Systemic pH modifies ketone body production rates and lipolysis in humans

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HOOD, VIRGINIA L., ULRICH KELLER, MOREY W. HAYMOND, AND DIETER KÜRY. *Systemic pH modifies ketone body production rates and lipolysis in humans.* Am. J. Physiol. 259 (Endocrinol. Metab. 22): E327–E334, 1990.—To investigate whether changes in systemic pH influence ketone body production or utilization, total ketone body (TK) kinetics were measured with [3-^{14}C]acetoacetate and D-β-[1,3-$^{13}C_2$]hydroxybutyrate tracers in overnight fasted subjects during metabolic alkalosis ($NaHCO_3$ infusion) or acidosis [NH_4Cl ingestion or arginine (Arg)-HCl infusion]. Somatostatin, with insulin, glucagon, and growth hormone replacement, was infused in all studies. Blood pH and HCO_3^- (mM) increased from baseline (0–30 min) to 180–210 min by 0.08 ± 0.02 and 7 ± 1 with $NaHCO_3$ and decreased by 0.08 ± 0.2 and 7 ± 1 or 5 ± 1 with NH_4Cl or Arg-HCl (all $P < 0.005$). Over this period blood TK (μM) differed between the $NaHCO_3$ (+198 ± 65) and both NH_4Cl (−90 ± 53) and Arg-HCl (−154 ± 55) ($P < 0.05$). These changes resulted from parallel alterations in TK production rate of appearance (R_a TK, $\mu mol \cdot kg^{-1} \cdot min^{-1}$), because changes from baseline in R_a ^{14}C TK also differed between $NaHCO_3$ (+1.9 ± 0.8) and NH_4Cl (−1.0 ± 0.6) and Arg-HCl (−2.0 ± 0.5) ($P < 0.05$). R_a TK calculated with single- or dual-tracer techniques were similar. Blood free fatty acids (FFA) increased with $NaHCO_3$, and FFA and glycerol decreased with NH_4Cl and Arg-HCl, suggesting that FFA availability mediated the pH effects on hepatic ketogenesis. These results demonstrate that modest changes in systemic pH modify FFA availability and TK production rates.

ketone body turnover; acid-base balance; ^{13}C tracer

KETONE BODY PRODUCTION and utilization are regulated by a complex interaction of hormones, substrates, and other factors controlling the activities of a number of enzymes. One factor that has recently regained attention is pH. In humans, net ketone body production is influenced by changes in systemic acid-base balance during acute ketosis of starvation (12) and during chronic stable ketosis associated with ketogenic diets (11, 13). However, it is not known whether the alterations in ketone body concentrations result from changes in ketone body production or peripheral ketone body clearance rates. Furthermore, the effects of acid-base disturbances on lipolysis and thus on free fatty acid availability have not been determined. To clarify these issues, ketone body kinetics and plasma glycerol and free fatty acids were measured in overnight-fasted normal subjects whose acid-base balance was modified by the administration of $NaHCO_3$ (alkalosis), NaCl (control), or NH_4Cl or arginine-HCl (acidosis) during a "pancreatic clamp." The latter utilizes somatostatin infusions with replacement infusions of basal amounts of insulin, glucagon, and growth hormone to overcome the confounding effects of pH influence on ketone body regulatory hormones. Furthermore, the study also addressed the question of whether single-ketone body tracer infusions could provide similar estimates of total ketone body turnover as dual-ketone body tracer infusions.

METHODS

Subjects. Twenty-four studies were performed in eight women (postmenopausal or surgically sterilized) age 40–59 yr and six men age 42–59 yr. Weights of the volunteers ranged from 52 to 81 kg, averaging 65 kg. All subjects were in good health, had normal glucose tolerance, were not taking medications, and had given written informed consent. Seven participated on one occasion, four on two, and three on three occasions with repeated studies separated by at least 8 wk. The studies were approved by the Human Ethics Committee and carried out in the Kantonsspital, Basel, Switzerland. After an overnight fast, subjects were admitted to the Clinical Research Center at 7:30 A.M. and were placed in a semirecumbent position in bed. After an 18-gauge Teflon cannula was inserted into a left antecubital vein for infusions, a butterfly needle was inserted in a dorsal vein of the right hand for blood sampling, and the hand was placed in a box heated to 58°C to "arterialize" the blood (30).

Study protocols. In all studies, constant infusions of somatostatin (Stilamin; Serono) 108 $ng \cdot kg^{-1} \cdot min^{-1}$, human regular insulin (50 $\mu U \cdot kg^{-1} \cdot min^{-1}$; Novo, Copenhagen, Denmark), glucagon (0.4 $ng \cdot kg^{-1} \cdot min^{-1}$; Novo, Copenhagen), and human growth hormone (6 $ng \cdot kg^{-1} \cdot min^{-1}$; Crescormon, Kabi, Stockholm, Sweden) were delivered with Harvard pumps during the 60-min tracer equilibration period and the 210-min study period (0–210 min) to maintain constant and basal plasma hormone concentrations. Dilutions were made in 0.154 M NaCl and human albumin (Swiss Red Cross) was added to the insulin, glucagon, and growth hormone infusates to a final concentration of 0.8%. At 7:45 A.M., priming doses of Na-[3-^{14}C]acetoacetate ([3-^{14}C]AcAc; 20 μCi) and of D-β-[1,3-$^{13}C_2$]hydroxybutyrate (β-[1,3-$^{13}C_2$]OHB; 0.9 mmol) were administered followed by continuous

