Brown adipose tissue in humans

By M. E. J. LEAN, *Diabetic Clinic, Aberdeen Royal Infirmary, Woolmanhill Hospital, Aberdeen AB9 1GS*

Introduction

Recent scientific and public excitement about brown adipose tissue (BAT) in humans stems from initially speculative extrapolation from animal experiments described in foregoing papers. Recognition that variations in the activity of the tissue can determine the overall efficiency of energy utilization, strengthened by the demonstration of thermogenic responses to overfeeding, has directed interest towards the possibility that BAT could be of importance in energy balance and the aetiology of obesity in humans, and perhaps more importantly, as a novel route for treatment (Himms-Hagen, 1979; James, 1983).

These suggestions require that BAT should be present and functional in humans, capable not only of regulated heat production to maintain body temperature, but also of physiological stimulation to remove unnecessary energy ingested as food. If BAT is potentially active in adults, then energy wastage might be inducible by therapeutic intervention for the treatment of obesity. Recent work has explored some of the grounds for recent speculation about human BAT thermogenesis, to strengthen the analogies with animal models.

Anatomy: structural indications of function

BAT was originally identified on the grounds of its colour, a result of its high vascularity and cytochrome content reflecting high mitochondrial density, and its texture and microscopic appearance. Its appearances in the human neonate are similar to those in other species, cells characteristically containing multivesicular fat droplets and round nuclei, and it was first identified as analogous to the 'hibernating gland' of animals in the cervical region of embryos by Hatai (1902). The widespread presence of histologically detectable BAT in human infants, and in adults at a variety of locations in which it occurs in infancy, is now well established (Aherne & Hull, 1966; Heaton, 1972; Hassi, 1977). In common with other primates, the largest sites of BAT are the axillary–deep cervical and perirenal adipose tissues. The interscapular site is quantitatively unimportant in human infants, although readily detectable as a thin kite-shaped structure in most neonates. In the adult human there are essentially no histologically identifiable, typical, brown adipocytes in the interscapular region. The literature is consistent on this point, and our own findings from dissections in Cambridge, UK, concur with those of Astrup *et al.* (1984) that the interscapular site cannot be quantitatively important for thermogenesis in adult humans.

Human superficial or subcutaneous adipose tissue shows typical white adipose histology at all ages, whereas virtually all internal sites of adipose tissue contain typical multilocular brown adipocytes in infancy. In adults these internal sites contain at least isolated cells or islands of typical BAT adjacent to blood vessels (Heaton, 1972; Hassi, 1977). Under the electron microscope, the BAT cytoplasm is seen to be densely packed with active mitochondria (Plates 1 and 2). As in many other larger species, notably the pig (Holloway *et al.* 1985), the typical BAT histology becomes modified with age. Lipid accumulates so that the proportion of cells showing multivesicular lipid falls, and ultimately the tissue may become indistinguishable from subcutaneous (white) adipose

tissue. Aherne & Hull (1966) conducted large post-mortem surveys of infant BAT and concluded that the histology reflected functional capacity. Lipid accumulation, with increased tissue bulk and unilocular histology indicates reduced demands for thermogenesis. By comparison with histological appearances in experimental animals whose BAT thermogenic activity is known, Hull (1977) was able to conclude that the approximately 30 g BAT in the human infant (about 1% of body-weight) is sufficient to account for total thermogenic capability in response to cold or noradrenaline, equivalent to a doubling of metabolic rate (Bruck, 1961).

The sites of BAT in humans are all central and internal, in a distribution likened to a 'high-collared vest', arranged such that heat generated warms the blood supply to vital organs (Lean & James, 1986). Thus, the cervical and axillary sites can warm the blood supply of the head, and the perirenal fat protects the kidneys. This concept is strengthened by the demonstration of direct vascular links between the perirenal BAT and the kidneys (Merklin, 1974a) the supra-iliac BAT and the lumbar and azygos veins (Merklin, 1974b), and between the interscapular site and the spinal cord (Aherne & Hull, 1966). Merklin (1971) also demonstrated a vascular link between the anterior abdominal BAT and the liver, and the pericardial site is well placed to provide direct heating to the myocardium. These observations do not prove any function, and would be consistent with a view that BAT is of phylogenetic interest only.

There are, however, certain conditions under which the sites of BAT show histological changes to more 'active' histological appearances, indicative of a functional thermogenic role. Heim et al. (1968) observed lower BAT lipid content in infants kept at 22–27° than in others kept at 34–35°. Aherne & Hull (1966) and Huttunen et al. (1981) have produced evidence to suggest that exposure to cold environments may lead to reduction in lipid content and reversion to typical BAT histology and histochemistry. More convincing evidence for functional capability is that the noradrenaline-secreting tumour phaeochromocytoma is associated with more abundant 'brown'-type adipocytes in the perirenal fat (Melicow, 1957). This is a situation where metabolic rate is known to be elevated (Mager & Gifford, 1977), and where we now know that BAT is thermogenically activated both in animal models (Ricquier et al. 1983) and in adult humans (Lean et al. 1986a). In hypothyroidism, the lipid content of BAT is increased (Curling, 1850; Shattock, 1909), suggesting a reduced thermogenic activity which seems appropriate on clinical grounds.

Hibernoma, a tumour of BAT, and phaeochromocytoma

Tumours of BAT are rare, and usually mis-diagnosed as lipomata (relatively common and uninteresting benign tumours of white adipose tissue) until after surgical removal. They occur at sites of the BAT of infancy, and appear to be benign masses with typical BAT histology, hence the intriguing name hibernoma. The question of their metabolic or thermogenic function has not been addressed prospectively. Such benign tumours in other tissues are very often non-functional, but at least one case report (Allegra et al. 1983) describes very significant weight loss of 7 kg in a young man during a year before the removal of a single large and uncomplicated hibernoma. It had a high cytochrome c oxidase (EC 1.9.3.1) content and active-looking mitochondrial christae on electron microscopy, and the amount of BAT in the tumour (80 × 110 × 40 mm) would be sufficient to account for that weight loss (about 210 MJ (50 000 kcal)) by an increase in basal metabolism of about 5% (about 580 kJ (140 kcal)/24 h) if the thermogenic activity of BAT from a room-temperature mouse is assumed. Other factors may have played a part in that case, and in practice it would be difficult to detect an increase in basal

metabolism of this size, a fundamental problem in any attempt to quantify the role of human BAT.

