Effect of Ketone Bodies on Glucose Production and Utilization in the Miniature Pig

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Abstract. The effect of ketone bodies on glucose production (R_a) and utilization (R_d) was investigated in the 24-h starved, conscious unrestrained miniature pig. Infusing Na-DL-β-OH-butyrate (Na-DL-β-OHB) and thus shifting the blood pH from 7.40 to 7.56 resulted in a decrease of R_a by 52% and of R_d by 45%, as determined by the isotope dilution technique. Simultaneously, the concentrations of arterial insulin and glucagon were slightly enhanced, whereas the plasma levels of glucose, lactate, pyruvate, alanine, α-amino-N, and free fatty acids (FFA) were all reduced. Infusion of Na-bicarbonate, which yielded a similar shift in blood pH, did not mimick these effects. Infusion of equimolar amounts of the ketoacid, yielding a blood pH of 7.35, induced similar metabolic alterations with respect to plasma glucose, R_a, R_d, and insulin; however, plasma alanine and α-amino-N increased.

Infusing different amounts of Na-DL-β-OHB resulting in plasma steady state levels of ketones from 0.25 to 1.5 mM had similar effects on arterial insulin and glucose kinetics. No dose dependency was observed.

Prevention of the Na-DL-β-OHB-induced hypoalaninemia by simultaneous infusion of alanine (1 μmol/kg × min) did not prevent hypoglycemia.

Infusion of Na-DL-β-OHB plus insulin (0.4 mU/kg × min) showed no additive effect on the inhibition of R_a. Ketones did not inhibit the insulin-stimulated metabolic clearance rate (MCR) for glucose. Infusion of somatostatin (0.2 μg/kg × min) initially decreased plasma glucose, R_a, and R_d, which was followed by an increase in plasma glucose and R_a; however, on infusion of somatostatin plus Na-DL-β-OHB, hypoglycemia and the reduced R_a were maintained.

In the anaesthetized 24-h starved miniature pig, Na-DL-β-OHB infusion decreased the hepatic exchange for glucose, lactate, and FFA, whereas the exchange for glycerol, alanine, and α-amino-N as well as liver perfusion rate were unaffected. Simultaneously, portal glucagon and insulin as well as hepatic insulin extraction rate were elevated. Leg exchange for glucose, lactate, glycerol, alanine, α-amino-N, and FFA were decreased, while ketone body utilization increased.

Repeated infusion of Na-DL-β-OHB at the fourth, fifth, and sixth day of starvation in the conscious, unrestrained mini-pig resulted in a significant drop in urinary nitrogen (N)-excretion. However, this effect was mimicked by infusing equimolar amounts of Na-bicarbonate. In contrast, when only the ketoacid was given, urinary N-excretion accelerated.

To summarize: (*a*) Ketone bodies decrease endogenous glucose production via an insulin-dependent mechanism; in addition, ketones probably exert a direct inhibitory action on gluconeogenesis. The ketone body-induced hypoalaninemia does not contribute to this effect. (*b*) The counterregulatory response to hypoglycemia is reduced by ketones. (*c*) As a consequence of the decrease in R_a, glucose utilization declines during ketone infusion. (*d*) The insulin-stimulated MCR for glucose is not affected by ketones. (*e*) Ketones in their physiological moiety do not show a protein-sparing effect.

Introduction

Ketotic hypoglycemia is the commonest variety of childhood hypoglycemia and accounts for 30–50% of the cases encountered in western clinical practice (1, 2). It occurs not only in children, but also in adults (2). Although it is known that ketotic hy-

In conducting the experiments described in this report, the investigators adhered to the Guiding Principles in The Care And Use Of Animals approved by the Council of The American Physiological Society. The facilities are fully accredited by the Gesundheitsbehörde der Freien und Hansestadt Hamburg, and veterinary supervision of the animals was supplied for the entire experimental period.

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poglycemia is not a single disease entity, ketones have been proposed to be in some way causative for hypoglycemia. The infusion of exogenous ketones has been widely used in searching for specific roles of ketone bodies in glucose homeostasis. Infusion of Na-DL-β-OH-butyrate (Na-DL-β-OHB)[1] reduced the rate of hepatic glucose production (3, 4, 5) as well as peripheral glucose uptake and oxidation (3, 5, 6). As to the mechanism responsible for the decrease in hepatic gluconeogenesis, the following hypotheses have been put forward: (*a*) a diminished hepatic precursor, i.e., alanine supply, possibly due to the proposed protein sparing action of ketones (1, 2, 7, 8); (*b*) ketone body-induced insulin secretion (5, 9, 10). With respect to the reduced muscular amino acid release, recent data demonstrate that the ketone-induced hypoalaninemic effect represents an experimental artifact related to the alkalinizing effect of infusing Na-DL-β-OHB (11). In fact, on infusing the free ketone acid, a rise in plasma alanine was observed; however, blood glucose decreased again (11).

On the other hand, the effect of insulin mediating the ketone-induced hypoglycemia was questioned by some clinical data (1, 12, 13), e.g., the hypoglycemic action of Na-DL-β-OHB also could be demonstrated in diabetics (14).

The present paper sets out to investigate the physiological effect of ketone bodies on glucose homeostasis. Our data clearly demonstrate (*a*) that ketones inhibit hepatic gluconeogenesis, (*b*) that this effect is predominantly mediated by insulin and is independent of the alanine supply, (*c*) that the counterregulatory response to hypoglycemia is reduced by ketones, and (*d*) that the proposed protein-sparing action of ketone bodies represents an experimental artifact relating to the alkalinizing effect of Na-DL-OHB infusion.

Methods

Animals. 14 male castrated miniature pigs (specific pathogen free, 28–32 kg body wt, 12-mo-old) were purchased (Versuchsgut Domäne Relliehausen, Göttingen, Federal Republic of Germany) about 4 wk before the experiments were started. The pigs were held under controlled conditions and kept on a standard chow diet as described previously (15, 16).

Surgical procedures. For experiments in the conscious, unrestrained pigs, polyvinyl catheters were implanted into the aortic arch, the vena cava superior, and one vena jugularis externa 8 d before the experiments (16). The complete recovery from surgery was checked by standard laboratory parameters (16). For measurement of liver and muscle metabolic balance, experiments were performed in five anaesthetized, 24-h starved miniature pigs. Anaesthesia was induced by intraperitoneal injection of 1.25 mg/kg body wt azaperon (Stresnil, Janssen, GmbH, Düsseldorf, Federal Republic of Germany) and 10 mg/kg body weight metomidat (Hypnodil, Janssen) followed by intratracheal intubation; anaesthesia was maintained with N_2O/O_2 (3:1) and supplementary doses of metomidat (0.1 mg/kg body wt) as described (17). In these experiments, polyvinyl catheters were inserted into the aortic arch, the vena cava superior, the vena jugularis externa, at the superior bulb of the vena jugularis interna, into the portal vein, the main stem of the hepatic vein of the left lobe, and via the right vena iliaca interna into the vena femoralis (16, 17). After finishing the operative procedures, the abdomen was closed and experiments were started after a recovery period of 60 min. In these experiments, an increase in serum creatine kinase (up to 70 μ/l) and lactate dehydrogenase (up to 240 μ/l) was observed. At the end of the experiments, animals were sacrificed, and the position of all catheters was verified (16, 17).

