 to 59±1 bpm ($P<0.05$ *vs.* protocol 1). This effect was not observed when sodium lactate was infused together with propranolol.

Discussion

The present data confirm the observations by Jenssen *et al.* (1990) that infusion of lactate in healthy humans does not increase hepatic glucose production. In addition, the measurement of the fractional rates of glycogenolysis and gluconeogenesis in the present experiments allow us to gain major insights into the mechanisms involved in the autoregulation of hepatic glucose production during infusion of lactate. The methodology used for this purpose differs markedly from the methods previously used to quantitate gluconeogenesis. It assesses the contribution of glycogenolysis to overall EGPR from the measurement of plasma ^{13}C glucose, and offers an direct assessment of hepatic ^{13}C glycogen enrichment; gluconeogenesis is then calculated as EGPR-glycogenolysis. This method provides estimates of the contribution of gluconeogenesis to EGPR which are substantially higher (Gay *et al.*, 1994b) than those previously reported using other techniques. Splanchnic catheterisation studies estimated that gluconeogenesis contributed approximately 20% of EGPR in healthy humans (Felig *et al.*, 1978). This technique however fails to assess gluconeogenesis from precursors released by the gut or within the hepatocytes. It also did not assess splanchnic glutamine uptake. Methods based on the rate of labelled glucose synthesis during infusion of labeled gluconeogenic precursors also provide lower estimates of gluconeogenesis (Jenssen *et al.*, 1990; Jahoor *et al.*, 1990). These methods, however, assess only gluconeogenesis from one out of

several endogenous gluconeogenic substrates at a time. Furthermore, for substrates entering the gluconeogenic pathway at the level of pyruvate, these approaches lead to substantial underestimation because part of the labelled oxaloacetate formed in the pyruvate carboxylase reaction is oxidized to CO_2 in the Krebs cycle (Hetenyi, 1982). On the other hand, Magnusson *et al.* (1992) measured glycogenolysis from the decrease over time in hepatic ^{13}C glycogen (measured with *in vivo* ^{13}C NMR). They estimated that gluconeogenesis, calculated as EGPR−glycogenolysis, was about 70% of EGPR in fasted healthy subjects.

The major observation of this study is that autoregulation of hepatic glucose production during lactate infusion is attained without any decrease of the contribution of hepatic glycogenolysis to EGPR. Surprisingly, total gluconeogenesis (as measured from indirect calorimetry and breath ($^{13}CO_2$) failed to increase significantly when lactate was infused. This contrasts with the report by Jenssen *et al.* (1990) which indicated that lactate conversion into glucose (as documented from plasma ^{14}C glucose synthesis during infusion of ^{14}C lactate) increased markedly during lactate infusion. The most likely explanation for this apparent discrepancy is that the methodology used in the present experiments provides an estimate of total gluconeogenesis (i.e. from glycerol, amino acids, exogenous lactate and from part of endogenous lactate as discussed below), whereas the ^{14}C lactate infusion only measures glucose synthesis from the labelled lactate. Consequently, the constancy in total hepatic gluconeogenesis during lactate infusion, observed in the present study, may indicate that an increased lactate conversion into glucose was compensated by a decrease in glucose synthesis, from amino acids and glycerol. These observations support the hypothesis by Jahoor *et al.* (1990) that an increased gluconeogenesis from one particular gluconeogenic substrate suppresses gluconeogenesis from other substrates, and that such a feedback mechanism takes part in the autoregulation of hepatic glucose production. Because the number of subjects tested was small, and considerable inter-individual variations were observed, we cannot exclude that inhibition of glycogenolysis did occur to some extent. Such an inhibition, although small, was indeed apparent during administration of lactate and β-adrenergic blockade.

