SA26

Effect of diet–gene interactions on energy balance

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The prevalence of obesity in the Nordic countries has increased from about < 1% shortly after the second world war to 15–20% today. As the gene pool has not changed the causes are entirely environmental, and due to factors such as increased food availability, low levels of physical activity, and a diet promoting passive overconsumption through a high content of fat, sugar-rich soft drinks, beer and wine.

However, there is good evidence to suggest that those becoming obese possess a genetic susceptibility that makes their energy balance more vulnerable to the environmental stimuli.

Genetic epidemiological studies have found that overweight subjects with a familial history of obesity are more likely to gain weight on a high-fat diet than those without this background.

This effect might be mediated through a preference for fat, a weaker satiating power of fat, a blunted thermogenic effect, and an impaired capacity to mobilise and oxidise fat during periods of negative energy balance.

Experimental studies in formerly obese subjects have found that their 24 h energy expenditure and fat oxidation adjust more slowly to increases in dietary fat content than matched never-obese controls, factors that are likely to contribute to the increased susceptibility to weight gain and resistance to weight loss. An impaired fat oxidation capacity in formerly obese subjects has been traced to oxidative enzyme systems in skeletal muscle, which are not explained by differences in physical activity. The genetic counterparts of these findings are unknown. However, in this context the peroxisome proliferator-activated receptor-γ (PPAR-γ) gene is interesting. This gene produces two proteins, one of which, PPAR-γ, is found in adipose tissue, where it plays a key role in the regulation of adipocyte differentiation. Activation of these receptors causes recruitment of pre-adipocyte fibroblasts to form mature cells, which then accumulate fat. The endogenous PPAR-γ ligands are fatty acids, eicosanoids and prostaglandins, which suggests the possibility that hyperplasia might be induced (and inhibited) by dietary factors, such as specific fatty acids (trans fatty acids, CLA, etc.). The importance is supported by reports that polymorphisms and mutations in the PPAR-γ gene or the encoding region of the gene have been associated with obesity and diabetes.

Single mutations in the genes encoding for the adipose tissue hormone leptin and its hypothalamic receptor exert a very powerful effect on energy balance in humans through a marked hyperphagia, which is difficult to limit unless there is a shortage of food. By contrast, genes that are expressed only when the lifestyle is sedentary, and the diet is fat and energy dense, are more likely to be operating in most obese individuals. High levels of plasma leptin, adjusted for body fat mass, are associated with resistance to weight loss during marked energy restriction, which might be an expression of hypothalamic leptin resistance or secondary to a low fat oxidation (Verdich *et al.* 2001).

The PPAR-γ2 gene also seems to be important for fat oxidative responsiveness during weight loss and propensity to regain weight after a diet-induced weight loss (Nicklas *et al.* 2001), and the Pro12Aala variant of this gene may influence the susceptibility for weight regain and obesity. Similarly, genes, and perhaps also environmental factors controlling the proton leak and uncoupling protein (UCP) expression, are important determinants of fat oxidation capacity, and influence weight loss outcome during energy restriction (Harper *et al.* 2002).

Ongoing and future research in genes and variants with effect on appetite regulation and metabolic efficiency during weight gain, and weight loss, should give us a better insight into body weight regulation in obesity, and perhaps make it possible to design individualised diets based on personal genetic make-up.

