Effect of mixed meal ingestion on fuel utilization in the whole body and in superficial and deep forearm tissues

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Six healthy lean male adults, given a mixed meal containing 3190 kJ (16 % from protein, 44 % from carbohydrate and 40 % from fat) were studied for the next 370 min using arteriovenous cannulation techniques across superficial and deep forearm tissues. The meal produced no significant change in forearm blood flow or skin temperature. The major differences between superficial and deep forearm tissues were (a) creatinine release by deep tissues but not superficial tissues; (b) the release of non-esterified fatty acids (NEFA) by superficial tissues and uptake by deep tissues; and (c) the more prolonged large positive arteriovenous concentration difference for glucose across deep than superficial tissues. The similarities were (a) general pattern of individual amino acid exchanges and transient positive amino acid N balance after meal ingestion; (b) consistent uptake of glutamate and release of glutamine (the main carrier of N out of superficial and deep forearm tissues); (c) the magnitude of the arteriovenous concentration differences for glucose, NEFA and total amino acids were related to the changes in their circulating concentrations and to the oxidation of carbohydrate, fat, and protein in the whole body; and (d) increases in the arterio–deep venous and arterio–superficial venous differences for glucose did not result in increased release of lactate, alanine or pyruvate, implying no increase in the activity of glucose–lactate and glucose–alanine cycles between forearm tissues and the liver. This study suggests that in a number of ways superficial and deep tissues can be regarded, at least qualitatively, as behaving as a ‘single metabolic unit’.

Metabolite exchange: Muscle: Skin: Adipose tissue

Arteriovenous catheterization techniques provide one of the few ways in which the metabolism of human tissues can be studied in vivo. Although substantial information exists about the exchange of metabolites between blood and splanchnic tissues (Felig et al. 1973), kidneys (Tizianello et al. 1980), brain (Hagenfeldt et al. 1980) and muscle (Elia et al. 1988, 1989), there is very little information about the exchange across skin and associated subcutaneous adipose tissue. A few studies have been performed on superficial tissues of the forearm (Baltzman et al. 1962; Pozefsky & Tancredi, 1972; Pozefsky et al. 1976; Jackson et al. 1987), but none has been comprehensive enough to measure a wide range of metabolites simultaneously. In addition none has made measurements in both the fed and fasted states, apart from one study in which the exchange of metabolites was measured before and after a protein meal (Elia & Livesey, 1983; Elia, 1991).

The present study aimed to determine the similarities between superficial and deep limb tissue metabolism and address three specific issues. First, it was postulated that both deep and superficial tissues are involved in the exchange of a wide range of intermediary metabolites that are linked to fat, carbohydrate, and protein metabolism, and that the exchange is markedly responsive to mixed food ingestion. Second, although muscle is involved in the glucose–lactate and glucose–alanine cycles, little attention has been given to the possibility that such cycles could also involve other tissues such as skin and adipose tissue in vivo. It was hypothesized that after meal ingestion, glucose uptake in these tissues would increase and stimulate lactate and alanine release. Third, it was postulated that superficial limb tissues (mainly skin plus subcutaneous adipose tissue), like muscle, release glutamine in both the fed and fasted states, and that they are also involved in a variety of other amino acid exchanges. The interest in glutamine arose from measurements of the interorgan flux of this amino acid after an overnight fast (Elia, 1991, 1992, 1993, 1996). The total combined uptake of glutamine by human splanchnic tissues, kidneys, and brain, is greater than the amount considered to be released from muscle (Elia, 1993, 1996). Therefore, it was suggested that other tissues release glutamine, at least after an overnight fast. The human lungs have

Abbreviations: A–DV, arterio–deep venous difference; A–SV, arterio–superficial venous difference; NEFA, non-esterified fatty acids.

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been reported to be a possible site of glutamine release (Plumley et al. 1990). Skin and adipose tissue (Frayn et al. 1991b) retain potential sites of release, although in vitro studies with adipocytes have reported that both uptake (Cooney et al. 1986; Kowalchuk et al. 1988) and release of glutamine are possible (Tischler & Goldberg, 1980: Ardawi, 1988), depending on the composition and pH of the incubating medium. By undertaking simultaneous measurements of gaseous exchange in the whole body and of metabolite exchanges across superficial and deep forearm tissues, it is possible to assess the extent to which they behave as a single ‘metabolic unit’, and the extent to which the exchange of metabolites relates to fuel selection in the whole body.

