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Why do people get fat: is adipose tissue guilty?

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The prevalence of obesity within the UK is increasing. Comparable surveys conducted in 1980 and 1986/7 (Knight, 1983; Gregory *et al.* 1990) showed that, even in the more health-conscious 1980s, the proportion of men and women with body mass indices (BMI) greater than 30 rose from 6 to 8% (men) and from 8 to 12% (women). Interest in the question 'Why do people get fat?' is, therefore, as great as ever. The present paper addresses seven questions relating to the particular role that adipose tissue might, or might not, play in the aetiology of obesity.

DOES A GENE DEFECT PLAY A ROLE IN OBESITY?

Many studies have addressed the question of whether there is a gene defect in obesity. Family studies give some evidence for a genetic component, but these can also be explained by a shared environmental upbringing (for review, see Ashwell, 1975). Better evidence comes from studies of adopted children (e.g. Stunkard *et al.* 1990) and studies of families containing monozygotic and dizygotic twins (e.g. Bouchard, 1990). Biological inheritance accounts for only 5% of the BMI value and the amount of subcutaneous fat, but accounts for 20–30% of the relative fat distribution. The amount of internal fat seems to be influenced by heredity more than the amount of subcutaneous fat.

Excess energy is only stored as fat in white adipose tissue (WAT) when the energy intake of an individual exceeds his or her energy output. A genetic defect could be responsible for an inherited tendency for an excessively high energy intake or for an excessively low energy output. The possibility that there is a defect within WAT which causes either an excessive accumulation of energy stores or a limited breakdown of these energy stores is not often considered. A primary defect within the energy stores might be responsible for secondary changes in energy input or output. The present paper will be devoted to these considerations and will also consider whether an intrinsic defect in brown adipose tissue (BAT) might play a role in determining energy expenditure.

IS THERE AN INTRINSIC DEFECT IN WHITE FAT CELLS?

The use of the adipose tissue transplantation technique to study the nature and nurture of adipose tissue in genetically obese rodents was first suggested by Sir Peter Medawar.

Experiments were performed with Sir Peter's group between 1971 and 1981 at the MRC Clinical Research Centre in Harrow, and then between 1981 and 1986 at the MRC Dunn Nutrition Unit in Cambridge. Immunological rejection of grafts was avoided by using effectively syngeneic rodents. The kidney capsule was a suitable transplantation site since the grafted tissue was soon revascularized and because it was easy to differentiate the fat graft from the surrounding perirenal adipose tissue when the grafts were removed from the host mice (usually after 1 or 2 months).

A defect in lipolysis? The first question to be answered using this technique was: do intrinsic abnormalities cause the increased size of adipocytes in WAT of genetically obese mice? At that time, there were several indications from studies of in vitro lipolysis and of individual enzyme activities (for review, see Ashwell & Meade, 1979), that intrinsic factors might be of prime importance. Several forms of genetically obese mice were studied: the obese mouse (ob/ob; Ashwell *et al.* 1977), the diabetic mouse (db/db), the adipose mouse (db^{ad}/db^{ad}) and the 'yellow obese' mouse ($A^{y}/+$; Meade *et al.* 1979).

Fig. 1 summarizes the results of experiments with the obese mouse (ob/ob). Gonadal adipocytes of obese mice were five to six times larger (on a weight basis) than those from their lean counterparts. At 1 month after transplantation into lean mice, they decreased to a size which was not statistically different from that of the host gonadal adipocytes. Conversely, the smaller adipocytes from lean mice increased in size substantially when transplanted into obese mice so that the resultant adipocyte size in the grafts was not significantly different from the obese mice. In all obese mutants studied, extrinsic factors

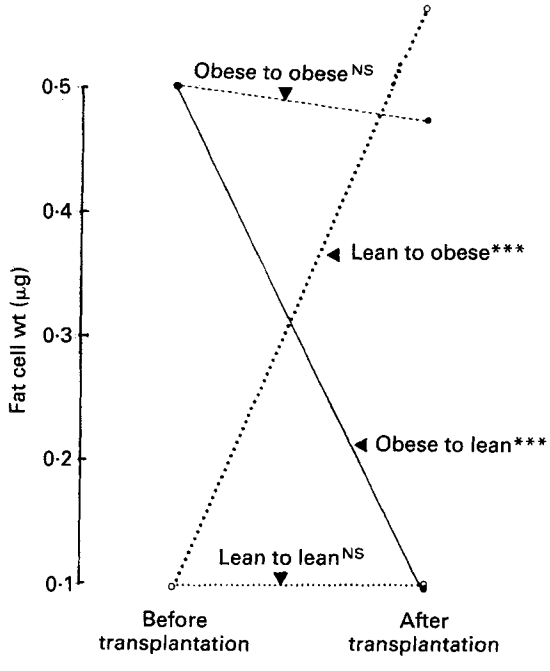

Fig. 1. Fat cell size in grafts between genetically obese mice (ob/ob) and their lean counterparts. Obese fat transplanted into obese mice (●-----●); obese fat transplanted into lean mice (●——●); lean fat transplanted into lean mice (○·····○); lean fat transplanted into obese mice(○·····○). The change in fat cell weight was significant: ***$P<0{\cdot}001$. NS, not significant.

associated with the lean host could 'normalize' (i.e. decrease) adipocyte size in the obese mutants. Therefore, there was no evidence for an intrinsic defect in lipolysis within the adipocytes of genetically obese mice.

