Clinical Physiology (1992) **12**, 537–552

Critical evaluation of the 'heated-hand-technique' for obtaining 'arterialized' venous blood: incomplete arterialization and alterations in glucagon responses

M. A. Nauck, H. Ließ, E. G. Siegel, P. D. Niedmann and W. Creutzfeldt

Division of Gastroenterology and Endocrinology, and Division of Clinical Chemistry, Department of Medicine, Georg-August-University, Göttingen, Germany

(Received 28 October 1991; accepted 4 February 1992)

Summary. In order to test the degree of 'arterialization' and the occurrence of arterio- (or capillary-) venous differences in glucose concentrations for commonly used blood sampling sites (including the retrogradely cannulated dorsal hand vein with application of dry heat to this hand/arm — the 'heated-hand-technique'), oxygen partial pressure (oxygen saturation) and plasma glucose was determined in blood drawn from different venous sites before and after an oral glucose load (75 g). Experiments with and without heating (hot air 68°C) were compared in nine healthy volunteers. Basal pO_2 (and oxygen saturation) increased in the order cubital fossa vein < superficial forearm vein < dorsal hand vein. Heating raised pO_2 by ~20 mmHg; $P = 0{\cdot}008$) and oxygen saturation ($P = 0{\cdot}008$–$0{\cdot}02$) at all sites, including those on the contralateral arm. Capillary-venous glucose differences after the glucose challenge were significantly related to the sampling site ($P < 0{\cdot}0001$). They were reduced by ~50% in response to heat exposure ($P = 0{\cdot}008$–$0{\cdot}011$) and could be correlated to pO_2-values ($r = 0{\cdot}92$; $P = 0{\cdot}01$). The lowest capillary-venous glucose concentration difference was measured with the 'heated-hand-technique' ($0{\cdot}4 \pm 0{\cdot}1$ mmol l^{-1}). Heating did not alter integrated incremental glucose (capillary values), insulin, and C-peptide-responses and late, counter-regulatory responses (120–240 min after glucose) of cortisol, growth hormone, and adrenalin. However, the late glucagon response was enhanced ($P = 0{\cdot}011$) by heating, concomitant with a significantly reduced 'reactive' decrement in glucose concentrations. In conclusion, the 'heated-hand-technique' provides blood more similar to arterial blood that can be obtained from other venous sampling sites. However, significant residual differences in pO_2 and glucose concentrations remain. In addition, altered counter-regulatory hormone responses may occur with heating.

Correspondence: Michael Nauck, MD, Division of Gastroenterology and Endocrinology, Department of Medicine, Georg-August-University, Robert-Koch-Strasse 40, D3400 Göttingen, Germany

Key words: arterio-venous glucose difference, insulin secretion, glucagon secretion, heat exposure, vasomotor response.

Introduction

For the study of glucose metabolism, arterial concentrations of glucose (and other substrates) determine insulin secretion and the amount of glucose presented to peripheral tissues. Tissue uptake of glucose from the circulation leads to arterior-venous concentration differences that vary with different sampling sites, depending on the metabolic activity of the tissue drained by the respective vein. Since arterial cannulations carry a small, but definite risk (Hall, 1971; Machleder *et al.*, 1972), substitutes for arterial blood have been sought to avoid arterial punctures, if they do not appear justified for ethical reasons. As early as 1925 the increase in skin blood flow after exposure to heating (Goldschmidt & Light, 1925; Somogyi, 1948; Roddie *et al.*, 1956; Astrup *et al.*, 1988) has been used to obtain 'venous blood similar to arterial blood in gaseous content' (Goldschmidt & Light, 1925). Later this was confirmed (Forster *et al.*, 1972), and attempts were made to use similar approaches (the 'heated-hand-technique') for metabolic studies (Jackson *et al.*, 1973; McGuire *et al.*, 1976; Astrup *et al.*, 1988). Although some limited information is available regarding residual differences in glucose concentrations when comparing arterial and 'arterialized venous' blood, the studies that have attempted to validate the 'heated-hand' methodology have primarily concentrated on the kinetics of radioactive tracer amounts of glucose (McGuire *et al.*, 1976), amino acids (Abumrad *et al.*, 1981) and ketone bodies (Sonnenberg & Keller, 1982).

Preliminary experiments in our laboratry using the 'heated-hand-technique' did not produce satisfactory results regarding blood gas analyses and glucose concentrations. Since numerous studies have made use of the 'heated-hand-technique, the present study was designed to systematically determine the influence of different sampling sites and the effect of heating on blood gases and plasma glucose values. Since arterial plasma glucose values are not systematically different from capillary measurements (Whichelow *et al.*, 1967; Förster *et al.*, 1972), capillary-venous concentration differences were determined both in the basal state and after an oral glucose load that should increase arterio-venous glucose concentration differences (Somogyi, 1948; DeFronzo *et al.*, 1979; Ferrannini *et al.*, 1985; Jackson *et al.*, 1987). The possibility that elevations in body temperature may occur when using the 'heated-hand-technique' (Roddie *et al.*, 1956; Astrup *et al.*, 1988; Gallen & McDonald, 1990), which in turn has been shown to alter counter-regulatory hormone responses (Collins *et al.*, 1969; Marreiro Rocha *et al.*, 1973; Rayfield *et al.*, 1973; Christensen *et al.*, 1984; Tatar *et al.*, 1986; Møller *et al.*, 1989), has prompted the measurement of glucagon, growth hormone, cortisol, and catecholamines in the course of these experiments. Preliminary data have been presented in abstract form (Nauck *et al.*, 1990).

Subjects and methods

SUBJECT CHARACTERISTICS

Nine healthy male volunteers participated in the study, which had been approved by the ethics committee of the Medical Faculty at the Georg-August-University on December 22, 1988. None had a family history of diabetes or took any medication. All were right-handers. They were 26 ± 5 (mean ± SD) years old, and their body-mass-index was 21·6 ± 1·5 kg m^{-2}.