Hibernomata are also described in patients with extreme sympathetic stimulation from a phaeochromocytoma. In that situation the 'tumours' must result from prolonged trophic stimulation of the tissue by catecholamines, and are almost certainly thermogenically active (Ricquier, 1988), contributing to the elevated metabolic rate and weight loss which are characteristic of phaeochromocytoma (Mager & Gifford, 1977). They may be found in the periadrenal adipose tissue adjacent to the phaeochromocytoma, but can occur elsewhere, so phaeochromocytoma should be excluded in any patient with a hibernoma, particularly if there has been weight loss.

Prominent typical BAT histology in periadrenal adipose tissue removed at surgery for phaeochromocytoma was first reported by Melicow (1957), in contrast to the 'adult fat' at that site in cases of Cushing's disease, adrenogenital syndrome, sympathicoblastoma and renal carcinoma. Similar reports have followed and the association seems established, although Medeiros *et al.* (1985) argue that normal subjects may have similar histological appearances of BAT at this site, and that firmer criteria are required for a scientific conclusion to be drawn. The work of Ricquier *et al.* (1982), and our own work (Lean *et al.* 1986*a*), have demonstrated that the periadrenal adipose tissue from patients with phaeochromocytoma possesses the ultrastructural and biochemical features of BAT. We have shown the content of mitochondrial uncoupling protein, unique to BAT and the best marker of thermogenic capacity, to be very significantly increased in the presence of phaeochromocytoma compared with cases of sudden death, and also that its concentration is elevated in sites of BAT distant from the tumour (see p. 248; Lean *et al.* 1986*a*).

Temperature sensing

Astrup *et al.* (1984, 1985) have stressed that if human BAT is indeed thermogenic then it ought to be possible to detect its temperature as higher than that of other, non-thermogenic, tissues. A number of workers have reported measurements of skin temperature using thermocouples or infrared thermography over regions where BAT was believed to exist (Rothwell & Stock, 1979; Contaldo *et al.* 1981; Lev-Bari *et al.* 1982; Leibel *et al.* 1987). Most of these have concentrated on the nape of the neck, where skin temperature tends to be higher in infants (Silverman *et al.* 1964) and in adults (Rothwell & Stock, 1979). Sadly, it seems likely that any differences in skin temperature in fact represent alterations in non-specific heat loss from changing cutaneous blood circulation, dependent on the extent to which the experimental conditions provide insulation to other sites of regulatory heat loss. The interscapular region does not in any case contain detectable BAT in human adults, as discussed previously.

More direct attempts to measure heat production have involved placing thermocouples deeply in the intercostal (James & Trayhurn, 1981) or perirenal adipose tissues (Astrup *et al.* 1985). Both these experiments did show an elevation in temperature, by reference to skeletal muscle or rectal temperature, in response to thermogenic treatment with noradrenaline or ephedrine. There is a general problem with the interpretation of temperature gradients because of alterations in local blood flow and countercurrent effects at both the test site and the reference site. When BAT is stimulated for heat production, the same sympathetic mechanisms tend to increase its blood supply, so the actual rise in tissue temperature is minimized, and the diffuse nature of the tissue makes accurate assessment of blood flow impossible, other than on very local basis. The assumption that a skeletal muscle temperature is a stable reference point may be incorrect, let alone that of the fermenting large bowel, in view of redistributions of blood flow.

Biochemistry of human BAT

Hassi (1977) used a variety of enzyme histochemical methods on human post-mortem material to make an assessment of thermogenic activity. Brown adipocytes had high contents of the enzymes necessary for fatty acid synthesis, lipolysis, β-oxidation of fatty acids, the citric acid cycle and cytochrome c oxidase, which indicated a high oxidative capacity with lipid as an important substrate. The content of monoamine oxidase (EC 1.4.3.4) was high, presumably reflecting high noradrenaline turnover. Interestingly, the content of lactate dehydrogenase was also high, suggesting considerable potential for carbohydrate oxidation. The enzyme activities were all greater in brown than in white adipocytes and, on a cellular basis, tended not to fall with increasing age.

Hassi (1977) was unable to find previous publications on the biochemistry of human BAT, although there is an extensive literature relating to the biochemistry of BAT in a variety of experimental animals, which has recently been reviewed comprehensively (Nicholls & Locke, 1984; Nicholls *et al.* 1986). It may be relevant, however, to consider also the literature that has examined human adipose tissue at sites that are those of BAT in infancy. Hamosh *et al.* (1963) compared the biochemistry of omental and subcutaneous adipose tissues and found enormously greater metabolic activity in the omental fat. The rate of in vivo fatty acid synthesis was up twenty- to seventyfold higher in the omental adipose tissue. The omental adipose tissue is also more active than subcutaneous adipose tissue in glucose metabolism by a factor of about five (Fessler & Beck, 1965; McLean *et al.* 1968). Ostman *et al.* (1979) found threefold higher rates of lipolysis in omental than subcutaneous fat, and markedly greater responsiveness to noradrenaline. Chakrabarty *et al.* (1981) found high glycerol kinase (EC 2.7.1.30) activity in human BAT and increased lipogenesis in perirenal, as compared with subcutaneous, adipose tissue in children and some adults (Chakrabarty *et al.* 1985). BAT is very active in lipolysis and lipogenesis in many species, the rate of lipogenesis correlating roughly with thermogenic activity (McCormack & Denton, 1977), so this literature provides some evidence to suggest continued BAT function in the intra-abdominal adipose tissue of adult humans. Further support for this suggestion comes from the data of Nauman *et al.* (1988) showing much higher activity of type II thyroxine 5-deiodinase (EC 3.8.1.4) in human omental, compared with subcutaneous, adipose tissue. Their study also indicated, intriguingly, reduced activity in the omental adipocytes of obese subjects expressed per unit microsomal protein.

Cunningham *et al.* (1985) presented data from human perirenal adipose tissue obtained from a variety of subjects at post mortem or at surgery. The cytochrome c oxidase content was related to the in vitro rates of oxygen uptake of portions of tissue, and then by a series of extrapolations it was suggested that BAT is of minimal importance in humans. Unfortunately, this conclusion depends on in vitro estimations of tissue functions which tend to underestimate maximal in vivo rates by five- to tenfold (Hoffenberg, 1972). This is likely to be a particular problem in human BAT, whose function in vivo is highly dependent on blood flow. It also makes the assumption, which now seems incorrect, that the perirenal adipose tissue is representative of, and indeed contains, most of the brown adipose tissue present in humans.

