The effect of a 48 h fast on the physiological responses to food ingestion in normal-weight women

BY I. W. GALLEN, I. A. MACDONALD AND P. I. MANSELL

Department of Physiology and Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH

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The thermogenic, cardiovascular and metabolic responses to a 30 kJ/kg body-weight test meal were studied in eight normal-weight, healthy female subjects after a 6 or 48 h fast. There was no significant change in metabolic rate following the 48 h fast, but plasma glucose, insulin, noradrenaline and respiratory exchange ratio were all reduced, and plasma β-hydroxybutyrate was increased. Forearm blood flow was increased, with reduction in diastolic blood pressure. After the 48 h fast, there was a reduction in the metabolic rate response 40–90 min after food (control+0.54 (SE 0.05), 48 h fast+0.27 (SE 0.12) kJ/min, P < 0.01), and in forearm blood flow and diastolic blood pressure responses, but increases in heart rate, blood glucose and plasma insulin responses to the ingestion of the test meal. There was no significant relationship between plasma catecholamine concentration and food ingestion or metabolic rate. Fasting induced considerable adaptation in these subjects and altered some of the physiological responses to food ingestion.

Fasting: Food ingestion: Metabolic rate: Thermogenesis

The ingestion of food has marked physiological effects, some of which may be mediated through activation of the sympathetic nervous system (Acheson et al. 1984) or by release of adrenaline from the adrenal medulla (Astrup et al. 1985). Following ingestion of food there is a rise in metabolic rate (the thermic effect of feeding), which is now thought to consist of two components, the predictable ‘obligatory’ energy cost of nutrient storage (Flatt, 1978), and an additional ‘facultative’ component (Acheson et al. 1984). This facultative component is readily demonstrable after the infusion of glucose and insulin and may be mediated by activation of the sympathetic nervous system, in that it can be abolished by previous administration of the β-adrenoceptor antagonist propranolol (Acheson et al. 1983). It has been proposed that a reduction in energy intake may lead to reduced sympathetic nervous system activity (Landsberg & Young, 1978), and this may considerably affect the thermic response to food ingestion.

We have recently shown that underfeeding for 7 d in normal-weight women produces considerable physiological adaptation in the basal state, but does not alter the thermic or haemodynamic response to food ingestion (Mansell & Macdonald, 1988). By contrast, we have observed that a 48 h fast is associated with abolition of the thermic response to glucose and insulin infusion (Mansell, 1988). Thus, the present study was designed to see if a 48 h fast affected the metabolic, cardiovascular and thermic responses to ingestion of a mixed meal.

METHODS

Eight healthy, non-obese female subjects were recruited for the study; none was taking any medication other than the oral contraceptive pill. The ages and physical characteristics of the subjects are given in Table 1. All gave written informed consent to the study, which was
Table 1. Details of ages and physical characteristics of female subjects

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
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<tr>
<td>Age (years)</td>
<td>18-46</td>
<td>27:6</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>51-69</td>
<td>58:4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1:54-1:74</td>
<td>1:64</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>19:4-25:3</td>
<td>21:82</td>
</tr>
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approved by the University of Nottingham Medical School Ethical Committee. Subjects were studied whilst on their normal diet, 6 h after a light breakfast, and after a 48 h fast. During the fast, subjects were encouraged to drink water ad lib. and were given oral sodium (80 mmol/d) and potassium (60 mmol/d) supplements to avoid alteration in fluid and electrolyte balance, which may otherwise have affected sympatho-adrenal activity. Studies took place in the afternoon in a temperature-controlled room (30°) with the subjects resting supine, wearing a ‘T’-shirt and shorts only. On each occasion, subjects were studied in the first 14 d of the menstrual cycle to exclude any of the effects that this might have on metabolic rate (Webb, 1986). Studies in each subject were performed 4 weeks apart with one subject being studied initially in the 48 h fasted state.

Subjects rested supine for 30 min, during which time intravenous cannulas were inserted under local anaesthetic, and the monitoring equipment attached. Baseline measurements were made for 30 min. The test meal (30 kJ/kg body-weight) was then consumed within 10 min, and was a warmed (32°) liquid preparation (Fortisip Plus; Cow and Gate) containing (% by energy) 48 carbohydrate, 39 fat and 13 protein (manufacturer’s values). Measurements continued for 90 min after food ingestion.