A defect in lipogenesis? The adipose tissue transplantation technique was also used to investigate the differences in fatty acid composition of WAT from lean and obese mice (Enser & Ashwell, 1983).

The fatty acid composition of WAT from obese mice has a lower proportion of linoleic acid (L) and a higher proportion of palmitoleic acid (P) than that of WAT from lean mice, i.e. WAT from obese mice has a lower L:P value than WAT from lean mice (Enser, 1979). These differences probably arise from: (1) generally increased rates of endogenous fatty acid synthesis in the liver and adipose tissue of obese mice (Rath & Thenen, 1980) which causes a greater dilution of the essential dietary linoleic acid (18:2 *n*-6); (2) the increased activity of acyl-CoA δ-9 desaturase (Enser, 1975) which converts palmitic acid (16:0) to palmitoleic acid (16:1 *n*-9).

The second question to be answered using the transplantation technique was, therefore, 'Are these increases in lipogenic enzyme activity in the WAT of obese mice dependent on factors intrinsic or extrinsic to the adipocyte?

Subcutaneous adipose tissue was transplanted between lean and genetically obese mice (ob/ob). At the end of the transplantation period (1–2 months), the grafts and samples of perirenal and subcutaneous adipose tissue from donor and host mice were extracted with solvent and the fatty acid composition of the lipids determined by gas–liquid chromatography.

Grafts from lean donors in obese mice had a fatty acid composition that resembled the perirenal adipose tissue of the obese host after 1 month, i.e. the WAT had a lower L:P value.

Most grafts from obese donors into lean mice had fatty acid compositions which completely resembled that of the lean host, i.e. an increased L:P value. A few grafts had fatty acid compositions which had only partially changed. The use of grafts, prelabelled by feeding the donor mouse a metabolically inactive fatty acid, margaric acid (17:0), indicated that a lack of fatty acid turnover, rather than selective metabolic processes, was responsible for the failure of these few grafts from obese donors to fully acquire the fatty acid composition of the perirenal adipose tissue of the lean host.

Fig. 2 summarizes the results of these experiments excluding those grafts where the margaric acid content indicated that there was an abnormal turnover of fatty acids. The L:P value of all adipose tissue grafts was not significantly different from that of the host adipose tissue.

The main conclusion from this study was that it is the environment in which the adipocyte finds itself which is the major determinant of its content of linoleic acid and palmitoleic acid and of its lipogenic activity. No intrinsic differences in fatty acid metabolism of adipocytes from obese mice were apparent once the cells were transplanted into the environment of the lean mice.

A defect in esterification? In a further series of experiments (Ashwell & Meade, 1978), adipocytes from genetically obese mice transplanted into lean mice were shown not to over-enlarge when the host mouse was chemically stimulated to become obese with gold thioglucose. The abnormally large size of adipocytes in the WAT of the obese mouse was not, therefore, due to an intrinsic defect in fat esterification causing increased lipid synthesis.

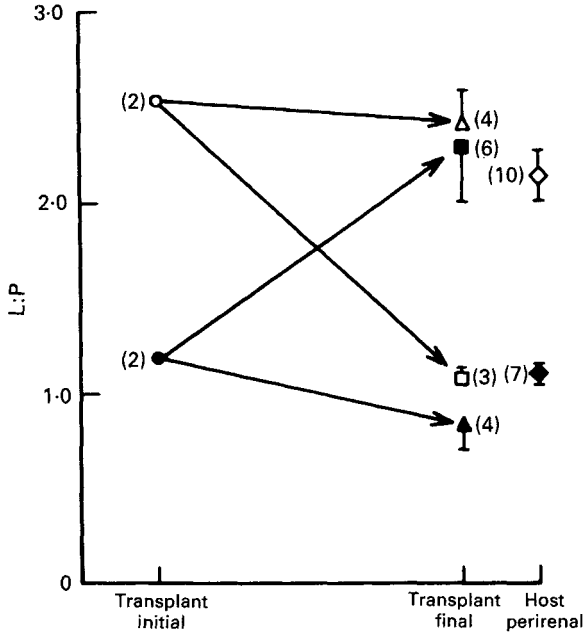

Fig. 2. Changes in the ratio of linoleic acid:palmitoleic acid (L:P) in the neutral lipids of adipose tissue transplanted between lean and obese mice. The results are means with their standard errors represented by vertical bars for the numbers of animals shown in parentheses. (●, Obese-donor fat; (○), lean-donor fat; (▲), obese-donor graft in obese host; (△), lean-donor graft in lean host; (■), obese-donor graft in lean host; (□), lean-donor graft in obese host; (◆), obese-host perirenal fat; (◇), lean-host perirenal fat. (From Enser & Ashwell, 1983.)

Conclusions. The transplantation experiments using WAT from genetically obese rodents showed that intrinsic defects in lipolysis, lipogenesis and fat esterification are unlikely to account for the inherited obesity of these animals (for more comprehensive reviews, see Ashwell & Meade, 1979; Ashwell, 1985*b*, 1987).

We can but assume that the same conclusion would apply to human WAT if it were possible to perform the same experiments.

