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**Metabolic abnormalities in advanced pancreatic cancer and their
modulation by an eicosapentaenoic acid-based preparation**

**A thesis in fulfilment of the requirements for the degree of M.D. in the
University of Glasgow**

by

Matthew David Barber

Department of Surgery, University of Edinburgh

Spring 2001

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He believed in science, in reason and understanding, in cause and effect. He loved elegance, and the sheer objective logic of scientific thought, which began by saying “Suppose...” but could then build certainty, hard facts from that unprejudiced, unrestricted starting point.

Iain Banks (1954-), *The Bridge*

For small erections may be finished by their first architects; grand ones, true ones ever leave the copstone to posterity. God keep me from ever completing anything. This whole book is but a draught - nay, but the draught of a draught. Oh, Time, Strength, Cash, and Patience.

Herman Melville (1819-1891), *Moby Dick*

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A thesis in fulfilment of the requirements for the degree of M.D. in the University of Glasgow

Matthew David Barber

Declaration

I declare that the work described in this thesis has been done and the thesis composed by myself and that the books and papers cited were all consulted by me personally.

Some work was performed in collaboration -

Analysis of dietary intake from diet diaries was performed by Alison Hinds and Rosemary Richardson at the University Department of Surgery, Royal Infirmary of Edinburgh.

Measurement of proteolysis inducing factor excretion and fatty acid analysis was performed by Bill Fields and Professor Michael Tisdale at Aston University, Birmingham.

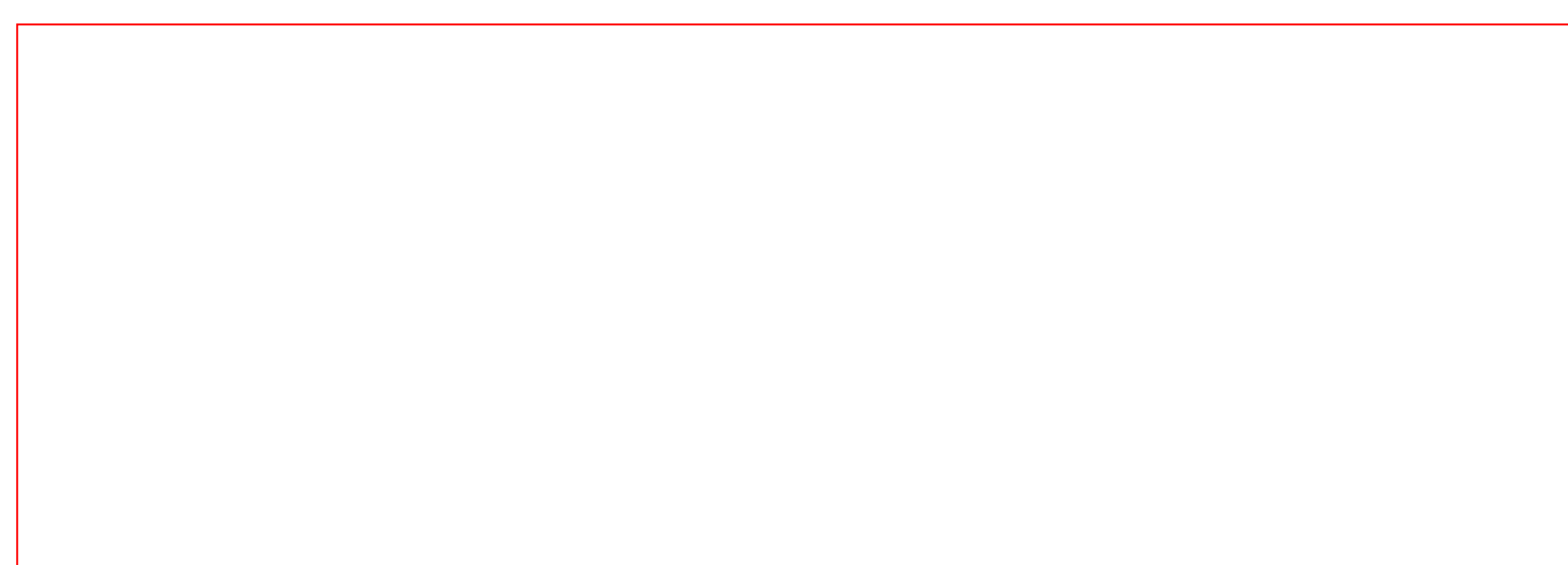
The majority of cytokine gene polymorphism genotyping was performed by Dr Susan Lynch and Dr James Powell at the University Department of Surgery, Royal Infirmary of Edinburgh.