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SA27

Uncoupling proteins and thermogenesis

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The concept that changes in adaptive thermogenesis can play an important role in the regulation of energy balance has been much debated. There is strong evidence from animal studies in support of such a proposition, particularly from work on rats consuming a cafeteria diet (diet-induced thermogenesis) and from genetically obese rodents (*ob/ob* and *db/db* mice and *fa/fa* rats). The extent to which thermogenesis is a component of energy balance in humans is still unclear, however, but there is little doubt that any role is at best minor. Several mechanisms for adaptive thermogenesis have been considered, but the only one for which there is compelling evidence is that associated with brown adipose tissue. Brown fat is specialised for heat generation through a regulated dissipation of the mitochondrial proton gradient. This is effected by a 32 000 molecular weight uncoupling protein (now termed uncoupling protein-1, or UCP1) located in the inner mitochondrial membrane. UCP1, which exists in active and inactive forms, is unique to brown fat and as such differentiates the two forms of adipose tissue (brown and white); it also appears to be restricted to mammals. A family of mammalian uncoupling proteins has now been identified – UCP1, UCP2, UPC3, BMCP1 (and perhaps UCP4) – with homologues in birds and plants. UCP2 has a wide tissue distribution, but is found particularly in white adipose tissue and cells of the immune system, while UCP3 is primarily expressed in skeletal muscle. Although these proteins were initially thought to act as uncouplers in a manner analogous to UCP1, it is increasingly clear that this is not the case. UCP2 and UCP3 may in practice be involved in lipid oxidation or play a role in anti-oxidant defence. A role for UCP1 and for brown adipose tissue as a locus for adaptive thermogenesis in relation to energy balance, as well as in thermoregulation, in rodents is well established. However, the extent to which brown fat thermogenesis normally occurs in adult humans remains problematic. Nevertheless, UCP1 is present in certain adipose tissue depots throughout life and increased levels (indicating activation of brown fat) are evident in patients with pheochromocytoma.

SA28

Fat metabolism in obesity and type 2 diabetes mellitus

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Obesity and type 2 diabetes frequently occur together, indicating that these conditions may share common pathological mechanisms. Increased circulating free fatty acid (FFA) concentrations have been indicated as an important risk factor in the etiology of both conditions and in the predisposition towards other chronic diseases like cardiovascular disease. An increased delivery of FFA to the liver may underlie many metabolic disturbances in obesity and type 2 diabetes like a decreased insulin binding to hepatocytes, a diminished insulin clearance, an impaired insulin-mediated suppression of hepatic glucose output and an increased very low density lipoprotein (VLDL)-triacylglycerol output. Additionally, it has been proposed that chronically elevated FFA concentrations may reduce insulin secretion in type 2 diabetes.

On one hand, the elevated FFA concentrations may result from an increased release from the expanded adipose tissue stores. There are indications that a decreased β-adrenergically mediated lipolysis may play an important role in the development or maintenance of increased triacylglycerol stores within (subcutaneous) adipose tissue (Blaak *et al.* 1994). This catecholamine resistance may be related to a decreased number or function of β_2-adrenoceptors, a dysbalance between α_2 and β-adrenoceptor function or a defect in the action of hormone-sensitive lipase. Additionally, it is hypothesized that defects in HSL translocation or perilipin function (proteins that coat the lipid droplet) may contribute to an impaired lipolytic response.

On the other hand, defects in skeletal muscle FFA uptake and oxidation exist in obesity and type 2 diabetes mellitus. Previously, we have shown a diminished skeletal muscle uptake of FFA in abdominally obese subjects during β-adrenergic stimulation (Blaak *et al.* 1994). Additionally, using the forearm balance technque in combination with infusion of the stable isotope tracer [U-^{13}C]-palmitate, it was shown that the uptake and oxidation of FFA were diminished in skeletal muscle of type 2 diabetic subjects during postabsorptive conditions and during β-adrenergic stimulation (Blaak *et al.* 2000; Fig. 1). The impairments in FFA utilization also extended to a condition of moderate intensity exercise (at 50% of $V_{O_2,max}$), where it was shown (using the stable isotope tracer [U-^{13}C]-palmitate) that the oxidation of plasma-derived fatty acids was significantly lower in type 2 diabetic subjects compared with controls, matched for body composition and maximal aerobic capacity (Mensink *et al.* 2001). This impaired capacity to take up and oxidize FFA during exercise persisted after weight reduction in type 2 diabetic subjects (Blaak *et al.* 2001) and was already present in obese subjects with impaired glucose tolerance (prediabetic state; Mensink *et al.* 2001; Fig. 2), indicating that these defects may play a primary role in the development of type 2 diabetes mellitus. We showed in a recent study that lifestyle intervention (a combined diet–physical activity intervention) can prevent a further deterioration of disturbances in (plasma free) fatty acid oxidation in subjects with IGT, which may be one of the mechanisms underlying the beneficial effect of a lifestyle-intervention programme on glucose tolerance and insulin resistance.