Experimental procedures. Experiments were performed after a starvation period of 24 h or up to 10 d. Labeled glucose was given via the vena cava superior, and all other substrates and hormones were given via vena jugularis externa. Patency of all catheters during the experiments was maintained by a constant infusion of 0.9% NaCl (0.1–0.2 ml/min); between the experiments, catheters were filled up exactly with a heparin solution (0.1 U/kg body wt). A primed (20 μCi)/continuous (0.20 μCi/min) infusion of [3-^{3}H] glucose (1 mCi/1 ml; New England Nuclear Corp., Dreieich, Federal Republic of Germany) was begun to permit isotopic determination of the rates of glucose production and utilization. Up to 120 min were allowed for isotope equilibration, steady state was reached usually between −20 min and 0 time as was reflected by the constancy in the concentration of labeled and unlabeled compounds in the blood. Na-DL-β-OHB (Sigma Chemicals, München, Federal Republic of Germany) was prepared immediately before the experiments as a 4% solution (pH 9.20; osmolality, 940 mosmol/kg H_2O) in sterile Ringer solution (Na^+, 147.2 mmol/liter; K^+, 4.0 mmol/liter; Ca^{++}, 2.25 mmol/liter; Cl^-, 155.7 mmol/liter; osmolality, 289 mosmol/kg H_2O). Using different concentrations of Na-DL-β-OHB, a 2, 4, 8, 12, or 16 g/100 ml solution was prepared; the total infusion volume was 226 ml/180 min or 450 ml/360 min. In some experiments, the pH of the ketone solution was adjusted to 7.4, 6.0, or 5.0 by HCl (0.1 M). The ketone solution was administered as a primed-constant infusion via a peristaltic infusion pump (Infusomat, Braun-Melsungen, Melsungen, Federal Republic of Germany). The priming dose was given over the initial 20-min period at twice the continuous infusion dose indicated in Figures and Tables. In order to produce increments in serum bicarbonate similar to that observed with the infusion of Na-DL-β-OHB, sodium bicarbonate was infused into four pigs at a dose of 1.5 mmol/kg × body wt. In addition, insulin (porcine insulin, Hoechst, Frankfurt, Federal Republic of Germany) or cyclic somatostatin (stilamin, Serono, Freiburg, Federal Republic of Germany) were given in a 250 mg/100 ml albumin solution (total volume, 50 ml) (porcine albumin, Sigma Chemical Co.) to prevent adherence to glassware and tubing. L-alanine (Merck, Darmstadt, Federal Republic of Germany) was prepared as a 1 g/100 ml solution in sterile Ringer-Solution (pH 6.17; osmolality, 395 mosmol/kg H_2O). All solutions were passed through a 22-μM filter apparatus (Millipore G.m.b.H. Neu-Isenburg, Federal Republic of Germany).

In long-term starvation experiments, pigs received Na-DL-β-OHB or Na-bicarbonate as indicated in figures and tables. Simultaneously urine was collected in refrigerated containers and cumulative nitrogen excretion (sum of urea plus NH_4^+) was measured as described (15, 16).

Hepatic blood flow was estimated in anaesthetized pigs using ^{133}Xe washout kinetics as described (17).

Analytical procedures. The methods for the determination of standard clinical and laboratory parameters, of glucose, lactate, pyruvate, glycerol, alanine, α-amino-N, free fatty acids (FFA), of the hormones insulin and glucagon, of pH, pO_2, and pCO_2 as well as of the specific activity of glucose have been described in detail previously (15, 16, 18, 19). Measurement of β-OHB and Acac in the blood as well as in the plasma revealed that neither ketones could be demonstrated in pig's erythrocytes; thus, plasma values were estimated to be higher than the concentrations

1. *Abbreviations used in this paper:* Acac, acetoacetate; β-OHB, D-β-OH-butyrate; MCR, metabolic clearance rate; MCR_G, MCR of glucose; MCR_K, MCR of ketones (ketones, sum of β-OHB plus Acac); R_a, rate of glucose production; R_d, rate of glucose utilization.

estimated for whole blood depending on the hematocrit (15). Osmolality was determined by a Halb-mikro-osmometer (H. Knaur, Berlin, Federal Republic of Germany).

Calculations. The rate of R_a and R_d was calculated from the equations of Steele (20) in their derivative form (21). In the basal state, when a dynamic equilibrium prevailed, the rate of glucose turnover (R_t) was calculated by the isotope dilution equation: $R_t = F/SA$ (F, the rate of infusion of the tracer; SA, specific activity of glucose). Under this condition, R_t equals R_a and R_d. In the nonsteady state, R_a and R_d were calculated according to $R_a = (F - [pVc_t\ dSA/dt])/SA_t$ and $R_d = R_a - (pVdc/dt)$, where c is the concentration of glucose, V represents the glucose distribution volume (calculated from the initial decline in the specific activity of glucose after the primed injection of the tracer), and p is the pool fraction (0.65 according to [21]). Polynomial curves were fitted to the SA and plasma concentrations of glucose vs. time curves and a smoothing tricubic spline algorithm was used for this purpose on a TR 440 Telefunken computer. The evaluation of the rates of glucose turnover using the primed constant infusion and the pool fraction has been validated for the steady and the nonsteady state (22, 23). The metabolic clearance rate (MCR) for glucose was calculated as the ratio of R_d to c that may reflect insulin-dependent glucose uptake, which is independent of the mass action of glucose. Recent data show that glucose uptake follows saturation kinetics. Thus, glucose clearance is not independent of plasma glucose concentration, indicating that the use of glucose clearance under some circumstances may be misleading (24, 25, 26). The MCR of ketone bodies (MCR_K) (the volume of blood irreversibly and completely cleared of ketone bodies) was calculated according to MCR_K = Na-D-β-OHB infusion rate/(Σ Ketone body concentration at equilibrium − basal ketone body concentration) (14). This calculation is based on the assumptions (*a*) that the presence of the unphysiological L-isomer did not affect the metabolism of ketones, (*b*) that exogenous ketones are metabolized in a similar way to endogenous ketones, and (*c*) that during infusion of ketone bodies, the metabolic changes with respect to endogenous ketone body production are negligible under our experimental conditions. The clearance of total ketones was used since β-OHB is rapidly converted to Acac (see Results). Assuming steady state concentrations, ketone body utilization was calculated from MCR and steady state ketone concentration. As shown in Table III, hepatic ketone body production was virtually unaffected by increasing circulating ketone bodies up to 1 mM.

Statistics. The data in text, Figures, and Tables are presented as the mean, or the mean±SEM. Their statistical significance were evaluated by either analysis of variance or two-tailed unpaired, and when appropriate, paired *t* test.

Results

Standardization of the experiments in non-anaesthetized miniature pigs (Fig. 1). Infusion of Na-DL-β-OHB increased plasma β-OHB as well as Acac, and the ratio of β-OHB to Acac was shifted to Acac indicating a rapid interconversion of both by tissue β-OHB-dehydrogenase. Steady state levels were reached between 45–145-min infusion time. The pH was shifted to alkaline, blood HCO_3^- was enhanced, and serum K^+ decreased. Heart rate increased from 62 to 72 per minute. For all other parameters (Fig. 1) as well as body temperature, blood pressure, hematocrit and serum protein, albumin, bilirubin, glutamate oxaloacetate transaminase, and alkaline phosphatase (data not shown), only minor alterations were observed.

Ketone body utilization at different ketone body levels (Fig. 2). On increasing the infusion of Na-DL-β-OHB, plasma concentration increased to a new steady state level. Up to a serum concentration of 1.2 mM, a linear relationship between ketone