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The majority of sample preparation for measurement of protein synthesis was performed by Dr Donald McMillan and Jason Donnelly at the University Department of Surgery, Glasgow Royal Infirmary.

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Matthew D. Barber

Metabolic abnormalities in advanced pancreatic cancer and their modulation by an eicosapentaenoic acid-based preparation

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Summary

The work presented in this thesis has explored the impact of cachexia on patients with advanced pancreatic cancer. This thesis has also explored the metabolic changes responsible for cachexia and the lack of response of cachectic subjects to apparently adequate nutrition. The effect of a fish oil-enriched nutritional supplement on the syndrome of cachexia was also studied. Particular emphasis was placed on the potential role of the inflammatory response in mediating aspects of cachexia. In order to evaluate this, the interaction of the inflammatory process and progress of disease was examined. The significance of the acute phase protein response and its potential to contribute to cachexia was also emphasised.

Progressive weight loss was seen in patients with pancreatic cancer and this accelerated with time. The plasma concentration of a variety of positive and negative acute phase proteins were increased or reduced respectively in advanced pancreatic cancer and these concentration changes became more pronounced with progress of disease (Chapter 8). Weight loss and progress of the acute phase protein response were associated with a deterioration in the ability of patients to perform normal tasks (Chapter 3). The presence of an acute phase protein response was also associated with a shortened survival (Chapter 5). Cachexia thus appeared to influence both morbidity and mortality in advanced pancreatic cancer.

Mediators of the process of cachexia were explored. The progressive acute phase protein response in advanced pancreatic cancer was associated with increased circulating concentrations and production of pro-inflammatory cytokines, particularly interleukin (IL)-6. These cytokines appear to have a role in mediating the acute phase protein response (Chapters 4 and 7). The relevance of the circulating cytokine concentrations and ex vivo cytokine production to the in vivo situation, however, remains unclear as levels were not different to those observed in controls.

Differences in the extent of cachexia and the inflammatory response in patients with apparently similar tumours suggest that systemic production of cytokines may be dependent not only on tumour phenotype but also on host genotype. This was investigated by studying a variety of cytokine gene polymorphisms which may influence cytokine production. The production of interleukin-1 β ex vivo was different in those patients with different IL-1 β gene polymorphisms and thus appeared to be genetically determined. The genotype of patients for an IL-1 β gene polymorphism also seemed to influence the presence of an acute phase protein response. IL-1 β genotypes associated with increased cytokine production and acute phase protein concentrations

were also associated with poor patient survival. By contrast, no evidence was found that two polymorphisms of the tumour necrosis factor (TNF) gene or a polymorphism of the IL-1 receptor antagonist gene affected ex vivo cytokine production, the in vivo acute phase protein response or survival (Chapter 5). Thus, IL-1 β (which can stimulate the production of other pro-inflammatory cytokines) may be an important early mediator in the cachectic process. TNF may still be important but the polymorphisms studied may not be relevant to this disease.

Advanced pancreatic cancer patients were found to have changes in the circulating concentration or excretion rates of various additional metabolic mediators which may contribute to catabolism and weight loss. These changes included a low circulating insulin concentration and the excretion in urine of proteolysis inducing factor, a novel cancer-associated catabolic factor (Chapter 7). A complex cascade of mediators including pro-inflammatory cytokines, tumour associated cachectic factors and neuroendocrine hormones thus appear to be involved in the development of cachexia and may give rise to an acute phase protein response.

The metabolic mechanisms underlying cachexia were then explored, with studies in the fasted and fed state. Cachectic patients had an increased resting energy expenditure in comparison with controls in association with changes in substrate utilisation and nitrogen excretion reflecting the weight losing state. However, there was no increase in the metabolic cost of digesting and absorbing a meal to explain the failure of conventional nutrition to reverse cachexia (Chapter 9).