infusions of Na-[3-^{14}C]AcAc (125 μCi total dose) and β-[^{13}C]OHB (5.85 mmol total dose) using Harvard pumps. ^{13}C tracer was given to six subjects in the $NaHCO_3$, five subjects in the NaCl, four subjects in the arginine-HCl, and three subjects in the NH_4Cl protocols. After a 30-min baseline period (0–30 min), the acid-base state was changed by the administration of $NaHCO_3$ (alkalosis), NaCl (control), NH_4Cl (acidosis), or arginine-HCl (acidosis) in doses of 4 mmol/kg. One molar solutions of $NaHCO_3$, NaCl, and arginine-HCl were infused intravenously at a rate of 41.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for 60 min and at 12.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for the remaining 120 min. The NH_4Cl was given orally in capsules in eight divided doses every 15 min because intravenous infusions caused acute nausea. The total fluid volume delivered during the 270 min was <450 ml and was similar in all groups.

Tracer preparation. Na-[3-^{14}C]AcAc was prepared by hydrolysis of [3-^{14}C]ethyl AcAc (Radiochemical Centre, Amersham, UK) using NaOH (18). The β-OHB infusates were synthesized from [1,3-$^{13}C_2$]ethyl AcAc (99% mole percent enriched) obtained from Tracer Technologies, Cambridge, MA. We prepared lithium [1,3-$^{13}C_2$]AcAc from ethyl AcAc (9) and subsequently converted it to D-β-[1,3-$^{13}C_2$]OHB (24) using a modification of the method of Passingham and Barton (27). After extraction into ethyl acetate and subsequent evaporation, the residue was redissolved in water, desalted with Dowex 50, and neutralized with NaOH to pH 7.2. This solution was again lyophilized, redissolved in water, and passed through Millipore filters into sterile vials, which were then autoclaved. The [$^{13}C_2$]sodium OHB concentration in the infusate was measured by enzymatic spectrophotometric assay. The stable isotope-labeled compounds were stored at −70°C.

Sample collection and analysis. Arterialized hand-vein blood was collected every 10 min during the baseline period and every 15 or 30 min during the experimental period and placed into iced 30% perchloric acid for measurement of blood ketone bodies, glycerol concentrations, ^{14}C radioactivity, and ^{13}C enrichment of ketone bodies. We neutralized these samples immediately using 20% KOH. Further aliquots were centrifuged and plasma was obtained for later measurement of glucose, immunoreactive insulin (IRI), glucagon, and free fatty acids (FFA). Partial pressure of CO_2 and O_2 in blood (P_{CO_2}, P_{O_2}), pH, and hemoglobin were measured in samples of cold heparinized blood, and blood bicarbonate and O_2 saturation were calculated automatically with an ABL blood gas analyzer (Radiometer; Copenhagen, Denmark). All analytical methods have been described previously (34). To summarize, blood AcAc was heat- and acid-decarboxylated and the newly formed and preexisting acetone were measured on the day of the study in neutralized filtrates by head-space analysis and gas chromatography. Blood β-OHB and glycerol were measured by microfluorimetric adaptations of standard enzymatic methods, plasma FFA with a radiochemical method, plasma glucose with a glucose analyzer (Yellow Springs), and IRI and glucagon with radioimmunoassays; ^{14}C radioactivity in blood AcAc and β-OHB were determined by a modification (18) of the method of Mayes and Felts (19).

Sample preparation for gas chromatography-mass spectrometry analysis of ketone bodies. Sample preparation was made by a modification of the procedure of Miles et al. (23). The deproteinized plasma supernatants were acidified with 2 N HCl, and 15 ml cold ethylacetate was added. The mixture was shaken and centrifuged at 450 g for 10 min. The organic layer was added to 1 ml Na_3PO_4 at 0°C. The mixture was again shaken and centrifuged. The aqueous layer was transferred into 2-ml crimp cap vials and lyophilized. *N*-methyl-*N*-(*t*-butyldimethyl)-silyltrifluoroacetamide and acetonitrile (100 μl each) were added to the residue. The vial was capped and sonicated for 15 min. The vials were stored at −70°C until the measurements were made.

Measurement of stable isotope enrichment. The analyses were made on a gas chromatograph with mass selective detector Hewlett-Packard 5890/5970. A 25 m × 0.2 mm 5% phenylmethylsilicone capillary column was used. The injector temperature was 250°C and that of the interface 270°C. With the use of electron impact ionization and the selected ion monitoring mode, the ions 275 and 277 were observed for β-OHB and the ions 273 and 275 for AcAc. Standard curves of increasing amounts of the ratios β-[^{13}C]OHB/β-[^{12}C]OHB and [^{13}C]AcAc/[^{12}C]AcAc were used to determine ^{13}C mole percent excess (MPE) of plasma ketone bodies (23). The peak height ratios for β-OHB (277/275 × 100) and for AcAc (275/273 × 100) in the samples were calculated.