Uncoupling protein in human BAT

In order to estimate the thermogenic capacity of human adipose tissue we have established a sensitive solid-phase radioimmunoassay, employing antisera generated against uncoupling protein purified from the mitochondria of human infant BAT (Lean & James, 1983), to measure the uncoupling protein content of mitochondria (Lean *et al.*

1986*b*). Samples were obtained at routine autopsy from forty-eight subjects of all ages, all of whom had died suddenly in Cambridge, UK, during the winter months. Uncoupling protein could not be detected in the mitochondria isolated from subcutaneous white adipose tissue, heart or liver. The concentration was significantly higher in axillary than perirenal tissue, and compared with infants there was significantly less in both adults and premature babies. The uncoupling protein contents of these human samples were quantitatively similar to those measured in young and adult experimental animals under normal environmental conditions (Fig. 1). Uncoupling protein content, indicating thermogenic capacity, of infant BAT in different sites correlated closely with tissue cytochrome *c* oxidase activity as an index of mitochondrial mass (r 0·97, $P<0·001$), and also with mitochondrial GDP binding as an index of thermogenic activity (r 0·85, $P<0·02$).

The different contents of uncoupling protein in the groups studied would be consistent with known physiological changes in thermogenic capacity and requirement, and support the view that BAT is an organ capable of regulated thermogenesis in humans. Low concentrations in premature infants would be in keeping with their reduced thermogenic responses to cooling (Bruck, 1961) and might also be expected in still-born infants, since synthesis of uncoupling protein will not have been stimulated by exposure to cold temperatures (Stirling & Ashwell, 1988). The highest concentrations were found in older infants and children, who may have greater exposure to cold temperatures with relatively less clothing than is customary amongst young infants. There was no correlation between uncoupling protein content and age or body mass index (weight/height^2, BMI) as an index of fatness.
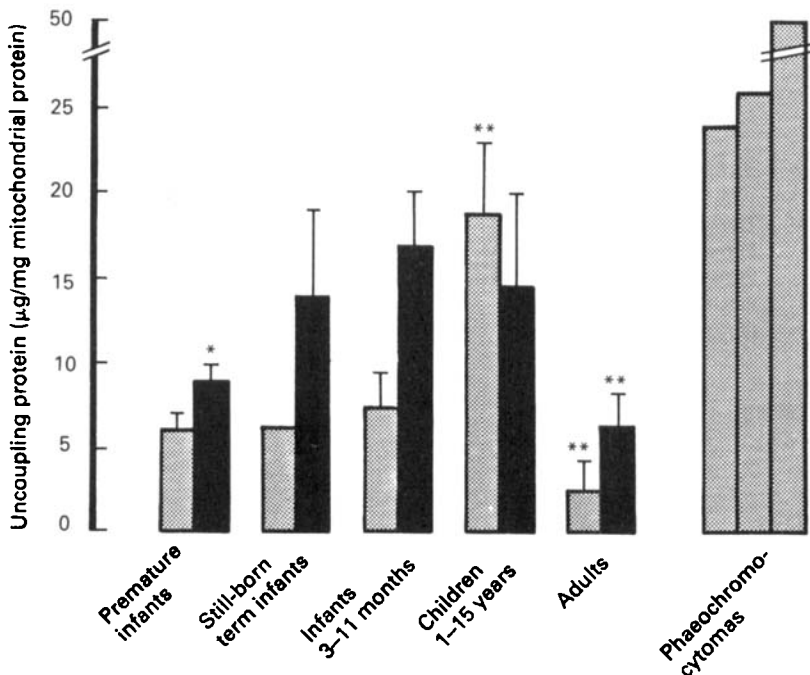

Fig. 1. Uncoupling protein content of perirenal (▨) and axillary (■) adipose tissue of humans of different age-groups, and of perirenal adipose tissue of three cases of phaeochromocytoma. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those of infants (3–11 months): *$P<0·05$, **$P<0·01$.

Intra-abdominal adipose tissue was also studied in three patients with phaeochromocytoma (Lean *et al.* 1986a). Isolated mitochondria showed a significant degree of uncoupled respiration, characteristic of BAT, which was inhibited by GDP (Table 1). The uncoupling protein contents of the four samples were significantly greater ($P < 0.02$) than those found in the sudden-death adults. Excess noradrenaline secretion thus has a general trophic effect on stimulating thermogenic capacity in adipose tissue of BAT origin, including the omental and perirenal sites, even when the tumour is situated elsewhere.

Bouillaud *et al.* (1988) have gone on to apply molecular-biology techniques in demonstrating the mRNA for human uncoupling protein in perirenal BAT in four cases of phaeochromocytoma and from a hibernoma. Its structure indicates a close homology with rat uncoupling protein mRNA, which strengthens our earlier findings of similarity of antigenicity and GDP-binding characteristics in concluding that human uncoupling protein has the same function as that of the rat. Measurement of the uncoupling protein, as a structural part of the BAT mitochondrion, is probably still the best indicator of thermogenic capacity, but it is possible that the more specific estimation of mRNA by Northern blot and c-DNA probing will be of value in identifying BAT in unstimulated human adipose tissue where the uncoupling protein content may be below the limit of

Table 1. *Biochemical findings in brown adipose tissue (BAT) from three cases of phaeochromocytoma and from two cases of cot-death*

			Cytochrome c oxidase (EC 1.9.3.1) activity (μmol/min per g tissue)	Uncoupling protein (μg/mg mitochondrial protein)	GDP-binding activity (pmol/mg mitochondrial protein)	GDP effect on oxygen uptake (% change)
Phaeochromocytomas						
Sex	Age (years)	Tissue				
♀	46	Perirenal	—	24	157	—
♂	22	Perirenal	9·7	50	43	−37·9
♀	21	Perirenal	6·8	26	616	−21·2
		Omental	4·5	22	103	−33·8
Cot-deaths						
Baby A: 9 months						
Axillary			246	22	147	—
Cervical			202	23	149	—
Perirenal			94	11	133	—
Interscapular			18	7	91	—
Baby M: 5 months						
Axillary			66	10	129	−23
Perirenal			23	9	104	−9
Mice, 3 months*						
33°			40	9	69	—
22°			178	43	200	—

*Means results obtained using the same methods on mitochondria from the interscapular BAT (the principal site) of mice housed at 33° (warm) and 22° (cool) (Ashwell *et al.* 1983).

detection by immunoassay (Lean *et al.* 1986*b*). Subcutaneous (white) adipose tissue does not contain the mRNA for uncoupling protein.