The acute phase protein response stimulated by mediators of cachexia has been noted above to be associated with poor outcome, however, a causative role has not been demonstrated. It has been suggested that production of hepatic acute phase proteins contributes to cachexia by increasing the breakdown of lean tissue to provide amino acids for their synthesis. In addition, the effect of feeding on such protein synthesis in cancer patients has not been studied previously. The present study found that the synthesis of negative acute phase proteins by the liver continued unchanged in advanced pancreatic cancer compared with controls (despite low plasma concentrations) in both the fasting and fed state. Moreover, there was a substantial increase in the production of positive acute phase proteins which was increased still further in the fed state in cancer patients (but not in healthy controls) (Chapter 10). Thus feeding stimulated further the acute phase response. Diversion of amino acids to meet increased demand for hepatic export protein synthesis on feeding may in part explain the block to the accretion of lean tissue in cachectic subjects provided with conventional nutritional supplementation. This may also provide a mechanism whereby the acute phase protein response is associated with progressive cachexia.

Eicosapentaenoic acid, a component of fish oil has been suggested to modulate aspects of the inflammatory process, specifically to downregulate pro-inflammatory cytokine production, interfere with the action of proteolysis inducing factor and to influence hepatic export protein production. It could, therefore, potentially influence the process of cachexia. Thus the combination of a nutritional supplement and eicosapentaenoic acid was given to patients with advanced pancreatic cancer to assess its effect on the ongoing cachectic process. The administration of a fish oil-enriched nutritional supplement providing 2g eicosapentaenoic acid and around 600kcal per day resulted in a significant change in the metabolic disposition of patients with advanced pancreatic cancer. Consumption of the supplement resulted in a reversal of weight loss and a gain in lean body mass in association with a reversal of negative nitrogen balance. Performance status and appetite improved. Production of interleukin-6 was reduced and the acute phase protein response stabilised, insulin concentration increased and proteolysis inducing factor excretion fell (Chapters 6, 7 and 8). These changes were associated with a rise in energy intake, a fall in relative resting energy expenditure and a normalisation of the metabolic response to feeding and substrate utilisation (Chapters 6 and 9). The increase in acute phase protein synthesis with feeding was abolished after the administration of the fish oil-enriched nutritional supplement (Chapter 10).

The progressive nutritional and functional deterioration of advanced pancreatic cancer patients thus seems to be associated with broadly pro-inflammatory metabolic mediators and a progressive acute phase protein response. This deterioration may in part be resistant to conventional nutritional supplementation because of further stimulation of acute phase protein production by feeding.

The provision of a fish oil-enriched nutritional supplement normalises the metabolic milieu, stabilises the acute phase protein response and abolishes the stimulation of acute phase protein production by feeding. One explanation to account for the effects observed after intervention would be that it allows dietary amino acids to be used for lean tissue anabolism with the consequent potential for quality of life and survival benefit.

These studies emphasise that by further investigation of the mechanisms and mediators of the cachectic process more rational and effective nutritional therapy can be designed. The benefits noted in the initial studies of the fish oil-enriched nutritional supplement are currently being subjected to a randomised controlled trial.

Introduction

Pancreatic Cancer

Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the Western world with an incidence of about 10/100000 person-years representing about 2% of all cancers and 5% of cancer deaths (Rosewicz & Weidenmann, 1997, Landis et al, 1999). Over 95% of patients will die of their disease. The 5-year survival rate is 1.3% with a median survival of 4.1 months (Ahlgren, 1996). More than 80% of cancers are unresectable at diagnosis and even in those patients suitable for surgical resection the 5-year survival rate is less than 25% in the best centres (Neoptolemos & Kerr, 1995).

Numerous studies of radiotherapy and chemotherapeutic agents in patients with pancreatic cancer have shown little benefit and significant toxicity (Thomas, 1996, Schnall & MacDonald, 1996). Current trials of combination chemotherapy and radiotherapy tend to concentrate on patients who have already had surgical resection (Neoptolemos & Kerr, 1995). This leaves little in the way of treatment options for the vast majority of patients with unresectable disease. New agents, such as the antimetabolite gemcitabine, are currently being tested in patients with advanced pancreatic cancer. These new agents have shown some benefits against surrogate disease markers despite little objective anti-tumour activity (Casper et al, 1994, Rothenberg et al, 1995, Moore et al, 1995, Carmichael et al, 1995, Parsons et al, 1997). A recent randomised controlled trial of gemcitabine versus 5-fluorouracil in patients with advanced pancreatic cancer has suggested a survival benefit for gemcitabine but only improved median survival from 4.4 to 5.6 months (Burris et al, 1997). These agents retain many of the side effects of traditional chemotherapeutic agents and thus their place in improving the well-being of this unfortunate group of patients remains unclear. A recent study in non-small cell lung cancer suggested that the patients would require a survival benefit of 4.5 months for a chemotherapeutic regime resulting in mild toxicity or of 9 months for severe toxicity to choose this over supportive treatment alone (Silvestri et al, 1998). Current chemotherapy regimes for pancreatic cancer cannot begin to approach this level of effectiveness and thus while continuing to explore the possibilities of cytotoxic treatment it seems important to study the mechanisms and mediators of decline in patients with advanced cancer in an attempt to maximise supportive treatment with the potential to improve quality and perhaps length of life. The work presented in this thesis concentrates on cachexia, the syndrome of metabolic changes observed in advanced cancer, and the modulation of the metabolic changes of cachexia by n-3 polyunsaturated fatty acids.

