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Food, obesity and non-insulin-dependent diabetes: are there molecular links?

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The concept that a relationship exists between food, obesity and non-insulin-dependent diabetes (NIDDM) is not a new one, but one that has in recent years become more refined. The understanding that body mass reflects to some extent the amount of food eaten must have been present since earliest times. Although Hippocrates noted that 'when more food than is proper is taken, it occasions disease', the specific association between obesity and NIDDM (and other diseases) was made more recently. Indeed, it was the observations of Vague (1947) that clearly defined this relationship, and extended them to include the importance of body fat distribution. It is now apparent that in addition to the contribution of the energy content of food to overall energy balance, food content or type may directly impinge on development of obesity and NIDDM. The present review focuses on three molecules that appear to be central to the food–obesity–NIDDM relationship. These molecules, leptin, tumour necrosis factor (TNF)-α and peroxisome proliferator-activated receptor γ (PPARγ), have been recognized recently to have roles in regulation of body weight, food intake, energy balance and insulin sensitivity. As understanding of these and other molecules increases, new therapeutic strategies for treatment of obesity and NIDDM are becoming apparent.

INTER-RELATIONSHIPS BETWEEN FOOD, OBESITY AND NON-INSULIN-DEPENDENT DIABETES

Food

The consumption of food in excess of requirements (i.e. positive energy balance) must lead to accumulation of stored energy. In mammals, this energy excess is largely in the form of fat, so if a situation of positive energy balance continues for long enough, obesity results. The situation is complicated slightly by differing thermogenic qualities of food type. Carbohydrate and protein are more thermogenic than fat, and thus, per unit energy consumed, dietary fat has a greater propensity to lead to obesity.

Based on a large number of studies, it is likely that dietary components may directly influence insulin sensitivity and insulin output, and hence contribute to an hyperinsulinaemic, insulin-resistant state independent of obesity. High concentrations of diet-derived triacylglycerols and free fatty acids (FFA) may contribute to the insulin-resistant state by promoting insulin secretion by pancreatic β-cells (Milburn *et al.* 1995), altering insulin binding to its receptor (Field *et al.* 1988), promoting hepatic glucose output (Rebrin *et al.* 1995) and impairing insulin signalling (Boden & Chen, 1995; Gumbiner *et al.* 1996;

Ikemoto *et al.* 1996; Kim *et al.* 1996). Similarly, arachidonic and other fatty acid metabolites contribute to the regulation of expression of a number of key proteins involved in adipose tissue metabolism and insulin signalling. These proteins include GLUT 4 (Long & Pekala, 1996), lipid transport proteins (Ailhaud *et al.* 1994), PPARγ (Vidal-Puig *et al.* 1996), lipogenic proteins (Hillgartner *et al.* 1995) including lipoprotein lipase (*EC* 3.1.1.34; Saxena *et al.* 1989), and the gene for leptin (Masuzaki *et al.* 1995).

Obesity

Obesity is a major risk factor for NIDDM, particularly if the excess adiposity is distributed in central (visceral) depots (Björntorp, 1991). Furthermore, visceral obesity is associated with hypertriacylglycerolaemia, which may be in part due to reduced FFA utilization by muscle (Colberg *et al.* 1995).

Fat mass may directly influence appetite and, hence, food intake. Adipose tissue secretes the peptide hormone leptin (Zhang *et al.* 1994), which in animal studies acts as a satiety factor mediating decreased food intake and increased energy expenditure (through an increase in BMR and in activity levels; Campfield *et al.* 1995; Halaas *et al.* 1995; Pelleymounter *et al.* 1995). Obese states are also associated with increased local and/or systemic concentrations of a number of hormones and cytokines known to influence insulin sensitivity. Principal among these are corticosteroids (shown in a number of epidemiological studies to be increased in obesity; Marin *et al.* 1992; Pasquali *et al.* 1993), androgens (shown to be elevated in obese females; Williams *et al.* 1993; Armellini *et al.* 1994) and TNF-α (Hotamisligil *et al.* 1995; Kern *et al.* 1995; Dandona *et al.* 1996). All three molecules directly induce insulin resistance via a number of mechanisms (Rizza *et al.* 1982; Hauner *et al.* 1995; Björntorp, 1996; Hotamisligil *et al.* 1996; Moghetti *et al.* 1996) and provide molecular links between obesity and insulin-resistance states and NIDDM.

