Molecular Basis of Disease-Related Anorexia

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MECHANISM OF WEIGHT LOSS IN CHRONIC DISEASE

Weight loss is a common accompaniment to many chronic inflammatory conditions, cancer and acquired immunodeficiency syndrome (AIDS). The potential causes of weight loss include reduced energy intake secondary to loss of appetite (anorexia), increased energy expenditure or in the case of gut inflammation, malabsorption of nutrients from the inflamed gut. Of these, reduced energy intake has been identified as the single most important factor leading to weight loss in inflammatory conditions and cancer. Early studies suggested that resting energy expenditure was increased in patients with AIDS and weight loss with wasting could be attributed to this. Further studies have shown however that total energy expenditure (the key determinant of the energy balance equation) is lower than expected in patients with AIDS even at times of rapid weight loss (1). Records of food intake showed that energy intake was significantly reduced during periods of rapid weight loss compared with periods of stable weight (Fig. 1). Thus, this study clearly showed that intake rather than total energy expenditure is the key determinant of energy balance in these patients. In contrast to results from some of the animal models of cancer, a reduction in energy intake is also thought to be the major mechanism leading to weight loss in patients with cancer. Loss of appetite was present in 85% of patients and was the second most frequent symptom in almost 300 consecutive cancer patients admitted to one unit (2). The energy intake of patients with cancer and weight loss has been documented from 24-hour dietary recall and shown to be markedly reduced at only about 50% of predicted values (3). However, it is accepted that the cachexia-anorexia syndrome also involves metabolic and immune changes and is associated with hypertriacylglycerolemia, lipolysis, and depletion of skeletal muscle. This profile differs from that observed during simple starvation, and the cancer-cachexia syndrome involves both anorexia and changes in metabolic pathways such as upregulation of the ubiquitin-proteasome catabolic pathway. This review will focus on the mechanisms of anorexia associated with cancer and inflammatory conditions.

CONSEQUENCES OF ANOREXIA

In the short-term anorexia might constitute an adaptive strategy in fighting infectious disease. For instance, forced feeding of mice during acute bacterial infection
FIG. 1. Total energy expenditure per kilogram (left hand panel) and energy intake per kilogram (right hand panel) in men with HIV infection who were losing weight rapidly (≥ 3 kg/month), slowly (<3 kg/month), or maintaining a stable weight. The reduction in total energy expenditure during weight loss could be attributed primarily to a reduction in expenditure that was related to activity. Adapted from Macallan et al. (1).
reduced survival time and increased mortality whereas food deprivation increased survival (4,5). Although beneficial in the short-term, a prolonged reduction in food intake will deplete body fat and protein reserves and lead to undernutrition and significant loss of body weight. Consequences of these deficits are compromised immunity, decreased wound healing and suboptimal response to therapeutic intervention. In children, reduced food intake and undernutrition leads to growth retardation and delayed puberty. In addition, extreme inanition may be a direct cause of mortality for some patients with advanced cancer and wasting.

MECHANISMS OF ANOREXIA

The Acute Phase Response and Anorexia

The acute phase of inflammation involves both anorexia and fever. Several analyses have suggested a linkage between meal size and body temperature and a number of studies have explored whether temperature changes are causal to anorexia in acute inflammation. Rats with colitis induced by intrarectal administration of trinitrobenzenesulfonic acid (TNB) develop anorexia that results specifically from a reduction in meal size. Although TNB-treated rats were hypothermic on the day of treatment, no other body temperature changes were noted (6). Injection of lipopolysaccharide (LPS) also reduced food intake and in addition elevated body temperature, but these two effects were not correlated temporally (6). Furthermore, administration of an antipyretic drug, sodium salicylate, to endotoxin-injected animals lowered rectal temperatures to control levels, but food intake was still suppressed (7). These findings clearly indicate that anorexia associated with the acute inflammatory response is not secondary to fever. Fatigue and muscle weakness are common symptoms of infectious and inflammatory diseases and reduced food intake might be due to reduced locomotor activity and reduced ability to gain access to food. In rats with intestinal inflammation, water intake is similar or increased compared with controls, suggesting that the reduction in food intake is not caused by nonspecific malaise or reduced locomotor activity that would also be expected to lower water intake (8,9). Aubert et al. have also provided evidence that difficulty in moving does not play a major role in anorexia during disease (9).

Pro-Inflammatory Cytokines and Anorexia

Experimental studies have implicated the pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α, previously called cachectin), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interferon-γ (IFN-γ) in causation of anorexia in these disease states. These cytokines may be derived from host inflammatory cells or in the case of malignancy, the tumor cells themselves (10). One of the cytokines secreted by tissue macrophages after injection of LPS is IL-1β. Intraperitoneal administration of IL-1β induces a reduction in calorie intake and a relative increase in carbohydrate ingestion when rats are given a choice of macronutrients, thus mimicking the effects...
of LPS (11). Mice, which are deficient in IL-1β, are completely resistant to the development of anorexia after injection of turpentine, which causes localized inflammation and tissue injury and is normally associated with anorexia (12). In contrast, Fantuzzi et al. found that food intake was normally suppressed in IL-1β-deficient mice after administration of LPS (13). They concluded that either IL-1β is not essential for the in vivo systemic response to LPS or that its role can be fulfilled by other cytokines with overlapping activities. Similar conclusions were made by Leon and co-workers who showed that intraperitoneal injection of LPS induced similar responses in IL-1 type 1 receptor wild-type and knockout mice (14). In contrast, knockout mice were resistant to the effects of turpentine, supporting a role for this receptor in the anorectic response to local inflammation. In rats with TNB-induced colitis, administration of an IL-1 receptor antagonist significantly increased food intake toward normal (Fig. 2), without affecting intestinal inflammation, suggesting a major role for IL-1 in the suppression of food intake in this model (15,16). Oldenburg et al. have suggested that the influence of IL-1 on food intake is mediated, at least in part, through IL-6 (17). In rats with a turpentine abscess, IL-1 receptor blockade reduced plasma concentrations of IL-6 and increased body weight and food intake. Administration of anti-IL-6 antiseraum had the same effect on food intake and body weight. Combined blockade of IL-6 and IL-1 receptor did not result in a further sparing of body weights or improvement of food intake (17). In the MCG 101 tumor model, anorexia was also attenuated by administration of anti-IL-6 antibodies or tumor implantation in IL-6 gene knockouts (10). Furthermore, indomethacin improved food intake to a similar extent in both wild-type and IL-6 gene knockouts, and suggests that eicosanoids may be a more important contributor to anorexia than host cytokines in this model of cancer.