EXPERIMENTAL PROCEDURES

In random order, one hand (the left hand in 5 and the right hand in 4 cases) was selected to be exposed to direct heating during one of two experiments (one with the application of heat, using a thermoregulated box maintained at a dry air temperature of 68°C, and one without heating). The 'heated-hand' and the distal half of the respective forearm were directly exposed to heat. On both occasions, the volunteers were studied after an overnight fast. They remained in a recumbant position, the ambient temperature being maintained at 22 ± 1°C. Teflon cannulae (Moskito 123, Vygon, D 5100 Aachen, Germany, 20 or 18 G) were inserted retrogradely into a dorsal hand vein on both hands, into a superficial forearm vein (located in the middle third of the forearm) and into a deeper vein punctured in the cubital fossa, on the contralateral arm (the one that was not directly exposed to heating in the experiment with the hot air box). The cannulae were kept patent using a slow drip of physiological saline. Saline infusions were stopped 2 min before drawing blood from these sites in order to avoid dilution. Before specimens used for analyses were drawn 1–2 ml of blood drawn from each cannula were discarded. Blood was drawn into heparinized tubes (10 ml, Sarstedt, D 5223 Nümbrecht, Germany) for the measurement of IR-insulin, C-peptide, glucagon, growth hormone, and cortisol, and 5 ml blood for the measurement of catecholamines was drawn into heparinized tubes containing 15 μl phosphate buffer, pH 7·5, containing 6 μg EGTA and 9 μg glutathione. Blood for gas analyses was sampled in pre-cooled 2 ml syringes that had been rinsed with a heparin solution (5000 U per ml). Blood for plasma glucose measurements was secured in NaF-coated tubes (Microvette CB 300, Sarstedt, D 5223 Nümbrecht, Germany). One ear lobe was made hypaeremic using Finalgon® 'extra stark' (Nonivamide, 1·7 mg per g, and nicoboxil, 10·8 mg per g; Thomae GmbH, D 7950 Biberach, Germany) for capillary plasma glucose measurements. Skin temperature on the back of both hands was measured using thermosensitive electrodes taped to the surface of the skin. Rectal temperature was also monitored using a temperature-sensitive electrode (Tele-thermometer, model 46 TUC, Yellow Springs Instruments, Ohio, USA). Heat exposure was started 30 min after placing indwelling venous cannulae at −60 min of the experimental protocol (see Figs 1 and 2). At 0 min, a glucose solution (in addition containing

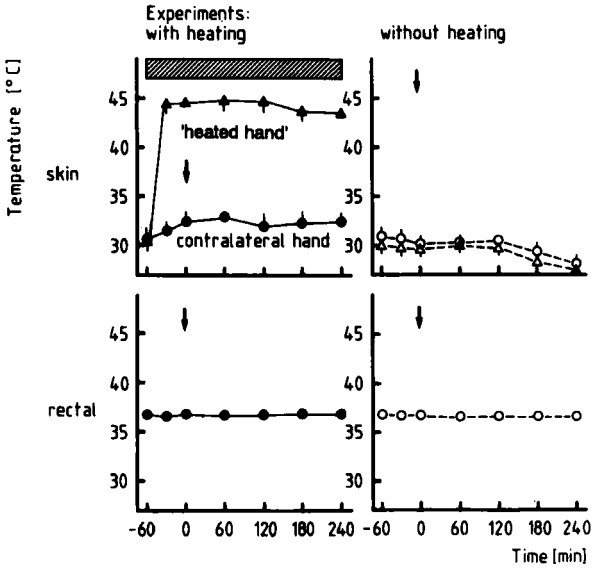

Fig. 1. Skin (dorsal hand) temperature (upper panels) and body (rectal) temperature (lower panels) in experiments using the 'heated-hand-technique' (closed symbols, left panels) or without heating (open symbols, right panels). The arrow indicates the oral glucose challenge (75 g). The hatched bar indicates the duration of applying heat. Triangles are used for the hand that was chosen to be heated in one of the experiments, circles are used for the contralateral hand/arm. Mean ± SEM; $n = 9$. Absence of error bars indicates an SEM smaller than the size of the respective symbol. See text for statistical evaluation.

maltose, maltotriose, and low molecular weight oligomers of glucose-equivalent to 75 g of glucose; Boehringer-O.G.T., Boehringer Mannheim, D 6800 Mannheim, Germany) was swallowed within 5 min.

LABORATORY DETERMINATIONS

Plasma glucose was measured in duplicate using a Beckman glucose analyser 2 (Beckman, D 8000 Munich, Germany). The coefficient of variation for multiple measurements on a given day was 1·0–1·5%. Blood gases were analysed using an automatic blood gas analyser (ABL 300, Radiometer Copenhagen, Denmark). No attempt was made to correct for differences in blood temperature, essentially because the increment possibly caused by the heating device was unknown. A temperature increment of up to 3°C would not alter the results. IR-insulin was determined using a commercial radioimmunoassay kit (Pharamacia, D 7800 Freiburg, Germany). C-Peptide was determined using the RIA-mat C-Peptid II assay kit obtained from Byk-Sangtec Diagnostica, D 6057 Dietzenbach, Germany. IR-glucagon was measured using glucagon kit, code 10904, from Biodata, obtained from Serono Diagnostika, D 7800 Freiburg, Germany. Human growth hormone and cortisol were assayed using double antibody RIA kits (Diagnostic Products Corporation) obtained from Hermann