IS THE NUMBER OF ADIPOCYTES IN WHITE ADIPOSE TISSUE FIXED IN INFANCY?

Evidence for the 'fat cell hypothesis'. The introduction of the needle-biopsy technique for sampling human adipose tissue caused an explosion in research into the size and total number of adipocytes in the human body. The most widely reported finding in the early 1970s was an inverse correlation between the total number of fat cells in the body and the age of onset of obesity (Brook *et al.* 1972; Salans *et al.* 1973). This finding heralded a much-needed campaign against the dangers of infantile obesity, but it tended to be widely misinterpreted (Poskitt, 1991) and resulted in a somewhat fatalistic approach to the treatment of child-onset obesity such as that epitomized in The Pre-School Child Unit of The Open University Course: 'Cells for storing fat in the body develop mostly in the first year of life. What's more important, fat storage cells never go away once you've

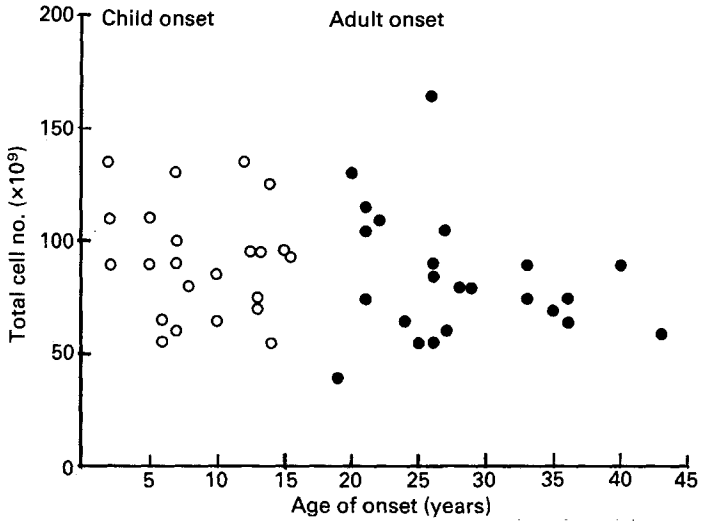

Fig. 3. Total cell number ($\times 10^9$) *v.* the age of onset of obesity for forty-six overweight and obese women (body mass index (weight/height^2) range 24·3–51·8 kg/m^2). There is no significant correlation between these variables (*r* 0·018).

got them. A fat baby becomes a fat adult with a large number of fat storage cells who puts on weight easily. He also finds it more difficult to slim than an adult who, as a baby, developed few fat storage cells.'

Evidence against the 'fat cell hypothesis'. Several research groups were subsequently unable to confirm this strong negative association between total fat cell number and the age of onset of obesity and they showed that total fat cell number is invariably increased with the severity of obesity, whatever the age of onset. Fig. 3 shows that in forty-six women (BMI range 24·3–51·8), there was no significant correlation between apparent fat cell number and the age of onset of obesity (Ashwell, 1979).

Experimental work on undernutrition (Widdowson & Shaw, 1973) supported the need for caution in the interpretation of fat-cell counts. If pigs were undernourished from 10 d of age so that they weighed only 5–6 kg, instead of 200 kg, when they were 1 year old, they had no detectable subcutaneous or deep body fat. They had plenty of fat cells at 10 d of age, but these cells were completely empty and did not register by conventional methods of cell counting at 1 year of age. However, as soon as plentiful food was supplied, the pigs became extremely fat. They were fatter indeed than animals that had never been undernourished, and the longer the period of deprivation the fatter they tended to become. This finding was directly opposed to the view that an excessive number of adipocytes are formed only when overfeeding takes place in infancy.

The main reason for the decline and fall of the 'fat cell hypothesis' was that one of the main proponents of the original hypothesis eventually agreed that an adult's total number of fat cells depended more on the severity of his obesity than on the age of onset of obesity (Hirsch & Batchelor, 1976).

Conclusions. As Sir Peter Medawar (1979) once remarked, in his *'Advice to a Young Scientist'*: 'I cannot give any scientist of any age better advice than this: the intensity of

the conviction that a hypothesis is true has no bearing on whether it is true or not. The importance of the strength of our conviction is only to provide a proportionately strong incentive to find out if the hypothesis will stand up to critical evaluation'.

There is currently much excitement and speculation about the relationship of early diet to the subsequent adult risk of coronary heart disease (CHD) and diabetes (Barker, 1991). Let us hope that Medawar's (1979) advice will be heeded and that research funds will be forthcoming to follow up the epidemiological associations with some experiments which focus on the mechanism of 'early programming' of later disease. A fatalistic approach to the prevention of heart disease and diabetes in adult life (i.e. assuming that the 'damage' has been done during pregnancy and infancy) would be disastrous. When interesting scientific results are communicated by the mass media, even if they are done in a very responsible manner as they were on BBC 2's *Horizon* documentary, they can become distorted by media misinterpretation. After the *Horizon* programme, my favourite television reviewer, Nancy Banks-Smith, wrote in the *Guardian* of 31st March 1992: 'In *Horizon* (BBC 2), Professor Barker of the Medical Research Council identified the poor nutrition of mill girls at the turn of the century as the origin of the North/South divide. By consulting old, but meticulously kept, records, touchingly saved from destruction by devoted keepers, a direct correlation appeared between the nutrition we get in the womb and the disease which kills us. Lancashire has a peculiarly high incidence of heart disease. This is a bit of a facer. From the moment we are born, it is already too late.

