

The effects of underfeeding for 7 d on the thermogenic and physiological response to glucose and insulin infusion (hyperinsulinaemic euglycaemic clamp)

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The effect of underfeeding for 7 d (at 60 kJ/kg ideal body-weight) on the thermic and physiological responses to glucose and insulin infusions (hyperinsulinaemic euglycaemic clamp) was studied in six healthy women. Underfeeding had no significant effect on baseline metabolic rate, heart rate, forearm blood flow, diastolic blood pressure, blood intermediary metabolites, plasma insulin or catecholamines, but reduced both respiratory exchange ratio (RER; control (C) 0.86 (SE 0.02), underfed (U) 0.75 (SE 0.01); $P < 0.01$) and systolic blood pressure (by approximately 10 mmHg, $P < 0.01$). Baseline forearm glucose uptake and oxygen consumption were similar in both states. During the final 30 min of the glucose and insulin infusion, metabolic rate rose by 0.43 (SE 0.05) kJ/min in the C state, but no rise was seen in the U state ($P < 0.01$). Glucose disposal rate (C 47.9 (SE 1.8), U 47.3 (SE 4.1) $\mu\text{mol/kg per min}$) and storage rate (C 27.5 (SE 2.4), U 31.6 (SE 3.6) $\mu\text{mol/kg per min}$) were similar in both states, but glucose oxidation rate was reduced in the U state (C 20.5 (SE 1.7), U 15.4 (SE 0.7) $\mu\text{mol/kg per min}$; $P < 0.05$). RER rose to a higher value in the C state than in the U state (C 0.97 (SE 0.2), U 0.80 (SE 0.01); $P < 0.01$). During hyperinsulinaemia, the forearm glucose uptake and O_2 consumption rose in both states. No significant differences were seen in the cardiovascular responses to hyperinsulinaemia in either state. Thus underfeeding abolishes the rise in thermogenesis and reduces glucose oxidation during glucose and insulin infusions in healthy women, but does not affect the glucose disposal or storage rates or the other measured responses.

Thermogenesis: Catecholamines: Glucose clamp technique

A reduction in food intake reduces resting metabolic rate (MR) (Dauncey, 1980) and the thermic effect of food ingestion is diminished in obese subjects on a very-low-energy diet (Alban-Davies *et al.* 1989). Total starvation for 48 h is accompanied by a reduction in the thermic response to food ingestion (Gallen *et al.* 1990) and abolishes the thermic response to the simultaneous infusion of glucose and insulin (Mansell & Macdonald, 1988). This latter effect occurred despite a maintained rate of insulin-mediated glucose storage in starvation. By contrast, the thermic response to the ingestion of food is not changed following more prolonged underfeeding with a less restrictive diet (Mansell & Macdonald, 1988; Alban-Davies *et al.* 1989). These studies suggest that the thermic effect of food or glucose infusion is variable and can be affected by alteration in nutritional state.

The cardiovascular response to food ingestion is reduced following a 48 h fast (Gallen *et al.* 1990) but not after underfeeding for 7 d (Mansell & Macdonald, 1988). The normal cardiovascular responses to food ingestion (rises in heart rate, limb blood flow and systolic blood pressure, with reduction in diastolic blood pressure) (Fagan *et al.* 1986) are also seen during hyperinsulinaemia (Christensen, 1983; Rowe *et al.* 1981; Scott *et al.* 1988). Thus the present study was designed to examine whether undernutrition for 7 d (at 60 kJ/kg ideal body-weight) has similar effects to a 48 h fast on the thermic response to glucose and insulin

Table 1. *Details of subjects*

Subject no.	Age (years)	Wt (C) (kg)	Body mass index (C) (kg/m ²)	Wt (U) (kg)
1	22	61.7	21.1	57.1
2	22	65.5	24.0	62.2
3	22	54.0	21.1	51.0
4	22	61.2	22.9	59.5
5	22	88.9	28.4	87.0
6	22	64.5	24.9	62.5

C, control; U, underfed.

infusions, and to study whether any such effect may be generalized to other physiological systems or confined to thermogenesis.

