Metabolism
Clinical and Experimental
VOL. XXXI, NO. 1 JANUARY 1982

Sampling of Arterialized Heated-Hand Venous Blood as a Noninvasive Technique for the Study of Ketone Body Kinetics in Man

G. E. Sonnenberg and U. Keller

To determine if sampling of arterialized-hand venous (HV) blood is a suitable alternative for arterial (A) blood to study ketone body metabolism, concentrations of unlabeled and labeled ketone bodies were measured during continuous infusion of 3-^{14}C-acetoacetate in simultaneously drawn samples from A and HV blood in normal subjects. The mean difference of acetoacetate between A and HV blood was in the basal state 1.5%, and that of β-hydroxybutyrate 6% (n.s.). Similarly, the ^{14}C-content of ketone bodies and their calculated rates of production and metabolic clearance were not significantly different between A and HV blood. Following induction of ketosis by acetoacetate loading infusions, the difference of concentrations and ^{14}C-content of total ketone bodies between HV and A blood remained insignificant (average 3%), and ketone body kinetics calculated from A and HV blood were similar. Furthermore, concentrations of glucose, lactate and pCO_2 did not differ significantly between the two sampling sites. In contrast, concentrations of ketone bodies, glucose and pO_2 were significantly lower, and the metabolic clearance rate of ketone bodies and pCO_2 higher in antecubital venous blood compared to heated-hand venous blood. Thus, the similarity of heated-hand venous and arterial blood suggests that the noninvasive technique is suited for kinetic analyses using tracer methods and for arteriovenous balance studies of ketone bodies.

IT IS GENERALLY agreed that samples of uniformly mixed blood, usually arterial blood, are required for the determination of substrate turnover rates using tracer infusions in whole animals or in man. Peripheral venous blood, although more obtainable in man, is influenced by the metabolism of the particular region which is drained, and metabolite concentrations may be considerably divergent from arterial blood.[1] This may lead to a distinct error in the computation of tracer-determined kinetics, particularly during non-steady state.[2] However, arterial catheterization carries the risk of complications.[3,4,5] Therefore, sampling of arterialized hand-venous blood has previously been evaluated as an alternative method, and it has been found suitable for the determination of glucose kinetics[6] and of blood gases[7] in man.

The present study was performed to assess the validity of using the sampling technique for the study of ketone body kinetics and of arteriovenous concentration gradients in man. Their results, and pO_2, pCO_2, blood glucose and plasma lactate were compared between samples obtained from a heated dorsal hand vein, an artery and an antecubital vein during low and during elevated ketone body levels.

MATERIALS AND METHODS

Subjects

15 healthy volunteers participated in the study. 10 were males, and 5 females. Their age was between 45 and 71 yr (58.7 ± 1.8 years), and their weight was within 20% of ideal body weight. Fasting blood glucose, hematocrit, serum electrolytes, urea, ECG, chest x-ray were within normal limits prior to study. The experimental protocol had been approved by the Local Ethical Committee, and written informed consent was obtained from all participants. The studies were started at 8 a.m. after a 14 hr fast.

Experimental Procedures

The brachial artery was cannulated in 9 subjects under lidocaine local anesthesia using a Plastimed® catheter (0.7 mmID) for blood sampling. A Venflo® catheter (0.8 mmID) was inserted into an antecubital vein for infusion of labeled and of unlabeled acetoace-

From the Division of Endocrinology and Metabolism, Department of Medicine and Department of Research, University Hospital, Basel, Switzerland.

Received for publication September 10, 1980.

Supported in part by Grant no. 3.849.–077 of the Swiss National Science Foundation.

Address reprint requests to Ulrich Keller, M.D., Department of Medicine, Kantonsspital, CH-4031 BASEL Switzerland.

0026-0495/82/3101-0001$0.100/0

tate. A Venflo was also introduced into the contralateral antecubital vein in 6 additional subjects for blood sampling. A dorsal vein of the hand was catheterized in all subjects by a Butterfly® needle (1.1 mmID) into proximal direction. The hand was placed into a thermostatic chamber while the subjects were resting in a 45° half-supine position. The chamber measured 55 × 33 × 28 cm, and was equipped with 3 air-shield openings, one of them fitting the subject's wrist. The temperature inside the chamber was maintained at 60°C by a stream of heated air controlled by a thermostat. This temperature was not associated with any discomfort to the subjects. According to our experience, human volunteers tolerate the chamber without discomfort for 4 hr. The catheter was connected to a three-way stopcock outside the chamber and kept patent by a slow drip of saline. Sampling was possible without using a tourniquet.