Methods
Subjects
Six healthy, lean, weight-stable male adults took part in this study. Their mean age was 34 (SE 2) years, weight 69 (SE 2) kg, height 1.77 (SE 0.03) m and BMI 22.1 (SE 1.1) kg/m². They were estimated to have 22 (SE 1)% fat from the sum of four skinfolds, measured by the method of Durnin & Womersley (1974). All subjects were studied in the morning after an overnight fast (12–14 h).

Clinical methods
An accurately timed overnight fasting urine collection was made into a bottle containing 25 ml 2 M-HCl. The collection began 4–6 h after the last meal. On the morning of the experiment each person was instructed to drink approximately 200 ml water in order to minimize errors associated with residual bladder volumes and small urine collections. Immediately before the meal was given, the bladder was emptied again, in order to start a new urine collection (0 to +370 min).

Three Teflon catheters were inserted into each person in a retrograde direction. The first was inserted into a superficial dorsal hand vein in order to obtain ‘arterialized blood’. The second was inserted into the antecubital vein of the contralateral forearm so that its tip would be close to the muscle bed of that forearm; and the third was inserted into a superficial vein of the same forearm so that it drained blood mostly from skin and subcutaneous fat. Arterialization of blood was achieved by placing the hand for a period of 20 min in a hot box maintained at 65–70°C (Kurpad et al. 1994; Kurpad & Elia, 1995). Patency of all three catheters was maintained by flushing them intermittently with saline (9 g NaCl/l). Before any measurements were made the subjects were asked to rest in the recumbent position for at least 30 min after catheterization, in a room maintained at an ambient temperature of 25 ± 1°C.

Resting energy expenditure was measured by indirect calorimetry (Crisp & Murgatroyd, 1985) whilst the subjects remained motionless in the recumbent position. The indirect calorimeter was an open-circuit ventilated-hood system which operated at a flow rate of 50 litres/min. Measurements of gaseous exchange (20 min) were made intermittently throughout the study (–60 to –40 min, –20–0 min, 30–50 mins, 60–80 min, 90–110 min, 120–140 min, 180–200 min, 240–260 min, 300–320 min and 360–380 min).

Forearm blood flow was measured by a Hg strain gauge plethysmograph with the hand circulation excluded for a period of 2 min. This was achieved by inflating a paediatric sphygmomanometer cuff fitted around the wrist to a pressure of 200 mmHg. Two temperature sensors, which were placed on the forearm above and below the strain gauge, were used to record skin temperature after excluding the hand circulation (inflation of sphygmomanometer cuff) for 2 min. This measurement was made immediately before measurement of forearm blood flow.

Arterialized, deep venous and superficial venous blood samples (approximately 6 ml from each site) were withdrawn after each measurement of blood flow at the following times: –40, 0, 40, 70, 100, 130, 160, 190, 250, 310 and 370 min. The blood samples were taken simultaneously at similar rates. The blood was placed into potassium EDTA tubes and deproteinized immediately by adding two parts of ice-cold 5-sulfosalicylic acid (35 g/l) to one part of whole blood (Khan et al. 1991). The sulfosalicylic acid contained 150 μmol norleucine/l which acted as an internal standard for subsequent amino acid analysis. Exact quantities of blood and sulfosalicylic acid were determined gravimetrically using an electronic balance sensitive to 0.0001 g. The remaining blood was centrifuged in order to obtain plasma, which was stored at –80°C.

The meal was eaten between 0 and +20 min. It consisted of 90 g boiled cod fillet with 50 g white sauce, 180 g boiled potatoes, 65 g boiled peas, 100 g apple crumble, 40 g double cream and a cup of tea or coffee. Its total energy content was calculated to be 3190 kJ (16.3% energy from protein, 43.5% from carbohydrate and 40.2% from fat; Paul & Southgate, 1978). Approximately 40% of the carbohydrate was in the form of simple sugars.