Another mechanism which may account for the autoregulation of hepatic glucose production is an alteration of the relative rates of glycogen synthesis and breakdown which are known to take place simultaneously in liver cells (Magnusson *et al.*, 1994; Tounian *et al.*, 1994). Net hepatic glycogen storage may occur through an increase in glycogen synthesis with no change in glycogenolysis. We have suggested that such a stimulation of glycogen synthesis may play a role in the autoregulation of hepatic glucose production during infusion of fructose (Tounian *et al.*, 1994). Such an enhanced glycogen synthesis, together with the afore mentioned inhibition of glucose synthesis from non-lactate precursors, may also participate in the autoregulation during infusion of lactate. This pathway was however not quantified in the present experiments.

The present results also indicate that changes in the activation of the β-adrenergic receptors are not involved in the autoregulation of hepatic glucose production during

the infusion of lactate. This conclusion is based on two sets of observations. Firstly, β-adrenergic blockade did not affect the basal rate of hepatic glucose production, nor the relative contributions of gluconeogenesis and glycogenolysis in the basal state (protocol 3); this suggests that basal SNS activity is not a major regulator of hepatic glucose metabolism in post-absorptive humans. Secondly, hepatic glucose production remained constant when lactate was infused, both without (protocol 1) and with (protocol 2) β-adrenergic blockade. A decrease in glycogenolysis was not involved in this autoregulation as shown by our isotopic procedure to measure glycogenolysis and gluconeogenesis. This clearly indicates that the autoregulation of hepatic glucose production is not attained by an inhibition of a β-adrenergically mediated glycogenolysis.

Infusion of lactate markedly increased resting energy expenditure, as already reported (Chioléro *et al.*, 1993; Ferrannini *et al.*, 1993). The present experiments indicate that a significant portion of lactate induced thermogenesis is sympathetically mediated because it was reduced by about 40% during β-adrenergic blockade. This is indirect evidence that SNS activity was increased during infusion of lactate and, therefore, is also evidence against the hypothesis that inhibition of basal SNS activity may have participated in the autoregulation of EGPR.

This interpretation is based on the assumption that the infusion of propranolol used in the present experiments effectively inhibited β-adrenergic receptors. This assumption is supported by the report that β-blockers at similar doses totally abolished the vasodilatory effects of β-adrenergic agonists (Randin *et al.*, 1994). In addition, a significant decrease in heart rate was observed during propranolol infusion.

In humans the metabolic effects of catecholamines on the liver are mainly mediated by β-adrenergic mechanisms (Deibert & DeFronzo, 1980; Rizza *et al.*, 1980). However, α-adrenoceptor stimulation has also been shown to stimulate EGPR (Rosen *et al.*, 1983), and the observations presented here do not exclude a participation of SNS in the regulation of hepatic glucose production through α-receptors during lactate infusion.

Some limitations of the methodology used to determine fractional glycogenolysis and gluconeogenesis are to be kept in mind. This methodology takes advantage of the fact that, under the experimental conditions used, endogenous glycogen is the only source of excess ^{13}C labelled substrate. It therefore allows us to estimate, with confidence, gluconeogenesis from unlabelled amino acids and glycerol. Lactate on the other hand, may originate from hepatic glycogen, through glycolytic degradation of plasma glucose in peripheral tissues. Gluconeogenesis from such a glucose derived lactate should therefore result in glucose with a ^{13}C enrichment close to plasma glucose (there is a loss of label at the level of oxaloacetate and at the level of the PEPCK reaction). This cycling between plasma glucose and lactate will therefore have little effect on plasma glucose enrichment, but will be accounted for in the determination of EGPR with 6,6-^{2}H glucose. Consequently, only the rate of glucose lactate cycling time, the fractional gluconeogenic rate, will be accurately measured as gluconeogenesis, while the remaining will be attributed to glycogenolysis. A numeric example may help to clarify this feature of our methodology. Let us assume that net glycogenolysis and gluconeogenesis from amino