Cancer cachexia

Importance of Cachexia

The term cachexia is derived from the Greek words *kakos*, meaning “bad,” and *hexis*, meaning “condition.” The syndrome is characterised by anorexia, early satiety, changes in taste perception, weight-loss, weakness, anaemia and oedema (Fearon, 1992). Cachexia is not exclusive to cancer but is also seen in a variety of inflammatory conditions such as acquired immunodeficiency syndrome and rheumatoid arthritis (Roubenoff et al, 1992, Grunfeld & Feingold, 1992).

Patients with advanced cancer frequently exhibit progressive weight-loss and this is associated with a shorter survival time and reduced quality of life and indeed, some patients appear to die of such severe wasting. Cancer patients with weight-loss live approximately half as long as those without and those with greater weight-loss appear to live a shorter time (DeWys et al, 1980). Those cancer patients with weight-loss have also been reported to have a reduced performance status and quality of life (DeWys et al, 1980, Ovesen et al, 1993a, O’Gorman et al, 1998). It has been suggested that around 20% of all cancer patients die of cachexia making it the most common cause of death, with this proportion being substantially higher in particular malignancies (Warren, 1935, Inagaki et al, 1974). Cancer cachexia is seen in the majority of patients with advanced cancer of the stomach, pancreas, lung and colon (DeWys et al, 1980). Other tumours, such as breast cancer and haematological malignancies, are rarely associated with cachexia. Similarly, patients with an apparently identical primary cancer and disease stage can vary considerably in terms of the development of cachexia suggesting variations in tumour phenotype and host response as important contributory factors.

A recent survey of patients with unresectable pancreatic cancer found that 85% of such patients have unintentionally lost weight by the time of diagnosis and that the median weight loss close to the time of death was almost 25% (Wigmore et al, 1997a). Clearly in a proportion of these individuals weight-loss contributed to their demise. The fundamental difference between the weight-loss observed in cachexia and that seen in, for example, starvation, is the lack of reversibility with feeding (Brennan, 1977, Nixon et al, 1981). This seems to be due to metabolic changes in cachexia, produced by the tumour or by the host in response to the tumour, which have a profound effect on the progress of the disease, the symptoms experienced by patients, and ultimately the survival of patients. Attempts to modulate the metabolic response to cancer therefore have the potential to improve quality and length of life and should form a part of the integrated care of patients with advanced cancer.

Mechanisms of cachexia

Many of the metabolic changes seen following surgery, trauma or sepsis share many features of cachexia and are driven, in part, by similar mediators.

Anorexia

Probably the majority of patients with pancreatic cancer have an inadequate nutritional intake which contributes substantially to weight loss (Perez et al, 1983, Wigmore et al, 1997b). In simple starvation patients undergo adaptations to reduce energy expenditure, conserve protein and to utilise fatty acids and ketone bodies derived from fat as an energy source (Grande et al, 1958, Leibel et al, 1995). It has been suggested that in cancer cachexia these adaptations are attenuated or absent such that energy expenditure may be increased and protein loss continues (Brennan, 1977). This also has implications for the pattern of weight loss in patients with cancer cachexia who may preserve visceral protein while muscle protein is lost preferentially (Heymsfield & McManus, 1985, Fearon & Preston, 1990).

