Glucose Metabolism Studied Isotopically in Diabetic Dogs: Effect of Restoration of Peripheral Normoinsulinaemia by the Artificial B Cell

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Summary. Normoglycaemia, peripheral normoinsulinaemia, and normoglucagonaemia were restored acutely in chronically diabetic dogs, using an extracorporeal artificial B cell with peripheral venous insulin administration. Glucose metabolism was analysed by a non-steady-state tracer technique with double-labelled glucose ($6\text{-}^3\text{H}$ - and $\text{U-}^{14}\text{C}$ -glucose), and the incorporation of the ^{14}C label into plasma lactate was determined. In the basal state, glucose turnover rates were not different from those in non-diabetic controls; but recirculation of the glucose-C label through the Cori cycle, and lactate labelling from glucose utilization were decreased. The glycaemic response to an intravenous infusion of non-labelled glucose was distinctly enhanced. This was based on a reduction

in the rates of glucose disappearance. Its rates of appearance (total endogenous glucose production) were, however, suppressed to a normal extent by the exogenous glucose. Accordingly carbon recycling was nearly totally suppressed during the glucose infusion as in the controls. It is concluded that metabolic recompensation in these fasting, resting diabetic dogs remained incomplete because the interval of normoinsulinaemia, which obviously applied only to the peripheral circulation, was not long enough.

Key words: Diabetic dog, artificial B cell, glucose metabolism, tracer kinetics in vivo, lactate, carbon recirculation.

Several hormonal and metabolic parameters in circulating blood can be restored to normal in insulin-dependent diabetic subjects by short-term closed-loop blood glucose control for up to 24 h using an extracorporeal artificial B cell, e.g. the fasting concentrations of ketones [1], some amino acids [17, 19], glucagon [2, 19] and the responses to different food or glucose stimuli [1, 2, 44]. Other metabolites, however, remain abnormal after such short-term restoration of normoglycaemia. Thus the concentrations of lactate, pyruvate [32], and alanine [1, 19] remain elevated, those of non-esterified fatty acids are frequently reduced [1, 2, 17], and ketone bodies respond more intensely to feeding [44].

It is not yet clear whether the isotopically measured glucose turnover in vivo is also normalized when normoglycaemia is acutely restored in diabetes. After meals it was distinctly elevated in dogs treated with an artificial B cell [37]. But in exercising diabetic patients on the artificial B cell, the pattern of endogenous glucose production was normal [43]. However, reduced rates of recirculation of the ^{14}C label from the blood-glucose pool after meals were reported by Nosadini et al. [32]. All these studies were performed during peripheral administration of insulin, and there was always a certain degree of hyperinsulinaemia, as reported by several au-

thors [1, 2, 19, 22]. Peripheral normoinsulinaemia during such treatment with an extracorporeal artificial B cell has been made possible by an algorithm for glucose-dependent insulin dosage based on regression analysis of the physiological glucose: peripheral plasma insulin relationship [10]. Thus the aim of this study was to analyse the degree of normalization of isotopically measured glucose turnover, of carbon recirculation, and of lactate metabolism in diabetic dogs with peripheral normoinsulinaemia restored and maintained by an artificial B cell.

Material and Methods

Animals

Nine diabetic Alsatian dogs (aged 34 ± 4 months, duration of diabetes 9 ± 2 months, body weight 24 ± 1 kg) and nine non-diabetic dogs (aged 36 ± 4 months, body weight 28 ± 1 kg) were studied. Three of the diabetic animals had been used previously in the control study before diabetes.

Experimental diabetes was produced by partial pancreatectomy and subsequent infusion of streptozotocin into the superior pancreaticoduodenal artery as detailed by Freyse et al. [16]. Based on sequential blood glucose measurements, these animals were treated with three SC injections a day of monocomponent porcine insulin

(dose $1.6 \pm 0.2 \text{ IU} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). The total loss of endogenous B cell function was proved by canine C-peptide analyses in peripheral plasma. The animals lacked anti-insulin antibodies. Production of diabetes and animal handling have been detailed by Fischer et al. [10].