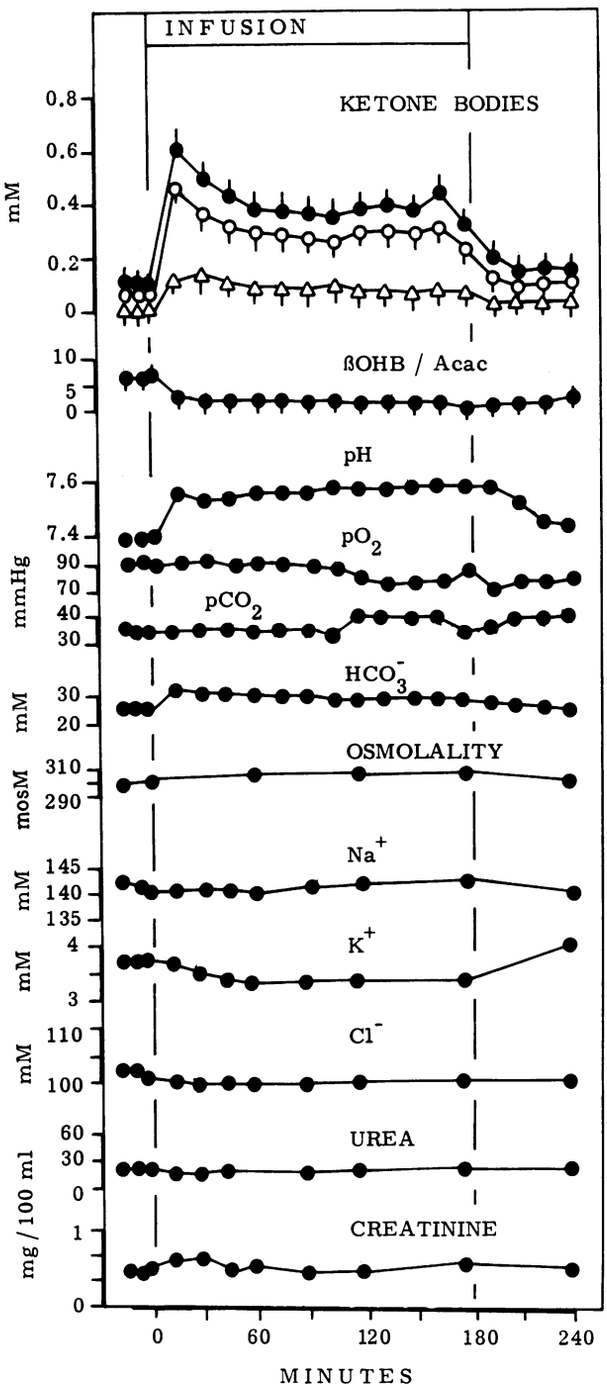

Figure 1. Effect of Na-DL-β-OHB infusion (15 μmol/kg × min, pH 9.2) on serum β-OHB (○), Acac (△), sum of β-OHB plus Acac (●), blood pH, pO_2, pCO_2, HCO_3^-, and various parameters in plasma. Data are given as means or means±SEM. (n = 4–8).

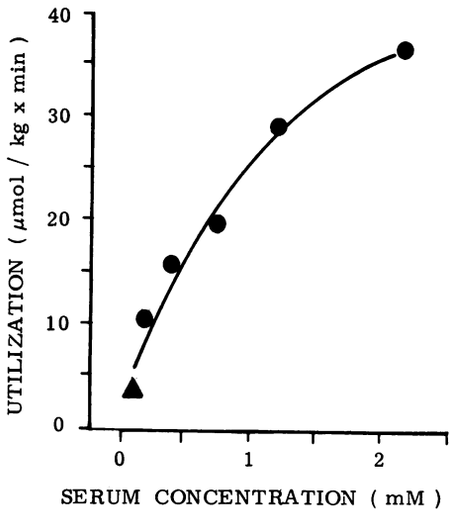

Figure 2. Correlation between ketone body concentration and utilization without (▲) and with (●) infusing increasing amounts of Na-DL-β-OHB. Infusion rate: 7.5, 15, 30, 45, or 60 μmol/kg × min; calculation of the utilization rate was performed when steady state levels were reached, i.e., between 45 and 135 min. Data are given as means (n = 2–5). For details, compare Methods.

body level and utilization was observed (Fig. 2) (for calculation, compare Methods).

Effect of ketone body infusion on arterial metabolites and hormones (Fig. 3). Infusion of Na-DL-β-OHB resulted in a rapid drop in the serum FFA and glycerol concentration, whereas the levels of plasma lactate, alanine, α-amino-N, and pyruvate (from 80 to 50 μM) showed a delayed decrease. Plasma lactate/pyruvate ratio was estimated to be 9 at zero time and showed only insignificant variations (from 8 to 10) during the infusion period. Arterial insulin and glucagon showed only small increases; however, the increases were pronounced when the portal concentrations were measured (see below and Fig. 7).

Effects of ketone body infusion on glucose turnover (Fig. 3). Infusion of Na-DL-β-OHB (15 μmol/kg × min) led to a drop in blood glucose, R_a (−50%), and R_d (−45%). The effects were statistically significant at the beginning of the infusion period, and alterations were observed for up to 135 min perfusion time. The MCR for glucose was transiently reduced (5–60 min) (data not shown) despite the elevated plasma insulin levels. On infusing different amounts of Na-DL-β-OHB, similar decreases in blood glucose concentrations, R_a, and R_d were observed (Table I).

Effects of ketone body infusion at different pH (Table II). In order to investigate whether ketone body-induced alteration in glucose homeostasis was due to the ketone-provoked shift in blood pH (compare Fig. 1), ketone solution was infused at different pH. The effect of infusing Na-DL-β-OHB (15 μmol/kg × min) at pH 9.2 (compare Fig. 3), 7.4, 6.0, or 5.0 was tested. At pH 5.0, blood pH decreased to 7.35 while a similar increase in plasma insulin and similar decline in glucose concentration, R_a, and R_d were observed. In contrast, plasma alanine level only declined after infusing Na-DL-β-OHB at pH 9.2 (Fig. 3).

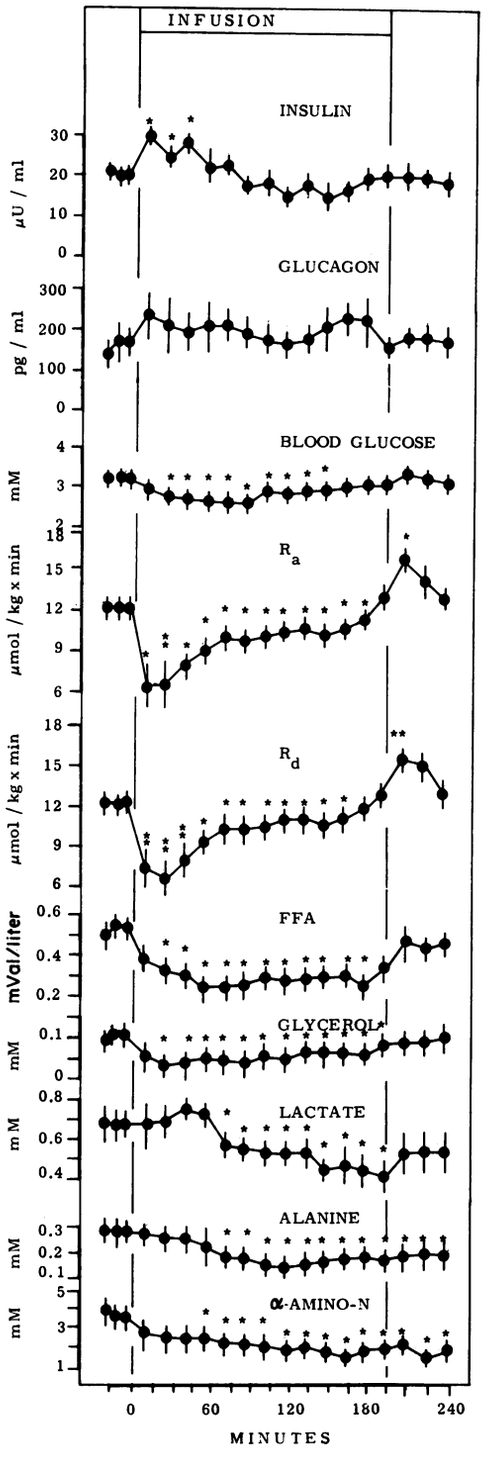

Figure 3. Effect of Na-DL-β-OHB infusion (15 μmol/kg × min, pH 9.2) on serum insulin, plasma glucagon, blood glucose, rate of glucose production (R_a) and utilization (R_d), plasma FFA, glycerol, lactate, alanine, and α-Amino-N. For the ketone body level, compare Fig. 1. The asterisks indicate levels of significance before and during infusion. *, $P < 0.05$; **, $P < 0.01$, n = 6.

Table I. Effect of Different Amounts of Na-DL-β-OHB Infusion on Blood Glucose Level, R_a, R_d, and Serum Insulin

Parameter	Infusion rate	Time (*min*) 0	+15	+30	+90
Ketone bodies (*mM*)	1	0.14	0.24	0.26	0.20
	2	0.13	0.62	0.51	0.40
	3	0.10	0.89	0.76	0.79
	4	0.14	1.70	1.53	1.23
Blood glucose (*mM*)	1	3.6	2.9	2.8	2.7
	2	3.9	3.6	3.3	3.3
	3	3.2	2.4	2.4	2.3
	4	2.8	2.7	2.4	2.4
R_a (*μmol/kg × min*)	1	13.3	10.0	10.0	10.0
	2	12.2	6.7	6.2	10.0
	3	12.7	9.4	8.9	9.4
	4	11.7	9.4	8.3	8.9
R_d (*μmol/kg × min*)	1	13.3	12.2	11.7	8.9
	2	12.2	7.2	6.2	10.6
	3	12.8	9.9	9.4	9.4
	4	11.7	10.6	10.5	8.3
Insulin (*μU/ml*)	1	14	34	17	11
	2	19	29	23	17
	3	17	16	22	19
	4	16	25	19	15

Data are given as means (n = 2–5). Rate of Na-DL-β-OHB infusion: 1 = 7.5, 2 = 15, 3 = 30, 4 = 45 μmol/kg × min; all at pH 9.2.