In addition, the increased circulating concentrations of triacylglycerols and FFA commonly seen in obese individuals may contribute to insulin resistance and hyperinsulinaemia as outlined previously. Again, elevation of these variables is characteristically associated with visceral obesity. Finally, it has recently become apparent that the ligand-dependent transcription factor PPARγ has a central role in the development of adipose tissue (Tontonoz *et al.* 1994*a*). Ligands for PPARγ promote adipogenesis *in vitro* (Kletzien *et al.* 1992), but paradoxically, the same compounds increase insulin sensitivity *in vivo* (Berger *et al.* 1996). This suggests that similar molecules and signalling pathways may be involved in the regulation of fat mass and insulin action.

Non-insulin-dependent diabetes mellitus

This condition is characterized in its early stages by insulin resistance and hyperinsulinaemia, and in later stages by (more severe) insulin resistance and insulinopaenia. It is commonly associated with obesity, hypertension and dyslipidaemia (syndrome X; Reaven, 1995), and the ontogeny of the metabolic abnormalities, or their cause–effect relationships within the condition, remain ill-defined. There is clear genetic predisposition (presumed polygenic) as a background, but diet and other environmental factors are able to significantly, and to some extent reversibly, modify the disease process. This is amply demonstrated by the improvement in measures of insulin sensitivity seen with weight loss. Insulin resistance, without diabetic-range blood sugar levels, is a common disorder, with the major risk factor or setting for its occurrence being obesity. Finally, the hyperinsulinaemia seen in this spectrum of disorders may in itself contribute to obesity

via a number of mechanisms. Insulin is the classical anabolic hormone, its deficiency inducing weight loss, and its excess inducing weight gain (disproportionately in the visceral depots; Inadera *et al.* 1993). Insulin promotes pre-adipocyte differentiation *in vitro* (Dixon-Shanies *et al.* 1975), and may have effects on food intake and metabolic rate by regulation of leptin production by adipocytes (Cusin *et al.* 1995), and of neuropeptide Y (a neurotransmitter involved in appetite regulation) action in the hypothalamus (Sahu *et al.* 1995).

THREE CANDIDATE MOLECULES

Leptin

Leptin is a peptide hormone secreted by adipocytes. A number of leptin-receptor subtypes have been identified (Tartaglia *et al.* 1995), but the only form with putative signalling capability is predominantly expressed in specific hypothalamic regions involved in weight control (Considine *et al.* 1996*a*; Mercer *et al.* 1996). This supports the concept that leptin forms the afferent arm of a 'feedback loop' between adipose tissue and central areas involved with regulation of appetite, activity and metabolic rate (Campfield *et al.* 1995). More recent work has also demonstrated the importance of the leptin signal in regulation of a number of neuro–endocrine axes, including the hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axis (Barash *et al.* 1996; Ahima *et al.* 1997). In evolutionary terms, the relative absence of leptin in states of underfeeding is likely to be as important as the elevated leptin seen in obesity (Ahima *et al.* 1996). In rodents, low leptin levels prevent ovulation (Chehab *et al.* 1997) and the onset of puberty appears to result from a transient increase in leptin levels (Cheung *et al.* 1997). This may relate to a critical fat mass or nutritional level being reached. Animal studies indicate that leptin is an important regulator of fat mass, as administration of the peptide induces weight loss in leptin-deficient as well as normal rodents (Campfield *et al.* 1995; Halaas *et al.* 1995; Pelleymounter *et al.* 1995; Chen *et al.* 1996; Muzzin *et al.* 1996).

Circulating leptin levels are increased in obesity (Considine *et al.* 1996*b*), giving rise to the concept of 'leptin resistance'. A co-existent relative secretory defect has not been excluded, and the issue is further complicated by the non-linear relationship between blood and cerebrospinal fluid (CSF) leptin concentrations as a function of weight. Thus, in obese individuals CSF leptin levels are relatively low in comparison with blood levels (Schwartz *et al.* 1996*b*). This suggests saturation (or regulation) of the system responsible for transport of leptin into CSF. Further evidence in support of this concept is the observation that diet-induced obese mice develop peripheral, but not central, resistance to leptin (Frederich *et al.* 1995; Van Heek *et al.* 1997).

