Leading article

Divergency of leptin response in intestinal inflammation

The identification of the *obesity* (*ob*) gene in 1994 signalled a new era of appetite research and for many brought hope for a quick and simple cure for obesity.¹ The biotechnology company Amgen, who have paid \$20 million for the rights to the gene product, must also hope that the *ob* protein will fulfil its promise as an effective appetite suppressant and treatment for obesity. At the other end of the weight spectrum, investigators have also examined the role of this protein in the metabolic response to starvation, and its potential role in the anorexia and weight loss that frequently accompany chronic inflammatory and infectious diseases.

The protein product of the *ob* gene, *ob* protein or leptin (from the Greek *leptos* meaning thin), is secreted into the circulation by adipose cells. A rising concentration of circulating leptin with increasing adiposity is proposed to serve as a negative feedback signal to the hypothalamus of the brain, resulting in decreased food intake, increased energy expenditure and return of body weight towards a predetermined set point. The key physiological role of leptin in the control of energy balance has been demonstrated in mice which have a mutation in the *ob* gene and lack functioning leptin (*ob/ob* mice), or have a mutation in the leptin receptor and are resistant to the action of circulating leptin² (*db/db* mice). These mice develop extreme obesity from an early age as a result of hyperphagia and a reduction in energy expenditure. Administration of recombinant leptin leads to decreased food consumption and weight loss in both normal and *ob/ob* mice, but not *db/db* mice.^{3,4} Leptin deficiency or resistance has also been identified as a cause, albeit extremely rarely, of extreme obesity in humans.^{5,6}

In healthy individuals, starvation results in a reduction in fat mass and a fall in circulating leptin concentrations. The normal physiological response during recovery from a period of starvation is a refeeding phase in which there is an increased drive to eat. Calorie intake during refeeding corrects the energy deficit and returns fat stores and body weight to the set point. It is thought that the drive to eat may be mediated, at least in part, by the fall in leptin concentrations.

Inflammatory bowel disease (IBD) is often associated with notable weight loss. Of adults attending an IBD clinic, one fifth weighed less than 85% of their ideal body weight.⁷ However, failure to gain weight has its greatest impact in children and adolescents with IBD. In 20–30% of these patients, it causes severe impairment of linear growth and delayed onset of sexual maturation with deleterious consequences which may continue into adult life.^{8–10} Prolonged anorexia has been identified as the single most important aetiological factor and intensive, prolonged nutritional support will restore weight and growth velocity to expected values.^{11,12} In patients with active IBD, the presence of continuing anorexia despite weight loss implies a failure of the normal compensatory responses which operate after a period of reduced feeding.

The anorexia and weight loss that occur in IBD and other chronic inflammatory conditions have been attributed to increased secretion of several pro-inflammatory cytokines. Exogenous administration of tumour necrosis factor α (TNF- α) and interleukin 1 (IL-1) has been shown to induce

anorexia and weight loss in animals.^{13,14} Mice engrafted with Chinese hamster ovary cells genetically engineered to produce TNF- α and IL-6 experience profound anorexia and wasting.^{15,16} Furthermore, administration of an IL-1 receptor antagonist to animals with colitis partially reverses the anorexia and weight loss that occur in untreated animals.¹⁷ Though evidence has mounted that inflammatory diseases mediate energy and weight loss through their associated cytokines, the mechanisms are unknown. Recently, investigators have set out to determine the role of leptin in the anorexia associated with IBD. The hypothesis in all of these studies has been similar: pro-inflammatory cytokines release leptin from adipose tissue leading to increased plasma concentrations which are inappropriately high for the percentage fat mass. Increased circulating leptin concentrations would then signal through the hypothalamus and lead to decreased appetite and food intake. In hamsters, administration of TNF- α and IL-1 leads to increased expression of leptin mRNA in adipose tissue associated with a 30% reduction in food intake. Furthermore, there was a strong inverse correlation between leptin mRNA expression and food intake.¹⁸ In fasted mice, administration of IL-1 and TNF increased plasma leptin concentrations in a dose related manner. TNF was the most potent and increased leptin concentrations to levels above those seen in the fed state.¹⁹ The effects were specific for these cytokines because IL-10 and IL-4, which generally exhibit anti-inflammatory characteristics, had no effect on serum leptin concentrations. In humans, infusion of TNF has also been shown to release leptin, resulting in a 60% increase in serum leptin concentrations.²⁰