METHODS

Six healthy 22-year-old female subjects were recruited for the study (their physical characteristics are given in Table 1). None was taking any medication other than the oral contraceptive pill. All gave written informed consent to the study, which was approved by the University of Nottingham Medical School Ethical Committee. Subjects were studied after an overnight fast, on two occasions 1 month apart either whilst on their normal diet (control, C) or after underfeeding for 7 d on a 60 kJ/kg ideal-body-weight diet (underfed, U). The diets were prescribed by a dietitian, and subjects weighed all food and recorded the values in a diary. Four subjects were studied first whilst on a normal diet, two following undernutrition. Measurements took place in a temperature-controlled room (30°) with the subjects resting supine, wearing light clothing only. The women were studied in the first 14 d of the menstrual cycle (Webb, 1986).

Subjects initially rested supine for 30 min, during which time intravenous cannulae were inserted under local anaesthetic and monitoring equipment was attached. Baseline measurements were made for 30 min, followed by a 90 min primed continuous infusion of insulin (100 mU/m² per min) through an intravenous cannula placed in a vein in the antecubital fossa. Blood glucose was maintained at 4.5 mmol/l by alteration of the rate of infusion of glucose solution (200 g/l; controlled by an IVAC 560 pump) through the same cannula (DeFronzo *et al.* 1979).

Continuous recordings of oxygen consumption and carbon dioxide production were made during the baseline and final 30 min of the study using an indirect calorimeter (Fellows & Macdonald, 1985). From the respiratory gas exchange values, calculations of MR (Weir, 1949) and respiratory exchange ratio (RER) were made. Heart rate was recorded from an electrocardiogram and brachial arterial blood pressure was measured by auscultation using a mercury sphygmomanometer, taking Korotkoff phase V as the diastolic pressure. Right forearm blood flow was determined by venous occlusion plethysmography using a mercury-in-rubber strain gauge (Whitney, 1953).

For blood sampling, a cannula was inserted retrogradely into a vein on the dorsum of the left hand and kept patent with a slow running infusion of saline (154 mmol sodium chloride/l). This hand rested in a warm-air box (55–60°) to obtain 'arterialized' venous blood samples (McGuire *et al.* 1976). Forearm effluent venous blood was obtained from a venous cannula inserted retrogradely into the deep muscular vein of the right

forearm (Andres *et al.* 1954), also kept patent with a saline (154 mmol NaCl/l) infusion. These samples were taken with the hand circulation occluded by a high-pressure cuff. After sampling arterialized and deep venous blood, glucose concentration (YSI 23 AM, Yellow Springs Industries, USA) and O_2 tension (Corning blood gas analyser, Corning Medical and Scientific Instruments, USA) were measured immediately. O_2 content was calculated from the values of O_2 tension and haemoglobin concentration. Forearm glucose uptake and O_2 consumption were then calculated from 'arterio-venous' difference in values of blood glucose and O_2 content and the forearm blood flow. Portions of the arterialized blood samples were deproteinized in perchloric acid, the supernatant fraction being stored at -20° for later analysis of lactate, glycerol and β -hydroxybutyrate (BOHB) (Lloyd *et al.* 1978). The remainder of the arterialized blood sample was centrifuged at 4° and the plasma separated. Plasma (3 ml) was mixed with 100 μ l EGTA-glutathione and stored at -80° for later determination of noradrenaline and adrenaline concentration using high-performance liquid chromatography with electrochemical detection (Macdonald & Lake, 1985). Plasma (1 ml) was stored at -80° for subsequent determination of insulin concentration by radioimmunoassay.

Timed samples of urine were taken so that protein oxidation could be estimated from nitrogen excretion. However, due to an accident with defrosting of the freezer, the urine samples were lost, and so N excretion during the final 30 min of the study has been estimated from the plasma urea and an assumed urea clearance rate for healthy subjects (urea clearance rate constant used 44.3 ml/min per m^2 at urine flow-rate greater than 2 ml/min; Brenner, 1986). From this estimation of N excretion rate a value for protein oxidation has been calculated (Livesey & Elia, 1988). Although this is not the best method of calculation of protein oxidation, and urea excretion is not synonymous with urea production, such calculation is likely to give a reasonable index of protein oxidation.