Experimental Design

The animals were well accustomed to the experimental conditions. During the tests they stood unrestrained in Pavlov frames of our own manufacture. On the morning of the experimental day, percutaneous prepared catheters were inserted (1) through a saphenous vein in the inferior caval vein for the infusions (Teflon Portex Intravenous Tubing, Hythe, Kent, UK); and (2) through a jugular vein in the superior caval vein for blood sampling (Cavafix RMT catheters, B. Braun-Melsungen, Melsungen, FRG).

In addition they had an indwelling polyethylene cannula (Volks-eigener Betrieb Kombinat Medizin-, Labor-und Wägetechnik, Kombinatsteil Medizinplaste, Lichtenberg, GDR) in a leg vein for glucose monitoring during the glucose-controlled insulin infusions. Perfusor E syringe pumps (B. Braun-Melsungen, Melsungen, FRG) were used for all infusions. The animals received a basal infusion of 0.5 ml/min sodium chloride (0.154 mol/l), supplemented according to protocol with the tracer solution (0.1 ml/min), with the test solution of unlabelled glucose (1.0 ml/min), and in the diabetic animals with the insulin solution (240 mU/ml). Insulin was delivered at 2.5 min intervals in pulses of constant rate ($0.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) but of variable duration by a laboratory extracorporeal artificial B cell of our own design [10]. The delay between sampling for glucose analysis and subsequent insulin dosage was 2 min . The algorithm parameters a_i for insulin dosage were obtained by regression analysis of normal glucose and plasma insulin data [10] according to

$$\text{dose} = a_0 + a_1(G_{t+\Delta t} - G_b) + a_2(G_{t+\Delta t} - G_t) \quad (I)$$

where G is the glucose concentration, and the subscripts denote the time t , the interval Δt , and the normal fasting basal state b . a_0 is the basal dose which was infused during each 2.5 min interval. Every other interval a new dose was calculated depending on $G_{t+\Delta t}$ and G_t and was infused in addition to a_0 .

Infusates

The tracer solution consisted of freshly chromatographed D-U- ^{14}C -glucose (specific activity 9.916 MBq/mmol) and D-6- ^3H -glucose (1.369 GBq/mmol), obtained from the Radiochemical Centre, Amersham, Bucks, UK. It was diluted with 50 ml NaCl (0.154 mol/l) to a final concentration of 185 kBq/ml ^{14}C and 740 kBq/ml ^3H . The unlabelled glucose was diluted with sterile distilled water to give the indicated dosage referred to the body weight (average concentration 250 mg/ml). The insulin solution contained 240 mU/ml of a short-acting monocomponent insulin (Novo Industri, Copenhagen, Denmark) in NaCl (0.154 mol/l) supplemented with 10% v/v sterile mixed normal homologous serum. All glucose solutions were freshly prepared at the beginning of the experiment. The insulin infusate was prepared 16 h before the experiment.

Protocol

The diabetic animals were treated with the artificial B cell from 06.00 h , i.e. 12 h after their last meal and last SC insulin injection. After normoglycaemia had been established, the constant tracer infusion ($18.5 \text{ kBq } ^{14}\text{C}$ - and $74 \text{ MBq } ^3\text{H}$ -glucose/min) began with a priming dose of $925 \text{ kBq } ^{14}\text{C}$ - and of $3.7 \text{ MBq } ^3\text{H}$ -glucose, respectively. The control animals were identically infused but without any insulin administration. One animal of each group was not infused with double-labelled glucose but with ^{14}C -labelled glucose only.

From 150 to 330 min after the beginning of the tracer infusion, an IV load of unlabelled glucose ($56.67 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was administered, followed by another control period of at least 75 min . Insulin

dosage by the artificial B cell was $0.32 \pm 0.05 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ during normoglycaemia before the glucose infusion test, and $320 \pm 34 \text{ mU} \cdot \text{kg}^{-1} \cdot (180 \text{ min})^{-1}$ to compensate for the glucose infusion test.