Biochemical measurements
The following metabolites in deproteinized whole blood were measured enzymically on a Cobas Bio centrifugal analyser (Roch Diagnostics, Welwyn Garden City, Herts., UK): pyruvate, lactate, acetoacetate, β-hydroxybutyrate, glucose, glycerol, alanine, glutamate, glutamine (Khan et al. 1991). Plasma was used to assay: triacylglycerol (Roch Diagnostics Kit 0710685); non-esterified fatty acids (NEFA) (Wako Chemicals GmBH Kit 994-75490; Alpha Laboratories, Eastleigh, Hants., UK); glycerol (Khan et al. 1991); urea (Roch Uni Kit II 0711144) and creatinine (Jaffe method) on the Cobas Bio centrifugal analyser. The triacylglycerol concentration was calculated by subtracting the free glycerol concentration from the total glycerol concentration measured after hydrolysis of triacylglycerol. Whole-blood amino acid concentrations were measured using an LKB 4151 Alpha Plus amino acid analyser (LKB Biochrom, Science Park, Cambridge, UK). Before analyses the deproteinized extract was centrifuged and passed through a 0.22 μm filter (Milllex GV filter unit, Millipore, Bedford, Beds., UK). The enzymic amino acid assays (glutamate, glutamine and alanine) were carried out within a few hours of sampling.
The packed cell volume was measured using a microcapillary system. The concentration and percentage saturation of haemoglobin in blood were measured with a hemoximeter (OSM 2 Hemoximeter, VA Howe, Radiometer Copenhagen, London, UK). Urine N was measured using the Kjeldahl method, and urinary urea and NH₃ by a standard kit (Boehringer Mannheim Urea Kit No 124770, Lewes, Sussex, UK).

Whole-body energy expenditure and fuel selection were calculated using the equations of Elia & Livesey (1992). Overnight fasting urine N excretion was used to calculate preprandial protein oxidation. Urine N excretion from the time of meal ingestion to +370 min was used to calculate postprandial protein oxidation.

Results are presented as means with their standard errors. The means of the two basal values were used for comparison with the values at the other time points. Statistical significance (P ≤ 0.05) was assessed using ANOVA and Student’s t test, which was applied to paired data.

**Ethics**

The study was approved by the local ethics committee, and subjects gave their informed consent.

**Results**

**Urinary nitrogen**

Urinary N was excreted at a rate of 7.11 (SE 0.35) mg/min before meal ingestion and 8.04 (SE 0.44) mg/min from 0 to 370 min (P ≤ 0.05; paired t test). Urea, creatinine and NH₃ accounted for 95% of the urinary N both before and after meal ingestion (in the ratios 91: 5: 4 and 91: 4: 5 respectively). The circulating urea concentration remained virtually unchanged between the basal period and the end of the study (see later).

**Energy expenditure and fuel selection in the whole body**

Basal resting energy expenditure (4.7 ± 0.7 KJ/min) increased significantly after meal ingestion, and reached a maximal value of 21% above the basal preprandial values between +50 and +70 min. The last value at 6 h after the meal was not significantly higher than the mean of the two basal values. The increase in resting energy expenditure over basal during the first 6 h after the meal corresponded to 6% of the energy content of the meal.

After the overnight fast, fat was estimated to contribute approximately 65% of the energy expenditure, but after the meal the contribution from fat decreased to 40–45%, whilst that from carbohydrate increased from a basal value that was a little less than 20% to a value of 35–42% between +70 and +250 min. The percentage contribution of protein before and after the meal was essentially unaltered (about 17–18%), although the actual oxidation rate increased. The blood urea concentrations were not significantly affected by meal ingestion (4.77 (SE 0.49) mmol/l before meal ingestion and 4.80 (SE 0.47) mmol/l at +370 min). Therefore changes in the urea pool had little or no influence on the estimated rate of protein oxidation, which was calculated from the rate of urinary N excretion.
Circulating concentration of metabolites

The concentrations of metabolites in arterialized blood are shown in Table 1. Glucose, lactate, pyruvate and triacylglycerol showed the expected rises, whilst NEFA and $\beta$-hydroxybutyrate decreased. The circulating concentrations of glycerol, acetoacetate, creatinine and $O_2$ showed no significant change. The concentrations of many amino acids increased particularly between 40 and 130 min, but the rise was usually less than 50 $\mu$mol/l and generally returned to basal values by the end of the study. The total amino acid concentration in the basal state (about 3100 $\mu$mol/l) increased