Hotpot . . . honeycomb tripe . . . meat pies which left shining ghostly circles on the greaseproof paper . . . vanilla slices whose airy pastry collapsed into solid custard . . . fish and chips in newspaper . . . pineapple chunks on Sunday.

My grandmother's carefully written recipes, and she was a turn-of-the-century mill girl, are interspersed with specimen letters of condolence: perhaps when we all meet in the Great Beyond, we shall realise the Wisdom of our Redeemer. It did not occur to any of us that the letters and the recipes might be related'.

If this fatalistic message becomes even more distorted by the tabloid press, it will be heartbreaking to see the recent Government thrust into Health Promotion (Department of Health, 1992) being totally wasted on a UK population who believe themselves to be doomed from the womb.

IS THERE AN INTRINSIC DEFECT IN BROWN ADIPOCYTES?

In the late 1970s and early 1980s, attention turned away from WAT and focused on BAT. The defective functioning of BAT in respect to its capacity to generate heat appeared to account for the reduced thermoregulatory energy requirements of genetically obese mice (Himms-Hagen, 1985; Trayhurn, 1986). The principal mechanism for BAT thermogenesis is through the operation of the mitochondrial proton conductance pathway; this pathway is associated with a specific membrane protein of molecular weight 32 000 commonly referred to as uncoupling protein (UCP). The amount of UCP in BAT mitochondria is closely correlated with the thermogenic capacity of the tissue. A sensitive radioimmunoassay (Lean *et al.* 1983) demonstrated that adult obese mice (ob/ob) had less UCP than their lean counterparts (Ashwell *et al.* 1985). The altered mitochondrial function in the obese mouse is also reflected in the mitochondrial ultrastructure. The cristae are less pronounced than those in lean mice and the matrix

tends to be denser. Decreased thermogenesis in obese mice can be demonstrated as early as 12 d of age and occurs before any other defect reported so far for this species.

The question arose, therefore, as to whether the abnormality in BAT mitochondria is intrinsic to the BAT of the obese mouse, or whether the BAT is receiving abnormal signals external to the tissue such that the changes in the mitochondria, both in UCP content and ultrastructure, are secondary to these environmental changes.

To answer this question, interscapular BAT was transplanted between genetically obese mice (ob/ob) and their lean littermates using the technique already described for WAT. Initially, the mice were kept at room temperature (23°) following transplantation (Ashwell *et al.* 1986).

A defect in lipolysis or lipogenesis? Examination of the adipocyte size in BAT by light microscopy revealed similar changes in cell size to those that had been seen with WAT. Thus, intrinsic defects in lipolysis in BAT were as unlikely as they were in WAT.

Likewise, the fatty acid composition of obese grafts changed towards that of the host lean BAT. Thus, the results confirmed that the physiological environment, rather than the source of the adipose tissue, was the major determinant of its fatty acid composition (Roberts *et al.* 1986).

A defect in thermogenesis and sympathetic innervation? When the BAT grafts were examined using electron microscopy, several intermediate forms of mitochondrial ultrastructure were seen. It was necessary, therefore, to devise a system for 'scoring' the mitochondrial ultrastructure on a scale between 1 and 7. Thus, the mitochondrial ultrastructure score (MUS) of BAT from lean donors was predominantly of types 1, 2 and 3 and that from obese donor mice was predominantly of types 5, 6 and 7 (Ashwell *et al.* 1986).

When lean BAT was transplanted into obese mice or when obese BAT was transplanted into lean mice, there was a partial shift towards obese type mitochondrial ultrastructure (see Fig. 4(a)). The adipocytes nearest the kidney in any graft were most likely to show MUS typical of the host, whereas the cells nearest the kidney capsule were the least likely to change. Could the partial changes in mitochondrial ultrastructure reflect incomplete or inconsistent sympathetic stimulation to some of the cells in the graft? Cold acclimation is known to increase sympathetic outflow to BAT and warm acclimation is known to decrease it. Therefore, host mice were subjected to either warm (33°) or cold (4°) acclimation after transplantation to see whether increasing or decreasing sympathetic outflow to the grafts could provide a more consistent change in mitochondrial ultrastructure. Fig. 4(b) shows that temperature acclimation after transplantation caused complete transformation which persisted even when the hosts were subsequently housed at ambient temperature (23°) after the period of temperature acclimation.

These results can be reconciled with the view that the difference in ultrastructure, and presumably function, of the obese BAT mitochondria is not of primary fundamental origin. Grafts in animals kept at ambient temperatures displayed partial mitochondrial transformation probably because the resultant sympathetic stimulation to BAT was at an intermediate level between that characteristic of lean and obese mice.