Effect of alkalinization of blood pH. Infusion of Na-DL-β-OHB induced a shift of the pH to alkaline (compare with Fig. 1). Therefore, in control experiments, alkalinization of the blood pH was performed by infusing Na-bicarbonate (1.5 mmol/kg × min) to give a pH similar to that observed after infusion of Na-DL-β-OHB (compare Fig. 1). This infusion affected neither blood glucose concentration, R_a, R_d, nor the plasma levels of insulin, alanine, and ketones within 180-min perfusion time (data not shown).

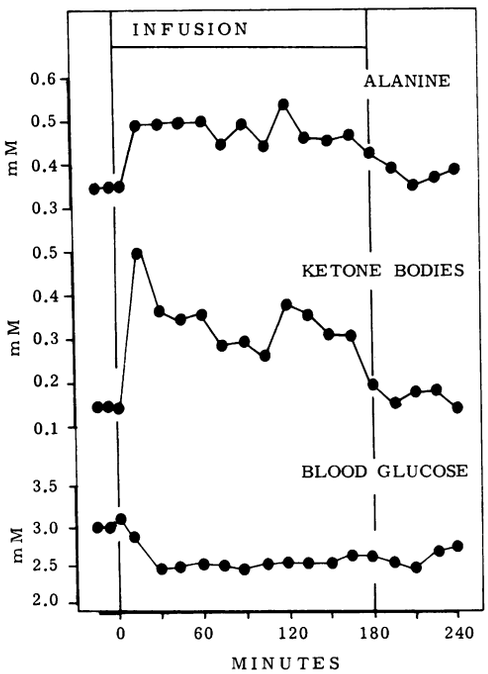

Figure 4. Effect of infusion of Na-DL-β-OHB (15 μmol/kg × min, pH 9.2) plus L-alanine (1 μmol/kg × min) for 180 min on plasma alanine, serum ketones (sum of β-OHB plus Acac), and blood glucose. Data given as means (n = 3).

Effect of ketone body plus alanine infusion (Fig. 4). To investigate whether ketone body-induced hypoglycemia was due to a limited alanine supply, ketones were infused together with low amounts of alanine. This resulted in steady state levels for β-OHB of ~0.35 mM and for alanine of ~0.5 mM. However, the supply of alanine did not prevent the ketone body-mediated decrease in blood glucose concentration.

Effect of ketone body plus insulin infusion (Fig. 5). Ketone body-insulin interaction was investigated by the simultaneous infusion of ketones and insulin. Infusion of small amounts of insulin alone resulted in physiological insulin levels of ~40 μU/ml. Steady state plasma insulin levels were unaffected by

Table II. Effect of Infusion of Na-DL-β-OHB (15 μmol/kg × min, from 0 to 180 min) at pH 5.0 on Blood pH, Ketone Bodies (Sum of β-OHB plus Acac), Blood Glucose, R_a, R_d, Plasma Alanine, and Serum Insulin

	Time (*min*) −15	0	+15	+30	+45	+60	+90	+120	+150	+180	+210
Blood pH	7.44	7.44	7.43	7.41	7.35	7.35	7.35	7.37	7.39	7.39	7.39
Ketone bodies (*mM*)	0.18	0.18	0.52	0.39	0.42	0.42	0.41	0.40	0.41	0.39	0.19
Blood glucose (*mM*)	3.5	3.5	3.1	3.2	3.1	3.0	3.1	3.2	3.1	3.0	3.7
R_a (*μmol/kg × min*)	16.1	16.1	9.9	10.1	11.5	12.5	13.2	13.7	15.1	14.8	15.8
R_d (*μmol/kg × min*)	16.1	16.1	15.1	14.9	14.8	14.0	12.1	12.9	14.2	14.1	15.6
Alanine (*mM*)	0.33	0.34	0.36	0.35	0.32	0.34	0.33	0.36	0.44	0.42	0.39
Insulin (*μU/ml*)	17	16	19	27	22	28	15	17	18	18	18

Data are given as means (n = 3).

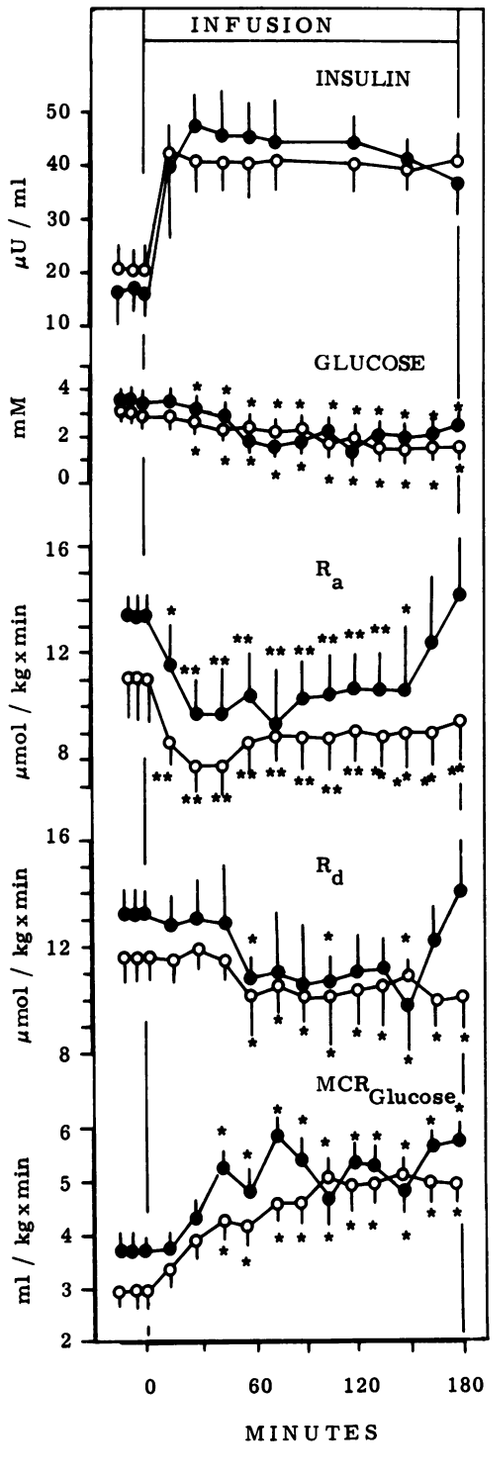

Figure 5. Effect of infusion of insulin (0.4 mU/kg × min) (○, $n = 5$) or insulin plus Na-DL-β-OHB (15 μmol/kg × min, pH 9.2) (●, $n = 4$) on blood glucose, R_a, R_d, and MCR of glucose. The asterisks indicate levels of significance before and during infusion. *, $P < 0.05$, **, $P < 0.01$.

the simultaneous infusion of Na-DL-β-OHB. This indicates that ketones did not affect the MCR of insulin (~20 ml/kg × min in the insulin-treated and insulin plus β-OHB-treated miniature pigs). Insulin at this infusion rate decreased blood glucose levels, and had pronounced effects on R_a. Only a minor, yet statistically significant reduction in R_d was observed. The addition of ketones to the infusion of insulin had no additional effect on blood glucose, R_a, and R_d. Furthermore, ketone body steady concentration after β-OHB infusion (15 μmol/kg × min) was identical in the presence and absence of insulin, indicating that insulin does not affect ketone body metabolic clearance rate.

Effect of ketone body plus somatostatin infusion (Fig. 6). Somatostatin was infused in order to investigate further the ketone body-insulin interaction. As a result, insulin and glucagon declined in parallel; blood glucose showed an initial slight decrease which was followed by hyperglycemia between 100 and 180 min. This effect was due to the fall in R_a as well as R_d, followed by a rise in R_a. In addition, MCR for glucose rapidly declined and plasma FFA, glycerol, alanine, and β-OHB continuously increased. Infusing somatostatin plus Na-DL-β-OHB also resulted in hypoglycemia, which persisted during the whole infusion period with concomitantly reduced rates of R_a. Infusion of ketones in the somatostatin experiment had no further effect on the other parameters, i.e., glycerol, FFA, and MCR of glucose.