Fig. 1. The effect of mixed meal ingestion on the total arteriovenous concentration differences of amino acids (■) and amino acid N (□) across (a) deep (A–DV) and (b) superficial (A–SV) forearm tissues in healthy male subjects. Values are means for six subjects, with their standard errors represented by vertical bars. Repeated measures ANOVA indicated significant differences ($P < 0.01$) for A–DV and A–SV. For both A–DV and A–SV the mean postprandial concentration, calculated from the area under the curve, was significantly different from the mean preprandial concentration ($P < 0.01$). All postprandial values were significantly greater than basal preprandial values.
by a mean of about 15% in the postprandial period (maximum increase about 25% at +70 min).

**Forearm blood flow and the exchange of metabolites across forearm tissues**

Skin temperature and forearm blood flow did not change significantly during the study period (mean value 32.4 × 4°C preprandial v. 32.2 × 2°C postprandial). Forearm blood flow also did not change following meal ingestion (32.5 × 0.5 v. 32.1 × 0.4 ml/l forearm per min in the pre- and postprandial periods respectively). The changes in the estimated flux of metabolites across deep forearm tissues generally paralleled the changes in the arterio–deep venous concentration differences (A–DV) because there was little change in blood flow. Therefore, significant changes in A–DV were almost always associated with significant changes in flux of metabolites. For simplicity and for comparison with arterio–superficial venous differences (A–SV), the exchanges across deep tissues are expressed as A–DV.

The mean A–DV for O₂ in the fasting state was 2760 μmol/l, and in the postprandial state 2820 μmol/l. The corresponding values for A–SV were 1430 and 1380 μmol/l respectively. The overall amino acid and N balance across both deep and superficial forearm tissues was negative in the basal state, became positive after meal ingestion, but reverted to being negative again towards the end of the study (Fig. 1). In the basal state the pattern of exchange of amino acids across deep forearm tissues (A–DV) was remarkably similar to that across superficial tissues in both the fed and fasted states (Fig. 2). Glutamate had the largest positive arteriovenous concentration differences and glutamine the largest negative concentration differences. Alanine was released in smaller quantities than glutamine. After meal ingestion, glutamate continued to be taken up by deep forearm tissues, and glutamine continued to be released. The A–DV for alanine and many other amino acids, which were negative in the basal state, became positive after meal ingestion, but reverted to being negative again by the end of the study. The branched-chain amino acids (leucine, isoleucine and valine) were consistently taken up for 3–4 h after meal ingestion (mean pre- v. postprandial arteriovenous difference, −22 v. +29 μmol/l; P < 0.01).

The effects of meal ingestion on A–DV and A–SV for selected metabolites are shown in Fig. 3. The A–DV for glucose increased 5–10-fold during the first 160 min after the meal. Between +40 and +100 min this was associated with a more positive A–DV for three-C glycolytic fragments in the form of lactate, pyruvate and alanine (see later). The pattern was similar with A–SV values but the A–SV for glucose showed a significant increase only at +40 min. After the meal there were early decreases in the A–DV for NEFA, acetoacetate and β-hydroxybutyrate. The integrated mean values for A–DV for creatinine showed little change between the pre- and postprandial periods (mean values, −9 (SE 2) and −7 (SE 2) μmol/min respectively). The most striking difference between A–DV and A–SV values of metabolites was that for plasma NEFA, which had a large...
and negative A–SV (−150 to −200 μmol/l) and a positive A–DV (+28 to 62 μmol/l). Meal ingestion caused the A–SV and A–DV to approach zero, but towards the end of the study the values for A–SV and A–DV deviated in opposite directions to re-establish the basal pattern. In the basal state the A–DV and A–SV for triacylglycerol were not significantly different from zero, but after meal ingestion they became more positive. The A–SV for creatinine was close to zero both pre- and postprandially (−2 (SE 2) and 0 (SE 2) μmol/l respectively, and neither significantly different from zero).

