

Liver protein and glutamine metabolism during cachexia

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The word cachexia is derived from the Greek words ‘kakos’ and ‘hexis’ meaning poor condition or bad health. This syndrome indicates a state of wasting of primarily peripheral (muscle) tissues in the presence of a variety of diseases, such as cancer, acquired immune deficiency syndrome or tuberculosis, in which the host’s inflammatory response plays an important role. This wasting results in loss of cell mass from the body. An important distinction exists, however, between depletion of body cell mass due to pure starvation, and depletion which is caused by the combined presence of starvation and disease, or more specifically a severe or prolonged inflammatory response. This latter category is referred to as cachexia and is the condition most frequently found in patient populations. One of the major differences is the fact that during pure starvation visceral organs such as the liver and the gut break down protein to furnish substrate for host protein metabolism while muscle protein is preserved at first (Heymsfield & McManus, 1985). Furthermore, protein synthesis rates and protein turnover are down regulated resulting in decreased N losses. In cachectic patients, on the other hand, starvation is usually accompanied by inflammation and muscle protein is broken down, leading to net release of amino acids which subsequently are taken up by viscera like the liver and spleen. Furthermore, N losses are significantly larger than during pure starvation.

In the past decade glutamine has received much interest especially in the diseased organism, not in the least because of its proposed effects on protein metabolism and the immune system. Therefore, the present article will be mainly focused on the changes occurring in protein and glutamine metabolism in cachectic patients, especially with regard to the liver.

CLINICAL ASPECTS OF CACHEXIA

Which are the clinical characteristics of cachexia? In addition to a history of weight loss and anorexia, fatigue and malaise are frequently accompanying symptoms. Moreover, the patient usually suffers from cancer or severe or chronic inflammatory diseases such as sepsis, rheumatoid arthritis, tuberculosis, malaria, etc. During physical examination atrophy of skeletal muscle and loss of subcutaneous fat are characteristic findings. Furthermore, muscle strength, e.g. tested by measuring the grip strength, is also diminished. In contrast to these catabolic signs, however, very often splenomegaly and sometimes enlargement of the liver is present in these patients. In short, cachexia is clinically characterized by wasting of peripheral tissues in contrast to the preservation or even hypertrophy of the visceral organs (liver and spleen).

The causes of cachexia can be categorized into two major groups: first of all anorexia which can be due either to the underlying disease (cancer, inflammatory mediators, pain, gastrointestinal tract obstruction, changes in taste perception) or to the therapy (e.g. chemotherapy, radiotherapy, surgery) and second, changes in host metabolism (Kern &

Table 1. *Summary of metabolic changes in cachexia*

Major metabolic changes in cachexia	
Protein metabolism	Increased whole-body protein turnover Decreased muscle protein synthesis Increased liver protein synthesis Increased muscle protein catabolism
Carbohydrate metabolism	Insulin resistance Increased gluconeogenesis Increased glucose turnover
Lipid metabolism	Increased lipolysis Decreased lipogenesis Increased free fatty acids and glycerol turnover Net loss of lipids from host

Norton, 1988; Laviano *et al.* 1996). These changes in host metabolism comprise changes in energy metabolism, carbohydrate, lipid and protein metabolism. The most frequently encountered changes are listed in Table 1. The main changes in protein metabolism are persistent muscle protein breakdown and increased hepatic protein synthesis rates. These changes will be discussed in more detail (see below).

Part of the C skeletons of the amino acids taken up by the liver are converted into glucose. This increased production of glucose seems contradictory to the insulin resistance frequently observed in cachexia, but the combination of these two changes could lead to an increase in availability of energy substrate for certain cell types such as immunocytes.

Several hormones and other mediators (glucagon, cortisol, interleukin 1, tumour necrosis factor etc.), which are also involved in the inflammatory response, can mediate the changes in intermediary metabolism found in cachexia.

The importance of recognizing the cachexia syndrome resides in the fact it is a predictor for the ability to tolerate the trauma of operative treatment and consequently also a predictor of post-operative complications and survival (von Meyenfeldt *et al.* 1992). Furthermore, since the catabolism in cachectic states can only be partly inhibited by providing artificial nutrition, a better understanding of the mechanisms underlying this problem is required to improve treatment.