In an initial study, six diabetic animals were subjected to the tracer-kinetic experiment for 3 h in the untreated fasting state (i.e. sub-decompensated). Only three received double-labelled glucose.

Blood samples for measurement of hormone and metabolite concentrations and specific activities were taken immediately before the tracer infusion and from 60 min at intervals of 15 min (exception: the glucagon concentration was analysed only five times during the experiment). The entire blood loss during one experiment was about 250 ml ; the hematocrit was 0.39 ± 0.08 and 0.37 ± 0.10 (mean $\pm$ SD) at the beginning and at the end of the experiment, respectively.

Analyses

Aliquots of blood (8 ml) were sampled in chilled tubes prepared with 500 U heparin (Weddel Pharmaceuticals, London, UK). When glucagon was to be determined, an additional sample of 8 ml blood was prepared with 100 U heparin, 4000 U of a proteinase inhibitor (Bayer, Leverkusen, FRG), and 200 ng NaN_3 . All samples were centrifuged immediately at 4°C and stored at -178°C until analysis. Glucose concentrations in plasma and urine (Beckman analyzer), blood total ketone bodies (measured as acetone) [29], and the immunoreactive insulin (IRI) [18] and glucagon (IRG) [42] activities in plasma were analysed. A double-antibody technique using guinea-pig anti-glucagon antiserum No. 43 (Zentralinstitut für Diabetes "Gerhardt Katsch", Karlsburg, GDR) and the World Health Organization pancreatic glucagon calibration standard No. 69/194 were used for the IRG assay. There was a 5% cross-reaction of the antibody used with porcine entero-glucagon-like immunoreactivity.

To calculate specific activities, the concentrations of glucose, lactate and pyruvate were determined spectrophotometrically [modified according to 3] in neutralized HClO_4 plasma extract and in the chromatographed samples (see below) using the following substances: hexokinase (E.C. 2.7.1.1., Boehringer, Mannheim FRG), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49, Ferak, West Berlin), lactate dehydrogenase (E.C. 1.1.1.27, Volkseigener Betrieb Arzneimittelwerk Dresden, GDR), NADP and NADH_2 (Volkseigener Betrieb Arzneimittelwerk Dresden, GDR), ATP (Boehringer, Mannheim, FRG).

Triplicates of the ^3H -glucose activity were determined from the extract of 1 ml plasma with 0.2 ml HClO_4 (3 mol/l). After freezing, 0.2 ml aliquots of the supernatant were lyophilized, re-dissolved with 1 ml distilled water, and mixed with 7 ml Bray's scintillator fluid [5]. For the determination of the ^{14}C -glucose and -lactate activities, 2.0 ml plasma were extracted with 2.0 ml HClO_4 (0.5 mol/l) and neutralized with 5 mol/l K_2CO_3 . Duplicates of 1 ml supernatants were chromatographed on minicolumns (5 ml , Quick-Sep, Panchem, Kleinwallstadt, FRG) with Dowex 1X8 ($200\text{--}400$ mesh, Serva, Heidelberg, FRG). The glucose was eluted with 4 ml 0.005 mol TRA-HCl buffer, pH 8.1 [21]. After an additional rinsing with the same buffer the lactate was eluted with 12 ml of a $1:1$ mixture of 0.15 mol NH_4Cl and 0.005 mol borate buffer, pH 8.1. Lactate activity was measured after lyophilization and re-dissolution of the eluate. All activity-counting was carried out over 10 min using a Tricarb Scintillation Counter (Liquid Scintillation Spectrometer Model 2650 Packard, Vienna, Austria) with internal standardization. Recovery after the entire procedure was $92 \pm 1\%$ ($n=25$) for glucose and $75 \pm 2\%$ ($n=28$) for lactate respectively. To determine the dose of labelled glucose really infused, $10 \text{ } \mu\text{l}$ of the infusate was added to 0.25 ml fasting plasma of the same animal, deproteinized, lyophilized, re-dissolved, and counted. The effective dose resulted from the ^{14}C and ^3H counts and the infusion rates.