Interpretation of arteriovenous differences across tissues is more difficult when the circulating concentration of metabolites is changing than when they are in a steady state. This is because during the time it takes for blood to reach the venous side, the arterial concentrations of metabolites change. If it is assumed that muscle blood flow is similar to forearm blood flow (2.5 ml/100 ml tissue per min; Table 1) and that 100 ml forearm muscle contains 2.5 ml blood (Snyder et al. 1975) (i.e. the transit time is ≈1 min), it can be calculated that the effect of a non-steady state (arterial and venous samples taken simultaneously) on the net flux of metabolites across the tissue is generally small: (a) glucose uptake may be overestimated by approximately 4% (between +70 and 100 min, with virtually no change on the calculated flux of glycolytic

Fig. 3. The arteriovenous concentration differences of various metabolites across deep (●) and superficial (○) forearm tissues in healthy male subjects. Measurements were made using whole blood, except in the case of non-esterified fatty acids (NEFA) which were measured in plasma. Values are means for six subjects, with their standard errors represented by vertical bars. ANOVA indicated significant changes for glucose, lactate, alanine, NEFA (deep and superficial) and glycerol (superficial only).
products (lactate, pyruvate and alanine) relative to glucose uptake; (b) the estimated exchange of acetoacetate should remain essentially unchanged but β-hydroxybutyrate may be overestimated by about 14% between +40 and 70 min; (c) the total fat uptake (NEFA, triacylglycerol and glycerol) may be overestimated by 5% between +40 and 100 min; (d) most amino acid balances would be hardly affected. The errors may be smaller than suggested since radiological studies indicate that the transit time from the arterial to the venous side of the resting forearm may only be a few seconds (Mottram et al. 1973). The errors for the estimated exchanges across superficial tissues are likely to be smaller, partly because the blood flow through skin plus subcutaneous tissue is as high or greater than for muscle (Elia & Kurpad, 1993), and partly because the blood content of these tissues is small (1.8–3.0 ml blood/100 g tissue; Snyder et al. 1975).

Discussion

In the present study an attempt was made to compare the pattern of metabolite exchange across superficial forearm tissues (skin plus adipose tissue) with that of deep forearm tissues (mainly muscle). Such an assessment is not possible without haemodynamic consideration.

Blood flow

Blood flow to superficial tissues was not measured and therefore it is not possible to quantitate the rate of exchange of metabolites across superficial tissues. However, we have previously shown that mixed meal ingestion produces little or no change in core or skin temperature (Elia et al. 1988, 1989) (the constancy of skin temperature is confirmed in this study) and therefore it is likely that there was no major change in skin blood flow. Similarly studies by Coppock et al. (1990) showed that a mixed meal, of similar size and composition as the present one, had little effect on blood flow to subcutaneous adipose tissue. Furthermore, by comparing the simultaneous arteriovenous concentration differences of metabolites across the same tissue, it is possible to establish relationships that are largely independent of blood flow. However, it is theoretically possible that blood from deep muscular tissues might be diverted to the superficial veins (or vice versa), so that the sample taken for analysis represents a mixture of blood draining superficial and deep tissues. Although the perforating veins at the wrist were occluded, the presence of a few communicating veins between the superficial and deep venous system of the forearm could allow this to occur. A theoretical upper limit to the contribution of deep venous blood to superficial venous blood must be between 0 and 50%. Inspection of the arteriovenous concentration differences for creatinine argue against significant ‘mixing’ effects. This is because creatinine is almost exclusively released by human muscle, and quantitatively excreted in urine at a rate of about 0.3 µmol/kg muscle per min (Graystone, 1968). This value is not very different from that obtained by multiplying forearm blood flow (Table 1) with the A–DV for creatinine. On the other hand the A–SV for creatinine is close to and not significantly different from zero, suggesting little or no contribution of deep venous blood to superficial venous blood. Therefore, the pattern of metabolite A–SV and A–DV values can be used to provide qualitative information about similarities and differences between superficial (skin and adipose tissue) and deep tissue (mainly muscle) metabolism.

Pattern of metabolite exchanges

Although there is considerable individual variability in the exchange of substrates such as amino acids, glycerol and glycolytic products (see also Frayn et al. 1991a; Elia et al. 1993), some general trends are evident from the present study, particularly if the mean values for metabolites are considered. The study demonstrates several similarities and differences in substrate exchanges across superficial and deep tissues, some of which are markedly responsive to meal ingestion.

