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Symposium on 'Predisposition to obesity: metabolic and/or behavioural factors'

Symposium 1
New insights into the development of obesity: obese genes and the leptin system

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The incidence of obesity is rising rapidly in a number of countries. In the UK, for example, the proportion of adult men and women classified as obese on the basis of a BMI >30 doubled in the decade between 1980 and 1991 (Prentice & Jebb, 1995). Despite the growing problems associated with the epidemic of obesity, current treatment modalities have remained stalled. This is also true of attempts to understand the aetiology of the disorder in terms of the fundamental underlying mechanisms. Much debate in the past has focused on whether the obese are hyperphagic, or have metabolic abnormalities relating to energy expenditure. This has now evolved, however, to the point where the issue is being discussed in terms of whether energy expenditure is falling (because of inactivity) faster than the documented decline in energy consumption in the population (Prentice & Jebb, 1995).

New insights into the regulation of energy balance and the fundamental causes of obesity are rapidly emerging following the recent application of molecular genetics. This paper summarizes these developments, focusing particularly on the biology of the system associated with leptin, the product of the *ob* (obese) gene.

GENETICS OF OBESITY

There is now little doubt that obesity has an underlying hereditable component. The genetic basis to the disorder is complex, however; the probability being that a number of interacting genes are involved (polygenic inheritance). There is clearly also a major interaction with the environment, particularly in relation to food intake and exercise. Work by Bouchard and colleagues (see Bouchard & Pérusse, 1993) has demonstrated that each of the main components of the energy balance relationship has a genetic basis. Thus, energy intake, BMR, the thermic effect of food, and physical activity all have a genetic element. There is also a genetic component to body fat distribution, as well as to total body fat *per se* (Bouchard & Pérusse, 1993).

Our information on a genetic basis to obesity in man comes primarily from studies on populations, families, twins, and from adoption studies (Bouchard & Pérusse, 1993). In

addition, certain inherited disorders such as the Prader-Willi syndrome (which occurs in approximately 1 in 20 000 children), Cohen's syndrome, Carpenter's syndrome and the Bardet-Beadle syndrome are associated with obesity (see Bray, 1991). There are, of course, clear examples in other species of a genetic basis to body fat content, and body composition in general, with major differences in the tendency to fatness between strains. In agricultural animals, for example, certain breeds of pig such as the Pietrain and the Landrace are lean, while the Chinese Meishan has a high body fat content (Trayhurn, 1992).

In attempts to identify certain genes which may be linked to obesity, candidate gene approaches have been employed. Genes such as those encoding the insulin receptor and the GLUT 1 facilitative glucose transporter have been investigated; there is, however, no clear evidence for a linkage between these genes and obesity (Trayhurn, 1992; Weaver *et al.* 1992). A DNA polymorphism in the uncoupling protein gene in brown adipose tissue has been associated with the proneness to increased body fat in a population in Quebec (Oppert *et al.* 1994), and this polymorphism is due to a point mutation in the 5'-flanking region of the gene (Cassard-Doulcier *et al.* 1996). A missense mutation (Trp 64 Arg) in the β_3-adrenoceptor gene has also been linked with a higher body fat content in a recent study in Japanese subjects (Kadowaki *et al.* 1995).

OBESITY GENES IN RODENTS

The clearest link between genes and obesity is evident in laboratory rodents. It has been recognized for many years that there are rat and mouse strains in which obesity results from a mutation in a single recessively-inherited gene (see Bray & York, 1979; Trayhurn, 1984). These animals have been used extensively as experimental models in obesity research and, although many abnormalities have been documented (including an increase in the efficiency of energy utilization, hyperphagia, hyperinsulinaemia, insulin resistance and thermogenic disorders), in each case the primary defect has remained elusive. The application of molecular genetics, however, has led in the past 2 years to the identification of some of these mutant genes. The *ob*, *db*, *fa*, *fat* and *tub* genes, mutations in which are responsible for the obesity of the obese (*ob/ob*) mouse, the diabetic (*db/db*) mouse, the Zucker (*fa/fa*) rat, the fat (*fat/fat*) mouse and the tubby (*tub/tub*) mouse respectively, have each been identified and sequenced (Table 1). The *fat* gene codes for the enzyme carboxypeptidase E (*EC* 3.4.17.10), which is expressed in the pancreas where it is involved in the post-translational processing of proinsulin (Naggert *et al.* 1995). Obesity in *fat* mice is mild and of late onset relative to both the *ob/ob* and *db/db* mutants, and this is also the case with *tub* mice (Coleman & Eicher, 1990). The nature of the *tub* gene and its encoded protein is unknown at the time of writing (Nasto, 1996) since the commercial company (Millennium Pharmaceuticals) which has identified the gene has chosen not to release the information.*

The key developments have been those stemming from the identification by Friedman and colleagues (Zhang *et al.* 1994) of the mutant gene in *ob/ob* mice, these animals being the most extensively studied models in obesity research. The elucidation of the *ob* (obese) gene has disclosed a key regulatory system in energy balance, based on the earlier lipostatic hypothesis (Kennedy, 1953). It has also resulted in renewed interest in the metabolic basis of obesity, offering the potential of new targets for the pharmacological treatment of the

* *Note added in proof.* The identity and expression pattern of the *tub* gene have now been reported (Noben-Trauth *et al.* 1996).