Effect of ketone body infusion on liver, splanchnic, and leg metabolite balance (Fig. 7, Table III). In order to investigate the ketone body-induced alterations in liver and muscle metabolism, experiments were repeated in five anaesthetized 24-h starved pigs by use of the catheter technique (compare with Methods). Infusing Na-DL-β-OHB into these animals resulted in similar alterations in blood pH, bicarbonate, and other standard parameters as was observed for the non-anaesthetized pig (Fig. 1). In addition, the concentrations of FFA, glycerol, α-amino-N, alanine, and glucose were comparable in both experimental models, with the exception of lactate and ketones (Table III). However, the higher steady state levels in arterial ketone bodies do not limit the conclusions, as it was shown that increasing the levels of circulating ketone bodies provokes similar alterations with respect to insulin and glucose turnover (compare Table I). In addition, recent experiments in anaesthetized and conscious dogs lead to the conclusions that narcotic-induced changes in portal vein plasma flow do not affect hepatic extraction of insulin and glucagon (27). Furthermore, anaesthesia and surgery did not alter basal insulin and glucagon or their secretion (e.g., induced by arginine) (27).

The effect of Na-DL-β-OHB infusion on arterial metabolite level as well as hepatic, splanchnic, and leg exchange is given in Table III. Estimation of hepatic blood flow revealed no significant alterations during our experimental conditions (mean, 29 ml/kg × min). During infusion of ketones, the hepatic ketone body exchange showed no significant alteration whereas the splanchnic exchange declined, indicating an enhanced ketone utilization by the intestine. Concomitantly, ketone body utilization of the leg increased paralleled by a reduced leg uptake of glucose; leg oxygen consumption remained constant. In ad-

dition, leg release of FFA, glycerol, alanine, α-amino-N, and lactate was decreased by Na-DL-β-OHB infusion. Hepatic FFA uptake decreased with a concomitant transient drop in hepatic oxygen consumption. Hepatic glucose output decreased during the infusion of ketones and intestine glucose exchange was slightly reduced. Despite the fall in gluconeogenic precursor release by the leg (compare alanine, α-amino-N, and glycerol), hepatic exchange of these compounds was unaffected by ketone infusion. In addition, average hepatic urea exchange did not vary during ketone infusion (data not shown). Hepatic lactate exchange showed an initial transient fall (15 and 30 min after ketone body infusion) but returned to the preinfusion level within the following hour. With respect to the hepatic exchanges, calculation was based on a portal venous/arterial perfusion ratio of 70%:30%. Assuming a value of 50%:50%, the observed effects on glucose output were even more pronounced, whereas pre-

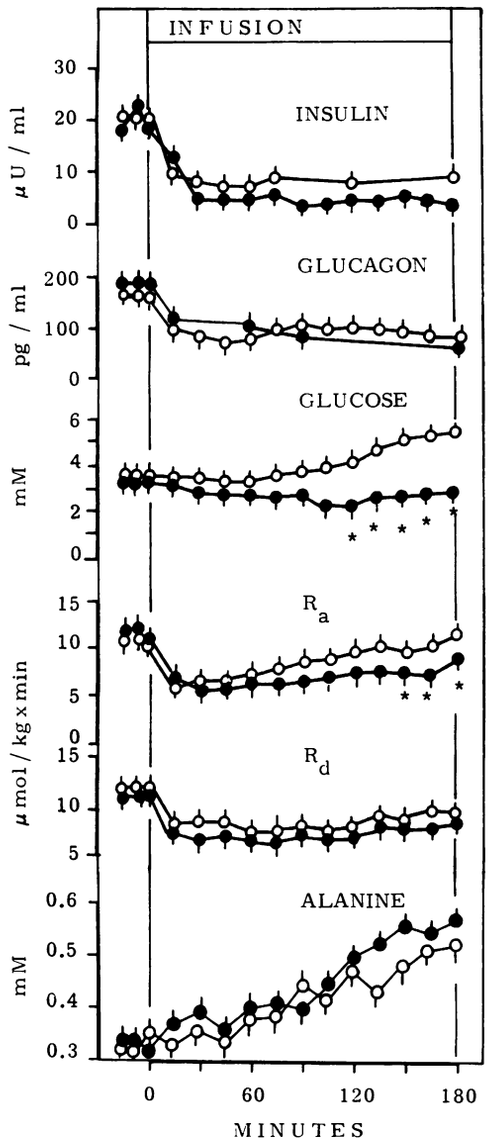

Figure 6. Effect of infusion of somatostatin (0.2 μg/kg × min) (○, $n = 5$) or somatostatin plus Na-DL-β-OHB (15 μmol/kg × min, pH 9.2) (●, $n = 4$) on serum insulin, plasma glucagon, alanine, blood glucose, R_a and R_d. The asterisks indicate levels of significance between the two experimental conditions. *, $P < 0.05$.

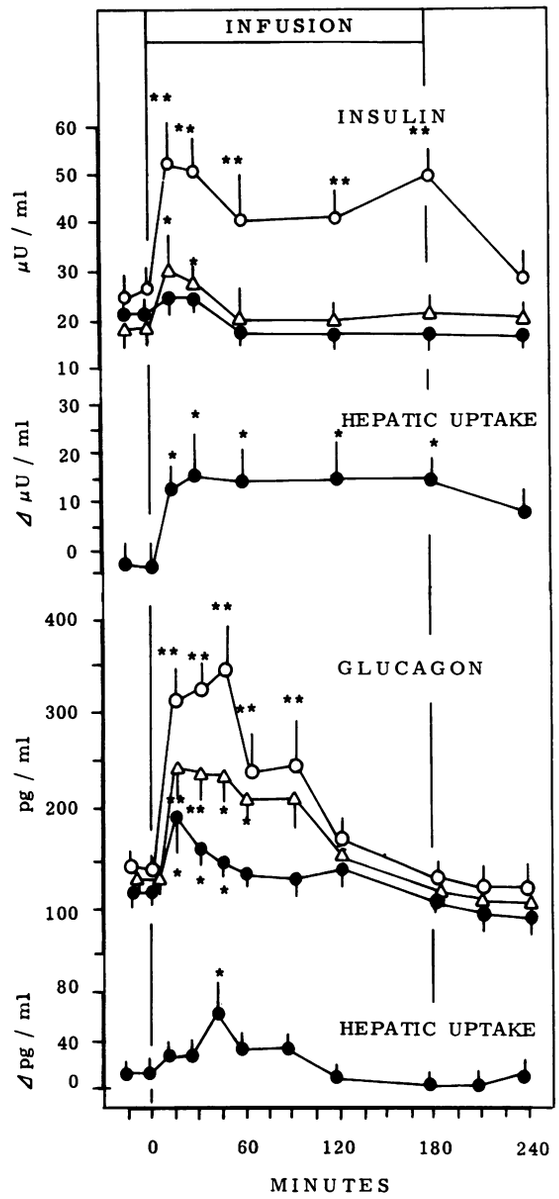

Figure 7. Effect of infusion of Na-DL-β-OHB (15 μmol/kg × min, pH 9.2) ($n = 3$) on the portal (○), hepatic venous (△), and arterial (●) level of serum insulin and plasma glucagon, and on hepatic hormone uptake. The asterisks indicate levels of significance before and during infusion. *, $P < 0.05$, **, $P < 0.01$.