Calculations

The measured concentration patterns of glucose and lactate, and of their radioactivities were stepwise (over five subsequent values) fitted to a third order polynomial, allowing for the inhomogeneities at the

Table 1. Metabolic parameters during the fasting steady state in normal and untreated diabetic dogs

	Control dogs (n=8)	Diabetic dogs (n=6)
Plasma glucose (mmol/l)	5.18 ± 0.16	21.60 ± 1.40 ^a
Plasma insulin (nmol/l)	0.043 ± 0.010	0.013 ± 0.007 ^{a,b}
Plasma glucagon (nmol/l)	0.018 ± 0.003	0.042 ± 0.007 ^{a,c}
Plasma lactate (mmol/l)	0.56 ± 0.07	0.98 ± 0.09 ^a
Blood total ketone bodies (mmol/l)	0.18 ± 0.03	1.37 ± 0.30 ^{a,c}
R _a - ¹⁴ C-glucose (μmol · kg ⁻¹ · min ⁻¹)	16.33 ± 0.94	17.06 ± 0.24 (n=3)
R _a - ³ H-glucose (μmol · kg ⁻¹ · min ⁻¹)	25.89 ± 2.47	47.23 ± 7.87 ^a
Recirculation of the C-label (%)	37 ± 3	58 ± 8
Lactate from glucose (%)	49 ± 7	100 ± 0 (n=3)

Results expressed as mean ± SEM

Average of four consecutive 15 min periods when the plasma glucose concentration did not differ by > 2%. Last meal and last SC insulin injection was administered to diabetic dogs 12 h before the experiment. ^a *p* < 0.05; ^b not significantly different from 0; ^c one fasting value per animal only

beginning and the end of the glucose infusion test. For calculating the glucose flux rates, Steele's monocompartmental model of non-steady-state kinetics was used [38, 39]:

$$R_a = [R^* - p \cdot V \cdot \bar{G} \cdot \Delta a / \Delta t] \cdot 1 / \bar{a} \quad (\text{II})$$

$$R_d = R_a - p \cdot V \cdot \Delta G / \Delta t \quad (\text{III})$$

where R_a, R_d = rates of glucose appearance and disappearance respectively within the assumed distribution space [μmol · kg⁻¹ · min⁻¹]; $\bar{G}$, $\bar{a}$ = averages of the concentration [mmol/l] and of the specific activity [disintegrations per minute/μmol] = [DPM/μmol] of glucose during the interval Δt = 15 min; ΔG, Δa = changes in the concentration and in the specific activity during the interval Δt = 15 min; R* = tracer dose infused [DPM · kg⁻¹ · min⁻¹]; V = apparent distribution space of glucose, 200 ml/kg according to own previous observations with no difference between diabetics and controls; *p* = 0.65,

pool fraction of the glucose space [7, 33]. The recirculation rates of the carbon level were estimated from the experiments using double-labelled glucose. It is the R_a-difference between the estimates from the ¹⁴C- and from the ³H-specific activities, given as percentage of the ³H-glucose flux rates [9, 24, 27, 28].

Lactate labelling resulting from glucose metabolism is according to Forbath et al. [12].

$$\text{Percentage lactate from glucose} = (a_{\text{lac}} \cdot 2 \cdot 100) / a_{\text{gluc}} \quad (\text{IV})$$

where "a" denotes the respective specific activities. All calculations were done on a C 8205 computer (Volkseigener Betrieb Cellatron, Meiningen, GDR). Results are expressed as mean ± SEM, and the non-paired t-test (*p* < 0.05) was performed if appropriate.

Results

Fasting Untreated Diabetic Animals (Table 1)

Twelve h after their last meal and last SC insulin injection, the diabetic animals had near-zero plasma IRI concentrations and hyperglycaemia of approximately 20 mmol/l which remained steady for at least 4 h. Plasma glucagon and lactate concentrations and blood acetone were also slightly but significantly increased. Under these conditions, the steady-state glucose turnover (R_a = R_d) was normal from the ¹⁴C-glucose specific activities but considerably enhanced when estimated from the ³H-glucose data. This resulted in a distinct increase in rates of recirculation of the carbon moiety through the Cori cycle and in an increased labelling of the circulating lactate by glucose.