Experimental support for these conclusions came from a fluorescent histochemical study of catecholamines in BAT from lean and obese mice (Ashwell & Dunnett, 1985). Lean BAT is characterized by dual innervation, i.e. innervation of the blood vessels plus innervation of individual cells. Obese BAT exhibits 'single innervation', i.e. innervation

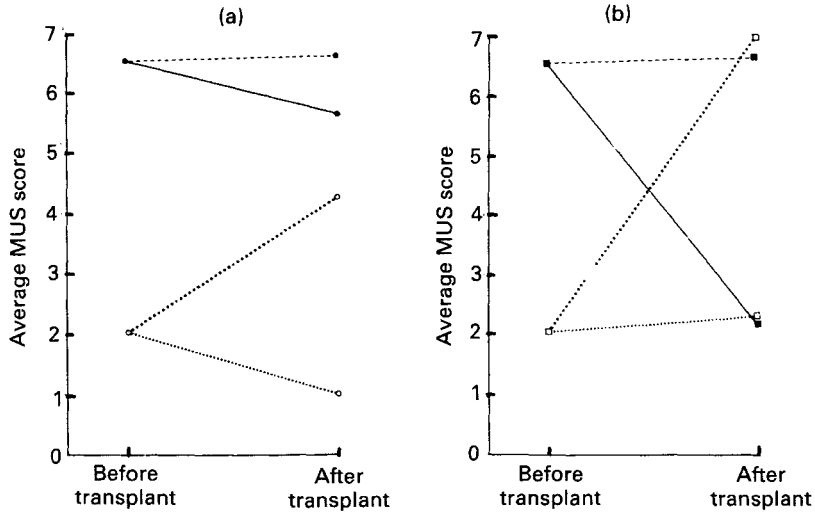

Fig. 4. (a) Summary of mitochondrial ultrastructure (MUS) changes in transplantation experiments performed at ambient temperatures (6 weeks at 23°). (●-----●), Obese donor in obese host; (●——●), obese donor in lean host; (○·····○), lean donor in lean host; (○·····○), lean donor in obese host. (b) Summary of MUS changes in transplantation experiments with subsequent temperature acclimation. (■-----■), Obese donor in obese host (1 week at 23°, 5 weeks at 33°); (■——■), obese donor in lean host (1 week at 23°, 5 weeks at 4°); (□·····□), lean donor in lean host (1 week at 23°, 5 weeks at 4°); (□·····□), lean donor in obese host (1 week at 23°, 5 weeks at 33°). (From Ashwell *et al.* 1986.)

of the blood vessels but not of the cells. When obese BAT was grafted into a lean mouse which was subsequently cold-acclimated, the BAT showed clear evidence of dual innervation. On the other hand, if lean BAT was grafted into obese mice which were subsequently warm-acclimated, there was no evidence of any functional cellular innervation, although the blood vessels were innervated as normal. Grafts between lean and obese mice that were kept at ambient temperatures showed normal innervation of the blood vessels in the grafts but only partial cellular innervation.

Results from several other groups using different methodology were consistent with the conclusion that there is no intrinsic defect in mitochondria in the BAT of obese mice (Himms-Hagen, 1985).

The current hypothesis for the defect in BAT of the genetically obese mouse is that it lies in the reduced activity of the sympathetic nervous system. Thus, thermogenesis in the obese mouse at ambient temperature is not as fully 'switched on' as it is in the lean mouse. This is reflected in decreased guanosine diphosphate binding to isolated mitochondria and decreased mitochondrial UCP content at ambient temperature. Maximum capacity can, however, be exhibited during cold acclimation indicating that the defect can be remedied by increasing sympathetic activity and is not due to a primary defect at the mitochondrial level (Trayhurn, 1990).

Conclusions. The BAT transplantation experiments showed that there is unlikely to be an intrinsic defect in the adipocytes in BAT of genetically obese mice, but that the defect is more likely to reside in the factors which regulate the sympathetic innervation of BAT.

The human adult appears to possess a substantial amount of tissue with the potential for uncoupled respiration and other characteristics of BAT, but in the unstimulated condition this tissue probably only accounts for 1–2% of overall energy expenditure (Lean, 1992). Research on sympathetic stimulation of human BAT via the novel β-3 receptor is proving very promising.

SHOULD WE HAVE A FAT CELL POOL CONCEPT?

The original 'fat cell pool concept' for WAT. Once it was established that total fat cell number was not fixed in infancy in either animals or man (see p. 356), there was increased interest in the question of pre-adipocyte development (see Cryer *et al.* 1992) and in the 'dedifferentiation' of mature fat cells in culture to cells which resemble adipocyte precursor cells.

In 1978, I suggested that we must think in terms of the 'fat cell pool' for adipose tissue instead of thinking only of the mature fat cells (Ashwell, 1978). I defined the fat cell pool as consisting of the following cell types: mature fat cells, dedifferentiating mature fat cells and fat cell precursors.

The new fat cell pool concept for WAT. I would like to update the concept I originally put forward to include the work of Hartrampf & Loffler (1992) who have recently presented evidence for cell division in mature cultured human adipocytes. Mature adipocytes from donors aged between 61 and 91 years were able to replicate their DNA and proliferate. The replication process was monitored by 5-bromodeoxyuridine (BrdU) labelling of DNA. There was a linear increase in BrdU incorporation with incubation time which was not influenced by insulin at any concentration and replication appeared to be under the control of growth factors such as insulin growth factor (IGF), epidermal growth factor (EDF) and basic fibroblast growth factor (FGF).