acids and glycerol both proceed at a rate of 5 μmol/kg/min. Fractional gluconeogenesis will be assessed at 0.5%. Let us further assume that cycling between plasma glucose and lactate/alanine proceeds at a rate of 2 μmol/kg/min. This process will not alter ^{13}C glucose enrichment, nor will it alter the estimation of hepatic ^{13}C glycogen; and hence will not alter fractional gluconeogenesis. Total EGPR is 5 μmol/kg/min net glycogenolysis+5 μmol/kg/min net gluconeogenesis+2 μmol/kg/min glucose cycling=12 μmol/kg/min. Using our calculations, glycogenolysis and gluconeogenesis will each be 12×0.5=6 μmol/kg/min. In other words, only a fraction of the cycling between lactate/alanine and glucose (equal to the rate of cycling×fractional gluconeogenesis) will be computed as gluconeogenesis. The same considerations apply for lactate issued from muscle glycogen degradation, although to a lesser extent, because muscle glycogen enrichment has been shown to be somewhat lower than hepatic glycogen enrichment in this experimental setting (Gay *et al.*, 1994a).

In spite of this limitation of the methodology, the absence of any detectable change in glycogenolysis and gluconeogenesis during β-blockade speaks against basal β-adrenergic activity being a major regulator of glucose/lactate cycling in healthy humans. Considerable day-to-day variation in fractional glycogenolysis and gluconeogenesis was observed in these experiments. The explanation for this variation remains unclear. It is possible that gluconeogenesis and glycogenolysis are extremely sensitive to relatively slight alterations of dietary composition and physical activity (two parameters which were not strictly controlled in this study). Alternatively, we cannot discard the possibility that minor inaccuracies in the determination of respiratory exchange ratio may have led to relatively large miscalculation of the ^{13}C hepatic glycogen enrichment. Such an error would lead to inaccurate estimations of the absolute rates of gluconeogenesis and glycogenolysis. It would not, however, not invalidate the assessment of relative changes of glycogenolysis and gluconeogenesis over time.

In conclusion, the results presented in this study suggest that autoregulation of hepatic glucose production during lactate infusion occurs without a reduction in glycogenolysis. An inhibition of gluconeogenesis from other gluconeogenic precursors occurs, and a stimulation of hepatic glycogen synthesis might possibly participate in this regulation. The significant diminution of the thermic effect of lactate during β-blockade indicates that sympathetic nervous system activity (SNS) is increased during lactate infusion. However, SNS activity does not seem to be involved in the regulation of hepatic glucose production as β-adrenergic blockade does not alter hepatic glucose production, neither in the basal state, nor during lactate infusion. Furthermore, β-adrenergic blockade does not alter glycogenolysis and total gluconeogenesis in healthy subjects.

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References

BURNIER P., TAPPY L., JÉQUIER E., SCHNEEBERGER D. & CHIOLÉRO R. (1992) Metabolic and respiratory effects of infused sodium acetate in healthy human subjects. *Am J Physiol*, **263**, R1271–R1276.

CHERRINGTON A. D., FUCHS H., STEVENSON R. W., WILLIAMS P. E., ALBERTI K. G. M. M. & STEINER K. E. (1984) Effect of epinephrine on glycogenolysis and gluconeogenesis in conscious overnight-fasted dogs. *Am J Physiol*, **247**, E137–E144.

CHIOLÉRO R., MAVROCORDATOS P., BURNIER P., CAYEUX M.-C., SCHINDLER C., JÉQUIER E. & TAPPY L. (1993) Effects of infused sodium acetate, sodium lactate, and sodium β-hydroxybutyrate on energy expenditure and substrate oxidation rates in lean humans. *Am J Clin Nutr*, **58**, 608–613.

DEIBERT D. C. & DEFRONZO R. A. (1980) Epinephrine-induced insulin resistance in man. *J Clin Invest*, **65**, 717–721.

DURNIN J. V. G. A. & 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.

FALOONA G. R. & UNGER R. H. (1974) Glucagon. In: *Methods of Hormone Radioimmunoassay* (eds Jaffe B. M. and Behrman H. R.), pp. 317–330. Academic Press, New York.

FELIG P., WAHREN J. & HENDLER R. (1978) Influence of maturity-onset diabetes on splanchnic glucose balance after oral glucose ingestion. *Diabetes*, **27**, 121–126.