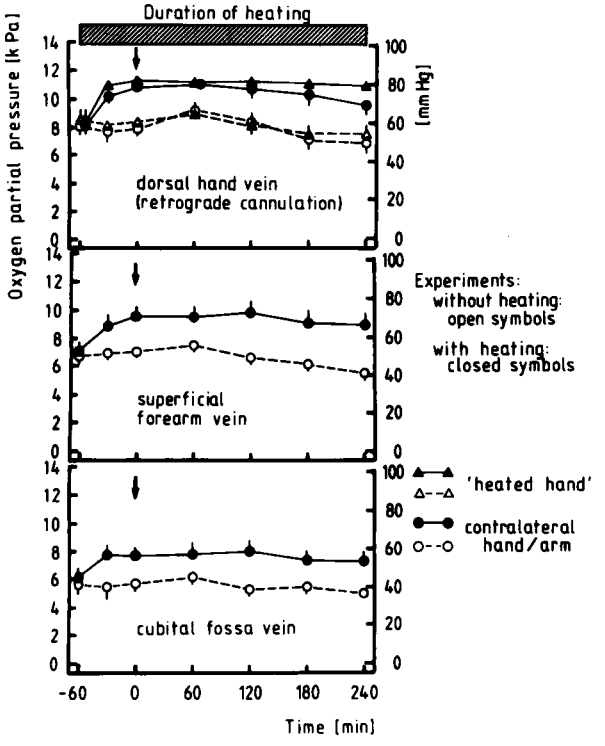

Fig. 2. Oxygen tension (pO_2) in dorsal hand veins (upper panel), superficial forearm veins (middle panel), and in cubital fossa veins (lower panel) in experiments using the 'heated-hand-technique' (closed symbols) or without heating (open symbols). The arrow indicates the oral glucose challenge (75 g). The hatched bar indicates the duration of applying heat. Triangles are used for the hand that was chosen to be heated in one of the experiments, circles are used for the contralateral hand/arm. Mean ± SEM; $n = 9$. Absence of error bars indicates an SEM smaller than the size of the respective symbol. See Table 1 for statistical evaluation.

Biermann Diagnostika, D 6350 Bad Nauheim, Germany. Plasma catecholamines were measured by HPLC analysis after derivatization with 1,2-dimethylethylendiamine and fluorescence detection as described by Mitsui and co-workers (Mitsui *et al.*, 1985).

CALCULATIONS

Values are presented as mean ± SD (subject characteristics) or mean ± SEM (experimental results) for 9 subjects. Integrated incremental responses were calculated according to the trapezoidal rule. In the case of plasma glucose responses, all increments above basal values and all decrements below basal values were calculated separately. Counter-regulatory hormone responses were calculated using the 120 min-value as baseline. After this time, plasma glucose concentrations had returned to basal values again and counter-regulatory responses started.

Table 1. Mean oxygen partial pressure and oxygen saturation (0–60 min after oral glucose), with and without application of the 'heated-hand-technique', for different venous sampling sites

Sampling site	Oxygen partial pressure [kPa]		Significance of the difference (Wilcoxon-test)	Oxygen saturation [%]		Significance of the difference (Wilcoxon-test)
	without heating	with heating		without heating	with heating	
Dorsal hand vein ('heated-hand')	4·7 ± 0·2	6·1 ± 0·2	$P = 0·008$	91·1 ± 1·0	95·8 ± 0·4	$P = 0·01$
Dorsal hand vein (contralateral)	4·5 ± 0·3	5·8 ± 0·1	$P = 0·008$	89·6 ± 1·7	95·2 ± 0·3	$P = 0·008$
Superficial forearm vein (contralateral)	4·0 ± 0·2*	5·2 ± 0·3*	$P = 0·015$	86·1 ± 1·8*	92·7 ± 1·3*	$P = 0·02$
Cubital fossa vein (contralateral)	3·3 ± 0·3*	4·2 ± 0·3*	$P = 0·02$	75·8 ± 4·1*	86·6 ± 2·3*	$P = 0·015$
Significance of the difference (Quade-test)	$P < 0·0001$	$P = 0·0001$		$P < 0·0001$	$P < 0·0001$	

Mean ± SEM; $n = 9$. The term 'heated hand' applies to the hand that was chosen to be warmed in a thermoregulated box in one of the two experiments performed with each volunteer. Asterisks denote a significant difference to the dorsal hand vein of the 'heated hand'.

Significances of differences were estimated using non-parametric tests for paired samples (the Wilcoxon-test if two conditions were to be compared, and the Quade-test if more than two conditions were to be compared simultaneously; Theodorsson-Norheim, 1987). Thus, two-sided *P*-values were corrected for multiple comparisons, and were considered to indicate significant differences if they were $<0{\cdot}05$.

Results

SKIN AND BODY TEMPERATURE

Heating rapidly increased the skin temperature on the back of the 'heated-hand' to 44 ± 1°C (Fig. 1). This temperature was maintained throughout the experiment. A small increase of skin temperature was also noted on the contralateral hand (up to $33{\cdot}0 \pm 0{\cdot}4$°C), whereas without application of heating skin temperatures on both hands were ~30°C, with a tendency to decrease 120 min after the glucose load. There was a non-significant ($P = 0{\cdot}14$) increment in (rectal) body temperature by $0{\cdot}1 \pm 0{\cdot}1$°C with heating, whereas the temperature tended to decline ($P = 0{\cdot}26$) without heating. Overall, there was a minor (~0·2°C) difference at 120–240 min ($P = 0{\cdot}008$–$0{\cdot}03$) between the experiments with and without heating. Heating did not change pulse (64 ± 2 vs. 64 ± 1 min^{-1}) or blood pressure ($9{\cdot}0 \pm 0{\cdot}1/6{\cdot}2 \pm 0{\cdot}1$ vs. $8{\cdot}9 \pm 0{\cdot}1/6{\cdot}0 \pm 0{\cdot}1$ kPa; $122 \pm 2/84 \pm 2$ vs. $121 \pm 2/81 \pm 2$ mmHg; Riva-Rocci-method).

BLOOD GAS ANALYSES

There were considerable differences in pO_2 between the sampling sites (Fig. 2, Table 1). In the basal state, they were highest in blood sampled from retrogradely cannulated dorsal hand veins ('heated-hand': $4{\cdot}6 \pm 0{\cdot}2$ kPa; contralateral dorsal hand vein $4{\cdot}2 \pm 0{\cdot}2$ kPa), and decreased in the order superficial forearm vein ($4{\cdot}0 \pm 0{\cdot}2$ kPa) > cubital fossa vein ($3{\cdot}3 \pm 0{\cdot}3$ kPa). Oral glucose did not considerably change these values in experiments without application of heat. With one hand and the lower half of the forearm directly exposed to heating, pO_2 values increased at all sampling sites within 30–60 min ($P = 0{\cdot}008$), i.e. also at the contralateral hand/arm. These higher pO_2 values were maintained throughout the experiments. However, the intra-individual variation was considerable (pO_2 5·2–6·9 kPa in the dorsal hand vein with heating).