Glucose Turnover on the Artificial B Cell

Hyperglycaemia was corrected in all diabetic animals within 2–3 h after connection to the artificial B cell (see prototype experiment Fig. 1). During the subsequent glycaemic steady state, the tracer infusions were begun. Subsequently there was also a stable glycaemic pattern

Fig. 1. Experimental protocol in a diabetic dog. Upper panel: plasma glucose concentration. Lower panel: insulin dosage by the artificial B cell (Δt = 5 min). Horizontal line = infusion of double labelled glucose, horizontal bar = duration of the intravenous glucose load (56.57 μmol · kg⁻¹ · min⁻¹). From 10.50 h blood samples were taken for the preparation of the specific activities (Δt = 15 min).

Fig. 2. Plasma insulin and pancreatic glucagon concentrations in nine control (○—○) and nine diabetic dogs (●—●) on the artificial B cell before, during, and after a glucose infusion test [shaded area]. The mean values are identical where semicircles are presented

Fig. 3. Plasma glucose concentration and glucose flux rates as estimated from the specific activities of U-¹⁴C-glucose in nine normal (○—○, □) and nine diabetic dogs (●—●, ■) on the artificial B cell before, during, and after a glucose infusion test [shaded area]. To show the endogenous R_a , during the infusion of unlabelled glucose, the dose ($56.67 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was subtracted from the estimated total R_a . Mean $\pm$ SEM, SEM is not given where smaller than the symbols. The horizontal bars denoting flux rates are smaller than $\Delta t = 15$ min where the estimates for control and diabetic dogs are identical

in all experiments when unlabelled glucose was infused for 3 h. After initial correction the plasma curves of insulin and pancreatic glucagon concentrations did not differ between the normal and diabetic animals on the artificial B cell during the entire experiment (Fig. 2).

The average glycaemia before glucose infusion test was also identical in the two groups of animals but during the steady-state phase of the exogenous parenteral glucose load, it was higher by 1–2 mmol/l, and normalization was also delayed in the diabetic dogs on the artificial B cell (Fig. 3). Thus the integrated glycaemic response area was 985 ± 92 in relation to $555 \pm 61 \text{ mmol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ in the normal animals ($p < 0.05$). This difference cannot be explained by any insufficiency in the suppression of endogenous glucose production by exogenous glucose in the diabetic animals (Fig. 3). It was nearly totally suppressed between 0 and 60 min of the glucose infusion test in both experimental groups. From 90 min the endogenous R_a was incompletely restored (significantly different from zero, $p < 0.05$) and finally a new steady state was established at a low level. There was again no difference between the two groups of animals in the post-glucose infusion test rates of glucose production.

However, R_d was slightly reduced in the diabetic animals during the glucose infusion test. It was 9.26 ± 0.60 in relation to $10.99 \pm 0.40 \text{ mmol} \cdot \text{kg}^{-1} \cdot (180 \text{ min})^{-1}$ during the entire test in the controls ($p < 0.05$). It was mainly the dynamics of R_d that were altered in the diabetic dogs (Fig. 3): both the initial increase and the post-glucose infusion test decrease are delayed. The differences in glucose utilization were not caused by differences in the renal handling of glucose. Glucosuria was 37 ± 11 (diabetic) and $17 \pm 5 \mu\text{mol/kg}$ (controls) respectively during the entire experiment of at least 8 h (NS) and thus contributed by $< 0.2\%$ to the total glucose disappearance from its distribution space.

Carbon Recirculation and Lactate Metabolism

There was a significant reduction in the basal rate of carbon recycling through the Cori cycle in the diabetic animals appropriately supplied with insulin. This was reproduced after the glucose infusion test. It was however totally suppressed in both groups of animals (Fig. 4) when endogenous glucose production was more or less inhibited during the glucose infusion test (Fig. 3).