Tumor necrosis factor (TNF) also induces anorexia and weight loss. Mice injected intraperitoneally with a cell line that secretes TNF developed severe cachexia and weight loss (18). Similarly in patients with advanced malignancies infusion of TNF,
as an anti-cancer agent, induced a range of side effects including anorexia (19). Porter and co-workers have shown that TNF also plays a major role in the suppression of food intake associated with LPS treatment (20). Intraperitoneal injection of pentoxifylline, a potent inhibitor of TNF-α production, completely eliminated the anorectic effect of intraperitoneally injected LPS (100 μg/kg body wt) and attenuated the anorectic effect of a higher dose of intraperitoneally injected LPS (250 μg/kg body wt). Concurrently, pentoxifylline pretreatment suppressed low-dose LPS-induced TNF-α production by more than 95% and IL-1β production by 39%. Similarly, high-dose LPS-induced TNF-α production was reduced by approximately 90%. Endogenously produced TNF is also one of the cytokines involved in the development of cancer anorexia. The reduction in food intake was partially reversed by intraperitoneal injections of TNF antiserum in rats bearing a methylcholanthrene-induced sarcoma (21).

It is likely that in most disease models one cytokine does not act in isolation to produce anorexia and several investigators have provided evidence that cytokines act synergistically. Intracerebroventricular microinfusion of IL-1β, TNF-α, and IL-8 to healthy rats decreased nighttime meal size by 42% and this was significantly greater than infusion of any one cytokine alone (22). Similarly, concurrent intravenous administration of TNF-α and IL-1α in rats had a synergistic effect in inducing anorexia (23). In summary a number of cytokines, particularly IL-6, IL-1, and TNF-α are implicated in the reduction in food intake in models of cancer anorexia and inflammatory and infectious conditions. In most models, with the exception of possibly turpentine abscess and IL-1, no single cytokine is solely responsible for induction of anorexia in any one model. Additional work is needed to determine the complex interaction between these cytokines.

IMMUNOMODULATORS AND SIGNALING TO THE CENTRAL NERVOUS SYSTEM

Observations by several investigators have shown that receptors for pro-inflammatory cytokines are expressed in the central nervous system (CNS), thus raising the possibility that cytokines act in the CNS to induce anorexia. Intracerebroventricular infusion of the IL-1 receptor antagonist attenuates the reduction in food intake occurring after intraperitoneal injection of IL-1β and indicates that IL-1β acts directly in the CNS to decrease food intake (24,25). Intracerebroventricular administration of the IL-1 receptor antagonist did not alter peripheral concentrations of IL-1β. In rats with TNB-induced colitis, intracerebroventricular administration of the IL-1 receptor antagonist increased food intake significantly more potently than peripheral (subcutaneous) administration, thus implicating central IL-1 receptors in the mediation of TNB-induced anorexia (15). These findings clearly indicate that IL-1 acts in the CNS to mediate anorexia during disease. Bloodborne IL-1 cannot passively cross the blood-brain barrier as a consequence of its molecular weight and hydrophilic profile. However, circulating cytokines can leak from the bloodstream into the circumventricular organs or gain access to the CNS via specific transport systems at
the blood-brain barrier (26,27). Although radioactively labeled IL-1 is found in the subfornical organ, one of the circumventricular organs, after intravenous injection, the physiologic relevance of IL-1 transport across the blood-brain barrier remains unknown (28). Circumventricular organs such as the arcuate nucleus and median eminence synthesize cytokines during peripheral inflammation. Peripherally administered LPS leads to upregulation of pro-inflammatory cytokines, TNF-α and IL-1β mRNA, in the pituitary, hypothalamus, cerebral cortex, and cerebellum (29,30). In contrast, LPS did not upregulate inhibitory (anti-inflammatory) cytokines, IL-1 receptor antagonist, and TGF-β in most brain regions examined (30). In another study, intraperitoneal administration of LPS induced a decrease in food intake associated with an enhanced expression of IL-1β, IL-6, and TNF-α mRNA in the hypothalamus (25). In the Lobund-Wistar rat model of prostate cancer, anorexia was associated with upregulation of IL-1β, IL-1 receptor antagonist and IL-1 receptor type I mRNA in the cerebellum, cortex, and hypothalamus (31).

In summary, brain synthesis of cytokines has been shown in response to peripheral induction of inflammation and tumour development and during peripheral cytokine administration. It is still unclear how cytokines of peripheral origin modulate CNS responses. Konsman and Dantzer have shown that inhibition of food intake by intraperitoneal administration of LPS or IL-1β is prevented by subdiaphragmatic vagotomy suggesting that cytokines might activate CNS structures via its action of the vagus nerve (32). They have shown that IL-1β activates afferent vagal fibers that terminate in the nucleus tractus solitarius, which in turn sends projections to a number of hypothalamic and limbic areas. Vagotomy attenuated the increase c-fos mRNA and protein in neuropeptide-containing neurons of the paraventricular nucleus of the hypothalamus after intraperitoneal injection of LPS or IL-1β (32). However, vagotomy only partially reversed anorexia in response to a higher dose of intraperitoneal LPS and vagotomy did not affect c-fos expression after intravenous administration of LPS. The data suggest that the vagus nerve activates central structures responsible for manifestation of anorexia after intraperitoneal injection of low doses of LPS. Porter et al. also showed that combined vagotomy and celiac superior mesenteric ganglionectomy had no effect on anorexia induced by intraperitoneal administration of LPS or IL-1β in rats and suggested that afferent nerves from the upper gut are not necessary for the feeding suppressive effects of these immunomodulators. They concluded that peripheral administration of LPS and IL-1β produces anorexia via a humoral pathway (33). Taken together these observations suggest that pathways other than the vagus nerve exist by which IL-1 can act on the CNS. The pathway may depend on the dose and route of administration of cytokine or LPS. However, pro-inflammatory cytokines are thought to be the primary signals to the orexigenic and anorexic pathways initiating the anorexia and wasting observed in inflammatory and cancer models.