Glucose disposal rate was calculated from the glucose infusion rate with corrections made for changes in the body glucose pool (DeFronzo *et al.* 1979). Glucose oxidation rate was calculated from O_2 consumption and RER during the final 30 min of the glucose clamp, with corrections made for protein oxidation (Livesey & Elia, 1988). Glucose storage rate was calculated by subtraction of glucose oxidation from glucose disposal rates. The net energy cost of glucose storage was derived from the increase in MR above baseline in the final 30 min of the infusion divided by the energy content of total glucose stored (Acheson *et al.* 1984).

Statistical analysis of the results was performed by two-way analysis of variance with repeated measures (ANOVA) using the statistical package BMDP. Time effects represent changes from baseline values during the infusion, treatment effects being differences in responses due to the effects of underfeeding. Where a significant treatment time effect was indicated, paired Student's *t* test for individual time points was performed. Results are means for baseline values, and of mean changes from baseline during the glucose clamps. Values of statistical analysis are presented as the degrees of freedom for the factor (f_1 and f_2) and the corresponding *F* ratio, together with the probability. Results given graphically are the means with their standard errors for each time-point. Values for MR and RER during the baseline and final 30 min of the glucose and insulin infusions were analysed by Student's paired *t* test, as were values for glucose metabolism and disposal during the final 30 min of the glucose and insulin infusions. These results are given as means with their standard errors.

Table 2. *Metabolic rate (MR) and respiratory exchange ratio (RER) of healthy female subjects on a normal diet (control) or after underfeeding at 60 kJ/kg ideal body-weight (underfed) for 7 d*

(Mean values with their standard errors)

	Control		Underfed	
	Mean	SE	Mean	SE
Baseline values				
MR (kJ/min)	4.25	0.312	4.08	0.28
RER	0.86	0.02	0.75**	0.01
Final 30 min of glucose and insulin infusions				
Δ MR (kJ/min)	+0.43	0.05	+0.10**	0.07
RER	0.97	0.02	0.80**	0.01

Mean values were significantly different from these for controls: ** $P < 0.01$.

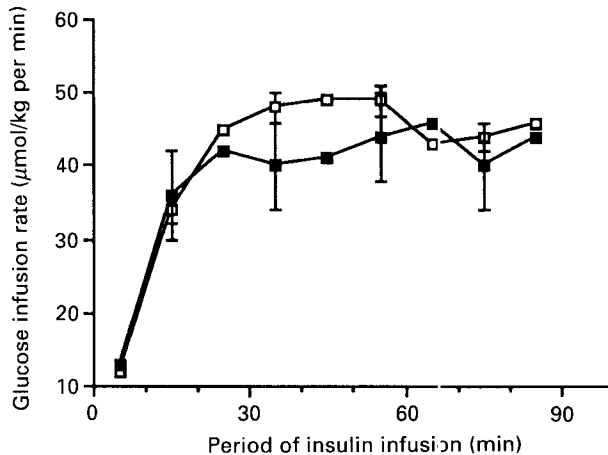


Fig. 1. Glucose infusion rate during insulin infusion in six healthy female subjects fed on a normal diet (control, □) or after underfeeding at 60 kJ/kg ideal body-weight (underfed, ■) for 7 d. Values are means with their standard errors represented by vertical bars.