Pharmacological assessment of human BAT

Physiological regulation of BAT thermogenesis involves sympathoadrenal and probably thyroid function peripherally, and a complex system of neuroendocrine mechanisms centrally (Trayhurn & Nicholls, 1986). Several drugs have been used to manipulate its function experimentally, some with rather specific actions on BAT. These provide tools to assess function in humans, and may have applications for the management of obesity and type II diabetes.

Noradrenaline itself (Jung *et al.* 1979), and its orally available isopropyl-analogue (Contaldo *et al.* 1981), certainly stimulate thermogenesis. The less problematic sympathomimetic, ephedrine, has direct effects on animal BAT, and produces thermogenesis of 15–25% above resting metabolism (Astrup *et al.* 1985). Our 6-week whole-body calorimetry study of the noradrenaline-reuptake blocker, ciclazindol, was undertaken because this drug is concentrated in BAT, and it stimulates BAT in animals. It had a significant and prolonged effect on energy expenditure, equivalent to increasing metabolic rate by about 5%, in young men (Dallosso *et al.* 1984; Lean, 1986). It has been difficult in these studies to attribute thermogenic effects in man to BAT because of the possible contributions of mixed α- and β-adrenergic stimulation in a variety of tissues, but Astrup *et al.* (1985) have combined measurements of temperature gradients and ^{133}Xe-clearance, to assess local blood flow, to estimate that the perirenal adipose tissue of young men is generally of low thermogenic importance, although it produced in one case up to 25% of the total thermic effects from oral ephedrine. This is remarkable from a tissue which comprises only 10 g/kg body-weight, considering that the entire muscle bulk at 500 g/kg body-weight provided only 50% of thermic effects. This study, despite its relatively imprecise methodology and small numbers, is important as the only quantitative information about BAT thermogenesis in humans.

Whether a thermogenic function is specific to BAT, or whether it applies to all adipose tissue but more markedly in tissue of BAT origin, is currently an interesting physiological problem. It is hoped that new scanning techniques may provide the answers. For the purposes of obesity management it is more important that stimulant effects spare the cardiovascular and central nervous systems. The new class of lipolytic β-agonists, discussed previously (Cawthorne, 1989), probably acts on all adipose tissues. It has not yet been shown to what degree the thermogenic effects in humans are attributable to the greater lipolytic activity of the deep adipose tissue in former sites of BAT.

BAT and cot-death

Cot-death is probably a heterogeneous diagnosis, with a number of different mechanisms operating in different cases. Overheating is a well-recognized problem amongst small infants, particularly if over-clothed, and can lead to death, for example, in infants left in cars in direct sunlight. Stanton (1984) has implicated hyperpyrexia from defects in thermoregulatory control in some cot-deaths, but without considering the possibility of endogenous heat production. We have had the opportunity of studying two cases of typical cot-death where no other pathology was found, but in both cases the core temperature on arrival at hospital was >40° (Lean & Jennings 1989). The biochemical findings from samples of BAT are shown in Table 1. There was clear evidence of active uncoupled mitochondrial respiration which could be recoupled by GDP, with an activity similar to that of mice acclimated to warm-room conditions. If the rate of O_2 uptake is assumed to be that of 23°-acclimated mice under maximal stimulation (1·5 ml O_2/min per

g tissue, or 35–40 kJ (8–10 kcal)/min per g tissue), then the heat production from 30 g BAT in an infant (Hull, 1977) would be sufficient to raise core temperature by 5–6°/h. Even a considerably lower rate of heat production could be dangerous if it occurred suddenly in a well-clothed and insulated infant.

In unconnected work, Naeye (1974) interpreted increased amounts of BAT in cot-death as a result of tissue hypoxia, possibly indicating pulmonary disease. Hypoxia, however, causes a suppression of O_2 utilization for thermogenesis (Oliver & Karlberg, 1962–3), since it could only continue at the expense of vital organs, so acute hypoxia is more likely to be a result than a cause of BAT activity. The suggestion of increased amounts of BAT in cot-death has not been widely supported, and our own evidence on the uncoupling protein content of BAT in cot-death indicates no difference from other infants of comparable age.

It seems possible that defects of thermoregulatory control in some cases of cot-death might include sudden inappropriate stimulation of BAT thermogenesis, without necessarily invoking any long-term trophic stimulation to produce any increase in maximum thermogenic capacity.

Altered abdominal fat distribution in diabetes, Cushing's disease and phaeochromocytoma, reflecting BAT activity?

Increasing age (Rothwell & Stock, 1983), spontaneous diabetes (Goodbody & Trayhurn, 1981), the development of insulin resistance in young 'congenitally obese' animals (Mercer & Trayhurn, 1983) and corticosteroid administration (Aronson et al. 1954; Galpin et al. 1983) all reduce the thermogenic activity and capacity of BAT in animals. At the same time, its lipid content is selectively increased (i.e. with a lesser or even opposite effect on the white adipose tissue and total body fat content). The reduced thermogenic response of BAT to cold is particularly marked in the congenitally obese (and diabetic) *ob/ob* mouse. Noradrenaline treatment (Huttunen, 1979; Mory et al. 1984) and phaeochromocytoma (Ricquier et al. 1983) in animal models have the opposite effect, i.e. thermogenic activity is increased and lipid content of BAT is reduced. We have used computed X-ray tomography (CT) to explore the hypothesis that, since the intra-abdominal fat is a site of BAT in humans, alterations in its lipid content might reflect changes in BAT function in relation to some of the pathophysiological influences (age, diabetes, Cushing's disease and phaeochromocytoma) that are known to affect BAT function in animal models (Lean et al. 1987). The normal CT images of eighty-nine subjects (forty-eight women) were selected as 'controls'.