In the basal state, there also were minor differences in pCO_2 (lowest in dorsal hand veins, highest in the cubital fossa vein; $P = 0{\cdot}0002$) and pH (highest in dorsal hand veins, lowest in the cubital fossa vein; $P = 0{\cdot}0003$), but at none of the sampling sites these values changed significantly with heating (data not shown).

The blood gas values measured in samples drawn from a dorsal hand vein exposed to heating in most individuals studied fell short of truly arterial values (Wissenschaftliche Tabellen Geigy, 1979; Braunwald *et al.*, 1987; Astrup *et al.*, 1988).

GLUCOSE CONCENTRATIONS

Basal and post-glucose capillary glucose concentrations were typical for subjects with normal glucose tolerance. The time course of glucose concentrations was not different with and without heating (Fig. 3).

In the basal state, there were minor differences between capillary and venous plasma glucose concentrations (0·1–0·2 mmol l^{-1}) that showed no clear pattern regarding the sampling site and that did not change with heating. However, after glucose ingestion considerable differences occurred. They mainly fell into the period of 15–60 min after the glucose challenge, when plasma glucose and insulin values were high (Fig. 3). The mean difference averaged over this time period was lowest for the dorsal hand veins and increased with more proximal sampling sites (Fig. 4; Table 2). At all sampling sites, this capillary-venous concentration difference was reduced by ~50% during heating. Even with the *classical* 'heated-hand-technique', residual differences were 0·4 ± 0·1 mmol l^{-1}. There was a large inter-individual variation (range 0·3–0·8 mmol l^{-1}), and at individual time points differences up to 1·1 mmol l^{-1} were noted.

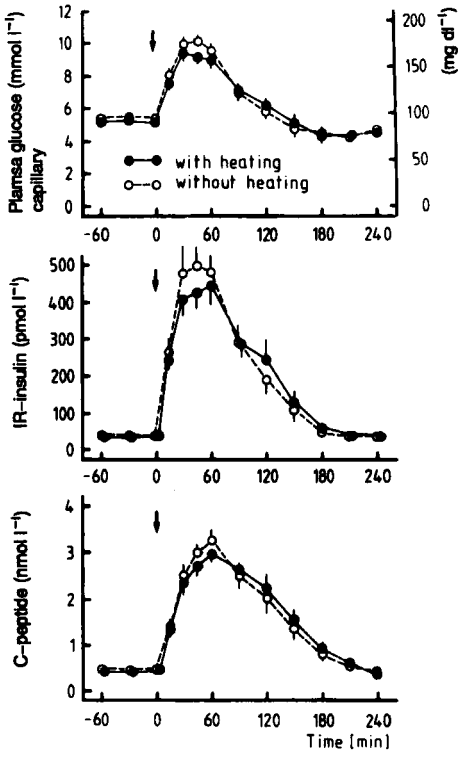

Fig. 3. Capillary plasma glucose (upper panel), 'heated hand'-venous insulin (middle panel), and C-peptide (lower panel) in experiments using the 'heated-hand-technique' (closed symbols) or without heating (open symbols). The arrow indicates the oral glucose challenge (75 g). Mean ± SEM; $n = 9$. Absence of error bars indicates an SEM smaller than the size of the respective symbol. See Table 3 for statistical evaluation.

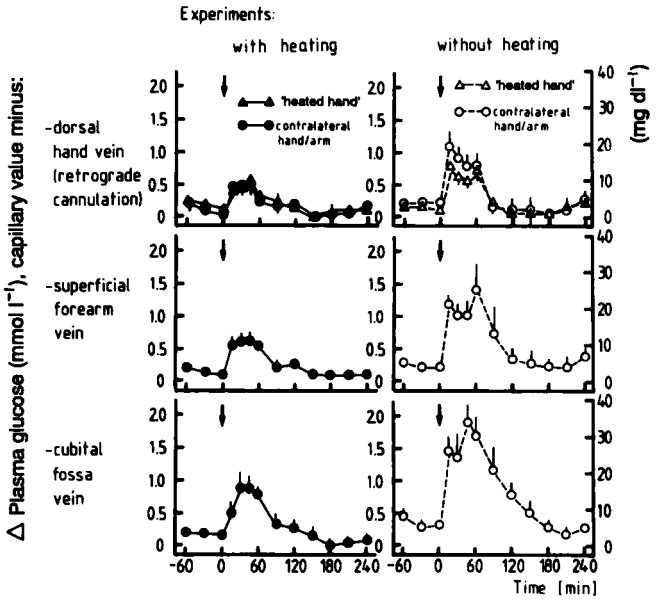

Fig. 4. Difference in plasma glucose concentrations between the capillary value and the value measured in dorsal hand veins (upper panels), superficial forearm veins (middle panels), and in cubital fossa veins (lower panels) in experiments using the 'heated-hand-technique' (closed symbols, left panels) or without heating (open symbols, right panels). The arrow indicates the oral glucose challenge (75 g). Triangles are used for the hand that was chosen to be heated in one of the experiments, circles are used for the contralateral hand/arm. Mean ± SEM; $n = 9$. Absence of error bars indicates an SEM smaller than the size of the respective symbol. See Table 2 for statistical evaluation.

Table 2. Mean capillary-venous plasma glucose concentration differences (15–60 min after oral glucose), with and without application of the 'heated-hand-technique', for different venous sampling sites

Sampling site	Glucose (mmol l^{-1}) without heating	Glucose (mmol l^{-1}) with heating	Significance of the difference (Wilcoxon-test)
Dorsal hand vein ('heated hand')	0·7 ± 0·1	0·4 ± 0·1	0·011
Dorsal hand vein (contralateral)	0·9 ± 0·1	0·4 ± 0·1	0·011
Superficial forearm vein (contralateral)	1·2 ± 0·2*	0·6 ± 0·1	0·008
Cubital fossa vein (contralateral)	1·6 ± 0·2*	0·8 ± 0·1*	0·008
Significance of the difference (Quade-test)	$P < 0·0001$	$P = 0·011$	

Mean ± SEM; $n = 9$. The term 'heated hand' applies to the hand that was chosen to be warmed in a thermoregulated box in one of the two experiments performed with each volunteer. Asterisks denote a significant difference to the dorsal hand vein of the 'heated hand'.