Table III. Effect of Na-DL-β-OHB Infusion (15 μmol/kg × min, infused from 0 to 180 min) on the Arterial Concentration, and the Hepatic, Splanchnic, and Leg Exchange for Oxygen, Glucose, Lactate, Alanine, α-Amino-N, Glycerol, FFA, and Ketones (Sum of β-OHB plus Acac) in the Anaesthetized, 24-h Starved Miniature Pig (n = 5)

		Time (*min*)			
		−15	0	+15	+30
O_2	Art.[1]	12.62±1.26	12.31±1.04	12.21±1.22	12.22±1.22
	Hep. exch.[2]	−2.81±0.29	−2.42±0.16	−1.64±0.59*	−2.15±0.18
	Sp. exch.[2]	−5.17±0.01	−4.68±0.07	−3.66±0.33**	−4.13±0.45
	Leg exch.[2]	−3.79±1.39	−3.64±0.14	−3.58±0.35	−3.83±0.35
Glucose	Art[3]	5.37±0.43	5.67±0.24	5.76±0.36	5.95±0.29
	Hep. exch.[4]	+0.80±0.19	+0.82±1.17	+0.22±0.06**	+0.30±0.17**
	Sp. exch.[4]	+0.53±0.25	+0.58±0.18	−0.04±0.18*	+0.10±0.14*
	Leg exch.[4]	−1.05±0.72	−0.95±0.41	−0.63±0.23	−0.44±0.10*
Lactate	Art[3]	3.22±2.58	3.48±1.42	3.35±1.24	3.11±1.57
	Hep. exch.[4]	−0.36±0.12	−0.48±0.28	+0.20±0.25*	−0.09±0.13*
	Sp. exch.[4]	−0.67±0.13	−0.71±0.45	0.00±0.27*	−0.04±0.11*
	Leg exch.[4]	+0.53±0.13	+0.58±0.22	+0.21±0.16	+0.16±0.02*
Alanine	Art[3]	0.66±0.08	0.66±0.07	0.63±0.08	0.60±0.07
	Hep. exch.[4]	−0.11±0.02	−0.13±0.04	−0.13±0.05	−0.11±0.01
	Sp. exch.[4]	−0.12±0.03	−0.15±0.05	−0.13±0.05	−0.11±0.04
	Leg exch.[4]	+0.15±0.01	+0.19±0.06	+0.15±0.03	+0.06±0.02*
α-Amino-N	Art[3]	6.83±0.54	6.72±0.37	6.43±0.43	6.60±0.67
	Hep. exch.[4]	−0.98±0.30	−1.00±0.35	−1.03±0.15	−0.94±0.22
	Sp. exch.[4]	−1.31±0.28	−1.32±0.30	−1.21±0.58	−1.25±0.36
	Leg exch.[4]	+1.43±0.74	+1.36±0.55	+1.02±0.59	0.96±0.42
Glycerol	Art[3]	0.36±0.14	0.39±0.15	0.39±0.14	0.34±0.19
	Hep. exch.[4]	−0.10±0.03	−0.10±0.02	−0.09±0.01	−0.12±0.06
	Sp. exch.[4]	−0.11±0.04	−0.14±0.05	−0.14±0.04	−0.13±0.04
	Leg exch.[4]	+0.14±0.04	+0.14±0.04	+0.13±0.05	+0.14±0.04
FFA	Art[5]	0.30±0.07	0.32±0.09	0.27±0.09*	0.22±0.09**
	Hep. exch.[6]	−0.10±0.04	−0.13±0.05	−0.07±0.03	−0.05±0.01
	Sp. exch.[6]	−0.08±0.01	−0.08±0.02	−0.08±0.03	−0.08±0.03
	Leg exch.[6]	+0.21±0.05	+0.29±0.05	+0.17±0.02*	+0.12±0.02*
Ketones	Art[3]	0.36±0.06	0.39±0.06	1.06±0.17**	1.02±0.16**
	Hep. exch.[4]	+0.06±0.02	+0.06±0.02	+0.07±0.02	+0.09±0.02
	Sp. exch.[4]	+0.06±0.02	+0.05±0.03	+0.04±0.02	−0.02±0.03*
	Leg exch.[4]	−0.10±0.07	−0.10±0.05	−0.15±0.04**	−0.14±0.03*

The asterisks indicate levels of significance before and during infusion. * $P < 0.05$, ** $P < 0.01$. 1, ml/min; 2, Δml; 3, mM; 4, ΔmM; 5, mmol/l; 6, Δmmol/l. Art, arterial concentration, Hep. exch., hepatic exchange; Sp. exch., splanchnic exchange; Leg exch., leg exchange.

cursor extraction rates remained still unchanged. In the pig, the ketone body infusion did not alter main glucose gradient.

Na-DL-β-OHB infusion increased insulin secretion, which was reflected by elevated portal venous and hepatic venous levels, as well as arterial insulin levels (Fig. 7). In addition, the hepatic insulin uptake was elevated during ketosis from 5 to 15 ΔμU/ml (Fig. 7). Since hepatic blood flow remained constant during ketosis, hepatic insulin extraction was also enhanced (Fig. 7). The fractional extraction of insulin increased from 23% in the basal period to ~41% during the infusion period (data not shown).

Concomitantly, portal venous, hepatic venous, and arterial glucagon concentrations were elevated by ketone infusion; the hepatic gradient of glucagon was transiently increased (Fig. 7). These data indicate an enhanced secretion of the α-cell together with a transient increase in hepatic glucagon extraction.

+45	+60	+90	+120	+180	+210	+240
12.30±1.36	12.60±1.34	12.22±1.27	12.24±1.32	12.47±1.34	12.38±1.40	12.50±1.30
−2.81±0.84	−2.34±1.24	−2.10±0.65	−1.98±0.22	−2.02±0.40	−2.72±0.07	−3.39±0.42
−4.96±1.25	−4.83±1.70	−3.97±0.66	−3.92±0.20	−3.88±0.61	−4.22±0.73	−5.53±0.40
−4.11±1.15	−4.64±1.47	−3.22±0.26	−3.44±0.23	−3.77±0.38	−4.15±0.52	−3.99±1.06
5.74±0.29	5.82±0.29	5.51±0.43	5.76±0.52	5.84±0.68	5.62±0.64	5.62±0.60
+0.38±0.26*	+0.28±0.11*	+0.39±0.21*	+0.40±0.14*	+0.41±0.30*	+0.37±0.14*	+0.44±0.31
+0.07±0.09*	+0.05±0.18*	+0.15±0.16	+0.04±0.05*	+0.03±0.18*	+0.25±0.38	+0.39±0.17
−0.62±0.26	−0.60±0.20	−0.48±0.08	−0.45±0.16*	−0.25±0.07	−0.58±0.22	−0.78±0.01
3.07±1.52	3.87±1.45	3.58±1.20	3.71±1.13	3.53±1.35	3.18±1.62	3.93±1.67
−0.92±0.39	−0.93±0.62	−0.65±0.46	−0.35±0.30	−0.31±0.17	−0.31±0.16	−0.45±0.37
−0.40±0.22	−0.27±0.07	−0.52±0.40	−0.50±0.36	−0.35±0.12	−0.47±0.15	−0.54±0.22
+0.12±0.42*	+0.09±0.15*	+0.34±0.30	+0.25±0.29	+0.54±0.26	+0.52±0.04	+0.48±0.39
0.56±0.07	0.55±0.07*	0.51±0.06	0.51±0.05*	0.50±0.04*	0.54±0.05	0.52±0.09
−0.10±0.02	−0.10±0.03	−0.11±0.01	−0.10±0.01	−0.10±0.01	−0.08±0.05	−0.09±0.01
−0.10±0.03	−0.12±0.03	−0.14±0.02	−0.11±0.03	−0.11±0.01	−0.09±0.03	−0.11±0.03
+0.06±0.02*	+0.08±0.04*	+0.08±0.03*	+0.06±0.04*	+0.04±0.03*	+0.08±0.02*	+0.12±0.05
5.87±0.38	4.98±0.37	5.06±0.48	5.15±0.53	4.64±0.49	4.79±0.61	4.72±0.58
−1.21±0.47	−1.15±0.35	−0.89±0.28	−1.03±0.41	−0.65±0.02	−0.72±0.23	−0.65±0.02
−1.47±0.42	−1.14±0.59	−1.18±0.43	−1.23±0.42	−1.32±0.28	−1.24±0.79	−1.07±0.30
+0.42±0.10*	+1.05±0.47	+1.03±0.18	+0.84±0.22	+0.60±0.23	+0.98±0.27	+1.11±0.23
0.31±0.09*	0.29±0.07*	0.27±0.06	0.26±0.04*	0.26±0.01*	0.26±0.02*	0.26±0.01*
−0.10±0.04	−0.08±0.03	−0.16±0.08	−0.14±0.06	−0.12±0.07	−0.20±0.06*	−0.12±0.06
−0.12±0.02	−0.10±0.02	+0.11±0.02	−0.09±0.02*	−0.15±0.01	−0.15±0.02	−0.15±0.04
+0.13±0.04	+0.11±0.02	+0.12±0.02	+0.11±0.02	+0.10±0.04*	+0.10±0.01*	+0.11±0.05
0.18±0.05*	0.21±0.05*	0.21±0.07*	0.19±0.05*	0.17±0.06**	0.17±0.05*	0.20±0.07*
−0.05±0.02	−0.06±0.03	−0.02±0.01*	−0.04±0.02	−0.01±0.01	−0.09±0.07	−0.07±0.04
−0.08±0.02	−0.09±0.01	−0.05±0.02	−0.04±0.02	−0.05±0.02	−0.05±0.02	−0.03±0.02
+0.05±0.01*	+0.08±0.02*	+0.06±0.03*	+0.02±0.01*	+0.05±0.02	+0.12±0.01	+0.12±0.02*
1.01±0.14**	0.98±0.21**	1.05±0.21**	1.16±0.2	1.14±0.32**	0.86±0.24*	0.57±0.10
+0.07±0.01	+0.13±0.07	+0.02±0.03	+0.01±0.00*	+0.07±0.04	+0.07±0.03	+0.07±0.04
−0.07±0.04**	−0.06±0.04**	−0.04±0.02**	−0.04±0.03**	+0.02±0.06	+0.04±0.09	+0.01±0.03
−0.20±0.04**	−0.17±0.04*	−0.17±0.04**	−0.23±0.07**	−0.15±0.04	−0.10±0.05	−0.07±0.04