Calculations. Total ketone body (TK) production (rate of appearance, R_a) was calculated using three different methods: *1*) TK ^{14}C specific activity, sp act (20), *2*) TK ^{13}C MPE (23), and *3*) an open two-pool model (29) using ^{14}C and ^{13}C results as applied by Miles et al. (24). In the single-isotope method, (*1*) and (*2*), the total ketone body production rates were calculated using non-steady state assumptions (16). The total ketone body ^{14}C sp act was obtained by dividing the sum of AcAc and β-OHB radioactivity by TK (AcAc and β-OHB) concentrations. In the dual-isotope method, (*3*), inflow and outflow of each ketone body and the interconversion rate between the two were calculated using a series of six simultaneous equations, which require for solution the infusion rates of the two isotope-labeled ketone bodies and the ^{14}C sp act and the ^{13}C MPE of each of the two ketone bodies. These calculations use steady state assumptions. This was assessed to be present by analysis of variance (ANOVA), indicating unchanged ^{14}C sp act, ^{13}C MPE, and concentration of the two ketone bodies during the periods of measurement. The metabolic clearance rate (MCR) was calculated as the ratio of the rate of total ketone body disappearance and the average total ketone body concentration. Urinary ketone body clearance was omitted in the calculation of the metabolic clearance as it has been demonstrated to be <5% of total ketone body clearance in similar situations (18).

Statistics. ANOVA with repeated measures (BMDP Statistical Software) demonstrated that ketone body kinetics had reached steady state during the baseline period (0–30 min) and during the last 30 min of the acid-base

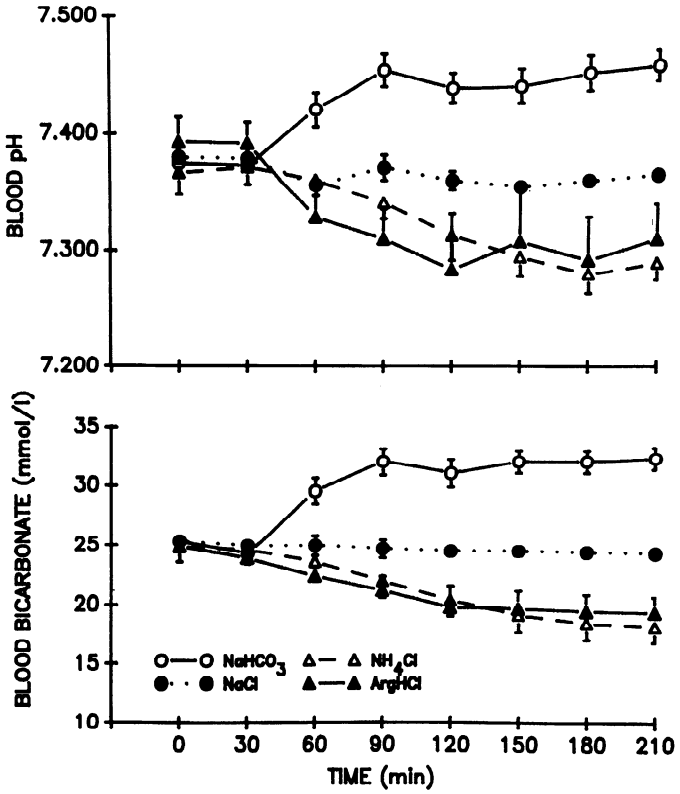

FIG. 1. Blood pH and bicarbonate during administration of $NaHCO_3$ ($n = 7$), NaCl ($n = 6$), arginine-HCl ($n = 5$), or NH_4Cl ($n = 6$) during 30–210 min. Blood pH and bicarbonate at 180–210 min differed from baseline (0–30 min) in all groups ($P < 0.01$) except NaCl (control) and were greater in the $NaHCO_3$ than either control or acidotic groups ($P < 0.01$).

infusion period (180–210 min). Wilcoxon tests and paired *t* tests, as appropriate, were used to compare the baseline and last 30 min test periods for all metabolic values. ANOVA with repeated measures and subsidiary comparisons with Bonferroni tests or Scheffé *F* tests using pooled variances were used to evaluate differences among groups in the final 30-min period and the changes from baseline to the last 30 min among the groups. Multiple regression analyses were performed to evaluate the influence of acid-base changes and plasma FFA concentrations on ketone body production rates and ketone body concentrations.