A positive correlation was found with age, but none with BMI, and higher proportions of intra-abdominal fat were demonstrated in men than in women. Intra-abdominal fat was found to be significantly increased in women with type II diabetes and in those with Cushing's disease, and reduced in subjects with phaeochromocytoma (Fig 2). Thus in humans the lipid content of internal fat, relative to subcutaneous fat, is increased with age, type II diabetes and Cushing's disease, and decreased with phaeochromocytoma. If the subcutaneous fat is accepted to be 'white' adipose tissue and the intra-abdominal fat is considered to be the 'brown compartment' in a transverse abdominal cross-section, then this alteration in its lipid content would imply that its thermogenic capacity is decreased with age, type II diabetes and Cushing's disease, and increased with phaeochromocytoma.

Altered metabolic responses to mild cold in obesity with type II diabetes and Cushing's disease: defective BAT function?

One of the most characteristic features of the congenitally obese animal models of obesity is their failure to generate a thermogenic response to cold, part of a generalized
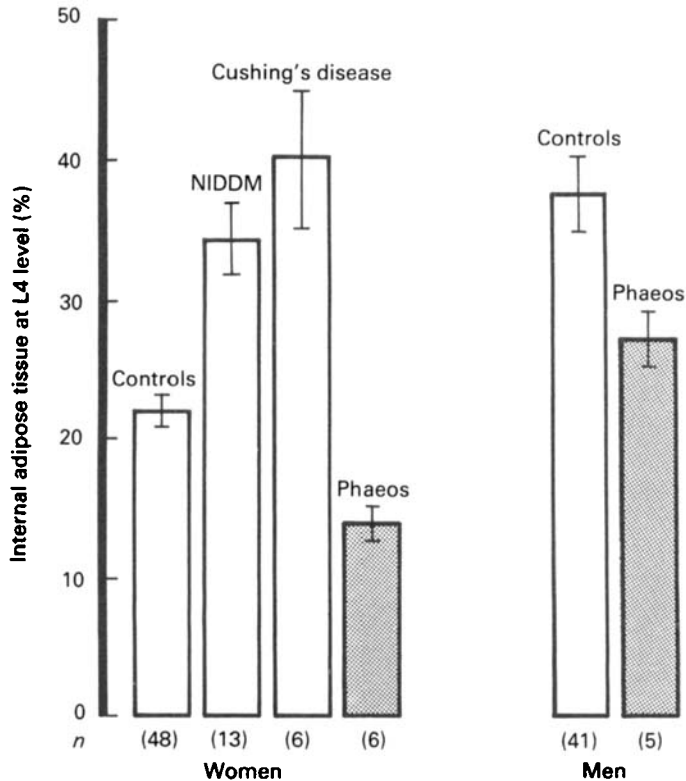
Fig. 2. Abdominal fat distribution by computed X-ray tomography at the L4 (umbilical) level, and comparison with age- and sex-matched controls. Internal fat is expressed as a percentage of the total fat pixels at this level (L4, umbilical). Wilcoxon's test was used to detect significant differences from age- and sex-matched controls, and values from the two sexes were combined for phaeochromocytoma (Phaeos). NIDDM, non-insulin-dependent diabetes. Values are means with their standard errors, no. of subjects given in parentheses.

defect of BAT regulation and related to insulin resistance. We therefore sought a similar defect in a highly selected group of obese women with high proportions of intra-abdominal fat (five with non-insulin-dependent diabetes and two with Cushing's disease) by examination of their metabolic responses to mild cold, using whole-body calorimetry (Lean *et al.* 1988*b*). Blaza & Garrow (1983) suggested that some obese women may have defective cold-induced thermogenesis, and there is also some evidence that Australian aborigines (Scholander *et al.* 1958) and Lapps (Andersen, 1963), who are prone to obesity and diabetes when 'Western' lifestyles are adopted, tend to drop their core temperature at night. This saving in energy expenditure might contribute to weight gain. Reduced diet-induced thermogenesis has been reported in obese diabetic humans (Golay *et al.* 1982), although it has never been suggested that this indicates impaired BAT activity. Reduced BAT thermogenesis has, however, been aetiologically related to the development of diabetes and obesity in animal models (Cawthorne *et al.* 1984).

Sleeping metabolic rates at 28° (thermoneutral) were closely related to body-weight (Table 2). In all six control women the sleeping energy expenditure was greater at 22° (mild cold) than at 28° ($P<0.05$), whereas the five overweight subjects with diabetes and

Table 2. *Non-shivering thermogenic responses to mild cold stimulus measured by whole-body indirect calorimetry at 22° as compared with 28°*

(Mean values with their standard errors)

Subjects	n	Age (years)	Wt (kg) Mean	SEM	BMI† (kg/m²) Mean	SEM	Sleeping energy expenditure at 28° (Watts) Mean	SEM	Differences at 22° with reference to 28° Aural temperature (°) Mean	SEM	Sleeping energy expenditure (% difference) Increase	Fall	Mean	SEM
Controls	6	31	64	4	23	1	66	3·3	-0·3	0·1	6	0	+3·8*	1·3
Diabetics	5	55	93	7	35	6	78	9·2	-0·6	0·2	0	5**	-3·5*	1·5
Simple obesity	4	41	93	4	36	1	77	4·5	-0·4	0·2	2	2	-2·0	1·0
Cushing's disease	2	37, 40	72, 82	—	27, 31	—	61, 66	—	-0·5, -0·9	—	0	2*	-4·2, -7·0	—
Adrenalectomized	2	37, 40	64, 75	—	24, 28	—	58, 66	—	-0·1, -0·7	—	1	1	+0·2, -5·2	—

Mean values were significantly different: *P<0·05, **P<0·001.
† Weight/height².

two with Cushing's disease all had lower energy expenditures at 22° than at 28° ($P<0.05$). Bilateral adrenalectomy, which restores BAT function in congenitally obese experimental animals (Holt & York, 1982), tended to normalize the responses of the two women with Cushing's disease when they were restudied 3 months after their operations, although interpretation should be cautious in view of the uncertain effect of previous pituitary surgery in these cases. The metabolic response to mild cold in these subjects was not simply reduced, but opposite and abnormal compared with controls ($P<0.001$ for diabetics, $P<0.05$ for Cushing's disease). These subjects all had high intra-abdominal fat distributions and were selected as cases most likely to exhibit insulin resistance. Four women with simple obesity, as in the study of Blaza & Garrow (1983), gave mixed responses. In the control women there were increased concentrations of thyroid stimulating hormone and of free triiodothyronine at 22° compared with 28°, and both of these measurements correlated with differences in sleeping metabolic rate. The diabetic women showed no such endocrine difference between the two temperatures (Lean *et al.* 1988*b*).