HORMONAL AND NEUROPEPTIDE MEDIATORS OF ANOREXIA

As was discussed in the introductory paragraphs of this chapter, weight loss in chronic disease results largely from a reduction in food intake secondary to anorexia.
In healthy individuals, weight loss due to underfeeding is a potent stimulus to food intake. Persistent anorexia in underweight animals with inflammatory or malignant conditions suggests that there is a failure in the regulatory mechanisms, which would normally lead to consumption of extra calories after a period of semistarvation. Central to most theories of appetite regulation is the hormone leptin, identified as the adipocyte-derived anorexic factor that provides information regarding peripheral fat stores to the brain. We have explored the hypothesis that anorexia in chronic inflammatory disease results from plasma concentrations of leptin that are inappropriately high for the percentage body fat (34). This hypothesis was developed from in vitro studies, which showed that pro-inflammatory cytokines released leptin from isolated fat cells (35,36). In our studies we found that patients with inflammatory bowel disease and AIDS had increased plasma concentrations of TNF, but serum leptin was not increased compared to healthy controls matched for body fat. In the AIDS patients, serum leptin was actually lower than expected suggesting a compensatory response to extreme weight loss (34). These results are thus in contrast to those predicted from the acute animal studies and do not support a role for leptin in mediating anorexia and weight loss associated with chronic inflammatory and infectious diseases. Similarly, animal and human studies have rejected the hypothesis that hyperleptinemia or increased binding of leptin to its receptor mediate anorexia associated with cancer (37–39).

Numerous factors have been implicated in the CNS control of food intake, including neuropeptide Y (NPY), serotonin (5-HT), melanin-concentrating hormone (MCH), and α-melanocyte stimulating hormone. Medial hypothalamic 5-HT is implicated in the anorexia associated with both inflammatory and infectious conditions and experimental cancer-cachexia. IL-1β stimulates local release of 5-HT after injection into the hypothalamus (40). In tumor-bearing rats inhibition of medial hypothalamic serotonergic activity improves food intake (41). In rats with TNB-induced colitis, we have measured a release of 5-HT from the paraventricular nucleus (PVN) of the hypothalamus using in vivo push-pull sampling (8). In healthy but food-restricted animals (i.e. pair-fed to match the hypophagia of the colitic rats) there was a 5-fold reduction in PVN 5-HT release compared with that in free-feeding controls; this is an expected and appropriate response of an anorexigenic neurotransmitter to food restriction. This reduction would remove any inhibitory influence on feeding behaviour and drive a feeding response once the animal has access to food. In contrast, in rats with TNBS-induced colitis, release of 5-HT from the PVN was 3-fold and 14-fold higher than in healthy free-feeding controls and pair-fed groups respectively. In comparison with the pair-fed group we can conclude that the increase in release of 5-HT is an inappropriate response to weight loss and continued 5-HT release would only serve to further inhibit feeding. In the later stages of colitis, the decrease in 5-HT release is associated with a reduction in anorexia. After depletion of brain 5-HT by administration of p-chlorophenylalanine, an inhibitor of tryptophan hydroxylase, food intake was increased in the colitic group although not to control values. These results suggest that anorexia associated with TNB-colitis is mediated in part by 5-HT, but other mediators must also play a role. In subsequent studies
we have shown that intracerebroventricular infusion of an IL-1 receptor antagonist resulted in an 18-fold reduction in PVN 5-HT release and an increase in food intake compared to vehicle-treated controls (Fig. 3). In contrast, intracerebroventricular administration of anti-tumour necrosis factor antibodies had no effect on either PVN 5-HT release or food intake in rats with TNBS-induced colitis (16). 5-HT is also implicated in anorexia induced by administration of LPS and in the development of cancer anorexia. In the LPS-model of acute infection, the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT attenuated LPS-induced anorexia in rats (42). In tumor-bearing rats, injection of the serotonin antagonist, mianserin, into the ventromedial hypothalamic nucleus selectively increased meal number leading to improved food intake (43). Other studies have suggested that both elevated 5-HT and low dopamine levels in the medial hypothalamus modulates food intake in cancer anorexia. In methylcholanthrene sarcoma tumor-bearing rats release of monoamines was measured by in vivo microdialysis via a probe placed in the ventromedial nucleus of the hypothalamus.

**FIG. 3.** Release of 5-HT from the hypothalamic paraventricular nucleus measured by in vivo push-pull sampling is significantly ($P < 0.001$) reduced in rats with TNB-colitis treated with an IL-1 receptor antagonist compared to vehicle (placebo) treated animals with TNB-colitis. Adapted from El-Haj et al. (16).
Onset of anorexia was associated with increased release of 5-HT and its metabolite, 5-hydroxyindoleacetic acid and reduced release of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid compared to non-tumor-bearing rats and pair-fed controls. Seven days after surgical removal of the tumor, food intake had increased and 5-HT, but no dopamine release, had returned to control values. The authors concluded that an increased 5-HT-to-dopamine ratio is related to the development of cancer-induced anorexia (44).