In states of low body fat such as anorexia nervosa, the low circulating levels of leptin support the concept that fat mass is reflected in leptin levels (Grinspoon *et al.* 1996). Animal studies signify the importance of low leptin as a signal to increase appetite, as the hyperphagia seen in *ob/ob* mice (which have an abnormal and bio-inactive form of the peptide) is reversed with leptin administration. Thus, leptin levels reflect fat mass, and directly influence appetite. To date, the influence of leptin, if any, on food preference has not been investigated. In addition to the effect of leptin on appetite, food intake and nutrition may in turn modulate leptin levels. Fasting reduces (Boden *et al.* 1996) and feeding increases (Saladin *et al.* 1995; Thompson, 1996) leptin levels, although the role of insulin in this regulation remains incompletely established (Saladin *et al.* 1995; Boden *et al.* 1996; Kolaczynski *et al.* 1996; Muscelli *et al.* 1996).

Leptin may also directly or indirectly influence insulin sensitivity and insulin secretion (Larsson *et al.* 1996). Insulin resistance is associated with increased leptin levels independent of body fat mass (Segal *et al.* 1996), and this may be due to the associated hyperinsulinaemia (Utrianen *et al.* 1996). Leptin receptors have been identified on pancreatic β-cells raising the possibility that leptin may directly influence insulin secretion (Kieffer *et al.* 1996). Leptin treatment of obese and insulin-resistant animals results in improvement of both abnormalities (Muzzin *et al.* 1996; Schwartz *et al.* 1996*a*), but whether the improvement in insulin sensitivity is independent of, or secondary to, the reduced fat mass has not been determined. Finally, leptin may be a regulator of fat distribution. Subcutaneous adipocytes express more leptin mRNA than do omental adipocytes (Montague *et al.* 1997), and circulating leptin levels reflect subcutaneous, but not visceral, fat mass (Dua *et al.* 1996). Thus, visceral adiposity may increase with relatively little increase in leptin levels, allowing 'unregulated' visceral obesity to occur. This may predispose individuals to a pattern of fat distribution more likely to be associated with NIDDM.

Tumour necrosis factor-α

TNF-α is a cytokine first characterized by its ability to kill cells. Its actions include regulation of cell division, differentiation and apoptosis, modulation of gene expression and protein production, and involvement in the immune response (Fiers, 1991). The effects of TNF are mediated by a large number of signalling pathways and signalling molecules, including prostaglandins. It is produced by a number of cell types including monocytes, macrophages and adipose cells. Production is increased by exposure to bacterial cell wall (lipopolysaccharide) and decreased by corticosteroids.

Adipose tissue TNF production is increased in obesity. In obese individuals, compared with lean controls, adipose tissue TNF mRNA is increased 2·5-fold (Hotamisligil *et al.* 1995; Kern *et al.* 1995), TNF protein is increased 2-fold (Hotamisligil *et al.* 1995) and circulating TNF levels are elevated up to 3·5-fold (Dandona *et al.* 1996). Furthermore, this overexpression is reversed with weight loss (Kern *et al.* 1995; Dandona *et al.* 1996). Thus, in obesity, adipose tissue-derived TNF may have endocrine, paracrine and autocrine effects.

TNF has numerous actions on adipose tissue which suggest that the cytokine may act as an 'adipostat' and, hence, reduce the tendency toward further weight gain in settings of positive energy balance (Petruschke & Hauner, 1993; Kern *et al.* 1995). TNF induces adipose tissue leptin production and, hence, may reduce appetite and increase metabolic rate (Grunfeld *et al.* 1996). TNF directly induces insulin resistance via multiple mechanisms, including inhibition of insulin-receptor tyrosine kinase activity (Hotamisligil *et al.* 1996), down-regulation of insulin-responsive glucose transporter GLUT4 mRNA (Hauner *et al.* 1995) and, potentially, by down-regulating PPARγ mRNA (Zhang *et al.* 1996*a* see p. 893). TNF has effects which lead to reduced adipose cell volume by promoting lipolysis through effects on lipoprotein lipase and hormone-sensitive lipase (*EC* 3.1.1.3) activity (Fried & Zechner, 1989; Green *et al.* 1994). Finally, TNF has effects which lead to reduced adipose cell number by reducing cell acquisition (by promotion of adipocyte de-differentiation and impairment of pre-adipocyte differentiation; Petruschke & Hauner, 1993) and by increasing cell loss (by induction of pre-adipocyte and adipocyte apoptosis; Prins *et al.* 1997).