The demonstration of DNA replication in cultured human fat cells only suggests the possibility of adipose tissue hyperplasia induced by mitotic activity of mature adipocytes, but the fact that a number of growth factors are able to modulate the replication rate in these cells is a strong indication for the importance of adipocyte multiplication in adult life. As yet, there has been no convincing demonstration of mature fat cell replication 'in vivo'.

In 1992, I would suggest that we must consider that the fat cell pool contains the following cell types: mature fat cells, dedifferentiating mature fat cells, fat cell precursors and replicating fat cells.

The fat cell pool concept for BAT. There has not been as much research on the types of fat cells within BAT, mainly because of the greater interest in the thermogenic capacity of BAT. There is good reason to assume, however, that the same types of cells exist within BAT as they do within WAT.

IS THERE A CONTINUOUS SPECTRUM OF THE ADIPOSE TISSUES?

On the basis of morphology and embryology, the adipose tissues are classified as connective tissues and have conventionally been divided into two main types: 'white' (WAT) and 'brown' (BAT).

The question of a common origin for WAT and BAT has caused much debate. On the one hand, scientists have emphasized the distinct differences between WAT and BAT

with regard to ultrastructure and innervation. On the other hand, scientists have regarded BAT as an arrested, embryonic, form of WAT and have suggested that a continuous spectrum exists (for review, see Ashwell, 1985*a*).

Cannon *et al.* (1978) suggested that 'it seems most realistic to envisage a spectrum of adipose tissues with perhaps epididymal WAT in the rat and BAT in the newborn forming the extremities, whereas perirenal adipose tissue in lamb and ox, and mesenteric and subcutaneous adipose tissue in dog and human, appear in intermediate positions'.

The functions of the adipose tissues can most conveniently distinguish the two extremes of the continuous spectrum. For simplicity, I called them 'storage' and 'thermogenic' adipose tissue and although they can still be contrasted with respect to their morphology, their distinction on the basis of function is much more informative (Ashwell, 1985*a*).

Movement along the spectrum. If the concept of a continuous spectrum is accepted, there are at least three different routes between one extreme and the other: Route A, 'thermogenic' adipose tissue develops into 'storage' adipose tissue by a gradual transformation of all the existing adipocytes, through various transitional states, such that all cells are in the same state at any one time; Route B, 'thermogenic' adipose tissue develops into 'storage' adipose tissue by the dedifferentiation of existing 'thermogenic' adipocytes through neutral precursor cells and their subsequent redifferentiation into 'storage' adipocytes; Route C, 'thermogenic' adipose tissue develops into 'storage' adipose tissue by the recruitment of 'storage' adipocytes from a pool of separate precursor cells. This may or may not be accompanied by the simultaneous involution of 'thermogenic' adipocytes. (N.B. 'storage' adipose tissue could become 'thermogenic' adipose tissue by parallel routes in the opposite direction.)

Thus, adipose tissue in the middle of the spectrum, which might be termed 'khaki' fat, could arise from 100% 'khaki' cells (route A) or 50% 'thermogenic' cells and 50% 'storage' cells (routes B and C). Of course, it is possible that movement along the spectrum could take place by routes A, B and C, either at the same time or in response to different stimuli. It is also quite possible that movement along the spectrum could occur in both directions, i.e. from 'storage' towards 'thermogenic' and vice versa. Some of the factors or stimuli which could affect movement along the spectrum are genetic, hormonal, neural, physiological, pathological and nutritional. Evidence for the involvement of these factors is discussed in detail elsewhere (Ashwell *et al.* 1987; Trayhurn & Ashwell, 1987; Trayhurn, 1990).

Conclusions. In the early 1990s, adipose tissue appears to be a much more exciting and interesting tissue than ever before. Fig. 5 summarizes 'the fat cell pool/continuous spectrum' concept of the adipose tissues. It is important to remember the possible interconversion of all cell types and the interaction of all influences when interpreting any experimental observations on adipose tissue or when speculating about their roles in the nature or risks of obesity.

IS ADIPOSE TISSUE DISTRIBUTION IMPORTANT IN DETERMINING THE RISKS OF OBESITY?

Even though the hypothesis was first proposed by Vague (1946) nearly half a century ago, it has only been in the 1980s that it has become widely accepted that the risks of obesity are associated more with the distribution of the excess adipose tissue rather than

Influence	Cell type	Influence
	Mature storage adipocyte	
	Replicating storage adipocyte	
GENETIC	Dedifferentiating storage adipocyte	PHYSIOLOGICAL
	Precursor storage adipocyte	
HORMONAL	Precursor thermogenic adipocyte	PATHOLOGICAL
	Dedifferentiating thermogenic adipocyte	
NEURAL	Replicating thermogenic adipocyte	NUTRITIONAL
	Mature thermogenic adipocyte	

Fig. 5. The fat cell pool/continuous spectrum of the adipose tissue and possible modifying influences.

the amount of adipose tissue. The greatest risks of obesity (i.e. predisposition to CHD, stroke, diabetes and some cancers) seem to occur when excess adipose tissue is stored in the intra-abdominal cavity rather than in subcutaneous depots (for review, see British Nutrition Foundation, 1992). What is not known yet, though, is whether such diseases, and the risk factors which cause them, are actually determined by the location of the adipose tissue or whether they just happen to be associated with an increase in fat storage in internal depots (for review, see Bjorntorp, 1990).