Recent studies have suggested that pro-inflammatory cytokines stimulate the central proopiomelanocortin system (POMC) resulting in the release of α-melanocyte-stimulating hormone (α-MSH) and activation of the melanocortin receptors (MC3R and MC4R) and in anorexia. Administration of the melanocortin-3/4 receptor antagonist, SHU9119 reversed anorexia induced by intracerebroventricular administration of IL-1β in rats (45). In the Lobund-Wistar rat model of prostate cancer, a 3-day course of treatment with this antagonist returned food intake to pre-tumor levels. The authors of this study also provided additional evidence that the effects of SHU9119 are downstream to the orexigenic peptides neuropeptide-Y and ghrelin (46). Further support for the role of MC4R in cancer-induced anorexia is provided by Marks et al., who showed that MC4R knock-out mice or mice administered the MC3-R/MC4-R antagonist, agouti-related peptide, also resist tumor-induced weight loss (47). LPS-induced anorexia may also be mediated by the central POMC system and activation of downstream feeding pathways. In LPS-treated rats, POMC and cocaine- and amphetamine-related transcript (CART), mRNA levels were increased in the arcuate nucleus associated with reduced expression of the orexigenic peptides, melanin-concentrating hormone (MCH), CART, and galanin in the lateral hypothalamic area. Levels of mRNAs for neuropeptide Y and galanin in the arcuate nucleus, and of MCH and CART in the zona incerta did not change significantly after LPS treatment. LPS-induced factors may mediate signaling to the POMC/CART neurons in the arcuate nucleus, which could lead to reduced food intake by decreasing MCH, CART, and galanin synthesis in target lateral hypothalamic neurons (48).

Neuropeptide Y (NPY) is thought to be a key physiologic mediator in the refeeding response after starvation. Reduced NPYergic activity has been reported in some, but not all rat models of cancer anorexia, although these rats are refractory to NPY-induced feeding (31,49–51). In contrast, after LPS administration or induction of TNB-colitis in rats, there was an appropriate increase in hypothalamic NPYergic activity but like the tumor-bearing rats intracerebroventricular administration of NPY did not induce a feeding response (48,52).

In summary, anorexia is a common and distressing manifestation of infectious, inflammatory, and malignant conditions. By necessity, almost all research to identify mediators of anorexia has been conducted in animal models. Pro-inflammatory cytokines are thought to be the primary signals to orexigenic and anorexigenic hypothalamic pathways. Most studies have not identified individual cytokines as the sole causative agents of anorexia and it is likely that multiple cytokines focus their actions on neurotransmitters and their receptors. Different mechanisms resulting in anorexia may operate in inflammatory and malignant conditions. Increased release of medial
hypothalamic 5-HT, induced by IL-1, mediates in part the anorexia associated with intestinal inflammation and malignancy. The specific receptor subtype(s) involved has not been characterized. There is increasing evidence that the central POMC system and melanocortin receptors play a key role in cancer-induced anorexia. The challenge is now to determine the signaling cascade between cytokine and feeding pathways and the interaction, if any, between the neurotransmitters that have been implicated in causation of anorexia.

REFERENCES


DISCUSSION

Dr. George A. Bray: I think I read in your paper about a macronutrient specific effect of some of the cytokines, is that correct? Do I remember a carbohydrate effect?

Dr. Anne Ballinger: Yes, that is correct. After both administration of lipopolysaccharide and toxin or administration of interleukin-1, the pattern of anorexia is similar in that the time course of anorexia is similar and also there seems to be a selection for carbohydrate. And that highlights one of the reasons that interleukin-1 has been thought to play a role in anorexia, because it does mimic the macronutrient selection and in LPS, it’s carbohydrate.

Dr. George A. Bray: I wanted to follow that with some studies we’ve done with serotonin and macronutrient specificity, which produced a confusion in my mind. In studies that Brenda Smith has published, where we microinject serotonin into the periventricular nucleus, we get almost a specific reduction in fat intake, and I had a hard time putting your model of carbohydrate effects of LPS peripherally with this highly specific macronutrient effect on fat by serotonin, and I wondered if you could help me rationalize some of that, because it didn’t quite fit.

Dr. Anne Ballinger: Well the first thing I should tell you is that we didn’t look at macronutrient intake in our model. Our model was the TNB colitis model, which may be a different mechanism from LPS, so in terms of 5-HT, we were only looking in the TNB model, and we didn’t look at macronutrient intake, so I can’t comment as to whether there was any alteration in fat or carbohydrate intake.

Dr. Mohammad Juffrie: It’s very interesting that some cytokines, specifically pro-inflammatory mediators such as interleukin 1 and 6, the TNFs and interferon-γ could reduce appetite. My question is what the other pro-inflammatory mediators such as interleukin-8 and interleukin-12 do, because both of these are also pro-inflammatory mediators.

Dr. Anne Ballinger: There is some evidence that interleukin-8 does reduce food intake and acts in a synergistic manner with IL-1 and TNF. I think it’s just with these four cytokines that I highlighted, and those are the ones that have been studied most and have been shown to have a role. I’m sure that there probably are other cytokines that contribute, but we were trying not to confuse the picture with too many cytokines. However, I certainly agree there is a role.

Dr. Gareth Williams: Your last point about there being a diversity of cytokines I think is a very important one. Not all of the reported models, particularly of cancer cachexia necessarily relate very well to human cancers, which also cause wasting. And some of the mediators that have been implicated from animal studies don’t appear to play much of a role in man. There’s
one called the MAC-16, which is a mouse adenocarcinoma, which is just about the only one that actually produces cachectic mediators which have also been characterized in human colonic and pancreatic cancer and in that particular model, the NPY system appears to respond normally. In other words, the expression of NPY is upregulated as the animals get thin from the cancer and at roughly the same rate, or very nearly the same rate, as if they were losing weight through food restriction. So that might either be due to a downstream effect or the fact that it's hitting some completely different system. So I think it is important to look in the various models, and to try to relate those as closely as one can to the sorts of mediators that you really do find in human diseases.

Dr. Anne Ballinger: I think that's a good point, particularly about some of the cancer models that aren't always a good replica of human disease and that the tumors are very large before the animals start to lose weight. Regarding the cachectic factor, which has been found in pancreatic patients particularly, I'm sure there is for some patients an element of something, which is basically dissolving fat and muscle, but I don't think there's any cancer patient who loses weight and eats normally, or if they were losing weight, their appetites should be in fact upregulated. So for any model where there is weight loss other than thyrotoxicosis and ketoacidosis, I think there is always an element of anorexia.