RESULTS

Acid-base changes (Fig. 1, Tables 1 and 2). After infusion of $NaHCO_3$, the pH rose from baseline values of 7.373 ± 0.007 to 7.455 ± 0.013 after 60 min and remained stable for the next 120 min, averaging 7.455 ± 0.014 in the final 30 min ($P < 0.005$). Blood bicarbonate followed a similar pattern, increasing from 25.0 ± 0.4 mM to 32.0 ± 1.2 after 60 min and averaging 32.1 ± 1.1 mM during the last 30 min ($P < 0.001$). The P_{CO_2} showed the expected modest rise from 43 ± 1 mmHg at baseline to 46 ± 1 mmHg during the last 30 min ($P < 0.005$). Changes of similar degree occurred in the opposite direction during NH_4Cl ingestion and arginine-HCl infusion. With NH_4Cl, blood pH decreased from 7.368 ± 0.013, leveling at 7.284 ± 0.015 in the final 30 min ($P < 0.01$). Blood bicarbonate decreased from 24.8 ± 1.1 mM to 18.3 ± 1.4 mM ($P < 0.005$) and P_{CO_2} from 44 ± 2 mmHg to 40 ± 2 mmHg at the end of the study ($P < 0.005$). During the arginine-HCl infusion, a metabolic acidosis was also produced as pH decreased from 7.390 ± 0.014 to 7.304 ± 0.033 ($P < 0.05$) and bicarbonate from 24.3 ± 0.2 to 19.4 ± 1.3 mM ($P < 0.05$) during the final 30 min whereas P_{CO_2} remained unchanged. Blood pH and P_{CO_2} remained constant throughout the NaCl infusions whereas bicarbonate decreased <1 mM ($P < 0.005$). There were no differences in blood pH, bicarbonate or P_{CO_2} among the subjects during the baseline periods, but blood pH, bicarbonate, and P_{CO_2} differed significantly between the $NaHCO_3$ and NH_4Cl or arginine-HCl groups in the final 30 min ($P < 0.005$, $P < 0.01$, $P < 0.05$), blood pH and bicarbonate between the $NaHCO_3$ and NaCl groups ($P < 0.001$, $P < 0.01$) and blood bicarbonate between the NaCl and NH_4Cl or arginine-HCl groups ($P < 0.005$, $P < 0.05$). Acid-base values were not different between the NH_4Cl and arginine-HCl groups.

Ketone body kinetics (Tables 1 and 2, Figs. 2 and 3). Blood TK concentrations increased with $NaHCO_3$ infu-

TABLE 1. *Acid-base values, blood substrate and hormone concentrations, and ketone body kinetics during 180–210 min alkalosis and acidosis*

	$NaHCO_3$	NaCl	NH_4Cl	Arg-HCl
n	7	6	6	5
pH	7.455±0.014	7.366±0.007	7.284±0.015*	7,304±0.033*
HCO_3^-, mM	32.1±1.1	24.3±0.5	18.3±1.4*	19.4±1.3*
P_{CO_2}, mmHg	46±1	43±1	40±2†	40±1†
AcAc + acetone, μM	140±27	99±25	80±16	79±15
β-OHB, μM	267±60	183±67	110±29	111±61
β-OHB/(AcAc + acetone)	1.8±0.2	1.6±0.4	1.1±0.3	1.3±0.3
TK, μM	407±86	282±89	191±43	190±54
R_a TK, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	5.8±1.6	4.0±1.0	3.2±0.8	2.9±0.7
MCR TK, $ml \cdot kg^{-1} \cdot min^{-1}$	13.8±1.2	15.9±1.6	18.3±3.0	16.7±1.6
[^{14}C]AcAC infusion rate, $dpm \cdot kg^{-1} \cdot min^{-1}$	11,149±1,432	8,857±1,632	11,065±1,327	10,277±992
FFA, μM	904±109	526±113†	521±34	473±153†
Glycerol, μM	76.3±9.5	44.7±6.2	45.6±5.5	57.9±11.3‡
Glucose, mM	9.9±0.6	8.5±0.8	9.9±0.7	9.6±0.3
IRI, μU/ml	12.5±1.8	14.7±1.8	17.1±2.6	12.5±1.7
Glucagon, pg/ml	50.2±5.0	40.1±4.7	34.5±6.1	56.7±8.7

Values are means ± SE. Values of R_a TK determined using [^{14}C]AcAc tracer. * $P < 0.01$ vs. $NaHCO_3$. † $P < 0.05$ vs. $NaHCO_3$. ‡ $n = 4$.

TABLE 2. *Changes in blood values in hyperketonemic subjects with superimposed alkalosis or acidosis*

	$NaHCO_3$	NaCl	NH_4Cl	Arg-HCl
n	7	6	6	5
ΔpH	+0.082±0.015[a]	+0.011±0.006	−0.084±0.019[a,d]	−0.086±0.025[a,d]
ΔBicarbonate, mM	+7.1±1.1[b]	−0.8±0.1	−6.5±0.9[b,d]	−5.0±1.3[b,d]
ΔR_a total ketones, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	+1.93±0.79	+0.05±0.41	−0.97±0.62[a,c]	−2.00±0.46[a,c]
ΔTotal ketones, μM	+198±65	+48±48	−90±53[c]	−154±55[c]
Δβ-OHB, μM	+142±47	+47±37	−71±41[c]	−95±35[c]
ΔFFA, μM	+197±71[b]	−136±52	−286±72[c]	−297±140[c]
ΔGlycerol, μM	+2±7	−16±5	−36±19[c]	−22±14[e]

Values are means ± SE. [a] $P < 0.05$, [b] $P < 0.01$ differs from control, [c] $P < 0.05$, [d] $P < 0.001$ differs from $NaHCO_3$, [e] $n = 4$. Changes (Δ) are between baseline 0–30 min and 180–210 min.