Experiments in humans with IBD and animal models of intestinal inflammation have produced more variable results. Rectal administration of trinitrobenzenesulphonic acid (TNBS) in ethanol to the rat produces a self-limiting distal colitis which causes notable suppression of food intake and loss of weight. The TNBS model shares some of the clinical and pathological features of human Crohn's disease, notably, skip-lesions, mucosal ulceration and "cobblestoning", and transmural inflammation with granulomas, Langhan's type giant cells, and an increase in mucosal IL-1 concentrations. Barbier *et al* have shown that on the first day after induction of colitis there was a three- to fourfold increase in plasma leptin concentrations compared with controls. Associated with the increase in plasma leptin, there was significant suppression of feeding and food intake dropped to only one third of that of controls. Furthermore, there was a significant correlation between the degree of intestinal inflammation, assessed macroscopically and by measurement of intestinal myeloperoxidase activity, and plasma leptin concentrations. At five days after induction of colitis, food intake had returned to control values. At this time point, animals had lost about 10% of their body weight and plasma leptin concentrations had fallen to below control values. Indomethacin induced ileitis and administration of endo-

Abbreviations used in this paper: IBD, inflammatory bowel disease; TNF, tumour necrosis factor; IL, interleukin; TNBS, trinitrobenzenesulphonic acid.

toxin also stimulated release of leptin, suggesting that the leptin response is not specific for the stimulus used to induce inflammation. The results in the TNBS–colitis model suggest that hyperleptinaemia in the early stages of colitis contributes to the suppression of feeding and subsequently as leptin concentrations fall, there is an associated and appropriate increase in feeding.²¹

In patients with IBD, plasma leptin concentrations have not been found to be increased. In 74 patients with either Crohn's disease or ulcerative colitis, we found that the strong positive correlation between percentage body fat and plasma leptin concentrations was preserved. Leptin concentrations were not, however, increased compared with healthy controls. Plasma concentrations of soluble TNF-receptor were increased in the patients with IBD but were not correlated with leptin concentrations. A subset of patients with IBD had a severe relapse of their disease, associated with anorexia, weight loss and an increase in inflammatory markers. Plasma leptin in these patients was similar to healthy and quiescent disease controls.²² Similarly, plasma leptin concentrations were the same as healthy controls in children with IBD.²³

The simple conclusion to these findings is that the leptin response to inflammation is different in rodents and humans. However, this is not the case because infusion of TNF has already been shown to induce release of leptin in humans.²⁰ The findings in the TNBS–colitis model may reflect a rapid adaption of the leptin response to inflammation with a transient increase in release during acute inflammation. A second possibility is that the severity of the inflammatory process influences leptin release. Intrarectal administration of TNBS in rats induces a severe distal colitis with extensive ulceration and sometimes full thickness bowel necrosis. The severity of the response is highly reproducible and associated with a notable reduction in food intake and loss of weight. It is unlikely that the severity of inflammation in any of the human IBD studies was comparable with that seen in this rodent model. This hypothesis would be supported by studies in patients after major surgery, in whom plasma leptin concentrations are raised for the first two days postoperatively, when there is usually a notable systemic inflammatory response.²⁴

In summary, these studies suggest that severe acute inflammation may induce release of leptin. Raised plasma concentrations would provide inappropriate signals to the hypothalamus regarding peripheral fat stores and result in inhibition of feeding. However, anorexia also frequently occurs with inflammatory disease when plasma leptin concentrations are normal or even reduced, suggesting dysregulation of other parts of the feeding system. Preliminary experiments suggest that alterations in hypothalamic neuropeptide Y, corticotrophin releasing factor and serotonergic neurones, may occur independently of leptin and contribute to the anorexia associated with inflammatory disease.^{25–26}

The leptin deficient *ob/ob* mouse is not only obese but also has neuroendocrine abnormalities similar to those of starvation including infertility and changes in the adrenal and thyroid axes. In both *ob/ob* mice and food restricted animals these changes are partially reversed by administration of exogenous leptin, suggesting that regulation of the neuroendocrine system during starvation could be a major physiological role of leptin.²⁷ Nutrition also has a well recognised influence over pubertal development and the attainment of a critical body weight and adipose tissue mass have been proposed to determine the onset of puberty. Experiments in mice suggest that leptin is the signal that informs the brain that energy stores are sufficient to support the metabolic demands of reproduction, and may be a major determinant of the timing of puberty.²⁸ In

this context, leptin may mediate the delay in puberty seen in adolescents with IBD. As discussed previously, reduced food intake in patients with IBD seems to occur independently of a leptin response. Anorexia leading to weight loss and reduced fat mass would in turn lead to a fall in plasma leptin concentrations. It is possible that in adolescents, a fall in leptin concentrations may be sufficient to result in inhibition of reproductive function.

In summary, plasma leptin concentrations in inflammatory disease are variable and may be dependent on the severity and chronicity of the inflammatory process. Depending on the response, leptin may have quite different systemic effects at various stages of the inflammatory process.

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