FERRANNINI E., NATALI A., BRANDI L. S., BONADONNA R., DE KREUTZEMBERG S. V., DEL PRATO S. & SANTORO D. (1993) Metabolic and thermogenic effects of lactate infusion in humans. *Am J Physiol*, **265**, E504–E512.

GAY L.-J., SCHUTZ Y., DIVETTA V., SCHNEITER P., TAPPY L. & JÉQUIER E. (1994a) Measurement of $^{13}CO_2$ in expired air as an index of compliance to a high carbohydrate diet naturally enriched in ^{13}C. *Int J Obes*, **18**, 591–595.

GAY L. J., SCHNEITER P., SCHUTZ Y., DI VETTA V., JÉQUIER E. & TAPPY L. (1994b) A non invasive assessment of hepatic glycogen kinetics and post-absorptive gluconeogenesis in man. *Diabetologia*, **37**, 517–523.

HERBERT V., LAU K. S., GOTTLIEB C. W. & BLEICHER S. J. (1965) Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab*, **25**, 1375–1384.

HETENYI G. J. (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.

HOHORST H. (1965). Lactate. In: *Methods of Enzymatic Analysis* (ed. Bergmeyer H. U.). pp. 266. Academic Press, New York.

JAHOOR F., PETERS E. J. & WOLFE R. R. (1990) The relationship between gluconeogenic substrate supply and glucose production in humans. *Am J Physiol*, **258**, E288–E296.

JENSSEN T., NURJHAN N., CONSOLI A. & GERICH J. E. (1990) Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. *J Clin Invest*, **86**, 489–497.

JÉQUIER E., ACHESON K. & SCHUTZ Y. (1987) Assessment of energy expenditure and fuel utilization in man. *Annu Rev Nutr*, **7**, 187–208.

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.

MAGNUSSON I., ROTHMAN D. L., KATZ L. D., SHULMAN R. G. & SHULMAN G. I. (1992) Increased rate of gluconeogenesis in type II diabetes mellitus. A ^{13}C nuclear magnetic resonance study. *J Clin Invest*, **90**, 1323–1327.

MAGNUSSON I., ROTHMAN D. L., JUCKER B., CLINE G. W., SHULMAN R. G. & MAGNUSSON G. I. (1994) Liver glycogen turnover in fed and fasted humans. *Am J Physiol*, **266**, E796–E803.

RANDIN D., VOLLENWEIDER P., TAPPY L., JÉQUIER E., NICOD P. & SCHERRER U. (1994) Effects of adrenergic and cholinergic blockade on insulin-induced stimulation of calf blood flow in humans. *Am J Physiol*, **266**, R809–R816.

RIZZA R. A., CRYER P. E., HAYMOND M. W. & GERICH J. E. (1980) Adrenergic mechanisms for the effects of epinephrine on glucose production and clearance in man. *J Clin Invest*, **65**, 682–689.

ROBERT J. J., KOZIET J., CHAUVET D., DARMAUN D., DESJEUX J. F. & YOUNG V. R. (1987) Use of ^{13}C-labeled glucose for estimating glucose oxidation: some design considerations. *J Appl Physiol*, **63**, 1725–1732.

ROSEN S. G., CLUTTER W. E., SHAH S. D., MILLER J. P., BIER D. M. & CRYER P. E. (1983) Direct alpha-adrenergic stimulation of hepatic glucose production in human subjects. *Am J Physiol,* **245,** E616–E626.

STEELE R. (1959) Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci,* **82,** 420–430.

TAPPY L., OWEN O. & BODEN G. (1988) Effect of hyperinsulinemia on urea pool size and substrate oxidation rates. *Diabetes,* **37,** 1212–1217.

TOUNIAN P., SCHNEITER P., HENRY S., JÉQUIER E. & TAPPY L. (1994) Effects of infused fructose on endogenous glucose production, gluconeogenesis and glycogen metabolism in healthy humans. *Am J Physiol,* **267,** E710–E717.

WEBBER J. & MACDONALD I. A. (1993) Metabolic actions of catecholamines in man. *Baillieres Clin Endocrinol Metab,* **7,** 393–413.