Invalidity of Hand Heating as a Method to Arterialize Venous Blood

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We have assessed in normal subjects the validity of using hand heating to obtain "arterialized" venous blood by biochemical comparison of results for "arterialized" venous and true arterial (radial artery) blood samples. The heating regimen involved placing one hand in an air-heated box at 45–50 °C for 45 min. This method produced blood that was "arterialized" for lactate, $P_{CO_2}$, HbO$_2$, and Hb but not for ammonia or $P_O_2$; it had no effect on determinations of pyruvate or glucose in plasma. Despite using a lower air temperature than previous workers, we observed thermal injury in one volunteer. Further, there was considerable between-subject variation in the effect of hand heating on blood gases. This suggests that blood gases should be measured in the "arterialized" samples at regular intervals from the start of hand heating in each patient to determine whether maximal "arterialization" has been achieved, to avoid making misleading biochemical measurements. Given the wide range in degree of observed "arterialization," we question the validity of this method.

Additional Keyphrases: blood gases • pyruvate • variation, source of

When repeated sampling of blood is required, an intravenous indwelling cannula provides the method least traumatic for the patient and one that is convenient for the observer. An indwelling cannula avoids repeated needle sticks, which may stress the patient and influence activity of the sympathetic nervous system, thereby causing changes in circulating metabolic analytes that may be of interest. Once inserted, a cannula also avoids the use of a tourniquet; the latter causes temporary stagnation of venous blood, with resulting changes in its biochemistry. However, one of the drawbacks of using venous blood for metabolic studies is that even free-flowing venous blood is influenced by the metabolism of the tissues from which it is draining. Given the regional differences in metabolism, it is difficult to interpret whole-body metabolism on the basis of metabolites measured in venous blood. Ideally, therefore, arterial blood should be sampled for metabolic studies. However, arterial cannulation is associated with a high (12–24%) morbidity (1). Complications include the loss of arterial pulse, damage to the arterial wall, thrombosis, clot formation, and loss of limb. We therefore consider arterial cannulation unethical for research purposes. Nevertheless, because clinical research potentially affects patient care, it is important that measurements made in blood are correctly interpreted. As is widely agreed, a reasonable alternative to arterial sampling may be the use of blood taken from a superficial dorsal vein of a heated hand. The increase in temperature causes an increase in blood flow to the hand as a result of both vasodilation of the forearm skeletal muscle and dilatation of the arteriovenous anastomoses in skin. This increased blood flow, together with the hand's negligible muscle mass, means that the venous blood so collected is supposedly similar in composition to an arterial sample.

The temperature and period of hand heating are critical factors in ensuring adequate "arterialization" of venous blood. There are two heating methods in current practice: the hand is placed between two pads heated to 45–50 °C for 20–30 min (1), or it is placed in a box in which the air is heated to between 60 °C (2) and 68 °C (4) for at least 25 min. Researchers using these methods present evidence for adequate arterialization of venous blood for assays of insulin, glucagon, glycerol, free fatty acids, 3-hydroxybutyrate, acetocetate, lactate, pyruvate, and $CO_2$. We were concerned that such high temperatures could damage the skin of individuals who have a high degree of vasoconstriction or sensitive skin. We wanted to check the validity of using arterialized blood from a vein in the back of a heated hand for measurements of glucose, pyruvate, ammonia, and blood gases, but we wanted to use a lower air temperature than previously described. In addition, most of the studies on hand heating to arterialize venous blood have involved sampling in a retrograde direction. We sampled in a proximal direction to facilitate blood sampling in individuals who already had low blood flow in their hands.

Materials and Methods

Subjects

Seven normal healthy volunteers from laboratory staff, two women and five men, ages 22–48 years, took part in these studies, which were approved by the Leeds Eastern Health Authority Ethics Committee. Subjects were fasted and refrained from vigorous activity for 4 h before the study.

Procedures

A small (21-gauge) cannula or butterfly needle, introduced into a vein in the back of both hands, was kept patent by flushing with 2 mL of heparinized isotonic saline ("Hepeal"; C.P. Pharmaceuticals Ltd., U.K.) after each withdrawal of blood. The first 2 mL of each withdrawal of blood was discarded, so that samples for biochemical analysis were not contaminated with Hepeal. After a baseline blood sample was taken from both hands (except in two subjects in whom peripheral vasoconstriction prevented sampling in one hand), each subject placed one hand on a 5-cm-thick foam pad in a Perspex (25 × 25 × 19 cm) box made of 0.5-cm-thick acrylic plastic. This box was heated with a commercial hair dryer to 45–50 °C, the temperature being continuously monitored by a thermometer housed under the air stream. Three 5-mL blood samples were taken at
15-min intervals during the 45-min period of hand heating.