RESULTS

Glucose metabolism and thermic response

Underfeeding caused a mean weight loss of 2.4 (range 0.5–4) kg (Table 1). Baseline blood glucose was reduced after underfeeding (C 4.40, U 3.95 mmol/l, treatment effect f_1/f_2 1/5, F 12.3, $P < 0.02$ ANOVA). RER was reduced from 0.86 (SE 0.02) in the C state to 0.75 (SE 0.01) in the U state ($P < 0.01$), but no significant change was seen in resting MR after underfeeding (C 4.25 (SE 0.3), U 4.08 (SE 0.3) kJ/min; Table 2). Steady-state glucose infusion rate was achieved after 50 min of the glucose and insulin infusions (Fig. 1). The mean blood glucose concentration achieved during the final 30 min of the glucose and insulin infusions was 4.45 mmol/l in both states, with a mean coefficient of variation of blood glucose concentration of 1.6 (range 0–6)% in the C state and 1.5 (range 0–3)% in the U state.

During the final 30 min of the glucose and insulin infusions MR rose in the C state by

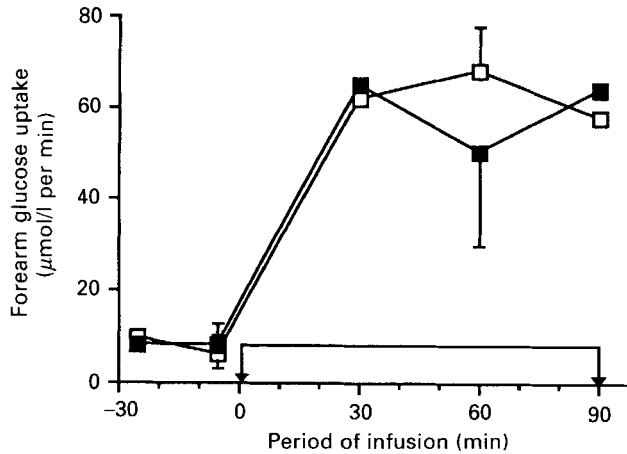


Fig. 2. Forearm glucose uptake in six healthy female subjects fed on a normal diet (control, □) or after underfeeding at 60 kJ/kg ideal body-weight (underfed, ■) for 7 d. ↓↓, Period of glucose and insulin infusions. Values are means with their standard errors represented by vertical bars. Forearm glucose extraction rose during the infusion in both states ($P < 0.01$, ANOVA).

+0.43 (SE 0.05; $P < 0.01$) above the baseline value, but no significant increase was seen in the U state (+0.1 (SE 0.07) kJ/min; Table 2). Furthermore, RER was lower in the U state than in the C state (C 0.97 (SE 0.02), U 0.80 (SE 0.01); $P < 0.01$).

During the glucose and insulin infusions, plasma urea was similar in both states (C 3.5 (SE 0.1), U 3.3 (SE 0.3) mmol/l). Both glucose disposal rate (C 47.9 (SE 1.8), U 47.3 (SE 4.1) $\mu\text{mol/kg per min}$) and glucose storage rate (C 27.5 (SE 2.4), U 31.6 (SE 3.6) $\mu\text{mol/kg per min}$) were unchanged after underfeeding, whereas glucose oxidation rate was significantly reduced from 20.5 (SE 1.7) to 15.4 (SE 0.7) in the U state ($P < 0.05$). Thus, the net energy cost of glucose storage derived from the change in MR above baseline values during the final 30 min of the glucose and insulin infusions, and the energy content of glucose stored during that period, was reduced from 9.3 (SE 1.3)% to 2.0 (SE 1.0)% by underfeeding ($P < 0.01$).

Baseline forearm glucose uptake (C 9.5, U 8.0 $\mu\text{mol/l per min}$) and O_2 consumption (C 1.9, U 2.25 ml/l per min) were not changed after underfeeding. During glucose and insulin infusions there were significant rises in both forearm glucose uptake (C +52.5, U +61.0 $\mu\text{mol/l per min}$, time effect f1/f2 4/20, $F 49$; $P < 0.001$, ANOVA; Fig. 2), and O_2 consumption with a significantly greater rise in forearm O_2 consumption in the U state (C +0.12, U +1.4 ml/l per min, treatment-time effect f1/f2 2/8, $F 7.3$; $P < 0.05$, ANOVA).