SUMMING UP – IS ADIPOSE TISSUE GUILTY?

There does not appear to be a good case against adipose tissue (either storage (WAT) or thermogenic (BAT)) as a crucial factor in the aetiology of obesity. Although most experiments reported here have been performed on genetically obese rodents, there is very little evidence to suggest that a primary defect in either WAT or BAT adipose tissue could be responsible for the genetic component of human obesity. So, adipose tissue is not guilty on this count.

The case against adipose tissue does become clearer if one considers the role of adipose tissue distribution in determining the risks of obesity. Adipose tissue could be considered guilty in that its distribution in internal depots is associated with various disease states. However, it is not known whether the adipose tissue directly determines the risk factors or the disease and so the case must remain not proven on this count.

Bearing in mind the two concepts summarized in the present paper, i.e. that of the fat cell pool and the continuous spectrum of the adipose tissue, I would predict that the most 'guilty' adipose tissue depot would be an internal storage depot which has the greatest potential to increase the size of its fat cell pool by precursor recruitment (and maybe by cell replication); thereby increasing its volume by changes in cell numbers as well as cell size. If the depot is fully 'storage' in function, rather than thermogenic, it will almost certainly exhibit increased insulin resistance, thus making it most likely to be associated with the risk factors for CHD and diabetes.

By the same token, the most 'innocent' adipose tissue depot must be one which is strongly thermogenic in function and one which has a very limited capacity to increase

the size of its fat cell pool. This depot might help to increase total energy expenditure of the individual without providing that person with any spare capacity for an increase in energy storage.

This paper is dedicated to the memory of Sir Peter Medawar who gave me so much help and advice when I was a 'young' scientist. In particular, he taught me to choose words carefully and not to waste them.

REFERENCES

Ashwell, M. (1975). The tendency to fatness. *Health Education Journal* **34,** 81–87.

Ashwell, M. (1978). The 'fat cell pool' concept. *International Journal of Obesity* **2,** 69–72.

Ashwell, M. (1979). How can research into fat cells and fat distribution help the treatment of obesity? *International Journal of Obesity* **3,** 188–189.

Ashwell, M. (1985*a*). Is there a continuous spectrum of the adipose tissues in animals and man? In *Metabolic Complications of Human Obesities*, pp. 265–274 [J. Vague, editor]. London: Elsevier Science Publishers.

Ashwell, M. (1985*b*). The use of the adipose tissue transplantation technique to demonstrate that abnormalities in adipose tissue metabolism of genetically obese mice are due to extrinsic rather than intrinsic factors. *International Journal of Obesity* **9,** Suppl. 1, 77–82.

Ashwell, M. (1987). Transplantation of white and brown adipose tissue in experimental animals. In *Biology of the Adipocyte: Research Approaches*, pp. 255–280 [G. J. Hausman and R. J. Martin, editors]. New York: Van Nostrand Reinholdt Co Inc.

Ashwell, M. & Dunnett, S. B. (1985). Fluorescent histochemical demonstration of catecholamines in brown adipose tissue from obese (ob/ob) and lean mice acclimated at different temperatures. *Journal of the Autonomic Nervous System* **14,** 377–386.

Ashwell, M., Holt, S., Jennings, G., Stirling, D. M., Trayhurn, P. & York, D. A. (1985). Measurement of radioimmunoassay of the mitochondrial uncoupling protein from brown adipose tissue of obese (ob/ob) mice and Zucker (fa/fa) rats at different ages. *FEBS Letters* **179**, 233–237.

Ashwell, M. & Meade, C. J. (1978). Obesity: do fat cells from genetically obese mice (C57B1/6J ob/ob) have an innate capacity for increased fat storage? *Diabetologia* **15,** 465–470.

Ashwell, M. & Meade, C. J. (1979). Adipose tissue in genetically obese rodents. In *Animal Models of Obesity*, pp. 107–130 [M. F. W. Festing, editor]. London: Macmillan.

Ashwell, M., Meade, C. J., Medawar, P. B. M. & Sowter, C. (1977). Adipose tissue: contributions of nature and nurture to the obesity of an obese mutant mouse. *Proceedings of the Royal Society of London* **195B**, 343–353.

Ashwell, M., Stirling, D., Freeman, S. & Holloway, B. (1987). Transformations within the continuous spectrum of the adipose tissues. In *Recent Advances in Obesity Research*, vol. 5, pp. 151–157 (E. M. Berry, S. H. Blondheim, H. E. Elliahou and E. Shafrir, editors]. London: John Libbey.

Ashwell, M., Wells, C. & Dunnett, S. B. (1986). Brown adipose tissue: contributions of nature and nurture to the obesity of an obese mutant mouse (ob/ob). *International Journal of Obesity* **10,** 355–373.

Barker, D. J. P. (1991). Programming of cardiovascular disease in fetal life and infancy. In *Early Diet, Later Consequences*, pp. 29–37 [D. M. Conning, editor]. London: British Nutrition Foundation.

Bjorntorp, P. (1990). Criteria of obesity. In *Progress in Obesity Research* 1990, pp. 655–657 [Y. Oomura, S. Tarui, S. Inone and T. Shimazu, editors]. London: John Libbey.