From three of the subjects we took an arterial blood sample by radial artery puncture from the nonheated arm, at the end of the study.

Clinical Biochemistry

**Plasma glucose, lactate, and pyruvate.** Plasma glucose and lactate were measured in samples from both hands, but pyruvate was measured only in blood from the heated hand. Whole blood was collected into ice-cold heparinized tubes and centrifuged without delay (2000 × g) at 0 °C for 10 min. The plasma was stored at −20 °C and analyzed for glucose within one week and for lactate and pyruvate within 24 h. We have observed variable measurements of lactate and pyruvate in blood that has been added to perchloric acid (60 mL/L) and subsequently neutralized with 6 mol L⁻¹ K₂CO₃ solution; we thus prefer to use plasma directly, as recommended in the analysis method we use. We have found no change in the concentration of lactate or pyruvate over 24 h when the samples have been handled as described above, and all samples were treated in this way during the present study. All three metabolites were assayed with an Analox PM7 automated analyzer (Analox Instruments Ltd., London, U.K.). The coefficient of variation (CV) for either repeated analyses was 1.7% for glucose, 0.9% for lactate, and 7.4% for pyruvate. Results of both the lactate and pyruvate assays were compared with results for an independent metabolite control (cat. no. S3005; Sigma Chemical Co., Poole, Dorset, U.K.). The metabolite control contained lactate at 2.23 mmol L⁻¹ (manufacturer's stated acceptable range 2.01–2.45 mmol L⁻¹) and pyruvate at 0.17 mmol L⁻¹ (acceptable range 0.15–0.19 mmol L⁻¹). In eight repeated measurements, the Analox method gave a mean lactate value of 2.34 (SD 0.02) mmol L⁻¹ and a mean pyruvate value of 0.17 (SD 0.01) mmol L⁻¹.

**Plasma ammonia.** Two milliliters of blood, collected into cooled Eppendorf tubes containing 200 units of NH₄⁻-free heparin, was immediately centrifuged and the plasma separated and placed on ice. We assayed ammonia within 15 min of separation by using an EIL ammonia probe (Kent Industrial Measurements Ltd., Stonehouse, Gloucestershire, U.K.) after the method of Park and Fenton (4). The within-run CV for this assay with plasma samples was 6% at a mean NH₄⁺ concentration of 55 μmol/L; day-to-day CV, determined with a Sigma standard NH₄⁺ solution (117 μmol/L), was 2.9%.

**Blood gases.** Blood gases were measured in the three subjects from whom an arterial sample was taken. Blood was collected into heparinized syringes and, after all the air had been expelled, placed in ice. The samples were analyzed within 1 h with a blood gas analyzer (ABL 3 Acid Base Laboratory; Radiometer, Copenhagen, Denmark) and a blood saturation analyzer (OSM 3 Hemoximeter; Radiometer) for Pₒ₂, P_CO₂, HbO₂, and reduced Hb. The CV for eight determinations of the same sample was 2.7% for Pₒ₂, 0.6% for P_CO₂, 0.5% for HbO₂, and 7.1% for reduced Hb.

**Statistics**

Statistical significance was assessed by using a one-way ANOVA for correlated means and a two-way ANOVA for repeated measures on one factor. To compare means, we subsequently performed a Tukey test.

**Results**

Six of the subjects reported that the hand heating was pleasant. However, one subject, who displayed marked peripheral vasoconstriction at the start of the study, reported thermal pain after 20–30 min of heating. Superficial skin scalding was apparent after 45 min. This subject subsequently repeated the study, with the hand resting on the base of the hot box (instead of on the foam pad) and this time the procedure was tolerable.

There was significant between-subject variability in the measurements of glucose (P < 0.01), lactate (P < 0.01), pyruvate (P < 0.01), and ammonia (P < 0.01) in plasma from the heated hand. However, there were no significant changes over time in values for glucose, pyruvate, or ammonia in plasma after heating. By contrast, measured lactate in plasma decreased systematically with heating in all subjects, there being significant differences between the baseline and all other values (P < 0.01). The maximum decrement in plasma lactate occurred after 30 min of hand heating (Figure 1). There were no significant differences between hands for plasma results for glucose or lactate (Figure 2).