Sodium 3-^{14}C-acetoacetate (250 μCi) diluted in saline was administered as a primed-constant infusion. Following priming injection of 20 μCi, the tracer was allowed to equilibrate for 40 min; later on blood samples were drawn in 9 subjects simultaneously from the artery and from the heated-hand vein in 10 min intervals. After a basal period of 30 min, unlabeled acetoacetate was infused at rates which were stepwise increased within 50 min from 5 to 25 μmole/kg/min. For comparison of heated-hand venous and antecubital venous blood, simultaneous blood samples were drawn in 6 subjects during the basal state.

Preparation of Infusates

3-^{14}C-ethylacetoacetate (250 μCi, S.A. 9.2 μCi/μmole, Radiochemical Center, Amersham) was added to 0.5 mmole freshly redistilled carrier ethylacetoacetate. After hydrolysis with a 10% excess of NaOH, incubation for 60 min of 40°C, and neutralization with 0.1 *N* HCl the solution was evaporated to approximately half of its initial volume. The radiochemical purity of the infusate was between 85% and 93% when determined as described previously.[12] The infusate containing unlabeled sodium acetoacetate was prepared by the same reaction from freshly redistilled ethylacetoacetate (Fluka, Switzerland) using Kreb's method.[8] Both infusates were sterilized by passing through a Millipore filter (0.22 μm), and kept frozen in sterile flasks at −60°C until they were used for infusion within 8 wk. The infusion syringes containing labeled and unlabeled acetoacetate were kept chilled with ice during infusion.

Analyses

The blood samples were drawn in heparinized syringes and collected in tubes containing EDTA. 4.5 ml of whole blood were immediately deproteinized by adding an equal volume of chilled 30% perchloric acid. The supernatants were neutralized with 20% KOH. Concentrations of acetoacetate (AcAc) and of β-hydroxybutyrate (βOHB) were determined enzymatically using a microfluorometric method.[9,10] The molarity of the acetoacetate infusate was determined prior and after the study using spectrophotometry. The acetone content in the infusate was less than 1% of the AcAc concentration. 0.3 ml of whole blood were pipetted into chilled 60 ml serum flasks for acetone determination using head space analysis by gas chromatography.[11] Concentrations of AcAc and acetone were determined within 3 hr after completion of the study because of their instability. ^{14}C-total ketone bodies were assayed in duplicates according to a previously described method[12] with a minor modification.[13] ^{14}C-content of total ketone bodies included AcAc, acetone and βOHB. The specific activity (S.A.) of total ketone bodies was calculated by dividing the concentration of total ketone bodies into the respective ^{14}C-radioactivity. Blood glucose[14] and plasma lactate[15] concentrations were determined enzymatically. pO_2 and pCO_2 were measured by an Eschweiler Blood Gas Analyzer (Kiel, FRG).

The rate of appearance (Ra) and the metabolic clearance rate (MCR) of total ketone bodies was calculated as follows:[2]

$$Ra = \frac{\text{Infusion rate} - p \times VD \times \text{Conc. (t)} \times \frac{dS.A.}{dt}}{\text{S.A. (t)}}$$

$$MCR = \frac{Rd}{\text{Conc. (t)}}, \text{ where } Rd = Ra - p \times Vd \times \frac{d\,\text{Conc.}}{dt.}$$

The infusion rate (dpm/min) and the volume of distribution (VD) were determined in each study as described previously,[16] and a pool fraction (p) of 1.0 was employed.[16] Ketone body kinetics were calculated from each time point using a sliding fit technique of 3 consecutive samples.[16] Results are presented as means ± SEM. For statistical analyses, Student's t test for paired data was used.