Patients with cancer cachexia frequently have specific problems which reduce nutritional intake, including physical obstruction of the gastrointestinal tract, nausea, constipation and debility, psychological problems including depression and pain and the side effects of treatment with opiates, radiotherapy and chemotherapy. Given the role of the pancreas in producing digestive enzymes, malabsorption may be particularly seen in patients with pancreatic cancer (Perez et al, 1983). However, even if these factors are well controlled, those with cancer cachexia often describe poor appetite, early satiety, changes in taste and classical anorexia (Fearon, 1992). These symptoms are similar to those seen in many groups of patients with inflammatory and infective illnesses and appear to constitute part of the metabolic response to the cancer-bearing state.

Hypermetabolism

A heterogeneous picture of energy expenditure has been described in cancer patients with resting energy expenditure varying between less than 60% and more than 150% of that predicted (Bozetti et al, 1980, MacFie et al, 1982, Knox et al, 1983, Dempsey et al, 1984, Hansell et al, 1986, Hylander et al, 1991, Fredrix et al, 1991). Longitudinal studies in a rat model of cancer cachexia has suggested that the animals are initially hypermetabolic before passing through a relatively normometabolic period to a preterminal hypometabolic phase (Zyclicz et al, 1990) (figure 1.1). Such longitudinal studies have not been performed in humans but this pattern would account for some of the variation in the results observed. Patients with cancer in sites