Data for blood gases are shown in Figure 3. Subject A in this figure was the individual who presented with marked peripheral vasoconstriction (clinical observation); the data shown are for the repeated study. This subject displayed initially low values for Pₒ₂ and HbO₂ but high values for
values in blood from the opposite hand before heating. In subject B, there was little difference between the values in the contralateral hand after 45 min of heating and any value in the heated hand.

Direct comparison of arterial and "arterialized" blood shows that the \( \text{P}_{\text{O}} \) was higher in arterial blood than in "arterialized" blood in both subjects, while the \( \text{P}_{\text{CO}} \) was higher, the \( \text{HbO} \) only slightly higher, and the reduced \( \text{Hb} \) only slightly lower. Other direct comparisons with arterial blood are shown in Table 1. In all subjects overall, arterial lactates were slightly higher than in the "arterialized" blood, the pyruvates and glucoses were similar, but the ammonia concentrations were considerably lower in arterial than in "arterialized" blood.

Discussion

Several interesting observations can be made from these data. It is evident that, of the variables we measured, hand heating produced significant changes only in plasma measurements of lactate. The fact that the arterial concentrations of lactate were actually higher than those of the "arterialized" samples suggests that this could be an effect of anxiety about the arterial stick.

The fact that similar changes in plasma lactate occurred in the contralateral hand provides evidence of reflex vasodilatation. However, in the subject in whom marked vasoconstriction was apparent (subject 2 in Table 1), such reflex vasodilatation was not evident in the results for the blood gases.

Interestingly, the "arterialized" plasma ammonia was considerably higher than the arterial ammonia. This may

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& \text{Cool hand} & \text{Warm hand*} & \text{Artery} & \text{Contralateral hand*} \\
\hline
\text{Lactate, mmol/L} & 1.25 & 1.00 & 1.20 & 1.25 \\
& 1.40 & 0.95 & 1.10 & 1.35 \\
& 0.80 & 0.70 & 0.75 & 0.70 \\
\text{Pyrurate, mmol/L} & 0.025 & 0.030 & 0.000 & — \\
& 0.170 & 0.000 & 0.000 & 0.000 \\
& 0.575 & 0.410 & 0.460 & 0.270 \\
\text{Glucose, mmol/L} & 5.20 & 5.45 & 5.30 & 4.90 \\
& 4.90 & 5.70 & 5.10 & 4.90 \\
& 5.65 & 4.55 & 6.05 & 4.90 \\
\text{Ammonia, \text{\textmu}mol/L} & 18 & 10 & 0 & 14 \\
& 34 & 32 & 4 & 24 \\
& 6 & 24 & 18 & 46 \\
\text{\text{P}_{\text{O}}} \text{ kPa} & — & 10.12 & 9.34 & — \\
& 4.57 & 8.15 & 11.91 & 5.12 \\
& 9.75 & 9.03 & 12.39 & 9.69 \\
\text{\text{P}_{\text{CO}}} \text{ kPa} & — & 4.83 & 5.03 & — \\
& 6.05 & 4.43 & 4.85 & 5.79 \\
& 5.26 & 4.99 & 5.27 & 4.72 \\
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* After 45 min of heating the warmed hand at 45–50 °C.
be related to a decreased uptake of ammonia associated with the high blood flow. There is a well-established negative correlation between peripheral blood flow and the uptake of ammonia by muscle (5), which would be consistent with the trend of an increase in plasma ammonia with hand heating.

A further observation of interest is the comparison between the two subjects in Figure 3. It is apparent that, although in one subject this heating regime had no effect on blood gases (presumably because the rate of blood flow did not change), in the other the partial pressures of both O2 and CO2 were still changing linearly after 45 min of heating. The 45-min values were, nevertheless, close to the arterial values.

The implications of these findings are, first, that it is not possible to guarantee that blood from a dorsal hand vein is comparable in composition with other venous blood and, second, that the time needed to maximally "arterialize" blood is highly variable between subjects. Moreover, because we observed minor thermal injury in one subject, even at this relatively low heating temperature, it may not be possible to attain maximal "arterialization" in all subjects, especially those with marked peripheral vasoconstriction. Ideally, therefore, the procedure should be validated for each patient under investigation by measuring the blood gases first, before measurements are made in "arterialized" blood. Failure to attain maximal "arterialization" in all patients in a clinical study could lead to misleading data and erroneous interpretation. We are not aware of any other studies that have shown attention to this individual variability. While this effect may be due to the lower temperatures used in this study, we believe that higher temperatures could pose a risk of thermal injury, as we demonstrated occurred in a normal volunteer with significant peripheral vasoconstriction.

References