RESULTS

Comparison of Heated-Hand Venous (HV) With Arterial (A) Blood

The concentrations of acetoacetate (AcAc) and of β-hydroxybutyrate (βOHB) were slightly but insignificantly lower in HV than in A blood (Table 1). The average difference calculated from the percent differences of individual studies was 1.2 ± 3.5% for AcAc, and 6.3 ± 4.7% for βOHB in the basal period. Infusions of unlabeled AcAc (Fig. 1) brought about a rapid elevation of ketone body levels to almost 10-fold their basal values. Acetoacetate concentrations peaked at 90 min, and total ketone bodies at 100 min. ^{14}C-total ketone bodies rose to a smaller extent, and they reached maximal levels at 120 min. During hyperketonemia, the average difference between HV and A was even smaller (2.5 ± 2.2% for AcAc, and 0.4 ± 1.4% for βOHB) than during the basal period (Table 1). The concentrations of acetone were slightly higher in HV than in A blood suggesting a small amount of acetone produced from AcAc decarboxylation as the blood passed through the hand. Similar to the ketone body concentrations, the ^{14}C-content of total ketone bodies (AcAc, acetone and βOHB) was slightly higher in A than in HV; the differences were 6.4 ± 3.9% in the basal state, and 2.6 ± 1.4% during hyperketonemia; neither reached statistical significance. The larger SEM in the basal state indicated a considerable variability of basal ketone body concentrations between individual subjects.

The specific activity of total ketone bodies derived from their concentration and radioactivity differed between A and HV blood by less than 3% in the basal state, and by less than 1% during hyperketonemia. Consequently, there were no significant differences of Ra or MCR of total ketone bodies calculated from A or HV blood.

When Ra was calculated from both vessels during the period of increasing acetoacetate concentrations

Table 1. Ketone Body Kinetics in the Basal Period and During Hyperketonemia Derived From Heated-Hand Venous (HV) and From Arterial (A) Blood Samples in 9 Normal Subjects (Means ± SEM)

	A-Blood	HV-Blood	A-HV Difference	Significance
Basal period* (0–30 min)				
Acetoacetate concentration (μmole/l)	136 ± 22	134 ± 23	2 ± 5	n.s.
β-Hydroxybutyrate concentration (μmole/l)	157 ± 21	144 ± 30	12 ± 6	n.s.
Acetone concentration (μmole/l)	17 ± 4	19 ± 5	2 ± 1	n.s.
^{14}C-Content of total ketone bodies (dpm/ml)	1031 ± 104	949 ± 90	82 ± 42	n.s.
Ra of total ketone bodies (μmole/kg/min)	6.2 ± 1.3	6.4 ± 1.3	0.2 ± 0.4	n.s.
MCR of total ketone bodies (ml/kg/min)	19.9 ± 3.6	20.5 ± 4.0	0.6 ± 0.3	n.s.
Hyperketonemia† (90–120 min)				
Acetoacetate concentration (μmole/l)	1225 ± 85	1192 ± 86	33 ± 23	n.s.
β-Hydroxybutyrate concentration (μmole/l)	886 ± 118	870 ± 111	16 ± 11	n.s.
Acetone concentration (μmole/l)	205 ± 17	213 ± 22	−8 ± 6	n.s.
^{14}C-Content of total ketone bodies (dpm/ml)	2250 ± 231	2190 ± 223	60 ± 39	n.s.
Ra of total ketone bodies (μmole/kg/min)	25.3 ± 2.1	26.8 ± 2.0	1.5 ± 1.0	n.s.
MCR of total ketone bodies (ml/kg/min)	6.7 ± 0.4	6.8 ± 0.5	0.1 ± 0.2	n.s.

*Mean of 4 consecutive samples.
†Mean of 3 consecutive samples.

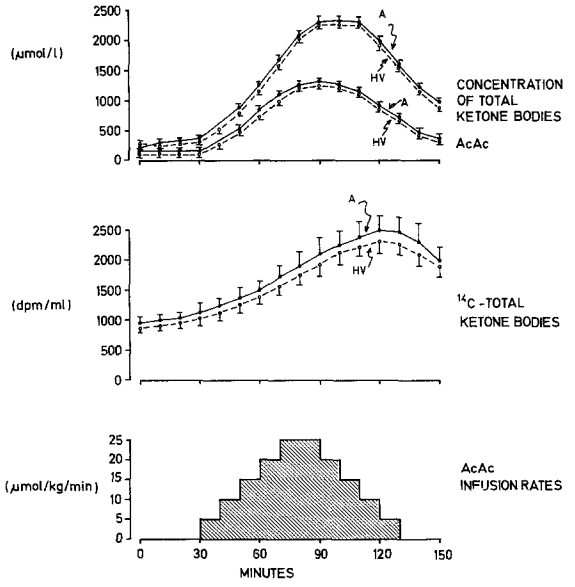

Fig. 1. Concentrations and ^{14}C-content of total ketone bodies and of acetoacetate, in arterial (A) and heated-hand venous (HV) blood during the basal state and during acetoacetate loading infusions. ^{14}C-acetoacetate was started at −40 min as primed infusion and administered throughout the study. Unlabeled acetoacetate was infused at stepwise increasing and decreasing rates from 5 to 25 μmole/kg/min. Results are means ± SEM of 9 normal subjects.