Several mechanisms may be responsible for the impaired capacity of skeletal muscle to take up or to oxidize plasma fatty acids. There are indications that muscle lipolysis may be increased in type 2 diabetic subjects (Blaak *et al.* 2000). An increased lipolysis may flood the muscle with FFA, thereby decreasing the blood–tissue concentration gradient, which is one of the primary determinants of plasma fatty acid uptake and oxidation. Furthermore, the biochemical characteristics of skeletal muscle for the capacity to take up or utilize FFA, like fibre type, oxidative enzyme capacity, the activity of carnitine palmitoyl transferease and the expression of various fatty acid binding proteins, may be altered in obese or obese type 2 diabetic subjects (Simoneau *et al.* 1999; Blaak *et al.* 2000). Further studies are required to elucidate the role of the disturbances in fatty acid metabolism in insulin resistance in obesity and type 2 diabetes mellitus and to obtain more information on the underlying mechanisms.

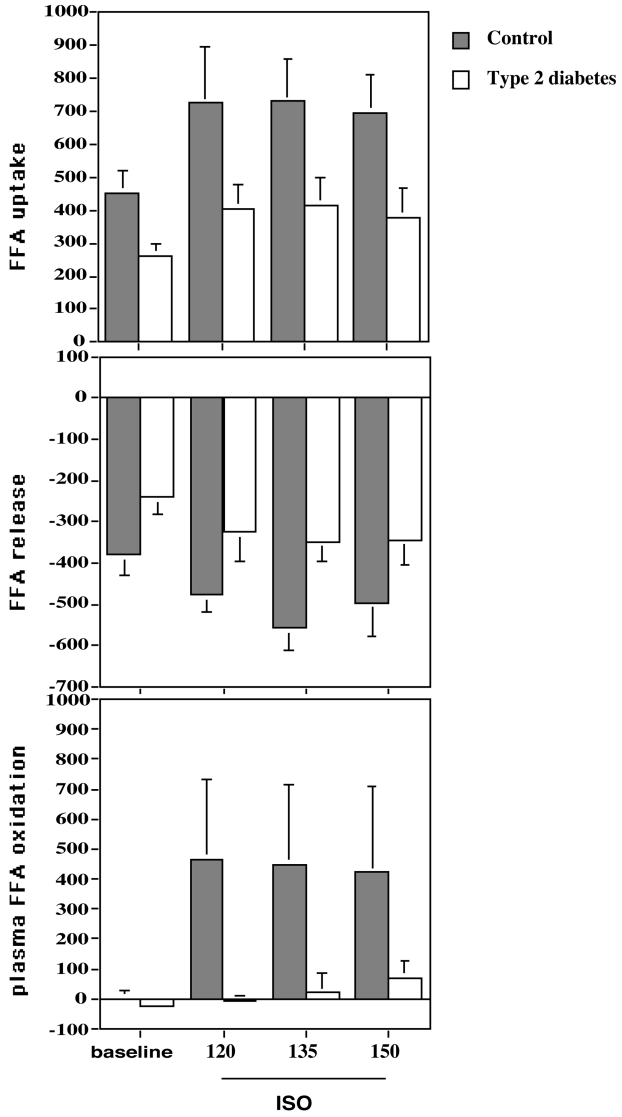

Figure 1. Forearm skeletal muscle free fatty acid (FFA) uptake, release and oxidation (in nmol per 100 ml forearm tissue per min) during baseline and β-adrenergically stimulated conditions (non-selective β-agonist isoprenaline, ISO) in control and type 2 diabetic subjects. For FFA uptake and release: $P < 0.05$ for ISO effect and for group effect. In plasma FFA oxidation, $P < 0.05$ for ISO effect and interaction of group × ISO effect (mean ± S.E.M.), adapted from Blaak *et al.* (2000).