Continuous recordings of oxygen consumption and carbon dioxide production were made using an indirect calorimeter (Fellows & Macdonald, 1985). From the respiratory gas-exchange findings, calculations of metabolic rate (MR) (Weir, 1949) and respiratory exchange ratio (RER) were made. Mean values (10 min) of MR and RER were used for statistical analysis and graphical representation. 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. The surface temperature of the skin was measured over the left mid-abdomen, left mid-thigh, left mid-calf, left mid-shin and dorsum of the left foot using thermocouples lightly taped to the skin, the readings taken from a Comark 6600 microprocessor-type thermometer. 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 similar in content to true arterial blood (McGuire et al. 1976). Forearm effluent venous blood was obtained from a venous cannula inserted retrogradely into the right deep muscular vein of the forearm (Andres et al. 1954), also kept patent with a saline (154 mmol NaCl/l) infusion. Immediately after each blood sample, arterialized and deep venous blood glucose concentrations (YSI 23 AM; Yellow Springs Industries, USA) and O₂ contents (Lex O₂ Con; Lexington Instruments, USA) were measured. Forearm glucose uptake and O₂ consumption were calculated from ‘arterio-venous’ differences in values of blood glucose and O₂ content, and the forearm blood flow measured at that time. A portion of each arterialized venous blood sample was deproteinized in 0:1 M-perchloric acid, the
supernatant fraction being stored at −20° for later analysis of lactate, glycerol and \(\beta\)-hydroxybutyrate (BOHB) concentrations (Lloyd et al. 1978). The remainder of the arterialized blood sample was centrifuged at 3500 rev/min for 10 min at 4°, and the plasma separated. Plasma (3 ml) was mixed with 100 \(\mu\)l EGTA–glutathione (antioxidant) and stored at −80° for later determination of noradrenaline and adrenaline concentration using high-performance liquid chromatography with electrochemical detection (Macdonald & Lake, 1985). Plasma was stored at −80° for subsequent determination of insulin concentration.

Statistical analysis of the results was performed by two-way analysis of variance (ANOVA) using the package BMDP. For each variable, analysis was initially undertaken on the baseline value obtained before the ingestion of the test meal to detect any difference between the 6 h (control) and 48 h fasted states. For each subject, mean baseline values were then subtracted from the subsequent values to calculate the magnitude of various responses to the test meal. These responses were further analysed by ANOVA. Where the \(F\) test in ANOVA indicated a significant treatment–time interaction, the nature of this difference was further tested by a \(t\) test on the difference between group means at individual time-points. In the absence of treatment–time interaction, treatment or time effects alone were ascertained. Values are presented as means. The values reported in the text for responses to the test meal are the maximum changes from mean baseline values; the values in the figures are the absolute values of the mean and the standard error of the mean of the responses at each time-point.

RESULTS

Haemodynamic responses (Fig. 1)
The 48 h fast had no significant effect on baseline heart rate or systolic blood pressure, but there was a significant reduction in diastolic blood pressure, from a control value of 72 to 64.5 mmHg (treatment effect \(f1/f2 = 1/7, F = 14.62, P < 0.01\); ANOVA). Baseline forearm blood flow was significantly increased by fasting (94 ml/l per min), above that seen in the control state (53) (treatment effect \(f1/f2 = 1/7, F = 12.35, P < 0.013\); ANOVA), although there was marked inter-individual variation in the magnitude of this effect.

Following ingestion of the test meal there was a greater increase in heart rate in the fasted state (+14.9 beats/min) than in the fed state (+12.1), (treatment time interaction \(f1/f2 = 1/28, F = 2.67, P < 0.05\); ANOVA). In the control state there was a drop in diastolic blood pressure (−6.9 mmHg), but no significant change was seen in the fasted state in diastolic blood pressure (treatment effect \(f1/f2 = 1/5, F = 4.37, P < 0.02\); ANOVA). Forearm blood flow increased in the control state (+22 ml/l per min), but did not rise any further in the fasted state (treatment effect \(f1/f2 = 1/5, F = 11.38, P < 0.01\); ANOVA).

Skin temperature
Fasting had no significant effect on baseline skin temperature at any of the five sites measured. After food, there was a significant rise in skin temperature of 1–2° at three sites: mid-abdomen (time effect \(f1/f2 = 3/18, F = 49, P < 0.02\); ANOVA), mid-thigh (time effect \(f1/f2 = 3/18, F = 5.2, P < 0.01\); ANOVA) and dorsum of foot (time effect \(f1/f2 = 3/18 F = 33, P < 0.05\); ANOVA), but no significant difference was seen in the fasted state in diastolic blood pressure (treatment effect \(f1/f2 = 1/5, F = 3.3, P < 0.05\); ANOVA) and dorsum of foot (time effect \(f1/f2 = 3/18 F = 33, P < 0.05\); ANOVA), but no significant difference in the response between the two states. No significant increase in skin temperature in either state after food was seen at mid-calf or mid-shin measurement sites.