General discussion and conclusions

It is now clear that the mitochondrial uncoupling protein necessary for regulated non-shivering thermogenesis can be demonstrated in the 'brown compartment' of human adipose tissue, and it seems biochemically homologous and functionally the same as that in experimental animals. There is, therefore, at least the biochemical potential in human adipose tissue in sites of BAT for the same thermogenic function as the BAT of experimental animals. In adults the uncoupling protein content is generally low, although usually measurable in the axillary fat; however, high circulating noradrenaline from a phaeochromocytoma stimulates thermogenic capacity in adipose tissue at sites of BAT. This finding raises two issues. First, adipose tissue in these sites retains in adulthood a biochemical distinction from the white subcutaneous adipose tissue (which does not contain uncoupling protein even in infancy). There is already evidence that intra-abdominal fat is more active than subcutaneous fat in lipolysis and lipogenesis, and in its response to catecholamines: such findings might be expected in BAT which is relatively inactive and lipid-replete. Second, the mechanism clearly exists for a possible therapeutic approach for obesity treatment if thermogenesis can be stimulated specifically.

The concept of a more metabolically active 'brown' adipose tissue compartment in human adults, as distinct from the subcutaneous white adipose tissue (even if there are regional variations in function within these compartments) is similar to that which is emerging in the adult dog, whose intra-abdominal adipose tissue can be shown to regain BAT characteristics after prolonged pharmacological stimulation (Holloway *et al.* 1985). The mass of adipose tissue in the adult human that is derived from sites of BAT in infancy is large, probably more than 10 g/kg body-weight, estimated by Astrup *et al.* (1985) to produce up to 25% of the thermogenic effect of ephedrine. The abdominal fat distribution studies, coupled with the calorimetry studies, suggest indirectly that, analogous to experimental animal findings, the intra-abdominal fat of adults may still show predictable responses to certain pathophysiological stimuli. It is an interesting and tenable hypothesis, in view of the studies on ephedrine, that BAT is able to increase metabolism by the 4–7%, which would account for the calorimetric responses to mild cold. The abnormal responses in diabetes and Cushing's disease would be expected if, as in experimental animal models, the regulation of their BAT function is impaired. There is no direct evidence for any involvement of BAT in physiological weight regulation, but responsibility for 1–2% of energy balance might be a reasonable possibility. Defects of

this order, if not otherwise compensated, could lead to weight gain (without overeating, compared with others) at about 1–2 kg (2–4 lb)/year. There is some evidence from whole-body calorimetry studies that women prone to obesity may have energetic defects of this size (Lean *et al.* 1988*a*).

While the role of BAT in thermogenesis and body-weight control in adult humans is gradually becoming clearer, and it is likely that at least some important subgroups of obese subjects have defects of BAT-type thermogenesis, the possibility is also raised that BAT may be involved in wider issues. The central fat distribution and the general observation that its intra-abdominal lipid content is increased relative to subcutaneous fat in type II diabetes and Cushing's disease allows another viewpoint on the intriguing clinical relations of central, 'android' or 'apple-shaped' fat distribution with ischaemic heart disease and hypertension.

REFERENCES

Aherne, W. & Hull, D. (1966). *Journal of Pathology and Bacteriology* **91**, 223–234.
Allegra, S. R., Gmuer, C. & O'Leary, G. P. (1983). *Human Pathology* **14**, 1044–1052.
Andersen, K. L. (1983). *Federation Proceedings* **22**, 834–839.
Aronson, S. M., Teodoru, C. V., Adler, M. & Schwartzman, G. (1954). *Proceedings of the Society for Experimental Biology and Medicine* **85**, 214–218.
Ashwell, M., Jennings, G., Richard, D., Stirling, D. M. & Trayhurn, P. (1983). *FEBS Letters* **161**, 108–113.
Astrup, A., Bulow, J., Christensen, N. J. & Madsen, J. (1984). *Clinical Science* **66**, 179–186.
Astrup, A., Bulow, J., Madsen, J. & Christensen, N. J. (1985). *American Journal of Physiology* **248**, E507–E515.
Blaza, S. & Garrow, J. S. (1983). *British Journal of Nutrition* **49**, 171–180.
Bouillaud, F., Villarroya, F., Hentz, E., Raimbault, S., Cassard, A.-M. & Ricquier, D. (1988). *Clinical Science* **75**, 21–27.
Bruck, K. (1961). *Biologia Neonatorum* **3**, 65–119.
Cawthorne, M. A. (1989). *Proceedings of the Nutrition Society* **48**, 207–214.
Cawthorne, M. A., Carrol, M. J., Levy, A. L., Lister, C. A., Sennitt, M. V., Smith, S. A. & Young, P. (1984). *International Journal of Obesity* **8**, Suppl. 1, 93–102.
Chakrabarty, K., Chauduri, B. & Jeffay, H. (1981). *Journal of Lipid Research* **124**, 381–390.
Chakrabarty, K., Manaligod, J. & Jeffay, H. (1985). *Proceedings of the 13th International Congress on Biochemistry* Abstr. TH463.
Contaldo, F., Presta, E., di Biase, G., Scalfi, L., Mancini, M., Maddalena, G., di Divinitiis, O. & Rocco, P. (1981). In *The Body Weight Regulatory System: Normal and Disturbed Mechanisms*, pp. 143–146 [L. A. Cioffi, W. P. T. James and T. B. van Itallie, editors]. New York: Raven Press.
Cunningham, S., Leslie, P., Hopwood, D., Illingworth, P., Jung, R. T., Nicholls, D. G., Peden, N., Rafael, J. & Rial, E. (1985). *Clinical Science* **69**, 343–348.
Curling, T. B. (1850). *Medico-Chirurgical Transactions* **33**, 303–306.
Dallosso, H. M., Davies, H. L., Lean, M. E. J., McIsaac, R. L. & James, W. P. T. (1984). *International Journal of Obesity* **8**, 413–425.
Fessler, A. & Beck, J. C. (1965). *Biochimica et Biophysica Acta* **106**, 199–201.
Galpin, K. S., Henderson, R. G., James, W. P. T. & Trayhurn, P. (1983). *Biochemical Journal* **214**, 265–268.
Golay, A., Schutz, Y., Meyer, H. U., Thiebaud, D., Curchod, B., Maeder, E., Felber, J. P. & Jequier, E. (1982). *Diabetes* **31**, 1023–1028.
Goodbody, A. E. & Trayhurn, P. (1981). *Biochemical Journal* **194**, 1019–1022.
Hamosh, M., Hamosh, P., Bar-Maur, J. A. & Cohen, H. (1963). *Journal of Clinical Investigation* **42**, 1648–1652.
Hassi, J. (1977). *Acta Universitatis Ouluensis* D21, **1**, 1–93.
Hatai, S. (1902). *Anatomischer Anzeiger* **21**, 369–373.
Heaton, J. M. (1972). *Journal of Anatomy* **112**, 35–39.
Heim, T., Kellermayer, M. & Dani, M. (1968). *Acta Paediatrica Academiae Scientarum Hungaricae* **9**, 109–120.
Himms-Hagen, J. (1979). *Canadian Medical Association Journal* **121**, 1361–1364.