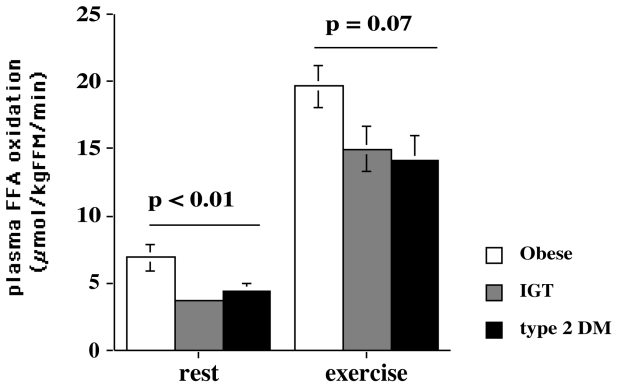

Figure 2. Plasma-derived fatty acid oxidation in obese, obese subjects with impaired glucose tolerance (IGT) and type 2 diabetic subjects during rest and moderate intensity exercise (means ± S.E.M.), adapted from Mensink *et al.* (2001).

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SA29

Interaction of habitual diet and physical activity in weight control

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Weight control is a reflection of energy balance (EB) and body composition. The maintenance of EB involves adaptations in physiology and behaviour. In turn, behaviour represents approximately 20–45 % of energy expenditure (EE) but 100 % of energy intake (EI). The interaction between these components is far from fully understood. Initially, the importance of habitual diet for weight control, and the impact of physical activity on EI will be considered separately. It is widely agreed that the macronutrient composition of the diet that people habitually consume has implications for EB and therefore for weight control. A high intake of dietary fat is a potent risk factor for body weight gain, but the relationship does not constitute a biological inevitability. There is considerable individual variability in responses to similar dietary intakes. Analysis of data bases such as the DNSBA (1990) indicates the existence of both lean and obese high fat consumers. This observation led to studies on individuals with similar BMIs but quite distinctive dietary patterns. These different consumers have been referred to as behavioural phenotypes. These high- and low-fat phenotypes (young adult males) have significantly different energy and fat intakes but similar BMIs. They display differences in RMR, RQ, resting and sleeping heart rates, plasma leptin levels, DIT responses to fat and CHO, control of meal size, tendency to passively overconsume fat, and hedonic responses to food. They do not differ in sensory responsiveness to fatty substances, energy cost of exercise and daily level of physical activity (though HF show a slight increase in the amount of time in sedentary activity). Our initial assumption was that the differences in EI (foods selected and eaten) of these phenotypes was countered by metabolic adjustments (i.e. thermogenesis). At the present time it has not been possible to detect the nature of this adjustment, although sleeping metabolism is a promising component. However, these phenotypes appear to achieve energy balance through quite distinctive profiles of behaviour and metabolism. A first attempt to genotype these groups has failed to find an association with either of two polymorphisms of the galanin-1 receptor.

In contrast to the HF and LF phenotypes, other groups can be identified who have similar habitual diets (> 43 % fat, > 120 g fat day^{-1}) but with high or low BMIs. These are the obese susceptible or resistant individuals. These individuals have been identified within databases and in the local community. Ultimately their characterization will involve measurement of all components of EB along with body composition. This investigation is at an early stage but a preliminary finding is that one difference between these groups (susceptible and resistant) may reside in the hedonic response to food and the impact of this on satiety.

More than 25 years ago it was argued that the driving of EI by EE constituted the basis for a mechanism controlling appetite. We have investigated this by imposing no (NEX), moderate (MEX) or high (HEX) exercise loads on groups of men and women, and monitoring changes in EI and activity. Initially there is a loose coupling between activity and EI with no immediate increase in either hunger or food intake to exercise loads of up to 5–6 MJ per day. Over periods of up to 16 days partial compensation occurs so that increased EI accounts for approximately 30 % of the increase in EE. There is evidence that it takes a considerable period of time for EI to adjust to elevations in EE, and that there is substantial individual variability in the time course of this process. Indeed, individuals in our studies can be divided into compensators and non-compensators. This feature may well help to explain why exercise is effective for inducing weight loss in some people but not in others. The physiological and behavioural characterization of these types of individuals is currently ongoing. However, in addition to the direct impact of physical activity on EB, habitual exercise can enhance the signalling sensitivity of the satiety control system and therefore adjust appetite control by improving meal to meal regulation.