Also, the basal lactate levels were not completely restored in the diabetic animals after the short duration of initial insulin administration. Their response to the glucose infusion test was, however, normal as the reactive areas of 115 ± 29 (diabetics) and $92 \pm 15 \text{ mol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ (controls, NS). This applies also to the lactate: pyruvate ratios. The part of circulating lactate arising from glucose metabolism was normalized in the diabetic animals before the glucose infusion test. During the test it was only transiently diminished when the glucose utilization R_d was distinctly reduced (Figs. 3 and 4).

Fig. 4. Percentage recirculation of the glucose carbon label ($n=8$), percentage of circulating lactate coming from glucose ($n=9$), plasma lactate concentration and lactate/pyruvate ratio (L/P) in normal (Δ — Δ) and in diabetic dogs on the artificial B cell ($\blacktriangle$ — $\blacktriangle$). The mean values are identical where semi-triangles are presented. *Significant differences between the groups. During the infusion of unlabelled glucose [shaded area], the kinetics of $6\text{-}^3\text{H}$ -glucose and of $\text{U-}^{14}\text{C}$ -glucose gave identical flux rates thus resulting in zero percentage recirculation

Discussion

There are contradictory data on the flux rates of glucose in vivo in hypoinsulinaemic diabetic dogs. Based on ^{14}C -glucose data, these have been found normal [26] but experiments with tritiated glucose have usually shown elevated levels [20, 25]. This corresponds to the results of this study: the untreated hypoinsulinaemic, hyperglucagonaemic dogs showed steady-state fasting hyperglycaemia as observed by other authors in patients [31], and they had an increased rate of appearance as calculated from the ^3H -glucose specific activities but a normal rate of appearance as calculated from the ^{14}C -glucose specific activities, thus resulting in increased recirculation rates. This resulted also in an increase of the lactate levels and of the contribution of glucose metabolism to lactate production as was similarly published by Hetenyi et al. [20].

The values for carbon recycling in healthy dogs are identical to the 40% given by DeFronzo et al. [8]. Reichard et al. [35] and Nosadini et al. [32] reported values of half that magnitude from their tracer injection experiments. Nosadini et al. [32] also showed a reduced carbon recirculation in diabetics under artificial B cell control. The differences may be explained by the different tracer techniques. Very similar rates of lactate carbon

formation from circulating glucose were given by Kreisberg et al. [30] for human beings and Forbath and Hetenyi [12] for intact dogs. But both groups pointed to great variations due to stress sensitivity of the lactate metabolism.

The model of animal diabetes used in this study is characterized by a total lack of pancreatic insulinogenic function but by an at least partially preserved exocrine pancreatic activity [16]. According to Blazquez et al. [4] it is, however, to be expected that in dogs the effective A cell function remains near-normal after this procedure, since gastric glucagon has apparently the same biological function. Accordingly there was a certain basal hyperglucagonaemia in these animals in the morning when they needed a new insulin supply. This hyperglucagonaemia was reversed during the glucose-controlled insulin infusion.

The main part of this study was performed after acute restoration and maintenance of peripheral normoinsulinaemia. Pre-programmed insulin infusion overnight can produce euglycaemia early in the morning. But the influence of initial hyperinsulinaemia can, however, be excluded, because after the glucose infusion test the same basal metabolic situation appeared as before.

So far it is not known whether normoinsulinaemia is really important for the long-term therapeutic effect of an artificial B cell, and in general hyperinsulinaemia has been observed in short-term studies [1, 2, 13, 19, 22, 41].