CHANGES IN PROTEIN METABOLISM

The basic metabolic changes found in cachexia mimic the changes in host metabolism which are found during a serious inflammatory response. Thus, data from studies in trauma models are also of interest when considering the pathophysiology of cachexia.

It was shown by Lust (1966) and others that during starvation the visceral organs are the primary sources of protein. Subsequently it was shown that this was not the case in inflammatory states. Clowes and others (Clowes *et al.* 1980; Rosenblatt *et al.* 1983; Clowes, 1986) showed that release of amino acids from the leg increased by 300% during sepsis or after trauma. The central plasma clearance rate (CPCR), a measure of the quantity of amino acids taken up by the 'central' visceral organs from the blood, which correlated significantly with liver protein synthesis *in vitro*, also increased by 300%. Furthermore, this was accompanied by an increase in liver synthesis of structural and secretory proteins such as C-reactive protein, serum amyloid-A and fibrinogen (Clowes, 1987). In patients with a decreased liver function increments in plasma concentrations of amino acids were

found as well as decreased CPR (Clowes *et al.* 1984), suggesting a central role of the liver in amino acid uptake after injury. Infusion of amino acids in septic and trauma patients was shown to decrease muscle proteolysis but had no effect on liver uptake of amino acids.

Furthermore, patients who had a low CPR were shown to have an increased risk of death (Pearl *et al.* 1985; Clowes, 1987), indicating the importance for recovery of an adequate liver response to stress. This finding thus suggests that the changes occurring in liver metabolism after injury are of crucial importance for survival of the host. Moreover, the ongoing loss of cell mass may eventually impair this response in cachectic patients, due to a lack of supply of amino acids by the depleted body cell mass.

The process of breakdown of muscle protein to furnish substrate for hepatic protein synthesis is inefficient in the sense that during this process considerable amounts of N are irreversibly lost. This is illustrated by Vilstrup (1980) and others who demonstrated that during steady-state conditions urea synthesis was linearly correlated with total blood amino acid concentration. During stress, blood levels of glucagon and cortisol, both known to be major regulators of urea synthesis, increase. The resulting increase in urea synthesis is partly responsible for post-operative N loss and decreases in body weight (Heindroff *et al.* 1988). There are several reasons which can explain this phenomenon: because there appears to be no negative feedback from the plasma amino acid concentrations to muscle amino acid release under catabolic circumstances, a vicious circle will develop during disease in which ongoing ureagenesis will lead to irreversible loss of N and to whole-body catabolism (Vilstrup, 1989). This, however, does not explain why the amino acids are not used for protein synthesis. A second reason could be the fact that the amino acid content of muscle protein is not equivalent to the amino acid content of acute-phase proteins; in particular phenylalanine, tryptophan and tyrosine are present in larger proportions in acute-phase proteins compared with skeletal muscle (Reeds *et al.* 1994). It is to be expected therefore that during an acute-phase response certain amino acids will be taken up in greater quantities by the liver than can be used for protein synthesis. This subsequently may lead to an increase in urea synthesis. This explanation, however, is difficult to understand in the light of the reported increases in serum phenylalanine and tryptophan concentrations during the acute phase of a variety of infections (Feigin & Rapaport, 1966; Wannemacher, 1977; Deutz *et al.* 1992). Finally, the major proportion of amino acids released from the muscle are released as glutamine and alanine after transamination of other amino acids (Elia, 1991). A considerable part of the C skeletons from glutamine and alanine is used for gluconeogenesis, leaving significant amounts of N to be removed from the body as urea.

In contrast to all catabolic features that are encountered in cachectic patients, the liver appears to be anabolic during these states, as illustrated clinically by the occurrence of hepatomegaly occurring in certain chronic inflammatory states, e.g. malaria.

This latter point is confirmed by results from a study with tumour-bearing rats. It was shown in these rats that arterial concentrations of total α -amino acids and essential amino

Table 2. *Changes in carcass and liver weight and in circulating concentrations of the acute-phase protein α_2 -macroglobulin in tumour-bearing rats v. controls*

Study group . . .	Control	Large tumour
Carcass wt (g)	200	181
Liver wt (g)	5.5	7.3
α_2 -Macroglobulin (μ g/ml)	< 50	5300

acids were increased leading to an increased uptake of these amino acids by the liver, resulting in higher tissue concentrations (de Blaauw *et al.* 1997).