The interaction between diet and exercise manipulations was examined in a study in which NEX and MEX treatments were combined with enforced high and low fat dietary regimes. Adaptation was observed in opposition to the direction of the perturbation in EB. Adaptation was stronger to negative than to positive energy balances. Enforced sedentariness (experimental suppression of physical activity in a human calorimeter) is not compensated by a reduction in EI, and therefore generates an immediate positive energy balance leading to weight gain. However, changes in body weight in response to changes in physical activity and diet are complex and can only be fully understood in the light of adjustments in body composition and the cost of energy storage.

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SA30

Genetics of obesity

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Recent advances in understanding the monogenic forms of human obesity have occurred at a rapid rate. Although rare, congenital leptin deficiency is now recognized as a treatable form of severe obesity. Mutations in the melanocortin 4 receptor are responsible for up to 6 % of cases of early-onset severe childhood obesity. We are also gaining increasing understanding of the mechanisms of obesity associated with mutations in pro-opiomelanocortin, prohormone convertase 1 and the leptin receptor. In addition, the genes underlying several forms of the Bardet-Biedl syndrome and that responsible for Alstrom syndrome have recently been cloned. In the case of these complex pleiotropic syndromes, the link between the precise gene involved in the obese phenotype is still obscure. In parallel with other human quantitative disorder, such as those affecting blood pressure and plasma glucose, dissecting the genetic basis for common obesity has been much more challenging. However, several genome-wide scans have provided applicated evidence for the involvement of particular chromosomal regions and it is only a matter of time before the genetic variants in those regions contributing to human variation in body fat mass will be identified.

SA31

From brown fat and obesity to cytokines in the brain

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The aim of this presentation is to illustrate how discoveries in one area of physiology may impact on other, seemingly unrelated fields, and in turn depend on multidisciplinary approaches.

Many animals and humans maintain body weight and body energy content within fairly close limits over long periods of time, lending support to the proposal that energy balance is regulated. Disruption of this regulation, due to a sustained but modest imbalance between energy intake and energy expenditure, leads to the conditions of obesity (positive energy balance) or cachexia (wasting). My research with Mike Stock demonstrated that rodents can adjust energy expenditure in response to increases in food intake through activation of diet-induced thermogenesis (DIT). DIT is highly dependent on age, genetic background and environmental factors, and in rodents results from sympathetic activation of brown adipose tissue (BAT) where heat is generated through the presence of the mitochondrial uncoupling protein (UCP). This process is regulated primarily within the hypothalamus, which responds to neural and humoral afferent signals, is influenced by endocrine systems most notably the hypothalamic-pituitary-adrenal axis and insulin and regulates the activity of the sympathetic nervous system as well as appetite.

Defective DIT in rodents leads to obesity, while excessive activation of brown fat thermogenesis leads to body weight and energy loss. We demonstrated the importance of brown fat thermogenesis in several protocols of experimentally induced cachexia including infection, injury and inflammation and cancer.

The relevance of DIT and brown fat to the development of obesity in adult humans is questionable, but may be important in cachectic status since BAT is markedly activated in cachectic children and the metabolic rate induced by an inflammatory/immune stimulation (typhoid vaccine) is prevented by β-adrenoceptor antagonists.

Pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNFα tumour necrosis factor α (TNFα1) have been identified as important mediators of cachexia. They inhibit appetite and feeding, stimulate thermogenesis and lead to depletion of fat and protein stores. These cytokines have local actions on adipose tissue, but are also synthesised and act within the brain (particularly within the hypothalamus), and in animals cause fever and hypermetabolism via the neuropeptide CRF. More recently we demonstrated that the hormone leptin, an important mediator of energy balance regulation, inhibits food intake and stimulates energy expenditure partly through induction of IL-1 in the hypothalamus. Mice lacking IL-1 or IL-6 show modest, maturity onset obesity, suggesting a role for these cytokines in normal physiology. Indeed leptin, like IL-6 and CNTF signals through the gp130 receptor family, and on the basis of some of its actions may be considered a cytokine rather than a hormone.