Cardiovascular responses

Baseline systolic blood pressure was reduced from the control value of 110 to 99.0 mmHg by underfeeding (treatment effect f1/f2 1/5, $F 7.3$; $P < 0.05$, ANOVA), but no significant changes were seen in baseline diastolic blood pressure (C 74.0, U 72.2 mmHg; Fig. 3), heart rate (C 73.5, U 72.0), or forearm blood flow (C 36.0, U 40.0 ml/l per min; Fig. 4).

During the glucose and insulin infusions, there were rises in systolic blood pressure (C +5.5, U +10 mmHg, time effect f1/f2 3/15, $F 5.11$; $P < 0.05$, ANOVA), heart rate (C +7.0, U +7.5, time effect f1/f2 3/15; $F 7.5$, $P < 0.01$, ANOVA) and forearm blood flow (C +12.0, U +24.0 ml/l per min, time effect f1/f2 3/15, $F 10.6$; $P < 0.01$, ANOVA). No significant changes from baseline values were seen in diastolic blood pressure.

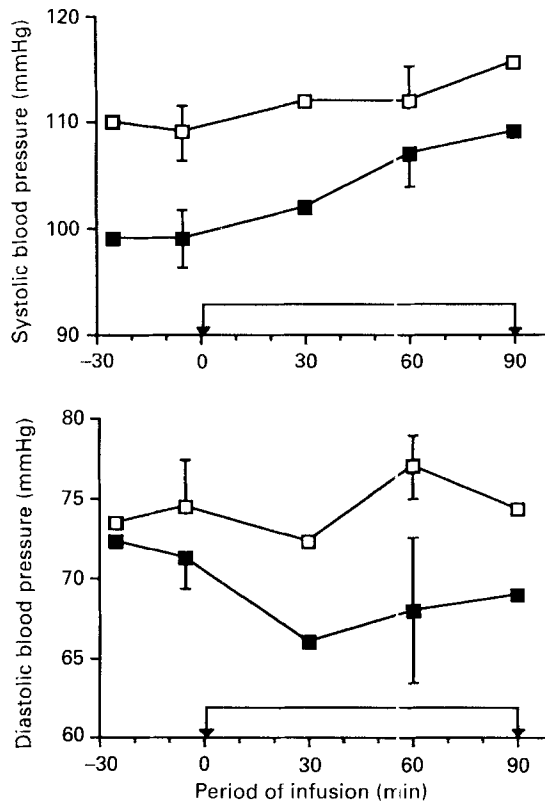


Fig. 3. Systolic and diastolic blood pressures in six healthy female subjects fed on a normal diet (control, \square) or after underfeeding at 60 kJ/kg ideal body-weight (underfed, \blacksquare) for 7 d. $\downarrow\downarrow$, Period of glucose and insulin infusions. Values are means with their standard errors represented by vertical bars. Baseline systolic blood pressure was lower ($P < 0.01$) in the undernourished state. During the infusion, systolic blood pressure rose ($P < 0.01$, ANOVA), but no change was seen in diastolic blood pressure.

Plasma insulin, catecholamines and blood BOHB, lactate and glycerol concentrations

Baseline plasma insulin was similar in both states (C 5.0, U 3.2 mU/l), as were plasma adrenaline (C 0.29, U 0.32 nmol/l) and noradrenaline (C 0.93, U 1.0 nmol/l). The insulin infusion achieved similar levels of hyperinsulinaemia in both states (C 245, U 258 mU/l). During the glucose and insulin infusions no change was seen in plasma adrenaline or noradrenaline concentrations.