The three major differences between superficial and deep limb tissues were the release of creatinine by deep tissues but not superficial tissues; the release of NEFA by superficial tissues and their uptake by deep tissues; and the more prolonged large positive arteriovenous concentration difference for glucose across deep tissues than superficial limb tissues. These differences can be readily explained by the presence of substantial amounts of adipose tissue in superficial tissues, and the presence in muscle of creatine and/or creatine phosphate and enzymes involved in glucose metabolism, particularly synthesis of glycogen (see later).

There were also at least four similarities in the patterns of metabolite exchanges across superficial and deep tissues. First, the overall amino acid and amino acid N balance changed from being negative in the fasted state to positive in the early postprandial state in both superficial and deep tissues. The overall negative amino acid N balance across superficial and deep tissues in the basal state implies net breakdown of protein in superficial tissues this would arise from skin or from the protein of adipose tissue (up to 5% of adipose tissue is protein; Snyder et al. 1975).

Second, the patterns of individual amino acid exchanges across superficial and deep tissues were also similar in both the fed and fasted states (see Fig. 2 for fasting pattern). It is notable that glutamate was consistently taken up by superficial and deep tissues (positive arteriovenous difference) in both the fed and fasted states, whilst glutamine was consistently released, carrying more N out of superficial and deep tissues than any other amino acid in both the fed and fasted states. Most other amino acids had an arteriovenous concentration difference close to zero in the basal state, and
were taken up between 40 and 100 min after meal ingestion (positive arteriovenous difference) both by superficial and deep tissues. Some amino acids may theoretically originate from the breakdown of circulating protein or from other amino acids. The disproportionately high release from forearm tissues of glutamine, which is not a major constituent of keratin, collagen, or muscle and adipose tissue protein (Christophe et al. 1966), or the major circulating proteins, implies that metabolic pathways for its formation must exist in these tissues. For example, branched-chain amino acid metabolism is known to occur in peripheral tissues (Elia, 1991), and to increase glutamine release from muscle and adipose tissue preparations in vitro (Tischler & Goldberg, 1980). Although branched-chain amino acids are released from both superficial and deep tissues in the basal state, it is likely that some branched-chain amino acids arising from proteolysis are metabolized there.

A third similarity between superficial and deep limb tissues concerns the uptake of glucose and exchange of glycolytic products. Both superficial and deep tissues released glycolytic products (sum of lactate + alanine + pyruvate) in the basal state, and both took them up in the early postprandial period. This uptake occurred despite a large positive arteriovenous difference for glucose and no major change in the arterialized blood concentrations of lactate, pyruvate and alanine between +40 and +130 min. These observations do not support the concept that increased glucose uptake by superficial and deep tissues will necessarily lead to increased release of glycolytic products. By contrast subcutaneous abdominal adipose tissue has been reported to be a source of lactate postprandially to a greater extent than in the basal state (Coppack et al. 1990), although this does not appear to be the case with alanine (Frayn et al. 1991b). It is unclear if these apparently discrepant results relate to differences in the composition of the meal, differences in the metabolic behaviour of adipose tissue at various sites or different contributions of blood flow from skin and/or other tissues. Parenthetically, the large uptake of glucose by deep limb tissues in the early postprandial period (0–130 min) is favoured by hyperglycaemia, hyperinsulinaemia and low NEFA concentrations. It is likely that a large portion of the glucose taken up during this time is stored rather than oxidized. This is because the amount of O$_2$ required to fully oxidize the glucose taken up (6 mol O$_2$ for every mol glucose oxidized) is 1.5–2.0 times greater than the observed O$_2$ uptake. The exchange of glycolytic products makes little difference to the calculations.

A fourth similarity relates to the exchange of glucose, NEFA and total amino acids across superficial and deep tissues, which approximately paralleled changes in their circulating concentration and in the oxidation of carbohydrate, fat and protein in the whole body. These observations are consistent with the view that the circulating concentrations of substrates are important determinants of their oxidation in the whole body and exchange across superficial and deep forearm tissues.

In conclusion this study suggests that there is substantial similarity in the metabolic behaviour of superficial and human forearm tissues in both the fed and fasted states.

References


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