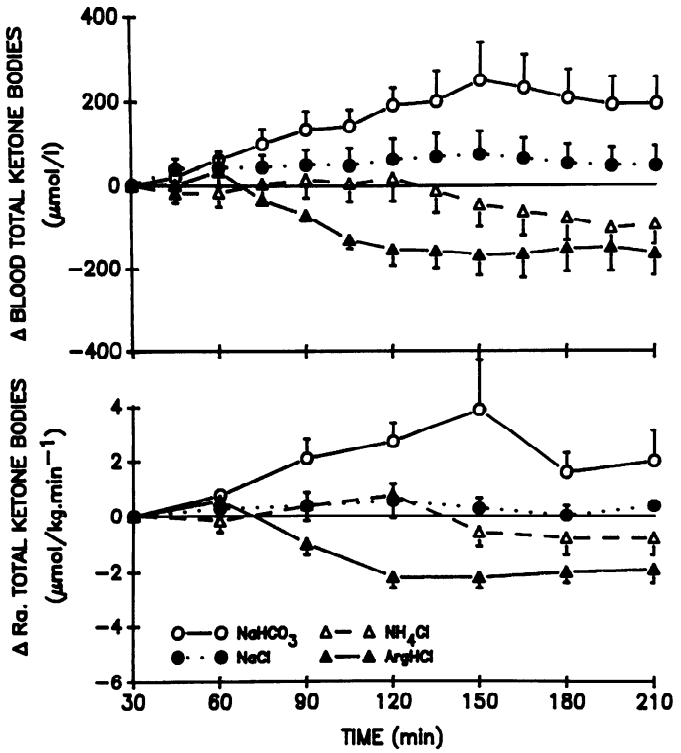

FIG. 2. Change (Δ) from baseline (0–30 min) for blood total ketone body (TK) concentrations and for rate of appearance of total ketone bodies (R_a TK) as measured by [^{14}C]AcAc tracer infusions during 180-min administration of $NaHCO_3$ ($n = 7$), NaCl ($n = 6$), arginine-HCl ($n = 5$), or NH_4Cl ($n = 6$). ΔTK and ΔR_a TK at 180–210 min differed between the $NaHCO_3$ group and both NH_4Cl ($P < 0.05$) and arginine-HCl ($P < 0.05$) groups.

sions from 209 ± 37 during baseline to 407 ± 86 μM in the last 30 min ($P < 0.03$). They decreased with arginine-HCl from 348 ± 88 to 190 ± 54 μM ($P < 0.05$) over the same period and were 241 ± 78 (baseline) and 191 ± 43 μM in the last 30 min in NH_4Cl group (NS). The change between baseline and the last 30 min differed between the $NaHCO_3$ and both the arginine-HCl ($P < 0.05$) and NH_4Cl ($P < 0.05$) groups (Fig. 2, Table 2). There were no significant changes in blood TK concentrations in NaCl control studies. The changes in TK concentrations were mainly the result of changes in blood β-OHB concentrations where changes between baseline and the last 30 min differed between the $NaHCO_3$ and the NH_4Cl ($P < 0.05$) and arginine-HCl ($P < 0.05$) groups (Table 2). Blood AcAc concentrations were not different among the groups, nor did they or the ratios of β-OHB to AcAc concentrations change significantly during pH modifications.

Neither metabolic clearance rates (MCR TK) at the end of the study (Table 1) nor their changes from baseline (Table 2) differed among the groups. There was a small increase in MCR TK from baseline in the arginine-HCl ($P < 0.05$) and NH_4Cl ($P < 0.05$) groups. TK production rates, assessed in all studies with [^{14}C]AcAc tracer (R_a TK ^{14}C), increased from 3.9 ± 1.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at baseline to 5.8 ± 1.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during the last 30 min with $NaHCO_3$ administration ($P < 0.02$), decreased from 4.9 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to 3.0 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the arginine-HCl group ($P < 0.01$) and was 4.2 ± 1.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (baseline) and 3.2 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (last 30 min) in NH_4Cl group (NS). In addition, the changes between baseline and the last 30 min differed between the $NaHCO_3$ and the arginine-HCl ($P < 0.05$) and the NH_4Cl ($P < 0.05$) groups (Table 2, Fig. 2). TK production rates were also determined using β-[^{13}C]OHB tracer (R_a TK ^{13}C) in most of the subjects (see METHODS). As shown in Fig. 3, there was a very close relationship between the values obtained for TK production rates using single-ketone body tracers ([^{14}C]AcAc, β-[^{13}C]OHB) and the dual-tracer methods.

Plasma FFA and glycerol concentrations (Tables 1 and 2, Fig. 4). Plasma FFA concentrations increased with $NaHCO_3$ infusion from baseline values of 706 ± 110 μM to values of 904 ± 109 μM during the last 30 min of infusion ($P < 0.05$). They decreased in the NH_4Cl group from baseline values of 807 ± 81 μM to 521 ± 34 μM ($P < 0.03$) and in the arginine-HCl group from 771 ± 92 μM to 473 ± 148 μM ($P < 0.05$) at the end of the study period. A small decrease also occurred in the NaCl group where values were 662 ± 154 μM at baseline and 526 ± 113 μM in the last 30 min ($P < 0.05$). The changes between baseline and the last 30 min differed between the $NaHCO_3$ group, and the NH_4Cl ($P < 0.05$) and arginine-HCl ($P < 0.05$) groups. Plasma glycerol concentrations decreased from baseline in NaCl ($P < 0.05$) and NH_4Cl ($P < 0.05$) and in the arginine-HCl and NH_4Cl ($P < 0.01$) groups. The change between baseline and the last 30 min differed between NH_4Cl and $NaHCO_3$ ($P < 0.05$).