There was a trend to increased baseline arterialized venous blood BOHB concentration in the U state (C 0.09, U 0.37 mmol/l) but this did not reach statistical significance (Fig. 5). Baseline arterialized blood glycerol (C 0.05, U 0.05 mmol/l) and lactate (C 1.1, U 0.8 mmol/l) were similar in both states (Fig. 5). During the glucose and insulin infusions, BOHB fell (C -0.06 , U -0.32 mmol/l, time effect f_1/f_2 4/20, F 3.3; $P < 0.05$, ANOVA) and, during the final 30 min of the infusions, BOHB concentration was low and stable in both states. Blood glycerol also fell during the infusions (C -0.02 , U -0.02 mmol/l, time effect f_1/f_2 4/20, F 6.8; $P < 0.01$, ANOVA), whereas arterialized venous blood lactate concentration rose by +1.2 mmol/l in the C state, but less in the U state (+0.8 mmol/l, treatment effect f_1/f_2 1/5, F 7.5; $P < 0.05$, ANOVA).

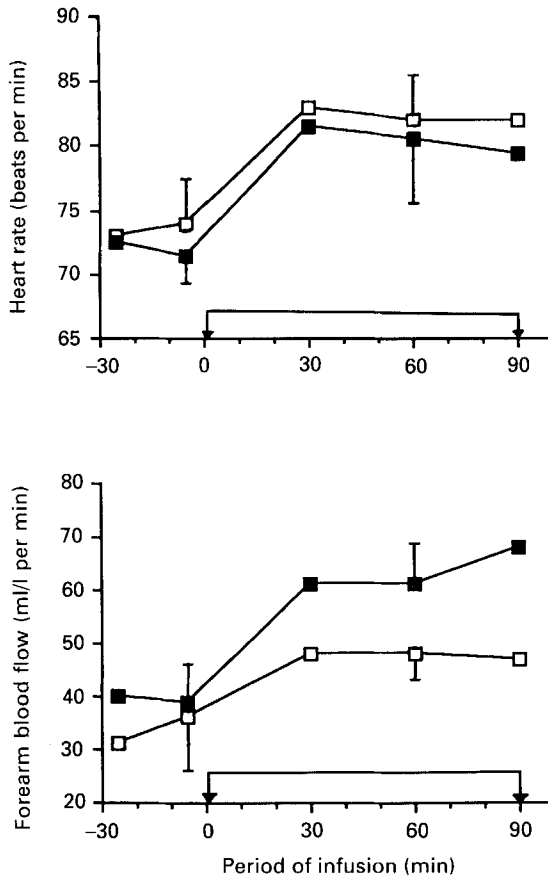


Fig. 4. Heart rate and forearm blood flow in six healthy female subjects fed on a normal diet (control, \square) or after underfeeding at 60 kJ/kg ideal body-weight (underfed, \blacksquare) for 7 d. $\downarrow\downarrow$, Period of glucose and insulin infusions. Values are means with their standard errors represented by vertical bars. Both heart rate and forearm blood flow rose during the infusion ($P < 0.01$, ANOVA).

DISCUSSION

The major finding of the present study was the marked difference in the thermic response to glucose and insulin infusion between the C and U states. Underfeeding for 7 d was associated with the absence of a thermic response. This finding is in accordance with the absence of a thermic response to a similar stimulus after a 48 h fast (Mansell & Macdonald, 1989), and with the reduction in the thermic response to food ingestion after a 48 h fast (Gallen *et al* 1990) or underfeeding on a very-low-energy diet (Alban-Davies *et al.* 1989). However, the present observation would appear to be at variance with the unaltered thermic response to food ingestion seen after underfeeding (Mansell & Macdonald, 1988; Alban-Davies *et al.* 1989) at similar levels of energy restriction. The alteration in the thermic effect of the infusions cannot be explained by differences in the amount of glucose infused, as glucose disposal rates were similar in both states. Glucose oxidation rate was significantly higher in the C state than in the U state, and this may in part account for differences in thermic response. Blood BOHB was suppressed to low levels during the early period of hyperinsulinaemia in both states. Thus during the final 30 min of the study BOHB will have made little or no contribution to total substrate supply. Protein oxidation