Blood glucose, BOHB, glycerol, lactate and plasma insulin concentration (Figs. 2 and 3)
Baseline blood glucose concentration was significantly lower after the 48 h fast (3.4) than in the control state (4.4 mmol/l) (treatment effect \(f1/f2 = 1/7, F = 29.42, P < 0.01\);
Fig. 1. Mean heart rate, systolic and diastolic blood pressure (BP), and forearm blood flow in the control (□) and fasted (●) states before and after food ingestion. Period of food ingestion indicated by the box. Values are means with their standard errors represented by vertical bars. Baseline fasting diastolic BP was lower ($P < 0.01$) and forearm blood flow greater ($P < 0.01$) in the fasted state. After food ingestion, the heart-rate increase was greater in the fasted state ($P < 0.05$). A rise in forearm blood flow and a fall in diastolic BP was seen in the control state only ($P < 0.01$; ANOVA). * $P < 0.05$, ** $P < 0.01$.

ANOVA), as was plasma insulin (fasting 1-9, control 9.5 mU/l) (treatment effect $f1/f2 = 1/7$, $F = 11.64$, $P < 0.01$; ANOVA). After the test meal there was a rise in arterialized blood glucose ($+20$ mmol/l) in the control state, but a significantly higher rise was seen in the fasted state ($+3.5$), (treatment–time interaction $f1/f2 = 4/28$, $F = 3.19$, $P < 0.02$; ANOVA), although the absolute values obtained were not significantly different. There was a greater rise in plasma insulin after food ingestion in the fasted state ($+45.4$ mU/l) than in the fed state ($+37.3$ mU/l) (treatment–time interaction $f1/f2 = 4/28$, $F = 4.45$, $P < 0.02$; ANOVA). Although the maximum absolute concentrations of insulin were not
In the fasted state the baseline forearm glucose uptake was 1.5 μmol/min per l, whereas the control value was 13.7 (treatment effect $f_1/f_2 = 2/7$, $F = 9.05$, $P < 0.025$; ANOVA).
Fig. 3. Mean blood $\beta$-hydroxybutyrate (BOHB), glycerol and lactate concentrations in the control (□) and fasted (●) states before and after food ingestion. Period of food ingestion indicated by the box. Values are means with their standard errors represented by vertical bars. Fasting increased BOHB concentration ($P < 0.01$). After food ingestion there was a greater fall in BOHB in the fasted state ($P < 0.01$; ANOVA), and a smaller rise in lactate concentration ($P < 0.01$). ** $P < 0.01$.

There was no difference in baseline forearm $O_2$ consumption (control 2.5, fasting 2.6 ml $O_2$/min per l). After food ingestion the increase in forearm glucose uptake was +21.2 $\mu$mol/min per l in the control state and 37.0 $\mu$mol/min per l in the fasted state (time effect $f^2/f = 4.28$, $F = 3.3$, $P < 0.03$; ANOVA). There was no significant increase in forearm $O_2$ consumption after food ingestion.

**Plasma adrenaline and noradrenaline concentrations** (Fig. 5)

Fasting had no effect on the arterialized venous plasma adrenaline concentration (control 0.30, fasting 0.29 nmol/l) but reduced the mean baseline concentrations of noradrenaline
Fig. 4. Mean forearm glucose and oxygen extraction in the control (□) and fasted (●) states before and after food ingestion. Period of food ingestion indicated by the box. Values are means with their standard errors represented by vertical bars. Forearm glucose extraction was reduced in the fasted state (P < 0.05), and rose after food in both states (P < 0.05; ANOVA). ** P < 0.01.

MR and RER (Fig. 6)

The baseline MR was 3.82 kJ/min in the control state and 4.06 kJ/min after the 48 h fast. The RER was reduced in the fasted state from 0.80 to 0.74 (treatment effect f1/f2 = 1/5, F = 7.44, P < 0.05; ANOVA). After food ingestion, there was a significantly different pattern of metabolic rate response between the two states, but with similar maximum increases (treatment–time interaction f1/f2 = 2/12, F = 2.38, P < 0.04; ANOVA). In the first 40 min of measurement after food ingestion, MR rose by 0.48 kJ/min in the control state and by 0.39 kJ/min in the fasted state. In the subsequent 50 min, the mean MR increase over baseline was +0.54 (SE 0.05) kJ/min in the control state, but only +0.27 (SE 0.12) kJ/min in the fasted state (Student’s paired t test, P < 0.01). The RER rose by 0.11...
in the control state, and by 0.05 in the fasted state (treatment effect $f_1/f_2 = 1/5$, $F = 3.98$, $P < 0.05$).