Effect of ketone body infusion during prolonged starvation. Since a protein-sparing action of ketones has been proposed (8, 9), which by decreasing precursor supply could contribute to the reduced hepatic output of glucose, Na-DL-β-OHB was infused at pH 9.2 at the fourth, fifth, and sixth day of the starvation period. Blood pH increased (7.45 to 7.56); blood glucose and plasma alanine values decreased (Fig. 8). N-excretion was reduced mainly due to a decrease in NH_4^+-excretion.

Infusing Na-DL-β-OHB at pH 5.0 at the fourth, fifth, and sixth day of the starvation period decreased blood pH from 7.43 to 7.35, but increased urinary N-excretion, both as urea and as NH_4^+ (Fig. 9).

On infusing Na-bicarbonate at the fourth, fifth, and sixth day of starvation, blood pH increased from 7.44 to 7.52, plasma glucose remained constant, and urinary N-excretion decreased (Fig. 8 and 9).

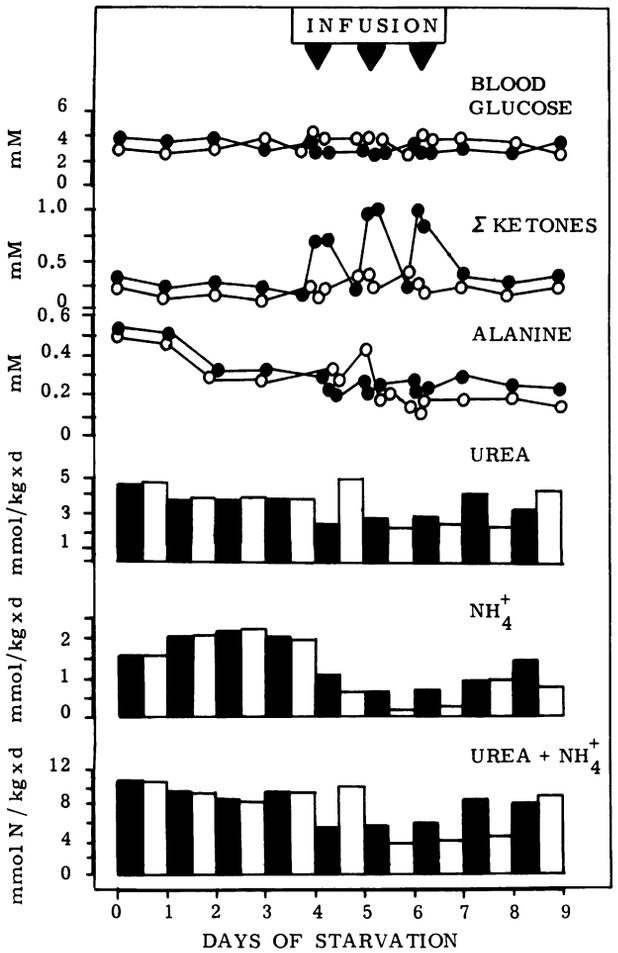

Figure 8. Effect of 6-h Na-DL-β-OHB infusion (15 μmol/kg × min) adjusted at pH 9.2 (●) or of 6-h Na-bicarbonate infusion (1.25 mmol/kg × min) (○) at the fourth, fifth, and sixth day of starvation on blood glucose, plasma ketone bodies (sum of β-OHB plus Acac), alanine (measured at 3- and 6-h infusion time), and on daily urinary urea and NH_4^+ excretion (n = 3).

Discussion

Ketone bodies and glucose production. The present data clearly demonstrate that physiological alterations of ketone bodies have a profound effect on the regulation of glucose homeostasis. From our data, ketone body-induced inhibition of gluconeogenesis is most probably due to alteration in portal serum insulin concentration. This assumption is based on the following lines of evidence: (*a*) Ketones stimulate pancreatic β-cell secretion as well as hepatic uptake of insulin (Fig. 7). Alterations in the arterial levels of this hormone were only small but nonetheless significant (Figs. 3 and 7, Table II). This is in accordance with the ketone-induced increase in arterial C-peptide level at simultaneously unaltered venous plasma insulin levels reported recently for man (5). (*b*) Infusion of increasing doses of ketone bodies, yielding serum levels between 0.3–1.7 mM, resulted in identical increases in arterial insulin, and correspondingly, identical decreases in R_a (compare Results). No dose dependent relationship between ketone body level and inhibition of R_a was observed (Table I). (*c*) Infusing physiological amounts of insulin together with Na-DL-β-OHB did not exceed the ketone-induced reduction of gluconeogenesis (Fig. 5). Nevertheless, assuming a direct effect of ketones, it may be possible that once R_a was reduced by insulin (Fig. 5), ketone could not provoke a further reduction. In fact, ketones infused at insulinopenia (during somatostatin plus Na-DL-β-OHB treatment) resulted in a significantly decreased R_a during the prolonged infusion time, which is possibly a direct action of ketones (Fig. 6). A direct effect of ketones could involve the following mechanisms: the ketone-provoked flux of pyruvate from glucose towards acetyl-Coenzyme A (CoA), as was demonstrated in the isolated perfused liver (28), and/or the ketone body-mediated reduction in FFA, which is suggested to stimulate hepatic gluconeogenesis. However, as ketones lowered R_a at constant levels of FFA (compare Table III), this mechanism seems unlikely under our conditions.

On infusing somatostatin, the late hyperglycemia (Fig. 6, from 90-min infusion period) is considered to be due to an enhanced catecholamine secretion stimulating glucose production (29). However, infusing somatostatin together with ketone bodies, the reduced rate of R_a persisted and consequently blood glucose decreased (Fig. 6). As well as a direct effect of ketones (compare above), this may alternatively or additionally be the result of the ketone-induced reduction of the adrenergic response. In fact, it is known that ketotic hypoglycemic children display a diminished adrenal medullary response (1, 2, 30, 31).

Ketone bodies and glucose utilization. The present data

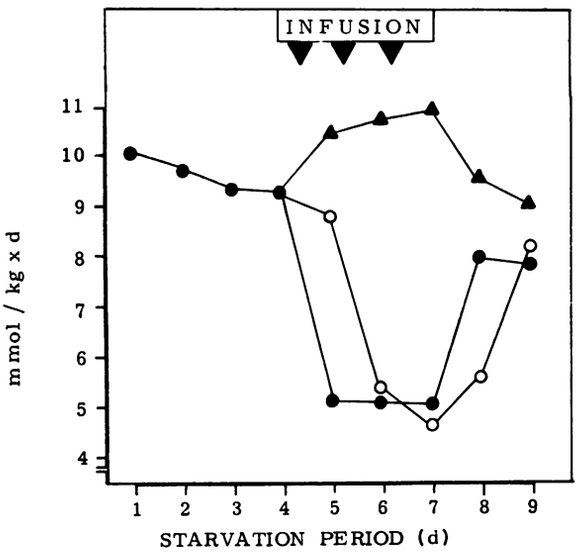

Figure 9. Effect of 6-h Na-DL-β-OHB infusion (15 μmol/kg × min) adjusted at pH 9.2 (●) or pH 5.0 (▲) or of 6-h Na-bicarbonate infusion (1.25 mmol/kg × min) (○) at the fourth, fifth, and sixth day of starvation on daily urinary N-excretion (n = 3).