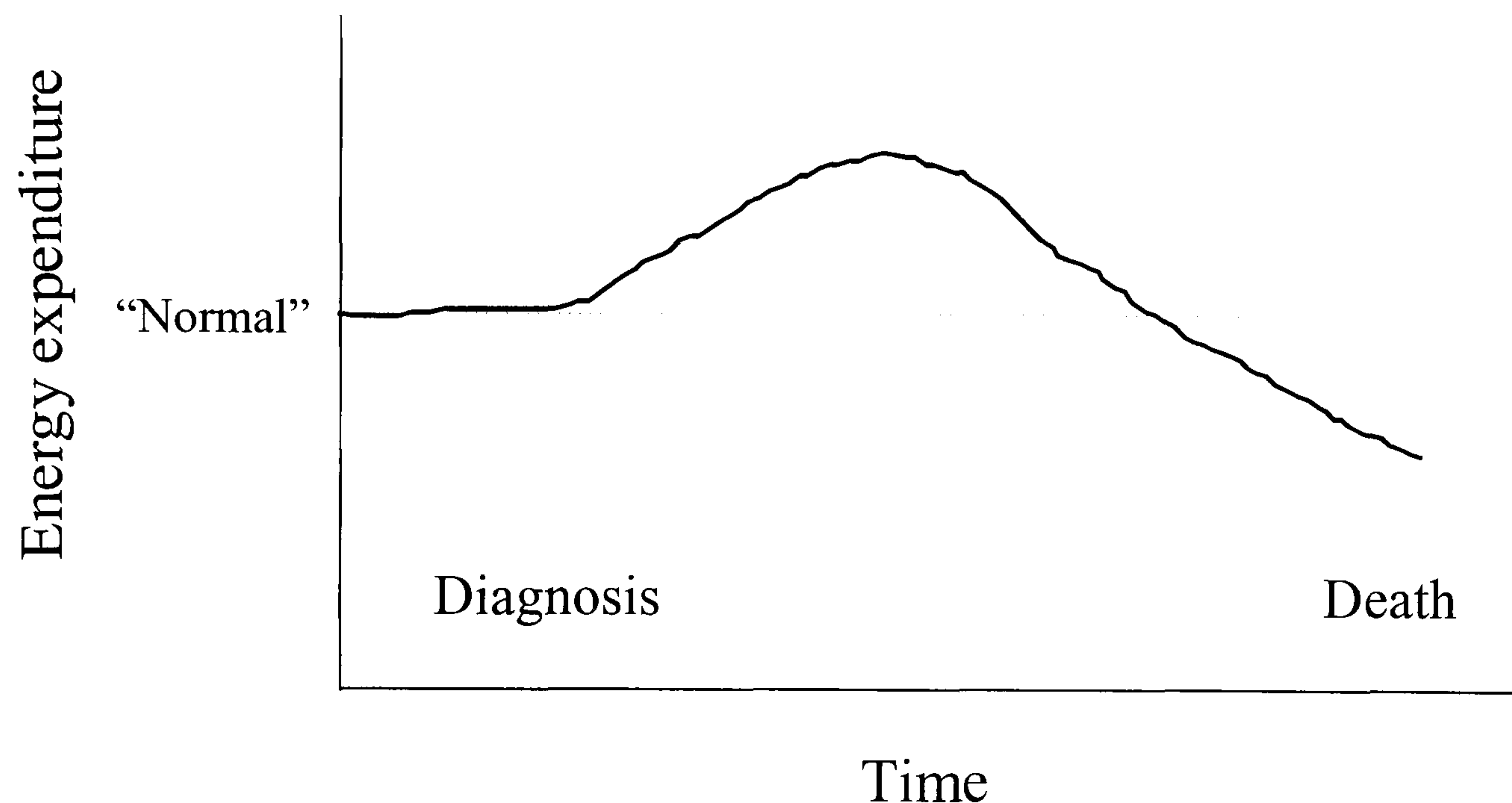


Figure 1.1. Possible pattern of change in resting energy expenditure through disease course in cancer cachexia (based on findings of Zyclicz et al, 1990).

frequently associated with cachexia (such as lung and pancreas) have more often been described as exhibiting elevated resting energy expenditure (Fredrix et al, 1991, Staal-van den Brekel et al, 1994, Falconer et al, 1994a). In models of inflammation sharing mediators with cachexia it has been suggested that this increased energy expenditure may be due to the induction of mitochondrial uncoupling proteins (Faggioni et al, 1998a). However, it has been suggested that, while resting energy expenditure is increased, total energy expenditure may be unchanged due to a fall in physical activity (Gibney et al, 1997) (figure 1.2). Thus overall energy balance may be maintained by a concomitant reduction in activity, however, this decreased physical activity may reflect a reduced quality of life.