Bouchard, C. (1990). Variation in human fat: the contribution of the genotype. In *Obesity: Towards a Molecular Approach*, pp. 17–28 [C. Bouchard, editor]. New York: Alan Liss Inc.

British Nutrition Foundation (1992). *Briefing Paper on The Nature and Risks of Obesity*. London: British Nutrition Foundation.

Brook, G. G. D., Lloyd, J. K. & Wolft, O. H. (1972). Relation between age of onset of obesity and size and number of adipose cells. *British Medical Journal* **2,** 25–27.

Cannon, B., Nedergaard, J., Romert, L., Sundin, U. & Srartergren, J. (1978). Biochemical mechanism of thermogenesis in brown adipose tissues. In *Strategies in Cold – Colon Natural Torpidity and Thermogenesis*, pp. 567–594 [L. C. Wang and J. W. Hudson, editors]. New York: Academic Press.

Cryer, A., Williams, S. E. & Cryer, J. (1992). Dietary and other factors involved in the proliferation, determination and differentiation of adipocyte precursor cells. *Proceedings of the Nutrition Society* **51,** 379–385.

Department of Health (1992). *The Health of the Nation.* London: H.M. Stationery Office.

Enser, M. (1975). Desaturation of stearic acid by liver and adipose tissue from obese-hyperglycaemic mice (ob/ob). *Biochemical Journal* **148,** 551–555.

Enser, M. (1979). The role of insulin in the regulation of stearic and desaturase activity in liver and adipose tissue from obese-hyperglycaemic (ob/ob) and lean mice. *Biochemical Journal* **180,** 551–558.

Enser, M. & Ashwell, M. (1983). Fatty acid composition of triglycerides from adipose tissue transplanted between obese and lean mice. *Lipids* **18,** 776–780.

Gregory, J., Foster, K., Tyler, H. & Wiseman, M. (1990). *The Dietary and Nutritional Survey of British Adults.* London: H.M. Stationery Office.

Hartrampf, R. & Loffler, G. (1992). Culture of mature human adipocytes: evidence for cell division induced by growth factors. *Experimental Cell Research* (In the Press).

Himms-Hagen, J. (1985). Brown adipose tissue metabolism and thermogenesis. *Annual Review of Nutrition* **5,** 69–94.

Hirsch, J. & Batchelor, B. (1976). Adipose tissue cellularity in human obesity. *Clinics in Endocrinology and Metabolism* **5 (Suppl. II),** 299–311.

Knight, I. (1983). *The Heights and Weights of Adults in Great Britain.* London: H.M. Stationery Office.

Lean, M. E. J. (1992). Brown adipose tissue and obesity. In *Obesity: Basic Concepts and Clinical Aspects*, vol. II. *Front Diabetes*, pp. 37–49 [F. Belfiace, B. Jeanrenaud and D. Papalia, editors]. Basel: Karger.

Lean, M. E. J., Branch, W. J., James, W. P. T., Jennings, G. & Ashwell, M. (1983). Measurement of rat brown adipose tissue mitochondrial uncoupling protein by radioimmunoassay: increased concentration after cold acclimation. *Bioscience Reports* **3,** 61–71.

Meade, C. J., Ashwell, M. & Sowter, C. (1979). Is genetically transmitted obesity due to an adipose tissue defect? *Proceedings of the Royal Society, London* **205B**, 395–410.

Medawar, P. B. (1979). *Advice to a Young Scientist.* New York: Harper and Row.

Poskitt, E. M. E. (1991). Early diet, later obesity? In *Early Diet, Later Consequences*, pp. 38–44 [D. M. Conning, editor]. London: British Nutrition Foundation.

Rath, E. A. & Thenan, S. (1980). Influence of age and genetic background on in vivo fatty acid synthesis in obese (ob/ob) mice. *Biochimica et Biophysica Acta* **618,** 18–27.

Roberts, J. C., Ashwell, M. & Enser, M. (1986). Fatty acids of brown adipose tissue of obese and lean mice: effect of transplantation. *Biochemical Society Transactions* **14,** 279.

Salans, L. B., Cushman, S. W. & Weismann, R. E. (1973). Studies of human adipose tissue. Adipose cell size and number in non-obese and obese patients. *Journal of Clinical Investigation* **52,** 929–941.

Stunkard, A. J., Harris, J. K., Pedersen, N. C. & McClearn, G. E. (1990). The body mass index of twins who have been reared apart. *New England Journal of Medicine* **322,** 1483–1487.

Trayhurn, P. (1986). Brown adipose tissue and energy balance. In *Brown Adipose Tissue*, pp. 299–338 [P. Trayhurn and D. G. Nicholls, editors]. London: Edward Arnold.

Trayhurn, P. (1990). Energy expenditure and thermogenesis: animal studies on brown adipose tissue. *International Journal of Obesity* **14**, Suppl. 1, 17–29.

Trayhurn, P. & Ashwell, M. (1987). Control of white and brown adipose tissues by the autonomic nervous system. *Proceedings of the Nutrition Society* **47,** 135–142.

Vague, J. (1946). Le Traitment des Obesities. *Marseille Medicine* **83,** 210–225.

Widdowson, E. M. & Shaw, W. T. (1973). Full and empty fat cells. *Lancet* **ii,** 905.

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