In addition to its action as a putative 'adipostat', TNF has been proposed to be a molecular link between obesity and NIDDM (Hotamisligil *et al.* 1993). Thus, as obesity

develops, adipose tissue TNF production increases with a net effect of inducing insulin resistance. This situation, whilst tending to reduce the rate of continued weight gain, has the deleterious effects of increasing circulating lipids, insulin and (potentially) glucose. Other mechanisms may be involved in the relationship between TNF and diabetes. First, TNF reduces insulin secretion (Chen & Wolf, 1996) and second, glucose promotes TNF production by monocytes (Morohoshi *et al.* 1996), and if adipose cell TNF production is similarly regulated, this could lead to elevated TNF levels in diabetes. These findings raise the possibility of intervention, and indeed, in animal studies, therapies aimed at reducing TNF levels have produced improvement in insulin sensitivity (Hotamisligil *et al.* 1993). In contrast, however, human trials of anti-TNF therapy (for diabetes) reported to date showed no improvement in insulin sensitivity despite apparently effective reduction in circulating TNF levels (Ofei *et al.* 1996; Scheen *et al.* 1996).

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ

PPARγ is an 'orphan' nuclear receptor which acts as a ligand-dependent transcription factor (Issemann & Green, 1990). PPARγ functions as an heterodimer with the retinoid X receptor (Kliewer *et al.* 1992), and is highly expressed in adipose tissue (Tontonoz *et al.* 1994*b*). PPARγ is expressed very early in the adipocyte differentiation process (Yeh & McKnight, 1995), and murine cell transfection studies suggest that expression induces determination of stem cells to the adipogenic lineage (Tontonoz *et al.* 1994*b*). Ligands identified to date include 15-deoxy-Δ^{12}, Δ^{14}-prostaglandin J_2 (PGJ2; Forman *et al.* 1995; Kliewer *et al.* 1995), an arachidonic acid metabolite (which may be the endogenous ligand), and thiazolidinediones (Lehmann *et al.* 1995), a class of compounds active as insulin sensitizers, which are under trial for the treatment of NIDDM.

PGJ2 and thiazolidinediones promote differentiation of murine pre-adipocytes and/or pre-adipocyte cell lines (Kletzien *et al.* 1992; Kliewer *et al.* 1995) and human pre-adipocyte differentiation *in vitro* (M. Adams, C. Montague, J. Prins, J. Holder, S. Smith, L. Sanders, C. Sewter, J. Digby, M. Lazar, V. Chatterjee and S. O'Rahilly, unpublished results). We have recently demonstrated that promotion of human pre-adipocyte differentiation by thiazolidinediones is depot-specific, with subcutaneous cells showing a dramatic response and omental cells being refractory. Studies have demonstrated that the relative potency of thiazolidinediones in promoting pre-adipocyte differentiation is similar to their potency in activation of PPARγ receptor constructs and to their potency as insulin sensitizers (Berger *et al.* 1996). Thus, it appears that the molecular mechanisms of the actions of the compounds to promote adipogenesis and promote insulin sensitivity may be shared. The mechanism by which PPARγ activation promotes glucose uptake has not been fully elucidated, but it appears that activation of both GLUT1 and GLUT4 (Ciaraldi *et al.* 1995; Tafuri, 1996) is involved, and the effect is present in skeletal muscle as well as adipose tissue. *In vivo* studies indicate a profound effect of thiazolidinedione treatment on markers of insulin sensitivity (Young *et al.* 1995; Kumar *et al.* 1996; Wasada *et al.* 1996), with significant decreases in insulin, glucose and low-glycated haemoglobin levels, despite modest concomitant weight gain. This paradox has yet to be explained, but our demonstration of the depot specificity of the adipogenic effects of the drugs may indicate that any fat accumulation in response to treatment may occur in the subcutaneous depot, with little resultant impact on insulin resistance.

PPARγ activation may also influence obesity and NIDDM via other mechanisms. Activation of the receptor down-regulates leptin gene expression (Nolan *et al.* 1996; Zhang *et al.* 1996*b*), an effect that may be contributory to the weight gain seen with treatment. In

addition, thiazolidinediones antagonize many of the effects of TNF on adipose cells (Szalkowski *et al.* 1995), raising the possibility that part of their insulin-sensitizing effect may be via this mechanism. Finally, PPARγ gene expression is regulated by nutrition, obesity and insulin (Vidal-Puig *et al.* 1996), further evidence for molecular links between food, obesity and NIDDM.

CONCLUSIONS

Whilst there is no doubt that food, obesity and NIDDM are strongly and intimately related, clear cause–effect links at the molecular level have only recently begun to be established. The present review has concentrated on three molecules that are currently subject to considerable research effort, largely because of their role in this three-way relationship.

As our understanding of the role of these and other molecules increases, it is hopeful that new strategies for treatment of obesity and NIDDM may evolve, strategies that may involve pharmacological and/or dietary manipulation.

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