It was shown also that in the tumour bearing rats, liver weight increased while carcass weight decreased. This change in weight was not due to changes in water content of the liver. Moreover, this finding was accompanied by an increase in a circulating acute-phase protein, α_2 -macroglobulin, an important acute-phase protein in the rat. The results are summarized in Table 2.

CHANGES IN GLUTAMINE METABOLISM

Our group has studied the metabolic changes during the first four postoperative days in a porcine model (Deutz *et al.* 1992). The pigs underwent surgery during which multiple catheters were inserted. By infusing *p*-aminohippuric acid as an indicator, flow as well as amino acid concentrations could be measured across various organs simultaneously.

As expected a significant increase in total amino acid release from the hindquarter was found on the first post-operative day, gradually recovering to almost baseline levels on day 4. Glutamine fluxes in the study are shown in Fig. 1. A decrease in arterial concentrations was observed despite an increase in muscle release of glutamine. The gut, under normal circumstances a major consumer of glutamine, took up less glutamine. This concentration dependency has also been observed in human subjects (van der Hulst *et al.* 1997); glutamine is 'pushed' into the gut by the arterial glutamine concentration. The liver and spleen, however, reacted in an opposite manner. Both organs consumed increased amounts of glutamine despite the decrease in arterial concentrations; they appear to actively 'suck' the glutamine out of the circulation. These findings are in line with the observation that stress hormones such as glucagon and cortisol increase the capacity for glutamine transport into hepatocytes (Low *et al.* 1992).

Data obtained in a tumour-bearing rat model which caused moderate cachexia were in line with the previously discussed results (de Blaauw, 1996). It was demonstrated that although arterial glutamine concentrations remained unchanged, portal glutamine concentrations increased, indicating lower intestinal glutamine consumption. The intracellular hepatic glutamine concentrations decreased as well. This was related to a change in net balance over the liver which decreased from net release to zero balance. These changes could be explained by an increased uptake and utilization of glutamine by the liver (Wannemacher, 1977).

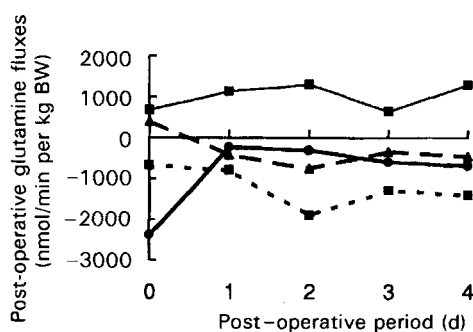


Fig. 1. Post-operative fluxes of glutamine across the hindquarter (■-■), spleen (▲-▲), liver (■---■) and small intestine (●-●). Positive fluxes indicate net release and negative fluxes indicate net uptake of glutamine. Control values were obtained 2–3 weeks after surgery. BW, body weight.

CONCLUSION

In conclusion, cachexia is the result of a large net efflux of amino acids from muscle protein which are largely taken up by the liver, the spleen and possibly the other cells of the immune system. The re-utilization of these amino acids for protein synthesis is inefficient, leading to considerable N losses. The conversion of the C skeletons of amino acids resulting in an increased gluconeogenesis may be useful in providing energy substrate for immune cells. Moreover, the liver and the spleen actively increase amino acid and specifically glutamine uptake despite lowered systemic concentrations. This at least suggests that the liver and the spleen are the driving forces in diseases which through a severe or prolonged inflammatory response finally result in the development of cachexia.

The hypothesis is that breakdown of peripheral tissues serves the purpose of providing substrate for acute phase protein synthesis and for synthesis of immune cells in the liver (i.e. the Kupfer cells), the spleen and possibly other parts of the immune system. This response in itself is essential for an appropriate host response to the inflammatory stimuli and for survival of the organism after injury or infection, but a sustained drainage of peripheral protein pools resulting in depletion of body cell mass in chronic diseases ultimately may lead to cachexia and limit chances of survival.

The previous discussion implies that knowledge of these changes in substrate utilization by the liver and immune system would allow us to adapt our nutritional interventions to make them more effective in preventing muscle protein breakdown and, thus, the development of cachexia.

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