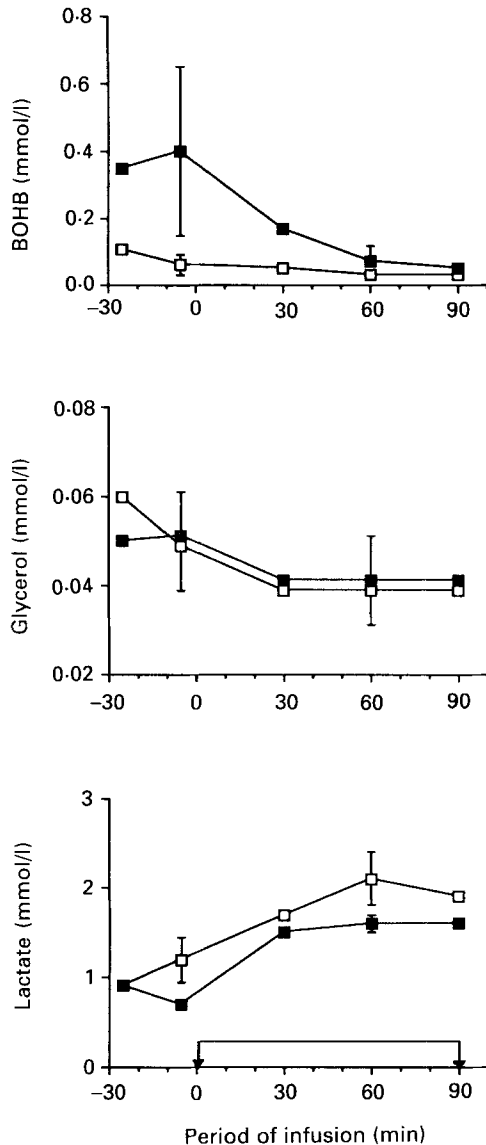


Fig. 5. Arterialized venous blood β -hydroxybutyrate (BOHB), glycerol and lactate concentrations in six healthy female subjects fed on a normal diet (control, □) or after underfeeding at 60 kJ/kg ideal body-weight (underfed, ■) for 7 d. $\downarrow\downarrow$, Period of glucose and insulin infusions. Values are means with their standard errors represented by vertical bars. During the infusion there were falls in both BOHB and glycerol ($P < 0.01$, ANOVA), and a rise in lactate concentration ($P < 0.01$, ANOVA).

estimated from plasma urea concentration during the final 30 min of the glucose and insulin infusions accounted for approximately 20% of O_2 consumption, and was similar in both states of nutrition. It is therefore unlikely that any differences in protein or ketone oxidation could have accounted for the marked alteration in the thermic response to glucose and insulin infusion.

The present findings confirm previous observations (Acheson *et al.* 1983), with an approximately 10% rise in MR above the baseline value during the final 30 min of the

infusions in the C state. From the RER it appears that there was no net *de novo* lipogenesis in either state and, therefore, the glucose was stored as glycogen. The net energy cost of glycogen synthesis from infused glucose has been calculated at 5.3% of the total energy content of the stored glucose (Flatt, 1978). An energy cost of glucose storage in excess of this value is evidence of the phenomenon of facultative thermogenesis (Acheson *et al.* 1983). The net energy cost of glucose storage during the C state in the present study was equivalent to approximately 9% of the energy stored. By comparison, during the final 30 min of the glucose and insulin infusions in the U state, there was no significant rise in MR above baseline and the net energy cost of glucose storage was reduced to about 2% of the energy equivalent of the glucose stored. This would be consistent with the absence of facultative thermogenesis after underfeeding.

In the present study the lower energy cost of glucose storage in the U state may represent activation of an energy-conserving mechanism induced by underfeeding. Such a phenomenon has been demonstrated during glucose and insulin infusions with acute lowering of blood glucose concentration (Gallen & Macdonald, 1989*a*). Another possible explanation of the reduced thermic response to glucose infusion may be that the rise in MR during glucose infusion is offset by a simultaneous fall due to inhibition by insulin of gluconeogenesis, lipolysis and ketogenesis in the U state. However, if this is the case, it is perhaps surprising that the resting MR was not elevated in the U state as is the case in early starvation (Elia *et al.* 1987; Mansell & Macdonald, 1989).