Table 1. *Obese genes identified in rodents*

Gene	Species	Encoded protein	Site of expression	Reference
ob	Mouse	Leptin	White adipose tissue (and brown?)	Zhang *et al.* (1994)
*db**	Mouse	Leptin receptor	Hypothalamus, choroid plexus; wide expression in peripheral tissues (several variants)	Chen *et al.* (1996), Chua *et al.* (1996), Lee *et al.* (1996)
*fa**	Rat	Leptin receptor	Hypothalamus, choroid plexus; wide expression in peripheral tissues	Chua *et al.* (1996)
fat	Mouse	Carboxypeptidase E	Pancreas	Naggert *et al.* (1995)
tub	Mouse	Tubby	Brain, eye, testis	Noben-Trauth *et al.* (1996)
agouti	Mouse	Agouti protein	Ectopic (normally acts on melanocytes; produced by cells in hair follicle)	Klebig *et al.* (1995)

* *db* and *fa* are homologues.

disorder. Recent work has established that the obesity of *db/db* mice, as well as that of the Zucker (*fa/fa*) rat, is caused by mutations in the gene encoding the receptor for the protein product of the *ob* gene (Chen *et al.* 1996; Chua *et al.* 1996; Lee *et al.* 1996).

As well as the identification of the genes responsible for several different forms of recessively-inherited obesity, the basis for the dominantly-transmitted disorder in yellow obese mice has been determined. This type of obesity follows from the ectopic expression of the agouti gene, this gene normally being expressed in cells in the hair follicle with the agouti protein interacting in a paracrine manner with melanocytes (Klebig *et al.* 1995; Manne *et al.* 1995). The ectopic expression of agouti appears to be the result of a mutation in the promoter region, the gene thus coming under the influence of ubiquitous promoters (see Klebig *et al.* 1995; Manne *et al.* 1995).

ob (OBESE) GENE AND LEPTIN

The primary importance of the identification of the mutant gene in *ob/ob* mice lies not in the mutation *per se*, but in the insight that it has provided on a normal regulatory system, as noted earlier. The original report identifying the *ob* gene indicated that it was expressed in white adipose tissue and encoded a protein with a relative molecular mass of 18 000 which did not resemble any known protein (Zhang *et al.* 1994). Subsequent studies have suggested similarities with the family of helical cytokines, which includes interleukin-2 and growth hormone (Madej *et al.* 1995). A putative signal sequence was identified and the mature protein has a molecular weight of approximately 16 000 Da (Zhang *et al.* 1994). It was originally proposed that the *ob* gene product, now termed leptin, acts as a satiety factor (Zhang *et al.* 1994). It was also envisaged that there are feedback signals to the adipocyte, regulating the expression of the gene.

Administration of recombinant leptin to *ob/ob* mice leads to a reduction in body weight,

body fat, and food intake (Campfield *et al.* 1995; Halaas *et al.* 1995; Pelleymounter *et al.* 1995). In addition, there is an increase in energy expenditure and a rise in body temperature, while both blood glucose and insulin levels fall towards normal (Halaas *et al.* 1995; Pelleymounter *et al.* 1995). Thus, a number of the key abnormalities of the *ob/ob* mutant are reversed. In contrast, recombinant leptin does not have a major effect on body weight or food intake in normal mice (Campfield *et al.* 1995; Halaas *et al.* 1995; Pelleymounter *et al.* 1995). This may be, in part, because of a suppression of endogenous leptin production. Importantly, the administration of the recombinant protein to *db/db* mice has no effect on body weight, body fat, or food intake (Campfield *et al.* 1995; Halaas *et al.* 1995), which is consistent with the concept developed from parabiosis experiments that the *db/db* mutant relates to a receptor system for a regulatory protein (Coleman, 1978).

Regulation of ob *gene expression* in vivo

The *ob* gene is expressed within white adipose tissue only in the adipocytes (Maffei *et al.* 1995*a*; Masuzaki *et al.* 1995; Murakami *et al.* 1995; Ogawa *et al.* 1995). It is unclear, however, whether expression also occurs in brown adipocytes. Most reports suggest that there is little or no expression in brown fat compared with white adipose tissue (Frederich *et al.* 1995; Maffei *et al.* 1995*a*; Murakami *et al.* 1995; Murakami & Shima, 1995; Ogawa *et al.* 1995; Trayhurn *et al.* 1995*b*), and that which is observed may reflect contamination or infiltration by white adipocytes. If the *ob* gene is indeed expressed by brown fat cells, then there are potentially important implications for our understanding of the physiological function of leptin, given the quite different roles that the two adipose tissues play in energy metabolism.