**DISCUSSION**

The present study demonstrates considerable metabolic and physiological adaptation to a short period of fasting, most of which has been previously reported. The major effects are on the cardiovascular system and on circulating metabolites. Diastolic blood pressure was reduced and limb blood flow increased after fasting, with no significant change in heart rate. Similar alterations in blood pressure and blood flow have been seen after a 48 h fast in healthy men (Bennett *et al.* 1984; Macdonald *et al.* 1984), but those studies did observe an increase in heart rate. The low diastolic blood pressure seen after a 48 h fast is likely to be due to limb vasodilation, rather than changes in blood volume (Macdonald *et al.* 1984) as subjects drank water *ad lib.* and had electrolyte supplements to avoid hypovolaemia. Skin temperatures were unchanged by fasting at all sites measured. There was no difference between baseline temperature recorded on sites overlying muscular calf or bony shin. Thus it would seem likely that vasodilation occurs mainly in the muscle rather than cutaneous
Fig. 6. Mean metabolic rate and respiratory exchange ratio (RER) in the control (□) and fasted (●) states before and after food ingestion. Period of food ingestion indicated by the box. Values are means with their standard errors represented by vertical bars. Fasting reduced RER \( (P < 0.05; \text{ANOVA}) \). After food ingestion, metabolic rate in the fasted state during the last 50 min was less than in the control \( (P < 0.01; \text{Student's paired } t \text{ test}) \). The rise in RER was greater in the control than in the fasted state \( (P < 0.05; \text{ANOVA}) \). * \( P < 0.05 \).

vasculature. Increased forearm blood flow would require either increased cardiac output— with an increased stroke volume, given that the heart rate was unchanged—or compensatory visceral or renal vasoconstriction.

In our previously reported study (Mansell & Macdonald, 1988), no changes were seen in the haemodynamic state or in plasma catecholamines after underfeeding for 7 d in normal subjects. Changes in heart rate, systolic and diastolic blood pressures and a fall in 24 h urinary catecholamine metabolite excretion have been previously reported in association with reduced energy intake in normotensive, obese subjects (Jung et al. 1979). In the present study we demonstrated no change in plasma adrenaline, but found reduced levels of noradrenaline after the 48 h fast. Thus a rise in plasma adrenaline cannot explain the increased forearm blood flow seen in the fasted state. In so far as ‘arterialized’ venous samples represent whole-body ‘spillover’ of noradrenaline released from sympathetic nerve terminals, they give an indication of sympathetic nervous system activity. A reduction in sympathetic nervous system activity after underfeeding has been reported previously (Jung et al. 1979; Schwartz et al. 1987), and the present study is consistent with this finding. Thus
it is possible that the increase in forearm blood flow is the result of a fall in muscle sympathetic vasoconstrictor activity.

The period of fasting significantly reduced blood glucose and plasma insulin concentrations, consistent with previous observations (Cahill et al. 1966). Forearm glucose uptake was reduced, as reported previously (Owen & Reichard, 1971). Forearm glucose uptake is largely insulin-dependent in the resting state, and the reduced glucose uptake in the fasted state probably reflects reduced plasma insulin. No change in forearm $O_2$ consumption was seen, which is consistent with studies by Owen & Reichard (1971), in which forearm $O_2$ consumption remained constant after both 3 and 24 d fasts.

The present study shows no significant change in resting MR after a 48 h fast, although there was a trend towards increased MR after the 48 h fast. This is consistent with our previously reported studies, which have observed a rise in resting MR after a 48 h fast in healthy men (Macdonald et al. 1984; Macdonald & Mansell, 1988). By comparison, there is a trend towards reduction in MR after 7 d undernutrition in healthy women (Mansell & Macdonald, 1988).

After the test meal, heart rate rose in both states, but the rise was significantly higher in the fasted state. Food ingestion was accompanied by a reduction in the diastolic blood pressure in the control state, but not in the fasted state. The greater rise in heart rate seen in the fasted state may be due to an augmented sympatho-adrenal response to food, although no significant difference was found in the plasma catecholamine response to food. In addition, we have shown that the heart-rate response to infused adrenaline is increased after a 48 h fast (Macdonald & Mansell, 1988). Thus, the increased heart-rate response seen in the present study may be due to an enhanced sympatho-adrenal effect after fasting. Another possible mechanism is of a more marked post-prandial suppression of the parasympathetic nervous system in the fasted state, although we have no evidence to support this. After food ingestion there was an increase in forearm blood flow in the control state, but no further increase in the fasted state. This may be because the forearm was already receiving a higher blood flow than seen post-prandially in the control state. However, the forearm is not fully vasodilated at this time, as it is possible to increase the blood flow further with an adrenaline infusion following a 48 h fast (Macdonald & Mansell, 1988).