Plasma pancreatic glucagon levels were also normalized in the animals reported here. Thus the differences observed in glucose metabolism between the control and diabetic dogs on the artificial B cell cannot be ascribed to any abnormality in peripherally available pancreatic glucoregulatory hormones. Basal glucose flux rates were also normalized in diabetic dogs under short-term artificial B cell treatment as shown by other authors [32, 37, 43]. But the recirculation of the carbon label was significantly lower than the 30%–40% observed in the control dogs in this study and of other investigations [8, 14, 15, 23, 28, 30, 32]. These findings and the slightly increased plasma lactate levels point to a decreased turnover of lactate. Thus the Cori cycle activity still seems to be inhibited in these resting diabetic animals, which were apparently sufficiently treated with insulin. Since the total rate of glucose utilization was essentially normal, one might speculate that the oxidative glucose metabolism and/or the formation of glycogen are relatively enhanced.

In addition, the utilization of the exogenous parenteral glucose load is also not completely normalized in these animals. On the one hand, endogenous glucose production is suppressed and finally directed to a new steady state level [24] as it is in non-diabetic controls. Accordingly, there is no recirculation of the carbon label [34]. On the other hand, the rate of disappearance shows a delayed and slightly diminished response to the

glucose infusion and to the related insulin administration, as has similarly been found by Kalant et al. [26] in non-insulin dependent diabetic patients.

The artificial B cell algorithm parameters used in this study were estimated before the experiments by regression analysis of simultaneously measured normal peripheral venous glucose and insulin data according to formula I [10]. They were used to control the glucose-dependent insulin dosage without having been individualized. The insulin dose pattern applied by the artificial B cell resulted in peripheral normoinsulinaemia but not in normal patterns of the isotopically studied glucose metabolism. One reason for this might be that the renormalization of glucose oxidation in diabetic patients is an inducible process which needs at least 3 days after connection to the artificial B cell [13].

In the experiments reported here administration of insulin into the peripheral circulation has clearly only normalized peripheral venous concentrations of insulin and glucagon. It can be assumed that there is also no normal portal-peripheral concentration gradient of insulin under these conditions since we have measured the concentration resulting both from peripheral administration and from catabolism, binding, and excretion of insulin. Thus we can expect portal venous insulin concentrations similar to those in the peripheral blood.

Thus the normal behaviour of endogenous glucose production in the diabetic animals reported on here might be explained additionally by the fact that the action of insulin on the rate of appearance is controlled by lower half-maximal plasma insulin concentrations as is its action on the rate of disappearance [36]. Accordingly, in terms of its action on the glucose disappearance rate, a certain peripheral insulin-insensitivity is to be assumed. The overall result of these conditions would be a hepatic insulinization sufficient to suppress the rate of glucose appearance to a normal extent and to reduce the lactate re-utilization to subnormal rates. But the normal peripheral insulin levels are insufficient – in this particular situation – to provide normal kinetics of utilization for an exogenous glucose load. It should be taken into consideration that the relation between hepatic and extrahepatic glucose utilization might be influenced by the route of insulin administration [40]. Thus peripheral-venous infusions, as performed in this study, allow for peripheral normoinsulinaemia but probably not for sufficient hepatic insulinization. This could have resulted both in a certain reduction of the hepatic part of the total rate of disappearance (Fig. 3) as shown by Cobelli and Ruggeri [6] employing computer techniques, of hepatic lactate utilization, and of the Cori cycle activity related to it (Fig. 4).

It has been shown by Goriya et al. [17] in a long-term study that these differences might be important in the practical application of insulin pumping systems. To achieve the same effect on the diurnal glycaemic profiles in diabetic dogs distinctly less insulin was required

via portal infusion than via the peripheral route. If, on the other hand, relatively high insulin doses are administered, no differences in the food-stimulated glucose flux rates appear between the two routes of glucose administration, independently of whether insulin dosage is closed-loop-controlled [37] or open-loop-administered in identical patterns [11]. From the data presented it is concluded that glucose turnover is not restored to normal by short-term restoration of peripheral normoinsulinaemia in insulin-dependent diabetes. Intra-portal insulin administration does not appear feasible with insulin pumps. Thus long-term observations are needed to determine whether complete normalization is actually necessary both in insulinaemia and glucose turnover, and – if so – whether individual adaptation of the insulin-dose parameters could compensate for the possible shortcomings of peripheral insulin administration.

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