There are distinct differences in the level of expression of the *ob* gene between adipose tissue depots. Strong expression is particularly evident in the epididymal and perirenal sites, while expression in the subcutaneous adipose tissue is low (Frederich *et al.* 1995; Maffei *et al.* 1995*a*; Masuzaki *et al.* 1995; Ogawa *et al.* 1995; Trayhurn *et al.* 1995*b*). These variations between depots may reflect site-specific differences in fat cell size, with larger adipocytes exhibiting greater expression of the gene. Such a view would be consistent with the concept of adipocytes signalling the state of energy stores at an individual level, this being integrated into an overall signal for the total body fat of the animal (Trayhurn *et al.* 1995*b*).

Increased *ob* mRNA levels have been reported for several types of obese animal, including *ob/ob* and *db/db* mice, the Zucker rat, and mice made obese through the administration of gold thioglucose or monosodium glutamate (Zhang *et al.* 1994; Frederich *et al.* 1995; Funahashi *et al.* 1995; Maffei *et al.* 1995*a*; Murakami & Shima, 1995; Ogawa *et al.* 1995; Trayhurn *et al.* 1995*b*). Obesity seems to be associated, therefore, with increased expression of the *ob* gene, and with the exception of the *ob/ob* mouse there is also a parallel elevation in circulating levels of leptin (Frederich *et al.* 1995; Halaas *et al.* 1995; Maffei *et al.* 1995*b*). It is, however, not certain whether the increase in *ob* gene expression is simply a consequence of larger adipocytes and the greater total fat mass. In the case of the obese mutants, there may be a continual stimulation of expression through the feedback signals to adipose tissue in the face of a defective gene product or receptor system.

Expression of the *ob* gene is subject to nutritional regulation, as would be predicted of a factor playing a key role in energy balance. Fasting induces a fall in the level of *ob* mRNA, which is rapidly reversed on refeeding, and the circulating level of leptin changes in a

parallel manner to the tissue mRNA (Becker *et al.* 1995; Frederich *et al.* 1995; MacDougald *et al.* 1995; Saladin *et al.* 1995; Trayhurn *et al.* 1995*b*). Insulin stimulates *ob* gene expression, as do glucocorticoids, with the effects of the latter being maintained during chronic treatment (Becker *et al.* 1995; De Vos *et al.* 1995; MacDougald *et al.* 1995; Saladin *et al.* 1995). Exposure to cold has a potent effect on *ob* gene expression, there being a rapid disappearance of the mRNA (Trayhurn *et al.* 1995*a*); cold exposure also leads to a fall in the circulating levels of leptin (L. J. Hardie, J. S. Duncan and P. Trayhurn, unpublished results). The response to cold exposure is likely to relate to the substantial increase in energy expenditure and fatty acid flux that low environmental temperatures induce in small mammals.

The effect of cold exposure on *ob* mRNA levels can be mimicked by the administration of noradrenaline, or the specific β-adrenoceptor agonist, isoprenaline (Trayhurn *et al.* 1995*a*). This suggests that there is a sympathetically-mediated suppression of *ob* gene expression, with the sympathetic system being a key component of the proposed feedback loop to adipocytes. The effects of sympathetic regulation are likely to be transmitted through changes in intracellular cAMP levels.

Cell culture

In addition to *in vivo* studies, cell culture has been used to investigate the factors which regulate the expression of the *ob* gene. The clonal cell lines, 3T3-L1 and F442A cells, have been employed, as well as primary culture of mature adipocytes. The *ob* gene is expressed late in differentiation in adipocyte cell lines, in parallel with the late marker adipsin (Leroy *et al.* 1996). Both insulin and glucocorticoids have been shown to have a stimulatory effect on *ob* gene expression, consistent with the *in vivo* studies (Murakami *et al.* 1995; Leroy *et al.* 1996; Rentsch & Chiesi, 1996). To date, however, the degree of expression of the *ob* gene in clonal cell lines is much lower than that which occurs *in vivo*, despite strong expression of late markers of cell differentiation (MacDougald *et al.* 1995; Maffei *et al.* 1995*a*; Murakami *et al.* 1995; Leroy *et al.* 1996). It would seem that either critical growth factors, or nutrients, are limiting, or that there is some inhibitory factor in the culture medium. In this regard, it is noteworthy that changing the serum in the medium increases the expression of the *ob* gene (MacDougald *et al.* 1995). It is also possible that the cells which are harvested in culture never approach the size of adipocytes *in vivo*, and that a relationship between cell size and *ob* gene expression would mean that the cultured cells do not express *ob* in a substantive way.