It has been proposed that the sympathetic nervous system may be involved in the regulation of the thermic response to glucose and insulin infusions (Acheson *et al.* 1983), and that in both animals and man, sympathetic nervous system activity may be reduced by undernutrition (Young & Landsberg, 1977; Jung *et al.* 1979). However, in the present study there was no evidence of a reduction in sympathetic nervous system activity after underfeeding. Furthermore, the present differences seen in the thermic response to glucose and insulin infusions in different nutritional states do not seem to be mediated by an altered sympathetic nervous system response, as plasma noradrenaline levels were similar in both studies. However, at best this is only an indirect index of sympathetic nervous system activity.

The hyperinsulinaemic glucose clamp as used here achieves plasma insulin concentrations and rates of glucose disposal which are in excess of those normally seen in the physiological state. These high concentrations of insulin cause near-complete suppression of endogenous glucose and insulin release, whence the glucose infusion rate approximates to the whole-body glucose disposal rate (DeFronzo *et al.* 1978). In this study, given a forearm glucose uptake of about 70 $\mu\text{mol/l}$ per min in either state and assuming that skeletal muscle is 40% of body mass, we can calculate that during hyperinsulinaemia about 70% of glucose disposal occurs in skeletal muscle, which is in agreement with previous values (DeFronzo *et al.* 1981). Skeletal muscle has been demonstrated as a site of adrenaline-mediated facultative thermogenesis in man (Astrup *et al.* 1987) and would therefore be a candidate for regulation of the thermic response to glucose. However, in the present study the increase in forearm O_2 consumption seen during glucose and insulin infusions does not account for the observed variation in MR response, as forearm O_2 uptake was greater in the U state than in the control state.

The arterial lactate concentration rose to a significantly higher level in the control state. We are not able to state whether this rise is due to increased skeletal muscle production of lactate or to reduction in hepatic lactate uptake during hyperinsulinaemia (Bjorkman & Eriksson, 1985). Net forearm lactate production has not been demonstrated during glucose and insulin infusions, and the source of the lactate may in fact be adipose or other non-muscle tissue (Frayn *et al.* 1989).

The present study was not designed to rediscover the effects of underfeeding, and as a result of the small number of subjects studied the expected biochemical features of undernutrition were not demonstrated (Mansell & Macdonald, 1988). However, underfeeding reduced blood glucose concentration and there was a trend towards increased blood BOHB concentration. The fall in both BOHB and glycerol concentration during hyperinsulinaemia was similar in both states of nutrition. This is consistent with the previous observation that fasting induces selective resistance to insulin action, but does not reduce insulin-induced inhibition of lipolysis or ketogenesis (Newman & Brodows, 1983).

Underfeeding caused a reduction in systolic blood pressure, and this is consistent with previous observations of the hypotensive response to energy restriction in obese women (Jung *et al.* 1979). No significant change was seen in either diastolic blood pressure, forearm blood flow or heart rate in the U state. The results for the cardiovascular response to glucose and insulin infusions are consistent with those of Scott *et al.* (1988) and our previous observations (Gallen & Macdonald, 1989*b*). Glucose and insulin infusions caused an increase in heart rate and forearm blood flow, but no significant change in diastolic blood pressure in both states. These responses may be due to the cardiovascular effects of insulin alone (Christensen, 1983), and were unaffected by nutritional state.

In summary the present study demonstrates that a period of underfeeding for 7 d at 60 kJ/kg ideal body-weight in healthy female subjects has no significant effect on resting MR, heart rate, forearm blood flow, diastolic blood pressure, blood metabolites, plasma insulin or catecholamines, but reduces both RER and systolic blood pressure. However, underfeeding has marked effects on the thermic response to glucose and insulin infusions and on glucose metabolism, although other responses to the glucose and insulin infusions do not appear to be altered by undernutrition. Underfeeding abolishes glucose- and insulin-induced facultative thermogenesis, with no evidence of alteration in sympathetic nervous system activity. This may represent an energy-conserving mechanism in man induced by underfeeding.

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