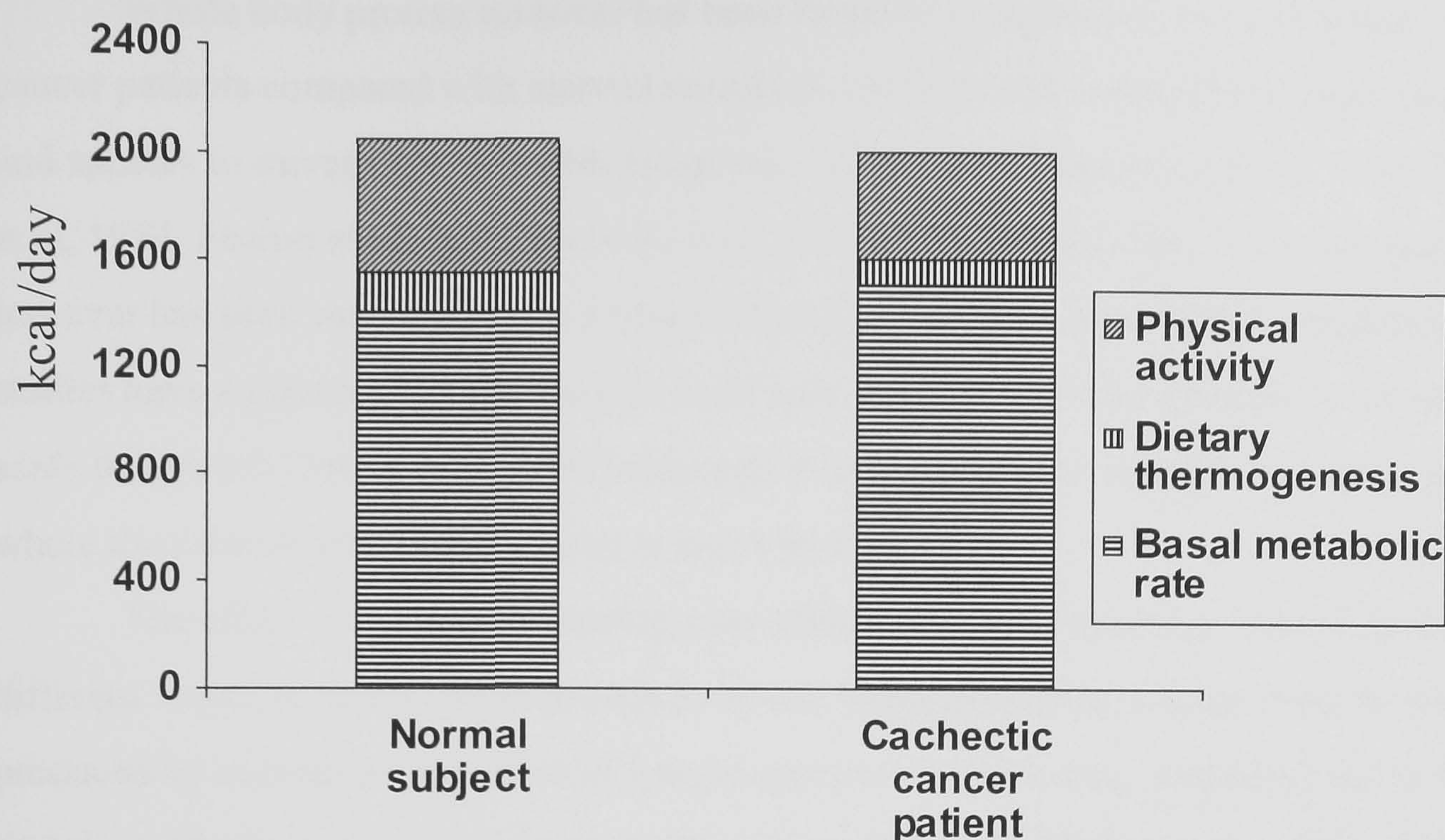


Figure 1.2. Energy expenditure in cancer patients. While basal metabolic rate may be increased, dietary thermogenesis and physical activity may be decreased resulting in little difference in total energy expenditure (based on findings of Gibney et al, 1997).

Substrate metabolism

A variety of changes in nutrient metabolism have been described in patients with cancer cachexia. Such patients exhibit relative glucose intolerance and insulin resistance with an increased rate of glucose production and recycling via lactate (the Cori cycle) (Rohdenburg et al, 1919, Waterhouse, 1974, Holroyde et al, 1975, Lundholm et al, 1978, Chlebowski et al, 1982, Holroyde et al, 1984, Edén et al, 1984, Shaw & Wolfe, 1987, Copeland et al, 1987). These changes may become more pronounced with progress of the disease (Chlebowski et al, 1982, Shaw & Wolfe, 1987). Most solid tumours seem to obtain their energy from the anaerobic metabolism of glucose (Holm et al, 1995) and there is debate as to what extent the above changes exist to supply the tumour with nutrients. It has been suggested that the increased glucose turnover observed in cancer has an energy cost of up to 260kcal per day (Edén et al, 1984).

Substantial loss of adipose tissue is observed in cancer cachexia (Heymsfield & McManus, 1985, Fearon & Preston, 1990) with significantly elevated fat oxidation rates (Hansell et al, 1986). Despite this, lipolysis rates are not significantly increased in cancer cachexia but rather lipogenesis appears to be reduced (Jeevanandam et al, 1986). This may be due to reduced levels of lipoprotein lipase in weight-losing cancer patients (Vlassara et al, 1986).

Whole body protein turnover has been found to be increased in the majority of advanced cancer patients compared with starved normal individuals and weight-losing non-cancer patients and appears to increase further with progression of disease (Carmichael et al, 1980, Jeevanandam et al, 1984, Fearon et al, 1988, Melville et al, 1990). The energy cost of this increased protein turnover has been estimated to be around 100kcal/day (Fearon et al, 1988). In addition, animal studies have suggested that the tumour itself may utilise circulating albumin as a source of amino acids for growth (Stehle et al, 1997) although it is not clear how relevant this is in human disease where the relative size of the tumour is much smaller.

The effect of the cancer-bearing state affects different aspects of protein synthesis in different ways. A model of the protein synthetic response to injury using stress hormone infusion produced an immediate reduction in lymphocyte protein synthesis, a delayed fall in muscle protein synthesis and a delayed rise in albumin synthesis (McNurlan et al, 1996). Loss of skeletal muscle protein is a prominent feature of cachexia, however, skeletal muscle protein breakdown rates in cancer patients have not been found to be different from controls. There is, however, a reduction in the rate of muscle protein synthesis (Emery et al, 1984, Dworzak et al, 1998). Thus in relative terms muscle protein breakdown is increased. In a rat model of cachexia it has been suggested that muscle wasting is associated with an activation of the ATP-dependent ubiquitin-mediated proteolytic pathway with no effect on calcium dependent or lysosomal proteolytic systems (Llovera et al, 1994, Baracos et al, 1995, Llovera et al, 1995, Lecker et al, 1999). The relevance in this system in human cachexia remains to be explored.