LEPTIN RECEPTOR

Following the identification of the *ob* gene and the recognition that leptin is secreted from fat cells, a critical issue has been the location of the receptor system with which the factor interacts. A leptin receptor has been cloned by screening a library from the choroid plexus (Tartaglia *et al.* 1995). The receptor has strong similarities to the class 1 cytokine receptors, particularly the gp130 signal-transducing component of the interleukin-6 receptor, the LIF receptor and the G-CSF receptor (Tartaglia *et al.* 1995). In addition to the choroid plexus, the gene for the leptin receptor was also found to be expressed in the hypothalamus, lungs and kidneys (Tartaglia *et al.* 1995). Its presence in the choroid plexus may relate to the transport of leptin across the blood–brain barrier, with the hypothalamus being the key site

of action of the protein. The presence of leptin receptors in the lungs and kidneys, however, is difficult to rationalize.

The cloning of a leptin receptor has been rapidly followed by the demonstration that the mutant gene responsible for obesity in *db/db* mice relates, as anticipated, to the same receptor (Chen *et al.* 1996; Chua *et al.* 1996; Lee *et al.* 1996). Thus, molecular genetics has shown that the long-held view that the *db* mutation is associated with a receptor for a specific factor in the regulation of energy balance (Coleman, 1978) is indeed correct. Intriguingly, several splice variants of the leptin receptor have been described on the basis of the reverse transcriptase–polymerase chain reaction, with different tissue distributions (Lee *et al.* 1996). One variant (Ob-Rb) is expressed at a high level in the hypothalamus, while several forms are expressed in adipose tissue. The latter suggests that there may be a paracrine role for leptin. The *fa* gene, a mutation in which causes the obesity of the Zucker rat, also relates to the leptin receptor; this gene is a homologue to the *db* gene in mice (Chua *et al.* 1996).

The multiplicity of tissues in which the expression of different variants of the leptin receptor occurs suggests that there are complex and multiple effects of the hormone. This does not preclude the possibility that there is a central target, probably the hypothalamus, for leptin. There is in practice evidence that leptin may interact with the hypothalamic neuropeptide Y system. Both the level of the mRNA for neuropeptide Y, and the concentration of neuropeptide Y itself in defined regions of the hypothalamus, are reduced following administration of leptin (Stephens *et al.* 1995; Bing *et al.* 1996). Since neuropeptide Y is a potent stimulator of food intake and it also affects energy expenditure (Billington *et al.* 1991; Egawa *et al.* 1991; Williams *et al.* 1991), a link between leptin and the neuropeptide Y system is entirely credible (Bing *et al.* 1996). Co-localization studies of leptin receptor mRNA and neuropeptide Y are required to substantiate the proposed interaction between the two regulatory systems; such studies have now been undertaken (Mercer *et al.* 1996).

CONCLUSIONS AND PERSPECTIVE; *ob* GENE AND THE LEPTIN SYSTEM IN HUMAN OBESITY

Considerable advances in our understanding of the regulation of energy balance and the causes of obesity in man are offered by the identification of mutant genes in obese rodents. The cloning of the genes responsible for obesity in *ob/ob* and *db/db* mice has led to the description of the leptin system. Mutations in either the gene coding for leptin or the receptor for leptin, led in rodents to a profound obesity with an early onset. Although many details remain to be established, the general concept of a circulating factor being produced by the adipocytes which signals the extent of the fat stores to the brain is clear. Pharmacological or nutritional modulation of the action of leptin may offer new therapeutic approaches to the treatment of severe obesity.

Mutations paralleling those in *ob/ob* and *db/db* mice and the Zucker rat are likely to be at best rare in humans; certainly, any such mutations will not be relevant to the generality of the obese population. Indeed, studies examining several obese patients have shown that the sequence of their leptin mRNA is identical to that of lean subjects (Considine *et al.* 1995; Hamilton *et al.* 1995). The small number of studies conducted to date suggest that obese subjects do not have a deficiency of leptin, both *ob* mRNA levels in white adipose tissue and the circulating levels of the protein being elevated relative to the lean values

(Hamilton *et al.* 1995; Lönnqvist *et al.* 1995; Maffei *et al.* 1995*b*; Considine *et al.* 1996). The concept of leptin resistance has rapidly evolved, and in this regard there is a distinct parallel with insulin and the aetiology of type II diabetes. However, such a concept should be viewed with caution in that there appears to be a close correlation between BMI and circulating leptin levels (Hamilton *et al.* 1995; Maffei *et al.* 1995*b*; Considine *et al.* 1996). This raises the question of whether leptin resistance is a consequence rather than a causative factor in most human obesity. The extent to which leptin truly represents the 'philosopher's stone' of obesity in man should become evident in the near future.

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