Dr. Antonius Hocky Pudjiadi: My first question is: how long did the pro-inflammatory mediators-induced anorexia last, as we know that in acute inflammation, the tumour necrosis factor and IL-1 continue to rise for several hours, but then how long it will last? The second question is: will the anorexia induced by the pro-inflammatory cytokine effect the deuterium absorption function of the gastrointestinal system?

Dr. Anne Ballinger: In answer to the first question, I think for the question of how long a pro-inflammatory cytokine works, there is evidence particularly from the models, where an antagonist has been administered and that's been done both for the tumour models and the colitis model. I showed you that if the antagonist is administered over a period of days, there is a reversal of anorexia so presumably, that particular cytokine is acting over a period of days. But I agree with you. You know it's not going to replicate the human situation exactly, where there may be anorexia for weeks, because you know the rodent models always live a shorter time. So I think the cytokines do help those antagonist models and have shown that they do have potential to act over a long period. In terms of cytokines altering GI function, I'm not sure if that's been looked at in detail. There has been a study, as Chris mentioned earlier, which has shown in Giardia infection that there is increased CCK release or increased levels of CCK in the plasma, and that's thought to be due to the inflammatory response produced by Giardia and presumably the cytokines having an effect directly on the cells, which produce CCK and presumably other endocrine cells in the intestine. In terms of alteration to other things such as of motility, there has been a suggestion in this TNB model that there is a delay in gastric emptying which contributes to the anorexia. In fact we found that gastric emptying was normal, so I'm not sure that a clear role has been shown to exist.

Dr. Christoph Beglinger: Do you have the chance to look at the effect of TNF-α antagonist treatment on appetite or on interferon treatment in hepatitis and their effect on appetite and weight?

Dr. Ann Ballinger: Your first question was about TNF antagonist. Okay, we haven't done any human studies looking at any of the TNF antagonists such as pentoxifylline and there are some others. In the colitis model, we didn't find any effect of TNF-α antibodies administered centrally on appetite, nor on intestinal inflammation. In terms of giving something like Infliximab to patients with inflammatory bowel disease, there would always be the problem, I
suppose, of trying to separate any effect on appetite, for just a general effect, from improvement in intestinal inflammation and trying to show whether there was just a specific effect on appetite. There is an observation of patients who are treated with interferon. They do get this flu-like illness with loss of appetite and weight loss. So there is definitely an effect on appetite. We haven’t studied that in detail to try and determine what that might be due to, or the length of that time.

Dr. B. Ramakrishna: My question is actually following Christoph’s question and your response to that. Rheumatoid arthritis is a condition where the TNF-α antagonists are used with great success. I believe by June last year 150,000 patients had been treated with Infliximab. Perhaps that would be a good procedure to adopt, to look at anorexia before and after treatment with Infliximab in the absence of any GI involvement. Would you like to comment on that?

Dr. Anne Ballinger. Yes, I think that’s a good point. My clinical impression is that for patients with bad rheumatoid, systemic rheumatoid, they aren’t generally over weight. I’m not sure how much has been studied in detail, because we’ve got to remember there’s a difference between local production of TNF in the inflamed joint, compared to a more general inflammatory response, where there are circulating inflammatory cells producing TNF either to have a local effect on the brain or otherwise. But yes, it would be an interesting study to do.

Dr. Farthing: I’m not sure there is perfect model, because you’re going to reduce pain, and I suspect pain might alter one’s interest in food.

Dr. Anne Ballinger. Yes, I guess that would be a confounding point.

Dr. Ramakrishna: I actually have one more question following your talking about the local effects of intestinal versus some systemic inflammation. One of the problems in the tropics, and probably Michael is the best person to talk about this, is tropical enteropathy. A lot of people in developing countries have an abnormal small intestinal mucosa. The villi are short, the crypts are longer and there is an intestinal mucosal inflammation. It’s fairly controlled. Now do you think that that might lead to reduced food intake in these people?

Dr. Anne Ballinger. Lead to a decrease in food, a reduction through an effect directly on the gut? I don’t know. I think again there may be a confounding influence there, but it has been suggested in tropical enteropathy, that there is increased translocation from the gut endotoxin. So yes, it may lead to reduction in food intake, but that may well be through the cytokine mechanism, and increased translocation from the gut and endotoxin and thereby an induction of cytokines, not through any direct effect on gut functional motility.

Dr. Aspi Irani: There are two questions. One, what is or would be the role of cyproheptadine in disease-related anorexia? Number two, in acute disease, where there is a loss of appetite, there is also a rise in the level of stress hormones and this would switch the body metabolism from an anabolic to a catabolic phase. How does this fit into the sequence of events leading to anorexia?

Dr. Anne Ballinger. 5-HT antagonists such as cyproheptadine do increase appetite, certainly in one disease model LPS, a number of 5HT antagonists have been tried and none of them has been shown to have a direct effect on increasing food intake. Whether that’s because there are, as I said, other things, which compensate, or whether it’s because the right receptor has not been characterized. So in answer to your question, 5-HT antagonists have been shown, particularly in some AIDS patients, to increase food intake. So that’s just an example of one inflammatory condition. I don’t think yet we’ve identified which receptor is likely to be playing a role and therefore which would be the best antagonist.
Dr. Aspi Irani: The second question was about the role of stress hormones in disease-related anorexia.

Dr. Anne Ballinger: Certainly CRF would be expected to go up, because of the stress. In some models, CRF antagonists have been shown to increase food intake. In the model that we use, which is the colitis model, we found that intraventricular administration of the CRF antagonist had no effect on anorexia, so I think it again brings into highlight that it’s unlikely to be one mediator, which is the same mediator that is causing anorexia in every disease model. In terms of CRF altering the stress response and effect on catabolism, it was what I was saying earlier, I think in many of the disease models, there is an alteration in fat and protein balance and the same with the ubiquitin proteasome pathway where there is an alteration in metabolism of protein. But in all these models, there is an element of anorexia. And if you correct the anorexia, if you overfeed them, you won’t always make the patient put on the same weight that they would put on without that inflammatory cascade. So I think there is an alteration of fat and protein, but at the same time, there is also a loss of appetite.