Insulin and glucagon. (Table 1, Fig. 5). As expected during somatostatin and insulin infusions, plasma insulin concentrations did not differ among the groups during the last 60 min. Except for a transient increase in plasma insulin concentrations (>10 μU/ml) early in the argi-

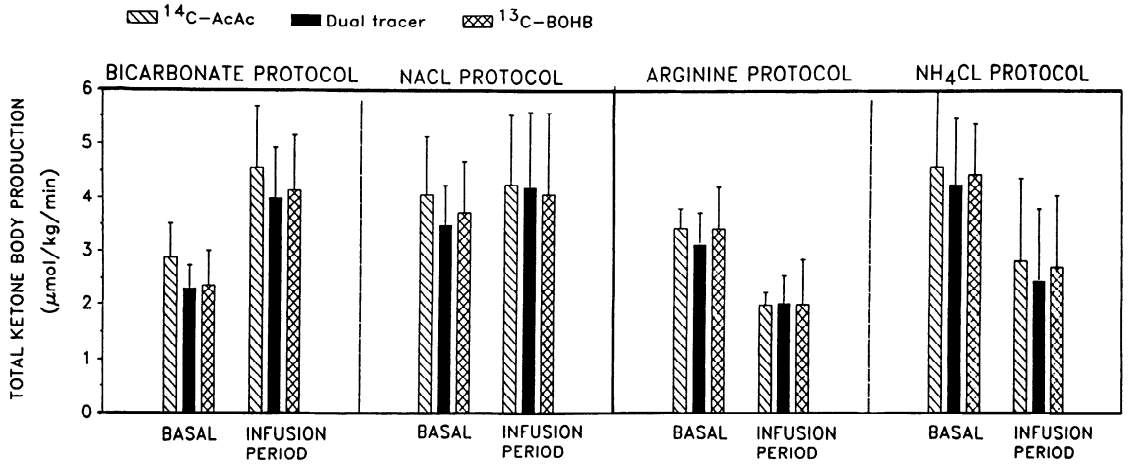

FIG. 3. Comparison of ketone body production rates calculated using [^{14}C]AcAc tracer infusion, β-[^{13}C]OHB infusion, or combination of both (dual-tracer method) during 150–180 min administration of $NaHCO_3$ (n = 6), NaCl (n = 5), arginine-HCl (n = 4), and NH_4Cl (n = 3).

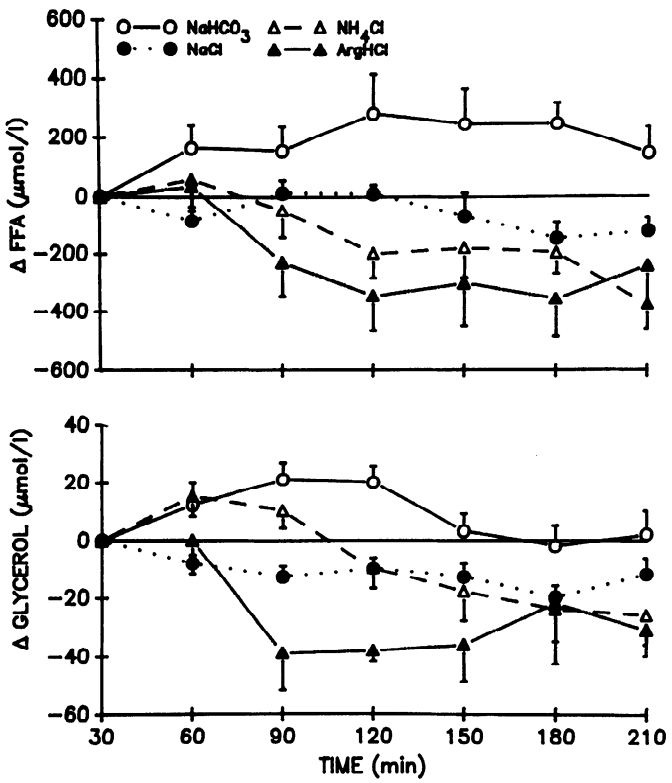

FIG. 4. Change (Δ) from baseline (0–30 min) for plasma free fatty acid (FFA) and glycerol concentrations during 180-min administration of $NaHCO_3$ (n = 7), NaCl (n = 6), arginine-HCl (n = 5), and NH_4Cl (n = 6). Values at 180–210 min differed between the $NaHCO_3$ and both NH_4Cl ($P < 0.05$) and arginine-HCl ($P < 0.05$) groups for ΔFFA and between $NaHCO_3$ and NH_4Cl ($P < 0.05$) groups for Δglycerol.

nine-HCl study in 3–5 subjects, the values remained stable during the last 60 min of the arginine-HCl infusions and throughout the infusions in the other protocols. Baseline plasma glucagon concentrations were 60.5 ± 7.3 pg/ml ($NaHCO_3$), 47.5 ± 5.0 pg/ml (NaCl), 47.5 ± 9.5 pg/ml (NH_4Cl), and 40.4 ± 4.2 pg/ml (arginine-HCl). Values remained unchanged during the infusions and did not differ among the groups.