(between 30 and 90 min) the values of Ra calculated from A and from HV blood differed by 3.5 ± 2% (n.s.).

Comparison of Heated-Hand Venous (HV) With Antecubital Venous (V) Blood

The basal state concentration of AcAc was 21.0 ± 7.3% ($p < 0.005$), and, that of βOHB 17.1 ± 4.8% higher ($p < 0.005$), in HV compared to V. The ^{14}C-content of total ketone bodies also differed significantly by 20.6 ± 6.9% ($p < 0.05$). This resulted in a similar specific activity of total ketone bodies in HV and in V blood, and thus in a similar Ra of total ketone bodies. In contrast, the MCR of total ketone bodies was significantly higher when calculated from forearm venous compared to hand-venous blood.

Glucose, Lactate, pCO_2 and pO_2

Blood glucose, plasma lactate and pCO_2 did not differ significantly between HV and A blood. Only pO_2 values were distinctly higher in A compared to HV by 14.0 ± 2.8% ($p < 0.005$). Contralateral venous blood glucose concentrations were 7.8 ± 1.1% ($p < 0.001$) lower than in HV blood, whereas plasma lactate was 22.0 ± 6.4% ($p < 0.025$), and pCO_2 11.0 ±

Table 2. Ketone Body Kinetics Derived From Heated-Hand Venous (HV) and From Antecubital Venous (V) Blood Samples in 6 Normal Subjects. (Means ± SEM of the Average of Three Consecutive Samples During the Basal Period)

	HV–Blood	V–Blood	HV–V Difference	Significance
Acetoacetate concentraton (μmole/l)	176 ± 32	151 ± 33	26 ± 6	< 0.005
β-Hydroxybutyrate concentration (μmole/l)	194 ± 34	164 ± 34	30 ± 12	< 0.05
^{14}C-Content of total ketone bodies (dpm/ml)	1712 ± 380	1405 ± 301	306 ± 121	< 0.001
Ra of total ketone bodies (μmole/kg/min)	4.8 ± 11	4.6 ± 1.0	0.2 ± 0.3	n.s.
MCR of total ketone bodies (ml/kg/min)	12.2 ± 1.0	13.8 ± 1.1	1.6 ± 0.3	< 0.01

2.9% ($p < 0.02$) higher in V than in HV. The pO_2 content was reduced by 31.6 ± 2.4% ($p < 0.001$) in V compared to HV blood. This represented a larger difference than that between HV and A blood ($p < 0.001$).

DISCUSSION

The results demonstrate that blood samples obtained from a heated dorsal hand vein yield similar concentrations and radioactivity content of ketone bodies as those obtained from an artery. This resulted in similar ketone body kinetics, both at basal steady state levels, and after ketone body concentrations had been rapidly increased by acetoacetate loading infusions. In addition, concentrations of glucose, lactate and pCO_2 differed only slightly between heated-hand venous and arterial blood. In contrast, all these parameters were significantly different between antecubital venous and heated-hand venous blood.

The present results of similar arterial and arterialized-venous blood concentrations are in line with previous reports on glucose,[6] as well as on pCO_2, pO_2 and lactate.[7] In the former study[6] it was demonstrated that results of glucose kinetics were transiently different when antecubital venous blood was employed instead of heated-hand venous blood. Elevation of the ambient temperature of the hand apparently produced arteriovenous shunting such that the arteriovenous difference of all substrates became insignificant. Only pO_2 as an index of overall oxygen consumption maintained a significant arteriovenous gradient. In contrast, the metabolite concentrations in a heated-

Table 3. Blood Glucose, Plasma Lactate, pCO_2 and pO_2

Comparison of arterial (A) and arterialized hand-venous (HV) blood in 9 normal subjects
(Means ± SEM of 4 consecutive samples during the basal period)

	A-Blood	HV-Blood	A-HV Difference	Significance
Blood glucose concentration (mg/dl)	92.3 ± 5.5	89.9 ± 5.8	2.5 ± 1.6	n.s.
Plasma lactate concentration (μmole/l)	911 ± 52	982 ± 52	−70 ± 39	n.s.
pCO_2, (mmHg)	39.9 ± 0.9	40.8 ± 0.9	−0.9 ± 0.4	n.s.
pO_2, (mmHg)	67.0 ± 2.9	57.7 ± 1.4	9.3 ± 1.9	< 0.005