Dr. N. K. Arora: What is the relationship between anorexia, nausea, and vomiting?

Dr. Anne Ballinger: I think that’s a good question, and I think there’s a very fine line between anorexia and nausea. I think there’s only one step beyond anorexia, which is nausea, so I don’t think I can put any new light on which mediators that specifically may be, only to say I think there is a very close relationship. It’s interesting that one of the initial studies looking at cholecystokinin, when cholecystokinin was administered albeit in high doses, it was shown that if at the same time an anti-emetic was given, that the food intake was restored to a more normal level. So I think that for many of these mediators, which induce anorexia, the next step is perhaps high concentrations in nausea.

Dr. Ravindra Chitted: You mentioned about melanocortin inhibitors causing hunger. Do they induce hunger in animals who are basically anorexic or induce greater appetite even in normal appetite?

Dr. Anne Ballinger: It certainly induces an increase in appetite in disease models. I’m sure those studies have been done and Gareth might know, but I can’t tell you the amount of the magnitude of the increase in food intake if they’re performed in healthy animals. I don’t know. Do you know, Gareth?

Dr. Gareth Williams: If you use an antagonist of the MC-4 receptor, you generally hit the MC-3 receptor as well, but something like AGRP is the peptide made by the NPY neurons. They have a very prolonged and active appetite-stimulating effect, which can act for some days after it’s been injected, and the antagonist that was mentioned in the cancer cachectic study, SAQ9119, is also a very powerful stimulus in feeding and the effect is very prolonged.

Dr. Abdul Majid Molla: Anorexia is a major cause of malnutrition in many childhood infections. Many studies have been carried out to measure the effect of anorexia and disease-specific conditions like rotavirus and hepatitis in children, but no study that I know has been made to look at how to break the effect of anorexia, particularly we are interested in gastroenteritis, rotavirus and hepatitis.

Dr. Anne Ballinger: I think that’s going to be the subject of a talk later on about things, which might break the cycle of anorexia. I mean there’s certainly a number of potential mediators, and ones which have been used in patients, so there are potential ways to tackle the problem of anorexia, but I think Wolfgang’s going to talk about that tomorrow.

Dr. Shrichandra Bhawnani: The classical undergraduate preaching used to be that increasing the appetite and weight loss are presenting two distinct categories of illnesses—eat more, lose weight, paradoxes, eat more, lose weight. On the other hand, there were two diseases like eat less, lose more, such as in malnutrition. Eat less, lose more letocracin malignancy.
Do your cytokine levels fall in accordance with these false states? I wish to know what are the cytokine changes in malignancy, diabetes mellitus, and thyrotoxicosis?

*Dr. Anne Ballinger:* I couldn’t comment about the cytokine changes in diabetes. I think one of the things we have to remember, when we’re talking about malignancy and in inflammatory conditions, is that most people have measured circulating levels of cytokines. And what we don’t know actually is whether the effect is not just by circulating TNF, but whether it’s a much more local effect, so that with stimulated inflammatory cells, macrophage and T-cells, actually the effect of cytokines is achieved by a local action, so they release their cytokines at the blood-brain barrier or the cytokines are bound at the blood-brain barrier. So it’s not all a local effect. So although measuring cytokines has been done in inflammatory and malignant conditions, and there have been variable results in terms of whether they’re always up or sometimes normal, we can’t always measure elevated levels of cytokines in malignancy and inflammatory conditions, but I don’t think that takes away the role, the potential role of cytokines in anorexia.

*Dr. Chourjit Ksherimayun:* What are the molecular changes in the psychologically caused anorexia?

*Dr. Anne Ballinger:* Well for anorexia-nervosa, as far as I know, almost every possible neurotransmitter, which influences appetite and the peripheral mediators of anorexia, have been measured in circulating blood, for instance CCK, and investigations also measured various neurotransmitters in the cerebrospinal fluid for patients with anorexia-nervosa. And often, they found the neurotransmitter of interest goes the way that one would predict. With CCK, for instance, there have been reported levels of elevated plasma concentrations in patients with anorexia or bulimia, suggesting that there is an inappropriate release of CCK and that this contributes to the anorexia. But the whole problem is that by the time people are measuring plasma concentrations or CSF concentrations of various appetite mediators, the whole system is completely disordered. They’ve got disordered gastric emptying. They’ve got disordered G.I. motility. Their whole psychology is disturbed, so I don’t think for anorexia any differences could really be shown to be the result. We couldn’t definitely say yes, this is the cause of, or the contribution to, the anorexia in psychological conditions.

*Dr. S. K. Mittal:* Short-term anorexia in an acute illness is a common problem, but patients tend to recover. Can a non-recovering anorexia be a signal of a serious problem, and if so, what is the time duration, say after acute diarrhea, if a child does not return to his normal appetite, in how much time can be taken as a sign of something is a problem in the child?

*Dr. Anne Ballinger:* I don’t know, to be honest. The indicator in patients who come into hospital, and I think this is the most I can comment on really, is that, if they’ve lost more than 10% of their body weight through inflammation or malignancy, that is when we may consider intervention, but often that patient’s weight would’ve been reduced, so they’re then getting to a worrying stage. So 10% is often what we take as a worrying level, but I’m not sure if anyone could really comment on how rapidly that weight needs to be lost before you worry.

*Dr. S. K. Mittal:* Majid Molla, you have excellent experience in this anorexia, diarrhea related anorexia. When would you consider non-recoverable anorexia as an important marker of the problem in a child with acute diarrhea?

*Dr. Abdul Majid Molla:* It will be better if I answer this from a clinical point of view. We have measured anorexia in different aetiological diarrhea and rotavirus remains in anorectic children for about three weeks, two weeks to three weeks. If a patient doesn’t return to his food in the usual time, it’s followed by a hyperappetite, but within two to three weeks,
everybody comes back. Cholera is the ugliest one, and *E. coli*, shigella also comes into a 10- to 12-day period, and rotavirus tends to be the slowest one, but these are all very clinical.