Plasma glucose concentrations increased from 7.4 ± 0.2 to 9.5 ± 0.3 mM during the infusions ($P < 0.001$),

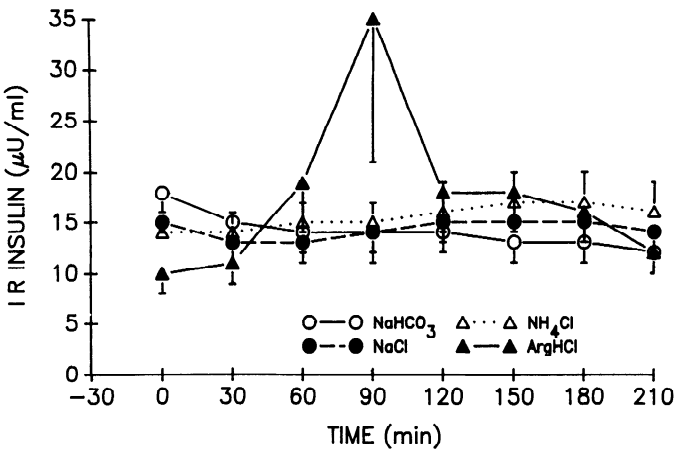

FIG. 5. Plasma immunoreactive insulin (IRI) in normal subjects infused with somatostatin and insulin at 50 $\mu U \cdot kg^{-1} \cdot min^{-1}$ for 210 min while $NaHCO_3$ (n = 7), NaCl (n = 6), arginine-HCl (n = 5), or NH_4Cl (n = 6) were administered during last 180 min. Values did not differ at baseline (0–30 min) or at 180–210 min among groups. Apparent increase in IRI at 90 min during arginine-HCl infusion was not statistically significant.

but there were no differences among the groups either during the baseline period or at any stage of the studies.

DISCUSSION

The present study demonstrates that modest changes in systemic acid-base balance, as seen in physiological or pathological situations, modify ketone body production rates. The superimposition of a mild metabolic alkalosis (+0.08 pH units) caused a 50% increase in total ketone body production compared with a 10% increase in the NaCl control study. In contrast, a mild metabolic acidosis of similar degree produced by either NH_4Cl or arginine-HCl caused 23 and 40% respective decreases in total ketone body production. Accompanying these alterations in production were parallel increases and decreases in blood total ketone body concentrations. As there were no significant differences in the ketone body metabolic clearance rates among the four protocols, blood ketone body concentrations changed as a result of changes in

TABLE 3. *Multiple regression analysis of factors influencing ketone body production during alkalosis and acidosis*

Dependent Variable	Independent Variables Entered	Step No.	Significant Independent Variables	F	R^2	P
R_a TK	FFA, pH, bicarbonate	1	Bicarbonate	21.1	0.49	<0.001
TK concentration	R_a TK, bicarbonate, MCR TK, pH	1	R_a TK	100.6	0.82	<0.001
		2	MCR TK	37	0.94	<0.001
		3	pH	4.1	0.95	<0.001
FFA concentration	pH, bicarbonate, TK concentration	1	Bicarbonate	25.6	0.54	<0.001

their production. The changes in blood total ketone bodies largely reflected the increases and decreases in blood β-hydroxybutyrate concentrations as blood acetoacetate concentrations did not change significantly. These observations provide the first direct evidence that ketone body production in vivo is enhanced during systemic alkalosis and inhibited during systemic acidosis. They also confirm previously made indirect observations that ketone body metabolic clearance rates are not significantly influenced by pH (6).

The rapid occurrence and direction of the changes in total ketone body production, although quantitatively small, are consistent with our previously stated hypothesis that modifications of ketoacid production brought about by changes in systemic pH can serve as a regulatory mechanism for preserving acid-base equilibrium (12, 14). The quantitative impact of the influence of pH on acid-base equilibrium is most apparent in situations where baseline ketoacid production is large (11, 12, 13). However, even in the present study where ketone body production rates were only modestly increased, the resultant rise or fall in ketone body production rates contributed a 15% offset for the base and acid infusions during the last 60-min steady state period.[1]

To assess the role of FFA substrates in mediating pH effects on ketogenesis, plasma FFA and glycerol concentrations were determined. FFA concentrations increased during the bicarbonate infusion and were greater in this phase than in the control or acidotic phases. Plasma glycerol concentrations decreased in the acidotic groups, and there was a similar pattern of divergence between the alkalotic and acidotic groups to that seen with FFA. These changes in FFA and glycerol suggest that the acid-base changes influenced lipolysis. Hepatic uptake of FFA is proportional to arterial FFA concentrations (8, 33), and FFA availability in the hepatocyte is one of the key regulating processes for ketone body production (17, 22). The contribution of the altered substrate availability to altered ketogenesis in this study is further emphasized by the multiple regression analysis that examined the relationship between ketogenesis and lipolysis, and pH, bicarbonate, and FFA concentrations where variation in blood bicarbonate accounted for 50% of the variability in R_a TK ($R^2 = 0.49$) and 54% of the variability in FFA concentrations ($R^2 = 0.54$) (Table 3). When plasma FFA were controlled for in the multiple regression analysis, there was no further significant effect of pH on ketogenesis.