Skin temperature rose after food at three of the sites measured, the response being similar in both states. At two sites, mid-calf and mid-shin, no increase in temperature was found. Skin temperature remained elevated by a similar amount during the final 50 min of measurement after the test meal in the fasted and control states, although there were different patterns of response in MR in the two states. We cannot comment on the implications of this for thermoregulation as we did not measure core temperature in the present study, but in a previous study diminished thermoregulation during cold-exposure after a 48 h fast was seen (Macdonald et al. 1984).

After the test meal, blood glucose and plasma insulin concentration showed a greater increase in the fasted state than in the fed state. This observation has been previously reported; glucose tolerance was reduced in healthy men following a 7 d fast (Cahill et al. 1966). The trend toward reduced forearm glucose uptake in the fasted state in the presence of elevated plasma insulin levels suggests that the glucose intolerance of starvation is due at least partly to peripheral resistance to the effect of insulin, and confirms the previous observations in underfeeding (De Fronzo et al. 1978; Bjorkman & Eriksson, 1985; Mansell, 1988). The insulin resistance appears to be specific to glucose metabolism, as the rate of decline in the concentration of plasma free fatty acid during insulin infusion is unchanged by fasting (Newman & Brodows, 1983). The mechanism of reduced insulin sensitivity in fasting may be through inhibition of insulin-induced glucose uptake by circulating ketones.
and fatty acids (Randle et al. 1963). Similar impairment of whole-body glucose tolerance (Gomez et al. 1972) and forearm glucose uptake can be induced in the non-fasted state by infusion of lipid (Rett et al. 1986). Studies with combined glucose and insulin infusion after 48 h fasting show that the observed reduced rate of whole-body glucose disposal is due to diminished glucose oxidation, the rate of glucose storage being unchanged (Mansell, 1988).

The ingestion of the test meal was followed by a small rise in adrenaline concentration in both states, but no significant difference in the responses between the two states was seen. A rise in plasma noradrenaline after glucose ingestion has been shown in healthy men, but in that study no rise in plasma adrenaline was seen (Welle et al. 1980). The stimulus to the release of noradrenaline by a mixed meal is carbohydrate, no response being seen after the ingestion of protein or fat (Welle et al. 1981). In so far as the changes in plasma noradrenaline and adrenaline concentrations are due to sympatho-adrenal activity, the present study indicates that this period of fasting does not affect the sympatho-adrenal response to food intake. However, we have no specific evidence of the physiological role of such a response.

The initial rise in MR after food ingestion was comparable in both states. This thermic response is likely to be due to the ‘obligatory’ energy cost incurred during nutrient assimilation and storage. In the fasted state there may be a component from the concurrent metabolism of both circulating ketone bodies and ingested nutrients. The energy cost of nutrient storage is constant, hence the similar initial increases in MR in both states. By contrast, in the next 50 min there was a further small increase in MR in the fed state, but no additional rise in MR in the fasted state. The diminished MR seen in the second 50 min may indicate a reduction in the ‘facultative’ thermic response to food. If the sympatho-adrenal component of the thermic response to food ingestion was increased after fasting, as was heart rate, it would have been expected that there would be an enhanced thermic response to food after fasting. The absence of such an effect would argue against a major role for the sympatho-adrenal system in mediating the thermic response to food in the first 90 min after food ingestion. However, we must be careful in our interpretation of the MR results, as the differences observed are small and we did not study the complete response. Astrup et al. (1987) have reported that there is a late secondary thermic response seen 3–4 h after glucose ingestion.

Thus the principal findings of the present study are that a 48 h fast reduces plasma insulin and glucose with a reduction in forearm glucose uptake. There is increased forearm blood flow with reduced diastolic blood pressure. A small reduction in plasma noradrenaline was seen, but no change in MR, forearm \( \bar{O}_s \) consumption, heart rate or systolic blood pressure was found. After a 48 h fast, ingestion of the test meal was associated with an increased insulin response, with insulin resistance and glucose intolerance. The heart-rate response was increased, but there were diminished forearm blood flow and diastolic blood pressure responses, and a reduction in the later thermic response to food ingestion. The catecholamine responses were unchanged. Thus a 48 h fast is associated with considerable physiological and metabolic adaptation and with alteration in some of the responses to food ingestion.

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