**Dr. George Fuchs:** I would endorse what you say that appetite, almost universally, comes back and they then exhibit catch up, intake and growth. The real problem is the numbers of episodes. Naturally, if they have frequent episodes in a course of a year or so, then that effect is one of more concern, but in an isolated single episode, almost all of them will come back.

**Dr. S. K. Mittal:** Maybe from a clinical viewpoint, I don't have any experimental data, but I don't know if it takes such a long time for a child with acute diarrhea to recover his appetite. I think most of them recover within 3 to 4 days, within a week. I mean after a week they're normally eating twice their normal level, so I'm not ready to accept, entirely as a clinician, that it takes almost 2 to 3 weeks for the appetite to recover.

**Dr. Shrichandra Bhawnani:** We're talking about the decreased anorectic appetite. I would like to know from the audience and also from the panelists, when do you begin and when should we start treating anorexia in a case of diarrhea? When do we start interfering? When do we treat? That's a really hard question. It's not important how much time will it take to recover. Treatment may begin at 1 month or 1 week, somebody else may try after 3 weeks, but my point is when do we treat anorexia and at what stage do we treat anorexia in a case of diarrhea? And if we need to treat, with what method and with what things?

**Dr. Anne Ballinger:** Well, I think the answer is probably as yet, there's no simple way that we can just treat anorexia. We can't just give a tablet, which will increase appetite. So when we talk about treating anorexia, we're really talking about treating undernutrition with food supplements. Now, largely from animal studies, there is the suggestion that feeding in the acute stage of an infection will have a detrimental effect, but this is not gastroenteritis, these are models of sepsis, but I don't think there's any recommendation particularly for gastroenteritis. I don't think one should follow those models and feel that they would have a detrimental effect, and I would say in adult practice for a patient coming into hospital, depending on their initial body weight, we would worry if they've 10% body weight loss and this would be an indication to consider supplementing nutrition. Also trying to predict how long they're going to have reduced feeding for is out of my sphere to try and comment for a young patient with gastroenteritis.

**Dr. S. K. Mittal:** The answer to this question is: you are not trying to feed anorexia, but trying to find the cause of anorexia. Most of the time you will find there is some associated systemic infection, which is causing the problem, and if you treat the systemic infection, the anorexia will recover and everything will be fine.

**Dr. Anand Pandit:** My point is again in the same direction. The only difference in this question is that, as Professor Mittal would agree with me, we see so many children in clinical practice, in whom we cannot pinpoint a cause, and they do falter on the growth chart. We usually blame the parents saying that they are not feeding this behavior properly. There's no question about it. At the end of it, there is a group of children which manages to thrive, but we just don't know how. Suppose we start looking at their cytokines. Do you think that would be beneficial? Would it be a good investigation?

**Dr. Ann Ballinger:** Certainly there's very good evidence that inflammation on its own has a detrimental effect on growth, so even if you feed, certainly in models that are starting to come through in human studies now, there is good evidence that the inflammatory process itself, independently from the nutrition, has a detrimental effect on growth, and that's both by an effect on peripheral insulin like growth factor-1 leading to reduction, and also by direct effect at the growth plate, particularly by a TNF-α and probably from IL-6, so I think yes.
I think some children can be fed, but if there is continuing inflammation and pro-inflammatory cytokines, then that in itself will inhibit growth in relation to how much they’re being fed.

Dr. Y. K. Amdekar: I think there are two groups that you are referring to. The other group referring to a change in the meal size includes children who have probably always been eating a small amount of food. They’re not anorexic strictly, and they’re probably also not faltering much on their growth, but if there is an anorexia, then I think we need to certainly find a cause for it. So some children will eat very low amounts of food and not be anorexic. Their metabolism probably demands a small amount of food intake.

Dr. Anand Pandit: Let’s talk of two different groups. This is a different group. With just small eaters we accept that I’m talking of children who falter on their growth in spite of no evidence in any form, in the form of any inflammation or any infection, but it is a continuous faltering of growth and endocrine-wise they’re normal, systemic investigations turn out to be normal. That’s why I’m asking, are there subclinical situations, where chronic inflammation cannot be detected by modern clinical science and cytokines would become sort of an answer to decide about the diagnosis?

Dr. Y. K. Amdekar: The cytokines won’t tell you the cause. They may at best tell you the mechanism of anorexia, but not the cause.

Dr. Anne Ballinger: I’m not sure that measuring the cytokines will necessarily tell you anything, if you took that group of children and decided to measure blood levels of cytokines. That’s what I was saying earlier really. Just simply measuring serum concentrations is not necessary the best indicator of whether there is an activated inflammation of cells, which may well have an effect at the local tissue level.

Dr. Eva Micskey: One short comment. Different types of inflammation can change the gastromyometric activity. It could be a change between nausea, vomiting and anorexia.

Dr. Anne Ballinger: And it’s certain that gastrointestinal inflammation can also disturb gut function, but I don’t know whether it’s worth looking at inflammation outside of the gut, which then impacts on gut function.

Dr. Eva Micskey: We have seen it by EGG.

Dr. Remy Meier: There are some data showing that omega-3 fatty acids improve weight loss in some cancer patients. Have you had the chance to look in your models on cytokine release or blocking, at giving omega-3 fatty acids?

Dr. Anne Ballinger: Did we block the omega-3 fatty acids?

Dr. Remy Meier: No, when you give omega-3 fatty acids you can improve weight loss in cancer patients. There are some small reports, some small studies in pancreatic cancer patients. Had you chance to look at this effect, fish oil components on cytokine release?

Dr. Anne Ballinger: No, well I haven’t. I do work with someone who’s particularly looking at it in intestinal inflammation, because it’s been suggested as a treatment for inflammation, particularly Crohn’s disease. So there are effects, but it seems to have, in these patients, an odd effect on cytokines in that there is a reduction in TNF-α but actually an increase in interferon-γ, but this is one particular group of patients. These are not cancer patients. I am aware of the work in the cancer patients, but my memory is that the control, in terms of how much the patients were eating, was not strict and it wasn’t entirely clear, whether it was all due to the omega fatty acids that their weight loss was decreased.