These results do not exclude the possibility that systemic acid-base disturbances can also directly influence hepatic ketogenesis. A decrease in extracellular pH from 7.4 to 6.6 inhibited β-OHB efflux from isolated rat hepatocytes (21). Similar observations were made in hepatocytes from fed rats (5) where both AcAc and β-OHB efflux increased during alkalosis and decreased during acidosis. When the same investigators produced both semichronic (acetazolamide-induced) or acute (HCl-induced) acidosis in rats in vivo, hepatic output of ketones was reduced, whereas when bicarbonate was infused into the portal vein, ketogenesis increased (5). At the intracellular level, decreased pH can inhibit and increased pH can stimulate carnitine palmitoyltransferase I (31), an important rate-limiting factor in ketogenesis, by modifying malonyl CoA binding to the enzyme (25).

The mechanism by which pH modifies lipolysis in humans remains to be investigated. Alteration in the sensitivity of lipolysis to endogenous catecholamines has been suggested by studies in isolated fat cells where acidosis has been shown to inhibit epinephrine-induced lipolysis and alkalosis to stimulate it (10, 32). In humans (12) and dogs (28), modest changes in systemic pH associated with metabolic acid-base perturbations did not demonstrate altered plasma FFA concentrations. When pH was reduced to 6.8 with ammonium chloride infusions in fasted rats, both FFA and glycerol concentrations were suppressed; insulin concentrations, however, were increased (1). Similarly, NH_4Cl administration to dogs inhibited, whereas a $NaHCO_3$, tris(hydroxymethyl)aminomethane, or hyperventilation stimulated norepinephrine-induced lipolysis (26). And in humans during exercise a superimposed metabolic acidosis was associated with a fall in plasma FFA concentrations (15), an event that was not observed when a respiratory acidosis was created during a similar exercise protocol (4). All these observations indicate that changes in systemic pH can influence fatty acid substrate availability with some variability among species.

Because insulin and glucagon concentrations did not differ among the groups during the last 60 min of the

[1] For example, during the first 2 h of the bicarbonate infusion, blood bicarbonate concentration increased by 7 mM as would be expected if the 3.25 mmol/kg ($41.7\ \mu mol \cdot kg^{-1} \cdot min^{-1} \times 60$ min + $12.5\ \mu mol \cdot kg^{-1} \cdot min^{-1} \times 60$ min) of administered bicarbonate was distributed in a space equaling ~50% body wt. In the 3rd h, with continued bicarbonate infusions of $12.5\ \mu mol \cdot kg^{-1} \cdot min^{-1}$, blood bicarbonate concentration would have been expected to increase (~1.5 mM), but it remained stable. This indicates that the infused bicarbonate must have been lost at the same rate in the urine, buffered in cells, or neutralized by increased acid production. The increased ketoacid production rates of $1.9\ \mu mol \cdot kg^{-1} \cdot min^{-1}$ compared with control during this period could have titrated 15% of the $12.5\ \mu mol \cdot kg^{-1} \cdot min^{-1}$ infused bicarbonate. Using a similar calculation for the arginine-HCl infusions, the decrease in ketoacid output of $2.05\ \mu mol \cdot kg^{-1} \cdot min^{-1}$ compared with control could have been responsible for 16% of the failure of the acid infusion to decrease plasma bicarbonate.

studies, their known regulatory influence on lipolysis and ketogenesis could not have caused the changes observed in this study. Metabolic acidosis can cause increased plasma insulin and glucagon concentrations and insulin resistance (3). However, insulin insensitivity cannot explain the results in the present study because lipolysis and ketogenesis were decreased, not increased, during acidosis.

Arginine-HCl produced a similar degree of systemic acidosis to NH_4Cl in this study, and the influence on ketone body production was remarkably similar. Although there was a transient increase in plasma insulin concentrations in the first 90 min of the arginine-HCl study period in three of the five subjects (probably because of breakthrough of somatostatin blockade during arginine stimulation), ketone body production was suppressed in all five subjects. Also, ketone body production rates remained lower even when insulin was suppressed during the last 60 min. This speaks in favor of acidosis-mediated suppression of ketone body production, but it is conceivable that arginine-HCl inhibited ketosis by a mechanism other than the acidosis it produced.

The present studies were performed using dual-ketone body tracer infusions. The second tracer, β-[^{13}C]OHB, was used to confirm the single-tracer method ([^{14}C]AcAc) for assessing total ketone body turnover, the validity of which has recently been questioned at least in situations where there is vast isotopic disequilibrium between individual ketone bodies (24). In addition, "pseudoketogenesis" may occur due to cycling of labeled carbon in precursor pools as demonstrated in the rat heart (7). As shown in Fig. 3, although there was a tendency for the dual-tracer technique to provide lower estimates of R_a TK, there was remarkable similarity in the ketone body production rates calculated using the AcAc or β-OHB tracers alone or in combination, and the direction and magnitude of the changes among all four protocols were almost identical using all three calculations. These results support the validity of the single-tracer method using the "combined" specific activity of individual ketone bodies in the calculation, in a study of the regulation of ketone body kinetics, at least during conditions of modest ketosis as observed in the present study. They confirm the previous findings that kinetics calculated with a single tracer, using non-steady state conditions (18), provide equivalent rates of total ketone production to those obtained by measuring net hepatic ketone output with arteriovenous catheterization (16), even though from a theoretical point of view the method is imperfect (2).

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