Comparison of arterialized hand-venous (HV) and mixed antecubital-venous (V) blood in 6 normal subjects
(Means ± SEM of 3 consecutive samples during the basal period)

	HV-Blood	V-Blood	HV-V Difference	Significance
Blood glucose concentration (mg/dl)	87.8 ± 2.1	81.5 ± 2.1	6.3 ± 0.9	< 0.001
Plasma lactate concentration (μmole/l)	516 ± 47	699 ± 56	−182 ± 51	< 0.025
pCO_2 (mmHg)	41.1 ± 1.6	46.2 ± 1.3	−5.1 ± 1.4	< 0.02
pO_2 (mmHg)	61.1 ± 2.2	42.5 ± 3.2	19.6 ± 2.9	<0.007

hand vein and in an antecubital vein indicated in agreement with previous studies significant exchange of ketone bodies,[17] glucose and lactate by forearm tissues.[1]

Catheterization of the brachial artery in man may be complicated by severe vascular injury. In an earlier study, 8 of 1400 patients undergoing arterial catheterization suffered from an arterial occlusion;[3] an impaired radial pulsation was noted in 24% of 204[4] and in 18% of 328 patients.[5] These figures appear to limit the unrestricted use of brachial artery catheterization for investigational purposes.

In view of the similarity of arterialized-venous and arterial blood samples, and on the other hand, the risks associated with arterial catheterization, the results indicate that sampling of heated-hand venous blood is a suitable method for the study of ketone body kinetics and arteriovenous concentration gradients in man.

REFERENCES

1. Baltzan MA, Andres R, Leder G, et al: Heterogeneity of forearm metabolism with special reference to free fatty acids. J Clin Invest 41:116–125, 1962
2. Steele R, Wall JS, De Bodo RC, et al: Measurements of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 187:15–24, 1956
3. Hall R: Vascular injuries resulting from arterial puncture or catheterization. Br J Surg 58:513–516, 1971
4. Campion BC, Frye RL, Pluth JR, et al: Arterial complications of retrograde brachial arterial catheterization. Mayo Clin Proc 46:589–592, 1971
5. Machleder HI, Sweeney JP, Barker WF: Pulseless arm after brachial artery catheterization. Lancet 1:407–409, 1972
6. McGuire EAH, Helderman JH, Tobin JD, et al: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 41:565–573, 1976
7. Forster HV, Dempsey J, Thompson J, et al: Estimation of arterial pO_2, pCO_2, pH, and lactate from arterialized venous blood. J Appl Physiol 32:134–137, 1972
8. Krebs HA, Eggleston LV: Metabolism of acetoacetate in animal tissues. Biochem J 39:408–419, 1945
9. Williamson DH, Mellanby J, Krebs HA: Enzymic determination of D-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J 82:90–96, 1962
10. Young DAB, Renold AE: A fluorimetric procedure for the determination of ketone bodies in very small quantities of blood. Clin Chim Acta 13:791–793, 1966
11. Van Stekelenburg GJ, Debruyn JW: A simple gas chromatographic determination of acetone and β-ketobutyric acid in blood serum by means of head space gas sampling. Clin Chim Acta 28:233–237, 1970
12. Mayes PA, Felts JM: Determination of ^{14}C-labeled ketone bodies by liquid scintillation counting. Biochem J 102:230–235, 1967
13. Keller U, Cherrington AD, Liljenquist JE: Ketone body turnover and net hepatic ketone production in fasted and in diabetic dogs. Am J Physiol 235:E 238–247, 1978
14. Schmidt FH: Die enzymatische Bestimmung von Glucose and Fructose nebeneinander. Klin Wschr 39:1244–1247, 1961
15. Bergmeyer HV: Methods of Enzymatic Analysis (ed 2), vols 3 and 4. New York, Academic Press, 1974, 266–270
16. Keller U, Sonnenberg G, Stauffacher W: Validation of a tracer technique to determine non-steady state ketone body turnover rates in man. Am J Physiol 240:E253–E262, 1981
17. Hagenfeldt L, Wahren J: Human forearm muscle metabolism during exercise. III. Uptake, release and oxidation of β-hydroxybutyrate and observations on the β-hydroxybutyrate/acetoacetate ratio. Scand J Clin Lab Invest 21:314–320, 1968