Hoffenberg, R. (1972). *Proceedings of the Nutrition Society* **31**, 265–272.

Holloway, B. R., Stribling, D., Freeman, S. & Jamieson, L. (1985). *International Journal of Obesity* **9**, 423–432.

Holt, S. & York, D. A. (1982). *Biochemical Journal* **208**, 819–822.

Hull, D. (1977). In *Scientific Foundation of Obstetrics and Gynaecology*, 2nd ed, pp. 545–550 [E. E. Philipp, J. Barnes and M. Newton, editors]. London: Heinemann Medical Books.

Huttunen, P. K. (1979). *Acta Universitatis Ouluensis* A80, **24**, 1–73.

Huttunen, P. K., Hirvonen, J. & Kinnula, V. (1981). *European Journal of Applied Physiology* **46**, 339–345.

James, W. P. T. (1983). *Lancet* ii, 386–389.

James, W. P. T. & Trayhurn, P. (1981). In *Nutritional Factors: Effects on Metabolic Processes, 13th Miles International Symposium*, pp. 123–138 [R. F. Beers and E. G. Bassett, editors]. New York: Raven Press.

Jung, R. T., Shetty, P. S., James, W. P. T., Barrand, M. A. & Callingham, B. A. (1979). *Nature* **279**, 322–323.

Lean, M. E. J. (1986). Brown adipose tissue in humans. MD Thesis, University of Cambridge.

Lean, M. E. J. & James, W. P. T. (1983). *FEBS Letters* **163**, 235–240.

Lean, M. E. J. & James, W. P. T. (1986). In *Brown Adipose Tissue*, pp. 339–365 [P. Trayhurn and D. G. Nicholls, editors]. London: Edward Arnold.

Lean, M. E. J., James, W. P. T. & Garthwaite, P. (1988a). In *Obesity in Europe 88*, pp. 281–286 [P. Bjorntorp and S. Rossner, editors]. London: John Libbey.

Lean, M. E. J., James, W. P. T., Jennings, G. & Trayhurn, P. (1986a). *International Journal of Obesity* **10**, 219–227.

Lean, M. E. J., James, W. P. T., Jennings, G. & Trayhurn, P. (1986b). *Clinical Science* **71**, 291–297.

Lean, M. E. J. & Jennings, G. (1989). *Journal of Clinical Pathology* (In the Press).

Lean, M. E. J., Murgatroyd, P. R., Reid, I. W. & Rothnie, I. (1988b). *Clinical Endocrinology* **28**, 665–673.

Lean, M. E. J., Trayhurn, P., Murgatroyd, P. R. & Dixon, A. K. (1987). In *Recent Advances in Obesity Research*, vol. 5, pp. 109–119 [E. M. Berry, S. H. Blondheim, H. E. Eliahou and E. Shafrir, editors]. London: John Libbey.

Leibel, R. L., Berry, E. M. & Hirsch, J. (1987). In *Recent Advances in Obesity Research*, vol. 5, pp. 117–123 [E. M. Berry, S. H. Blondheim, H. E. Eliahou and E. Shafrir, editors]. London: John Libbey.

Lev-Bari, E., Horwitz, C. & Shilo, R. (1982). *Israel Journal of Medical Science* **18**, 889–890.

McCormack, J. G. & Denton, R. M. (1977). *Biochemical Journal* **166**, 627–630.

McLean, P., Brown, J. & Greenbaum, A. L. (1968). In *Carbohydrate Metabolism and its Disorders*, pp. 397–425 [F. Dickens, P. J. Randall and W. J. Whelan, editors]. London: Academic Press.

Mager, W. M. & Gifford, R. W. (1977). *Phaeochromocytoma*. New York: Springer Verlag.

Medeiros, L. T., Katsas, G. G. & Balogh, K. (1985). *Human Pathology* **16**, 970–972.

Melicow, M. M. (1957). *American Medical Association Archives of Pathology* **63**, 367–372.

Mercer, S. W. & Trayhurn, P. (1983). *Biochemical Journal* **212**, 393–398.

Merklin, R. J. (1971). *American Journal of Anatomy* **132**, 33–34.

Merklin, R. J. (1974a) *Anatomical Record* **178**, 415.

Merklin, R. J. (1974b) *Anatomical Record* **178**, 637–646.

Mory, G., Bouillaud, F., Combes-George, M. & Ricquier, D. (1984). *FEBS Letters* **166**, 393–396.

Naeye, R. L. (1974). In *F. E. Camps Symposium on SIDS*, pp. 1–6 [R. R. Robinson, editor].

Nauman, A., Nauman, J., Sypniewska, J., Fiedorowicz, K. & Bielecki, K. (1988). In *Obesity in Europe 88*, pp. 177–184 [P. Bjorntorp and S. Rossner, editors]. London: John Libbey.

Nicholls, D. G., Cunningham, S. A. & Rial, E. (1986). In *Brown Adipose Tissue*, pp. 52–85 [P. Trayhurn and D. G. Nicholls, editors]. London: Edward Arnold.

Nicholls, D. G. & Locke, R. M. (1984). *Physiological Reviews* **64**, 1–64.

Oliver, T. K. & Karlberg, P. (1962–3). *Acta Paediatrica Scandinavica* **140**, Suppl., 51–53.