Dr. Ravindra Chittal: I have two questions. The first is: what is the mechanism of anorexia in acute hepatitis, viral or otherwise. The second question might sound very naїve, but how do babies on steroids have hyperphagia? What is the mechanism? Is it related to tumour necrosis factor being eliminated?

Dr. Anne Ballinger: I’m not sure I can answer your question on acute hepatitis, other than
to say there are various reasons people have measured cytokine levels in acute hepatitis and they're all very high, so whether it is related to that I don't know. I don't think I could adequately comment. In terms of why do steroids increase appetite, again that's not known. What is known is that the increase in appetite with steroids is seen before there is suppression in CRF, and CRF is an inhibitor of feeding, so it's been thought that steroids suppress CRF and, therefore, that's why there's an increase in appetite. However, it's not that simple, because there is a discrepancy in the time course of food intake and CRF, so I don't know if Gareth knows any more about steroids and hypophagia?

*Dr. Gareth Williams:* Glucocorticoids do turn on the NPY cells directly, but I think if I remember rightly, people are still tending to look at the suppression of CRF, as you implied.

*Dr. Y. K. Amdekar:* Is the outcome of anorexia dependent on the balance between pro-inflammatory and anti-inflammatory cytokines?

*Dr. Anne Ballinger:* One may guess it would be, but that hasn't been looked at. To be honest, that probably hasn't been looked at properly at all, I don't think.

*Dr. Y. K. Amdekar:* Is any anorexia, especially in an acute disease, beneficial, harmful or harmless?

*Dr. Anne Ballinger:* I'm sort of loathe to say. There are these animal studies, where with an acute infection, force feeding of the animal has been shown to increase mortality, but I'd be reluctant to support this, because clearly in young children with gastroenteritis who have lost their appetite, we wouldn't want to advocate stopping feeding, because that may increase their mortality, because I'd say these models, as far as I know, have been limited to disease models, acute disease models. So I don't think we should necessarily carry that over to the human situation, because the benefits of feeding may, in the human situation, outweigh any potentially adverse influence.

*Dr. Michael J. G. Farthing:* I think it's still a very controversial area, as to whether undernutrition does protect from infectious disease, but I think there is evidence in other human diseases, malaria is one, where a group of people were persisting with this idea that undernutrition protected from malaria and indeed one or two other disorders. I don't know. Perhaps Dilip and Majid will remember these data better.

*Dr. George A. Bray:* There are also the calorie restriction studies in rodents, which show substantial prolongation in life. Now this is not with infection, but just reducing their intake over a prolonged period of monitoring their data at least in two sets of studies in non-human primates in the US, where in one case, you will prevent the development of diabetes by clamping intake on rhesus monkeys over a 10-year period of time. So I think there is some value in lower intake, but certainly not in the presence of infection, but I think there are settings and settings in the US now being funded to look at calorie restriction in adults for its potential benefit for health.

*Dr. Dilip Mahalanabis:* I think the issue of anorexia in acute illness needs to be looked at from the clinician's point of view, and then we should go to the issues, which are related to anorexia. One is the treatment of, say, acute diarrhea or any other illness, including febrile illness. At the turn of the century, the first thing a doctor was supposed to do was to stop food and give them some diluted gruel or something like that. So basically starvation was one of the components of treatment. Today there is a major change and shift over time and basically what we are doing is not to restrict food during acute illness. That does not mean you put a tube down and feed as much calorie as they are supposed to eat when they're well. There is a restriction imposed by the patient for himself, by not taking more than he or she can really handle or thinks he/she can really eat or drink. And if you look at a situation like shigellosis in children, severe shigellosis, due to shigella dysenteriae and when we studied
their food intake we used the paradigm that they become severely malnourished as they’re not able to eat well for 10 to 15 days. I’m talking about really severe disease and that if we offer them frequent feeds, which are liquid enough to be eaten, especially children, then the total intake would be larger than what it would have otherwise been and that’s what really happened in our study. We could increase intake during the very acute phase, when they’re very anorexic, offering two hourly feeds during that period and the net effect, at least on the growth or the weight loss, was definitely beneficial. So it is one thing forcing feeds in an experimental context, but it’s a very different situation, when you are treating a patient and it’s a question of what food you are offering and how often. So this is where we are. I’d also like to touch on an issue you have raised about how long do you wait to treat anorexia. The real life situation was different. The real life situation was that if the child came for treatment. It’s a very acute disease. You probably have to send the patient out of the treatment centre as soon as the patient can be taken care of by the parents. They do not have enough time to feed them, but those were very severely malnourished at ICDDR, Dhaka. Let’s say less than even 55% weight for age of NCHS median were the kids who obviously needed prolonged feeding and they used to be admitted to a special rehabilitation unit. If you look there what happened, there was a fairly large proportion of these infants, who were very severely marasmic. They’re just wasted kids and these are very young infants of the age of maybe 6 months to 2 to 3 years. George has done another elegant study on feeding these children. There is a fear about how much food you are able to get into these babies. There’s a phase when weight is static, after some time, it starts gaining weight. We do not know the mechanism for this. They were started on a very large calorie intake. They simply did not grow. There is a phase when weight is static and it’s been reported in other centers also. So there you are really landing up with very different sets of situations. Here, we are treating a child with very severe marasmus; so maybe George, you would like to comment on your studies?

Dr. George Fuchs: I’m not sure what the mechanism is. There is a limit in marasmus, above which it’s not utilized. I’m not sure whether that’s really been looked at in terms of a mechanism. Its limit has been examined in a clinical situation and there is a limit. Of course that’s a different story, and there are some metabolic abnormalities and reasons that you go slower, and again there are all sorts of theories, but the mechanism for that increased risk has not really been completely defined. So the short answer is no, I don’t know exactly what the reason is, but it is an observation that holds.