Table 3. Integrated incremental responses (0–240 min) after 75 g oral glucose, of capillary plasma glucose and (dorsal hand) venous insulin and C-peptide concentrations

Parameter	[unit]	Without heating	With heating	Significance of the difference (Wilcoxon-test)
Glucose*	[mmol l^{-1} min]	347·7 ± 29·6	346·1 ± 19·8	$P = 0·77$
Insulin	[nmol l^{-1} min]	35·1 ± 3·9	33·0 ± 2·5	$P = 0·44$
C-peptide	[nmol l^{-1} min]	293·2 ± 28·0	309·1 ± 21·3	$P = 0·77$

Mean ± SEM; $n = 9$. *Only the increment above basal values was quantitated without subtraction of the reactive glycaemic excursion below basal values (which is shown in Table 4).

These average residual capillary-glucose concentration differences could be correlated to the pO_2 reached at the different sampling sites ($r = 0·921$; $P = 0·01$). This close relationship suggests different degrees of 'arterialization'.

INSULIN SECRETION

Basal values of insulin and C-peptide and the increment in these parameters of B cell secretion after glucose ingestion were not changed significantly by heating (Fig. 3; Table 3).

COUNTER-REGULATORY HORMONE RESPONSES

Capillary plasma glucose concentrations declined below basal values 120 min after the glucose load (glucose below basal, Table 4). Beginning at that time point, and in individual subjects temporally related to the glucose nadir, there were increments in circulating growth hormone and adrenalin concentrations, which were not changed in experiments with heating (Table 4). Glucagon concentrations were depressed initially after the glucose load and returned to basal levels later (Fig. 5). However, in experiments with heating a reactive increment of glucagon occurred at 210 min after the glucose challenge ($P = 0·024$ vs. the experiment without heating). The integrated response over the 120 min value also increased significantly with heating (Table 4; $P = 0·011$). With heating, the reactive glycaemic excursion below basal values was reduced by 19% ($P = 0·021$), while the lowest capillary glucose concentrations measured were not significantly different between experiments with (3·3 ± 0·2 mmol l^{-1}) and without (3·4 ± 0·2 mmol l^{-1}; $P = 0·21$) heating. In 8 out of 9 volunteers, glucagon increased and the reactive glycaemic excursion decreased with heating, whereas an inverse relationship was observed in one volunteer. In any case, a higher glucagon response was associated with a lower reactive glycaemic excursion or vice versa.

NORADRENALIN VALUES

Venous noradrenalin concentrations showed a decrement 30 min after starting to heat ($P = 0·008$), which was not maintained to the same degree later. After the oral glucose

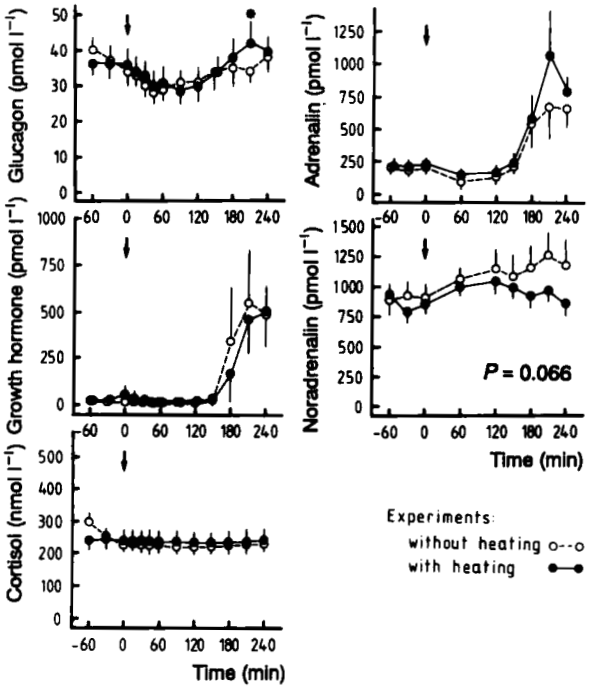

Fig. 5. Glucagon, human growth hormone, cortisol (left panels) and catecholamine (right panels) responses to oral glucose challenges in experiments using the 'heated-hand-technique' (closed symbols) or without heating (open symbols). The arrows indicate the oral glucose challenge (75 g). Blood was drawn from the dorsal hand vein. Mean ± SEM; $n = 9$. Absence of error bars indicates an SEM smaller than the size of the respective symbol. The asterisk indicates a significant difference ($P = 0{\cdot}011$) to the experiment without heating. Also see Table 4 for statistical evaluation.

Table 4. Reactive glycaemic excursion of capillary plasma glucose below basal values and integrated incremental counter-regulatory hormone responses (120–240 min) after 75 g oral glucose

Parameter	[unit]	Without heating	With heating	Significance of the difference (Wilcoxon-test)
Glucose below basal*	[mmol l^{-1} min]	105·1 ± 17·8	93·9 ± 17·8	$P = 0{\cdot}021$
Glucagon	[pmol l^{-1} min]	397·5 ± 158·1	826·0 ± 227·9	$P = 0{\cdot}011$
Adrenalin	[nmol l^{-1} min]	38·3 ± 8·7	51·0 ± 12·9	$P = 0{\cdot}26$
Growth hormone	[nmol l^{-1} min]	33·3 ± 18·4	25·3 ± 11·3	$P = 0{\cdot}68$
Cortisol	[nmol l^{-1} min]	3836 ± 2870	5603 ± 4030	$P = 0{\cdot}44$

Mean ± SEM; $n = 9$. *Glucose values are capillary values. Only the reactive glycaemic excursion below basal was quantitated. Blood for the determination of counter-regulatory hormones was drawn from the dorsal hand vein (retrograde cannulation) that was chosen to be exposed to heating in one experiment ('heated hand').

challenge, noradrenalin values increased over basal values. There was no clear temporal relationship of late increments in noradrenalin the glucose nadir. The integrated incremental noradrenalin responses after glucose (0–240 min) tended to be lower with heating ($P = 0{\cdot}066$). If a subgroup of experiments (five subjects), all samples of which were assayed in the same run, was looked at separately a more homogeneous result was noted (paired *t*-test: $P = 0{\cdot}01$).