demonstrate that during ketone body infusion glucose utilization is decreased (Fig. 3). A similar in vivo finding was shown previously by other authors (3, 5, 6). From in vitro data, it is known that Acac inhibits glucose uptake in rat soleus but not in extensor digitorum longus muscles or the perfused hindlimb of rats (32, 33). Circulating insulin levels of ~40 μU/ml primarily reduce R_a and thereby, the blood glucose level, whereas only minor effects on R_d are apparent (Fig. 5). It is known from the literature that insulin levels of ~40 μU/ml inhibit endogenous glucose production without affecting peripheral glucose utilization (34); accordingly, it was reported for man that the Michaelis constant (K_m) for the half maximal inhibition of R_a was ~30 μU/ml, whereas the half maximal stimulation of glucose utilization occurred at ~55 μU/ml (34). Infusion of insulin in the absence or presence of exogenous ketones resulted in identical R_d, indicating that insulin antagonized the ketone body-induced decrease in glucose utilization. Considering the insulin-mediated increase in MCR of glucose (MCR_G) (Fig. 5), our data confirm that ketones do not inhibit the insulin-stimulated glucose uptake (for the limitation of MCR_G, see Methods). However, the ketone-induced fall in R_d may simply be explained as a consequence of decreased R_a; thus, the effect of ketones can be explained entirely by their effect on R_a.

Ketone bodies and the islets of Langerhans. It is generally accepted that ketones provoke insulin secretion (for a review, see 35). This is supported by our data showing a ketone induced increase in portal insulin with a concomitant alteration of its hepatic extraction (Fig. 7). Accordingly, in man, ketones provoked an increase in arterial C-peptide at simultaneously unchanged insulin levels (5).

Only sparse data are available on the effect of ketones on α-cell secretion (35, 36). Glucagon release from isolated guinea pig islets of Langerhans incubated in vitro was unaffected by ketones (37). However, our in vivo data clearly demonstrate that physiological increases in serum ketones provoke α-cell secretion (Fig. 7). This may be simply due to hypoglycemia. Thus, the α-cell response may be responsible for the recovery from hypoglycemia observed during ketone infusion (Fig. 3) (compare 38, 39). In fact, the latter idea is supported by the sustained hypoglycemia observed during simultaneous infusion of somatostatin and Na-DL-β-OHB (Fig. 6). Despite the increased glucagon secretion, hepatic fractional glucagon uptake remained nearly constant (Fig. 7). This is similar to data observed after insulin, glucagon, arginine, or cholecystokinine infusion (40, 41). On the other hand, hepatic glucagon extraction was enhanced in dogs after meat ingestion (42) suggesting that neural or hormonal signals from the gut may be involved in hepatic glucagon extraction (42).

Ketone bodies and alanine. There are several lines of evidence that the β-OHB-mediated decrease in hepatic glucose output does not depend on alanine supply, as has been proposed by several authors (1, 7, 8, 14): (*a*) Infusing the ketoacid resulted in an increase rather than a decrease in plasma alanine, nevertheless hypoglycemia occurred (compare Results). Using stable isotopes, Miles et al. (43) also demonstrated recently an increased rate of alanine appearance in man, when infusing Na-DL-β-OHB at pH 7.0. (*b*) Although infusing Na-DL-β-OHB (pH 9.2) reduced leg alanine release (Table III) as well as arterial alanine concentration (Fig. 3), hepatic extraction of alanine was not affected (Table III). (*c*) Prevention of the ketone-induced hypoalaninemia by the simultaneous infusion of physiological amounts of alanine together with β-OHB did not prevent hypoglycemia (Fig. 4). However, it should be mentioned that using pharmacological amounts of alanine (2.8 mmol/kg body wt × 30 min), ketotic hypoglycemic children become euglycemic (1, 2). (*d*) Ketone bodies decreased hepatic gluconeogenesis by ~50%; plasma alanine levels decreased by only 25% (Fig. 3, Table III). (*e*) In the miniature pig, alanine contributes only ~13% of splanchnic glucose output (16); consequently, any alteration in alanine extraction could not produce a fall in gluconeogenesis between 30 and 50%; (*f*) Considering urinary N-excretion during starvation (Fig. 8), ~9 μmol N/kg × min were released accounting for 4.5 μmol glucose/kg × min, assuming a maximal conversion efficiency. However, Na-DL-β-OHB decreased urinary N-excretion by 4 μmol/kg × min (Fig. 9), which is equivalent to maximally 2 μmol/kg × min of glucose. Thus, the ketone-induced inhibition in amino acid conversion to glucose could not explain the decrease in R_a from 12 to ~7 μmol/kg × min (Fig. 3). (*g*) Infusion of somatostatin plus β-OHB resulted in an increase in circulating alanine; however, R_a decreased again (Fig. 6). (*h*) The ketone-induced decrease in glucose production preceeded hypoalaninemia (Fig. 3, Table III). In addition, recent data demonstrate that the initiation of a ketogenic diet in children produced hypoalaninemia independent of hypoglycemia (44). As alanine flux is reported to be decreased in ketotic hypoglycemic children (1), our data provide evidence that hypoglycemia and hypoalaninemia are independent metabolic events.

Role of "Alanine-Ketone Body Cycle." The existence of an "Alanine-Ketone Body Cycle" has been proposed by some authors (45, 46); thus, the rise in serum ketone acids may represent a signal to muscle that increases alanine output and restricts hepatic ketogenesis, while gluconeogenesis is maintained (46, 11). However, with respect to ketotic hypoglycemia, the regulatory significance of this cycle seems unlikely: keto-acids inhibited glucose production despite increasing alanine supply (Table III, Fig. 4). However, the functional significance of the "Alanine-Ketone Body Cycle" in the presence of high levels of glucagon and the absence of insulin, e.g. diabetes mellitus, cannot be excluded by our experiments.

Regulatory role of blood lactate/pyruvate ratio for muscle alanine release. Recently, it was suggested (45) that a shift of muscle pyruvate towards lactate as reflected by alterations in the lactate/pyruvate ratio in the blood may represent an important mechanism responsible for the decrease of alanine in ketotic states. However, in our experiments at elevated plasma ketones and simultaneously reduced alanine levels (Fig. 3), the blood lactate/pyruvate ratio was unaltered (compare Results). This finding questions the regulatory significance of the blood lactate/pyruvate ratio for reducing plasma alanine levels.

Protein sparing action of ketone bodies. As in man, in the miniature pig during starvation, hepatic ketogenesis and plasma ketones increase, whereas plasma glucose, alanine, and urinary N-excretion, an indicator of protein catabolism, decrease (15, 16). During fasting, the protein sparing effect has been ascribed to the action of ketones (7, 8, 47). Recently, Pawan and Semple (48) demonstrated a decrease in urinary-N and methyl-histidine excretion in obese fasted subjects when infusing minimal amounts of ketones (1.2 μmol/kg body wt $\times$ min). In contrast, our data clearly demonstrate that the ketone-induced inhibition of alanine release and reduction in urinary N-excretion represents an experimental artifact (Fig. 8) related to the alkalinizing effect of the sodium salt of β-OHB. Using equimolar amounts of the ketoacid, urinary N-excretion increases during the starvation period (Fig. 9). This could be due to the known protein wasting effect of acidosis leading to an increase in muscle alanine release (43, 49). On the other hand alkali-treatment reduces urinary N-excretion in prolonged fasted subjects (50, 51). Contrary to our data, it was reported that the infusion of sodium free β-OHB provoked a 50% decrease in renal ammoniogenesis in dogs (52). Yet, it should be mentioned that the ketosis here (4.5 mM) differed from our experimental conditions (0.5–1 mM) and that NH_4^+ only contributes up to $\sim$10% of total N-excretion (compare with Fig. 8).

Taken together, ketone bodies inhibit endogenous glucose production, and thereby, provoke hypoglycemia. This effect is most probably mediated by insulin; however, a direct effect of ketones on hepatic gluconeogenesis may contribute to the observed phenomenon. Furthermore, a ketone body-induced decrease in adrenergic response may maintain hypoglycemia. Ketotic hypoglycemia is not due to a limited alanine supply. Ketone-induced decrease in glucose utilization is most probably a consequence of decreased glucose production. In summary, our data offer a reasonable explanation for ketotic hypoglycemia and they demonstrate that ketones do not show a protein sparing action.

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