Ostman, J., Arner, P., Englefeldt, P. & Kager, L. (1979). *Metabolism* **28**, 1198–1205.

Ricquier, D., Mory, G., Nechad, M., Combes-George, M. & Thibauld, J. (1983). *American Journal of Physiology* **245**, C172–C177.

Ricquier, D., Nechad, M. & Mory, G. (1982). *Journal of Clinical Endocrinology and Metabolism* **54**, 803–807.

Rothwell, N. J. & Stock, M. J. (1979). *Nature* **281**, 31–35.

Rothwell, N. J. & Stock, M. J. (1983). *International Journal of Obesity* **7**, 583–589.

Scholander, P. F., Hammel, H. T., Hart, J. S., Le Mesurier, D. S. & Steen, J. (1958). *Journal of Applied Physiology* **13**, 211–218.

Shattock, S. G. (1909). *Proceedings of the Royal Society of Medicine* **2**, 207–270.

Silverman, W. A., Zamelis, A., Sinclair, J. C. & Agate, F. J. (1964). *Paediatrics* **33**, 984–987.

Stanton, A. N. (1984). *Lancet* ii, 1199–1201.

Stirling, D. M. & Ashwell, M. (1988). *Proceedings of the Nutrition Society* **47**, 75A.
Trayhurn, P. & Nicholls, D. G. (eds.) (1986). *Brown Adipose Tissue*. London: Edward Arnold.

EXPLANATION OF PLATES

Plate 1.   Haematoxylin- and eosin-stained sections of brown adipose tissue from a 22-year-old woman with a pelvic phaeochromocytoma (magnification × 52).

Plate 2.   Electron micrograph of brown adipose tissue from a 46-year-old woman with an adrenal phaeochromocytoma. Bar = 1 μm.
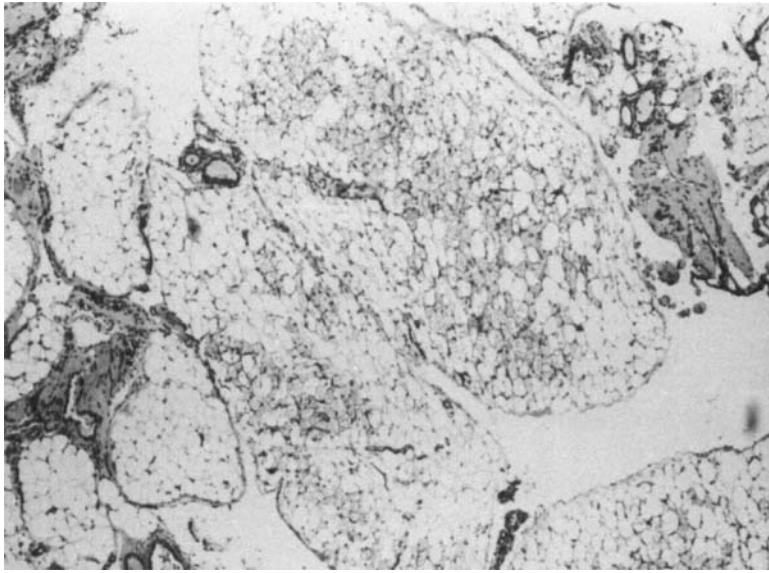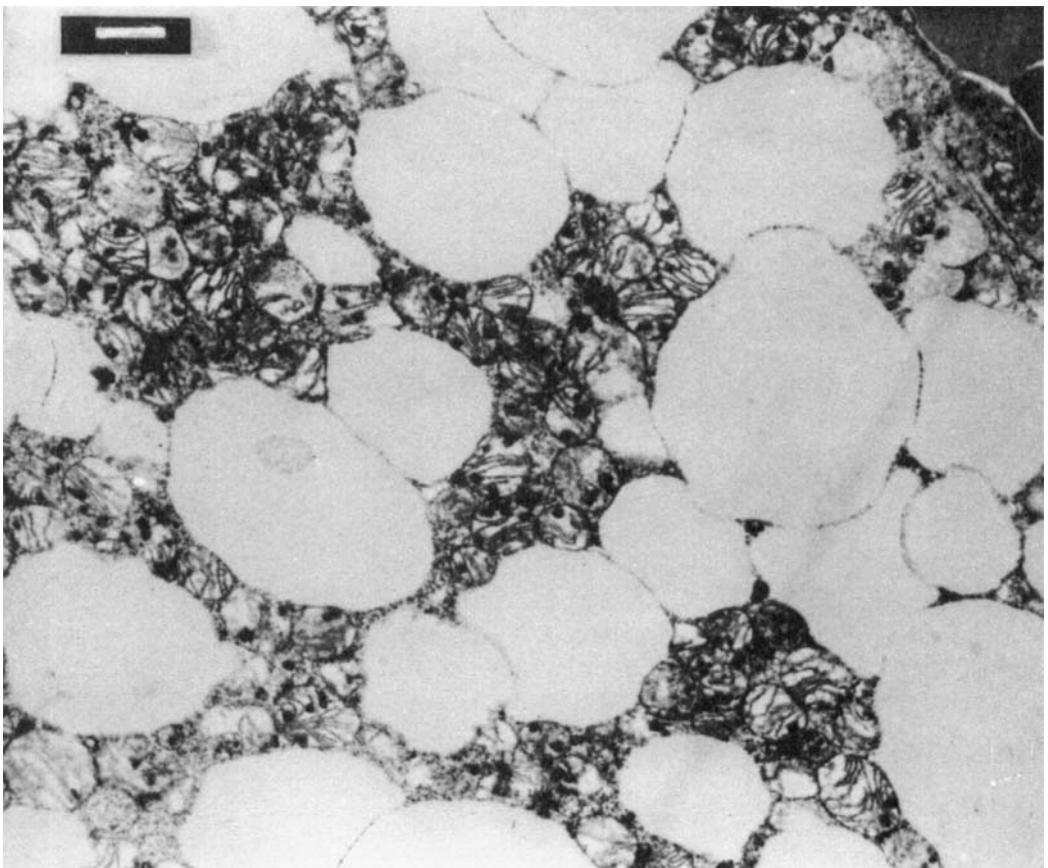
Printed in Great Britain

Plate 1

Plate 2

M. E. J. LEAN   *(facing p. 256)*