Discussion

The 'heated-hand-technique' to obtain 'arterialized' venous blood has been widely used since some studies have provided evidence for its effectiveness in certain regards, i.e. for tracer studies of glucose (McGuire *et al.*, 1976), amino acid (Abumrad *et al.*, 1981) and ketone body (Sonnenberg & Keller, 1982) turnover. With respect to blood gas values and glucose concentrations, the method has been looked at less thoroughly. With the correct use of the 'heated-hand' methodology, Forster and co-workers (Forster *et al.*, 1972) noted a mean difference in pO_2 values between arterial and 'arterialized venous' blood of ~1·2 kPa (16 mmHg). Jackson and co-workers reported only small residual glucose concentration differences ($0{\cdot}2 \pm 0{\cdot}1$ mmol l^{-1}) and an oxygen saturation of $91{\cdot}7 \pm 0{\cdot}3\%$ (Jackson *et al.*, 1973). The latter value certainly is not representative of arterial blood in healthy subjects (Wissenschaftliche Tabellen Geigy, 1979; Braunwald *et al.*, 1987; Astrup *et al.*, 1988), and in the present study values of $95{\cdot}8 \pm 0{\cdot}4\%$ were reached. McGuire and co-workers showed that residual glucose concentration differences between arterial and 'arterial-hand arterialized venous' blood remained during hyperglycaemic clamp experiments (McGuire *et al.*, 1976), but did not provide data on their exact magnitude and inter-individual variation, nor on correlations to blood gas analyses. Sonnenberg & Keller (1982) noted significant arterio-'arterialized venous' differences in pO_2 values of $0{\cdot}7 \pm 0{\cdot}1$ kPa ($9{\cdot}3 \pm 1{\cdot}9$ mmHg) in the basal state. Ipp & Forster (1987) considered an O_2-saturation of >90% evidence of sufficient arterialization. Jackson and co-workers reached a pO_2 of $5{\cdot}7 \pm 0{\cdot}4$ kPa ($77{\cdot}0 \pm 4{\cdot}9$ mmHg) in 'arterialized venous' blood (Jackson *et al.*, 1987). Kiens and co-workers reported an oxygen saturation of 94–96%, a value very similar to ours (Kiens *et al.*, 1989; Table 1).

Taken together, the results reported in the study described here and the available literature point to the fact that 'heated-hand' arterialized venous blood is often not identical to arterial blood in composition. This is also true for plasma glucose concentrations (Fig. 4). The magnitude of the residual glucose difference is likely to depend on the metabolic situation and should be greater at times of a significant glucose uptake into peripheral tissues, like during hyperglycaemic (or hyperinsulinaemic) clamp experiments or after meals (Somogyi, 1948; Jackson *et al.*, 1973, 1987; Defronzo *et al.*, 1979; Ferrannini *et al.*, 1985), or after an oral glucose load (this study). Recent experiments support this conclusion (Krarup *et al.*, 1990). Furthermore, arterio-

venous insulin gradients exist which are also present between arterial and 'heated-hand' arterialized venous blood (Madsbad *et al.*, 1988; Krarup *et al.*, 1990).

The use of capillary instead of arterial blood for the determination of plasma glucose concentrations should not have influenced the conclusions, because both sampling sites provide blood with similar glucose levels (Whichelow *et al.*, 1967; Förster *et al.*, 1972).

Counter-regulatory hormone responses after oral glucose coincident with the reactive glycaemic excursion below basal values have been noted and described previously (Kleinbaum & Shamoon, 1982; Tse *et al.*, 1983; Astrup *et al.*, 1986, 1990), but the possible influences of heating have not been dealt with in detail. Circulating glucagon concentrations are lowered by glucose ingestion, and one (Astrup *et al.*, 1986), but not all previous studies (Kleinbaum & Shamoon, 1982; Tse *et al.*, 1983) have noted a reactive increment in glucagon later in the time course. In the study described here the initial depression of glucagon values was followed by an increase to higher than basal values 210 min after the oral glucose load (Fig. 5), but only in the experiment with heating. This response was rather small in magnitude, although statistically significant. The integrated incremental response from 120 to 240 min was also significantly higher with heating (Table 4). At the same time, reactive glycaemic excursions below basal values were lower with heating (Table 4), while the glucose nadir was similar under both conditions. This excludes differences in glucose concentrations as the cause of differences in glucagon responses. Rather, the minor improvement in the recovery from low plasma glucose concentrations could be the consequence of the higher glucagon response. Such a relationship is further suggested by the fact that in all volunteers studied a higher glucagon response in one of the two experiments was always associated with a smaller reactive glycaemic excursion below basal values. However, the effect on both parameters was small. It may be more important when studying glucagon secretion at lower glucose concentrations.

It is interesting to note that previous studies have described higher circulating glucagon concentrations under conditions characterized by increased body temperature. This was true in the case of febrile illnesses (Marreiro Rocha *et al.*, 1973; Rayfield *et al.*, 1973), during hyperthermia in a sauna (Tatar *et al.*, 1986) and during prolonged bathing in a hot tub (Møller *et al.*, 1989).

However, the higher glucagon response in the experiment with heating need not be the consequence of enhanced secretion. If one assumes peripheral extraction of glucagon as it has been observed for insulin (Madsbad *et al.*, 1988; Krarup *et al.*, 1990), 'arteriailization' of venous blood alone may raise glucagon concentrations.

The obvious difference to the study by Astrup and co-workers in the noradrenalin response after glucose (Astrup *et al.*, 1988) needs to be explained. Venous noradrenalin values have to be analysed with caution, because a considerable proportion of venous noradrenalin is thought to stem from the part of the body drained by the respective vein (negative arterio-venous concentration differences; Halter *et al.*, 1980). Certainly, heating may affect the noradrenergic sympathetic tone, and this

effect may depend on variables like ambient and body temperature. During the initial phase, heating reduced noradrenalin in both the study by Astrup and co-workers (Astrup *et al.*, 1988) and in our experiment (Fig. 5). The opposite results at later sampling points could be the consequence of more profound effect of heating on body temperature in that study (Astrup *et al.*, 1988).

The study described here with indirect evidence (increment in pO_2 and oxygen saturation; Fig. 2; Table 1), confirms the old observation that heating, probably via vasomotor reflexes, changes subcutaneous blood flow (Roddie *et al.*, 1956; Mottram *et al.*, 1961; Astrup *et al.*, 1988). It did so, although there was no clear elevation in body temperature in the present study. The consequences for the limited usefulness of the 'heated-hand-technique' for the determination of 'arterio'-venous concentration differences have been pointed out by Astrup and co-workers (Astrup *et al.*, 1988).

It is our impression that the method used in carrying out the 'heated-hand-technique' in different laboratories varies considerably. Thermoregulated boxes (McGuire *et al.*, 1976; Abumrad *et al.*, 1981; DeFeo *et al.*, 1988), heating blankets or pads (Förster *et al.*, 1972; Jackson *et al.*, 1973; Kiens *et al.*, 1989), and electric lamps (Paolisso *et al.*, 1988) have been used. The temperature applied to the skin also shows wide variation. The degree by which skin and body temperature have been raised have rarely been reported, so that the efficiency of 'arterializing' venous blood cannot always be judged. It is likely that the clear increase in body temperature noted by Astrup and co-workers (Astrup *et al.*, 1988), on the one hand, and the minor effect observed in the present study, can be traced to the different ways of applying heat. This is supported by a recent study comparing a heating blanket to a thermoregulated box (Gallen & McDonald, 1990).

In conclusion, the present study demonstrates that it is not possible to obtain completely 'arterialized' venous blood by means of the 'heated-hand-technique' when using a hot air box. This may limit the widespread use of this methodology. Furthermore, heating may alter counter-regulatory hormone responses, notably glucagon secretion (or clearance). The effectiveness (on glucose and blood gas concentrations) and side effects (raised body temperature, altered endocrine responses) of using the 'heated-hand-methodology' should be examined carefully and reported in each study.

Acknowledgments

Supported in part by the Deutsche Forschungsgemeinschaft, grant Na 203/2-1, 5300 Bonn 2 (Bad Godesberg), Germany. We thank A. Möhle, C. Winzker, C. Lange, W. Kopp, S. Born, and T. Brasching for excellent technical assistance.

References

ABUMRAD N. N., RABIN D., DIAMOND M. P. & LACY W. W. (1981) Use of heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism*, **30**, 936–940.

ASTRUP A., BÜLOW J., CHRISTENSEN N. J., MADSEN J. & QUAADE F. (1986) Facultative thermogenesis induced by carbohydrate: a skeletal muscle component mediated by epinephrine. *Am J Physiol,* **250** (Endocrinol Metab 13), E226–229.

ASTRUP A., SIMONSEN L., BÜLOW J. & CHRISTENSEN N. J. (1988) Measurement of forearm oxygen consumption: role of heating the contralateral hand. *Am J Physiol,* **255** (Endocrinol Metab 18), E572–578.

ASTRUP A., ANDERSEN T., CHRISTENSEN N. J., BÜLOW J., MADSEN J., BREUM L. & QUAADE F. (1990) Impaired glucose-induced thermogenesis and arterial norepinephrin response persist after weight reduction in obese humans. *Am J Clin Nutr,* **51,** 331–337.

BRAUNWALD E., ISSELBACHER K. J., PETERSDORF R. G., WILSON J. D., MARTIN J. B. & FAUCI A. S. (1987) Appendix, Table A-9 In: *Harrison's principles of internal medicine,* 11th edn, McGraw-Hill, New York.

CHRISTENSEN S. E., JØRGENSEN O. L., MØLLER N. & ØRSKOV H. (1984) Characterization of growth hormone release in response to external heating: comparison to exercise-induced release. *Acta Endocrinol,* **107,** 295–301.

COLLINS K. J., FEW J. D., FOREWARD T. J. & GIEC L. A. (1969) Stimulation of adrenal glucocorticoid secretion in man by raising body temperature. *J Physiol,* **202,** 645–660.

DEFEO P., GALLAI V., MAZZOTTA G., CRISPINO G., TORLONE E., PERIELLO G., VENTURA M. M., SANTEUSANIO F., BRUNETTI P. & BOLLI G. B. (1988) Modest decrement in plasma glucose concentration causes early impairment on cognitive function and later activation of glucose counterregulation in the absence of hypoglycaemic symptoms in man. *J Clin Invest,* **82,** 436–444.

DEFRONZO R. A., TOBIN J. D. & ANDRES R. (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol,* **237** (Endocrinol Metab 3), E214–223.

FERRANNINI E., BJORKMANN O., REICHARDT G. A., Jr, PILO A., OLSSON M., WAHREN J. & DEFRONZO R. A. (1985) The disposal of an oral glucose load in healthy subjects; a quantitative study. *Diabetes,* **34,** 580–588.

FÖRSTER H., HASLBECK M. & MEHNERT H. (1972) Metabolic studies following oral ingestion of different doses of glucose. *Diabetes,* **21,** 1102–1108.

GALLEN I. W. & MCDONALD I. A. (1990) Effect of two methods of hand heating on body temperature, forearm blood flow, and deep venous oxygen saturation. *Am J Physiol,* **259** (Endocrinol Metab 22), E639–643.

GOLDSCHMIDT S. & LIGHT A. B. (1925) A method for obtaining from veins blood similar to arterial blood in gaseous content. *J Biol Chem,* **64,** 53–58.

HALL R. (1971) Vascular injuries resulting from arterial puncture or catheterization. *Br J Surg,* **58,** 513–516.

HALTER J. D., PFLUG E. & TOLAS A. G. (1980) Arterial-venous differences of plasma catecholamines in man. *Metabolism,* **29,** 9–12.

IPP E. & FORSTER B. (1987) Sparing of cognitive function in mild hypoclycemia: dissociation from neuroendocrine response. *J Clin Endocrinol Metab,* **65,** 806–810.

JACKSON R. A., PETERS N., ADVANI U., PERRY G., ROGERS J., BROUGH W. H. & PILKINTON T. R.-E. (1973) Forearm glucose uptake during oral glucose tolerance test in normal subjects. *Diabetes,* **22,** 442–458.

JACKSON R. A., HAMLING J. B., SIM B. M., HAWA M. I., BLIX P. M. & NABARRO J. D. N. (1987) Peripheral lactate and oxygen metabolism in man: influence of oral glucose loading. *Metabolism,* **36,** 144–150.

KIENS B., LITHELL H., MIKINES K. J. & RICHTER E. A. (1989) Effects of insulin and exercise on muscle lipoprotein lipase activity in man and its relation to insulin action. *J Clin Invest,* **85,** 1124–1129.

KLEINBAUM J. & SHAMOON H. (1982) Selective counterregulatory hormone response after oral glucose in man. *J Clin Endocrinol Metab,* **55,** 787–790.

KRARUP T., NÜRNBERG B., MIKKELSEN K., ASTRUP A., MADSBAD S., HENRIKSEN J. & HOLST J. J. (1990) How arterialized is blood sampled from a heated superficial hand vein (abstract)? *Diabetes,* **39** (Suppl. 1), 88A.

MCGUIRE E. A. H., HELDERMAN J. H., TOBIN J. D., ANDRES R. & BERMAN M. (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol,* **41,** 565–573.

MACHLEDER H. I., SWEENEY J. P. & BARKER W. F. (1972) Pulseless arm after brachial-artery catheterization. *Lancet,* **i,** 407–409.

MADSBAD S., JEPPESEN J., BONESGAARD S., KRARUP T. & TRONIER B. (1988) The importance of arterial versus venous sampling in evaluation of plasma glucose, IRI and C-peptide responses after oral and intravenous glucose (abstract). *Diabetes Res Clin Pract* (Proceedings of the 13th Congress of the International Diabetes Federation, Sidney, Nov. 20–25, 1988), 5359.

MARREIRO ROCHA S., SANTEUSANIO F., FALOONA G. F. & UNGER R. (1973) Abnormal pancreatic alpha-cell function in bacterial infections. *New Engl J Med,* **288,** 700–703.

MITSUI A., NOHTA H. & OHKURA Y. (1985) High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylendiamine as precolumn fluorescence derivatization reagent. *J Chromatogr,* **344,** 61–70.

MØLLER N., BECKWITH R., BUTLER P. C., CHRISTENSEN N. J., ØRSKOV H. & ALBERTI K. G. M. M. (1989) Metabolic and hormonal responses to exogenous hyperthermia in man. *Clin Endocrinol,* **30,** 651–660.

MOTTRAM R. J. & BUTTERFIELD W. H. J. (1961) The human forearm as a preparation for metabolic investigation. *Proc R Soc Med,* **54,** 549–552.

NAUCK M., LIEß H. & CREUTZFELDT W. (1990) Critical evaluation of the "heated-hand-technique" for obtaining "arterialized" venous blood for metabolic studies (abstract). *Eur J Clin Invest,* **20,** A11.

PAOLISSO G., SGAMBATO S., RORELLA R., VARRICCHIO M., SCHEEN A., D'ONOFRIO F. & LEFEBVRE P. J. (1988) Pulsatile insulin delivery is more efficient than continuous infusion in modulating islat cell function in normal subjects and patients with Type 1 diabetes. *J Clin Endocrinol Metab,* **66,** 1220–1226.

RAYFIELD E. J., CURNOW R. T., GEORGE D. T. & BEISEL W. R. (1973) Impaired carbohydrate metabolism during a mild viral illness. *New Engl J Med,* **289,** 618–821.

RODDIE I. C., SHEPERD J. Y. & WHELAN R. F. (1956) Evidence from venous oxygen saturation that the increase in forearm blood flow during body heating is confined to the skin. *J Physiol,* **134,** 444–450.

SOMOGYI M. (1948) Studies of arteriovenous differences in blood sugar. I. Effect of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation. *J Biol Chem,* **174,** 189–200.

SONNENBERG G. E. & KELLER U. (1982) Sampling of arterialized heated-hand venous blood as a non-invasive technique for the study of ketone body kinetics in man. *Metabolism,* **31,** 1–5.

TATAR P., VIGAS M., JURCOVICOVA J., KVETANSKY R. & STREC V. (1986) Increased glucagon secretion during hyperthermia in a sauna. *Eur J Appl Physiol,* **55,** 315–317.

THEODORSSON-NORHEIM E. (1987) Friedman and Quade tests: Basic computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comp Biol Med,* **17,** 85–99.

TSE T. F., CLUTTER W. E., SHAH S. D., MILLER J. P. & CRYER P. E. (1983) Neuroendocrine responses to glucose ingestion in man; specificity, temporal relationships, and quantitative aspects. *J Clin Invest,* **72,** 270–277.

UNGER R. H. (1971) Glucagon physiology and pathophysiology. *New Engl J Med,* **285,** 443–449.

WISSENSCHAFTLICHE TABELLEN GEIGY (Physikalische Chemie, Blut, Humangenetik, Stoffwechsel und Xenobiotika), 8. Auflage, Ciba-Geigy Ltd., Basel 1979.

WHICHELOW M. J., WIGGLESWORTH A., COX B. D., BUTTERFIELD W. J. H. & ABRAMS M. E. (1967) Critical analysis of blood sugar measurements in diabetes detection and diagnosis. *Diabetes,* **16,** 219–226.