The balance of liver export proteins is altered in many cancer patients such that while albumin synthesis remains unchanged while fibrinogen synthesis rates are significantly increased (Fearon et al, 1998, Preston et al, 1998). These changes occur on a background of a decrease in the circulating concentration of albumin and an increase in the concentration of fibrinogen.

The acute phase protein response

These latter changes reflect aspects of the acute phase protein response, a reprioritisation of liver protein synthesis often seen in trauma, inflammation and infection (Fleck et al, 1985, Baumann & Gauldie, 1994). The acute phase protein response is characterised by a fall in the concentration of negative acute phase proteins such as albumin and a rise in positive acute phase proteins including C-reactive protein and fibrinogen. An acute phase protein response may be seen in a significant proportion of patients with cancer of the pancreas, lung, kidney and oesophagus (Falconer et al, 1994a, Staal-van den Brekel et al, 1995, Blay et al, 1992, Wayman et al, 1997). The proportion of pancreatic cancer patients exhibiting an acute phase response

increases with disease progression (Falconer et al, 1994a). An acute phase response has been related to increased weight-loss in lung and pancreatic cancer and melanoma (Staal-van den Breckel et al, 1995, Scott et al, 1996, Wigmore et al, 1997b, Harvie et al, 1998). Moreover, the presence of such a response in cancer patients is strongly associated with a reduced quality of life in gastrointestinal cancer patients (O’Gorman et al, 1998) and a shortened survival in renal, pancreatic and colorectal cancer (Blay et al, 1992, Falconer et al, 1995, Nielsen et al, 1998). In pancreatic cancer those with an acute phase protein response were found to have a median survival of 66 days while those without had a median survival of 222 days (Falconer et al, 1995).

During an inflammatory response there are altered demands for amino acids. Primary sources of amino acids are the diet and structural protein (largely skeletal muscle), with potential contributions from the gut and plasma proteins. The cachectic cancer patient may have an insufficient nutritional intake to provide the required amino acids and consequently there may be relatively increased breakdown of skeletal muscle to supply sufficient amino acids. This breakdown may be further exaggerated as there is an imbalance between the amino acid composition of skeletal muscle and acute phase proteins (Reeds et al, 1994). During inflammation, the acute phase proteins represent only one possible destination of amino acids, others would include structural proteins including muscle, gluconeogenesis, transport proteins and processes such as repair and the immune response.

In general the acute phase protein response aids tissue repair, blood clotting, the prevention of ongoing tissue damage and the destruction of infective organisms (Baumann & Gauldie, 1994). The value of the acute phase protein response in patients with cancer is not clear and it may be that it occurs simply as part of a stereotyped response to inflammation. The acute phase protein response is stimulated at least in part by pro-inflammatory cytokines, notably interleukin (IL)-6 (Castell et al, 1990). Some tumour cell lines produce pro-inflammatory cytokines (Wigmore et al, 1994) and it is conceivable that the tumour may benefit from changes associated with the acute phase response such as altered energy substrate metabolism (Edén et al, 1984, Holroyde et al, 1975) which may aid its nutrition. Alternatively, the strong association between an acute phase protein response and survival in some malignancies (Falconer et al, 1995) may be due to a link between pro-inflammatory cytokine production and the phenotypic behaviour of the tumour. For example, IL-1 receptor blockade will reduce the metastatic potential of melanoma cells in vivo (Vidal-Vanaclocha et al, 1994).

It would appear that these alterations in substrate metabolism are designed to provide a ready supply of nutrients and proteins for host defense and tissue repair. While this may be beneficial in relatively short term insults such as infection or trauma, in the cancer-bearing patient

it seems that such alterations are likely to exacerbate weight loss and may preferentially provide additional nutrients for the tumour.

Mediators of cachexia

It has been suggested that a tumour constitutes a new metabolically active organ requiring its own sustenance and so increasing demand for nutrients and causing weight loss if these are not forthcoming. However, the presence and severity of cachexia often correlates poorly with the size of the tumour, it is often seen early in the course of the disease and manifestations such as alterations in appetite and nutrient metabolism cannot easily be explained by this hypothesis (Tisdale, 97). Parabiotic studies in rats where the tumour-bearing animal induces cachexia in the non-tumour-bearing partner suggest circulating mediators are more likely to be responsible (Norton et al, 1985). Thus it appears likely that the metabolic changes seen are the result of mediators produced by the tumour or by the body in response to the tumour (figure 1.3). Potential mediators are discussed below.

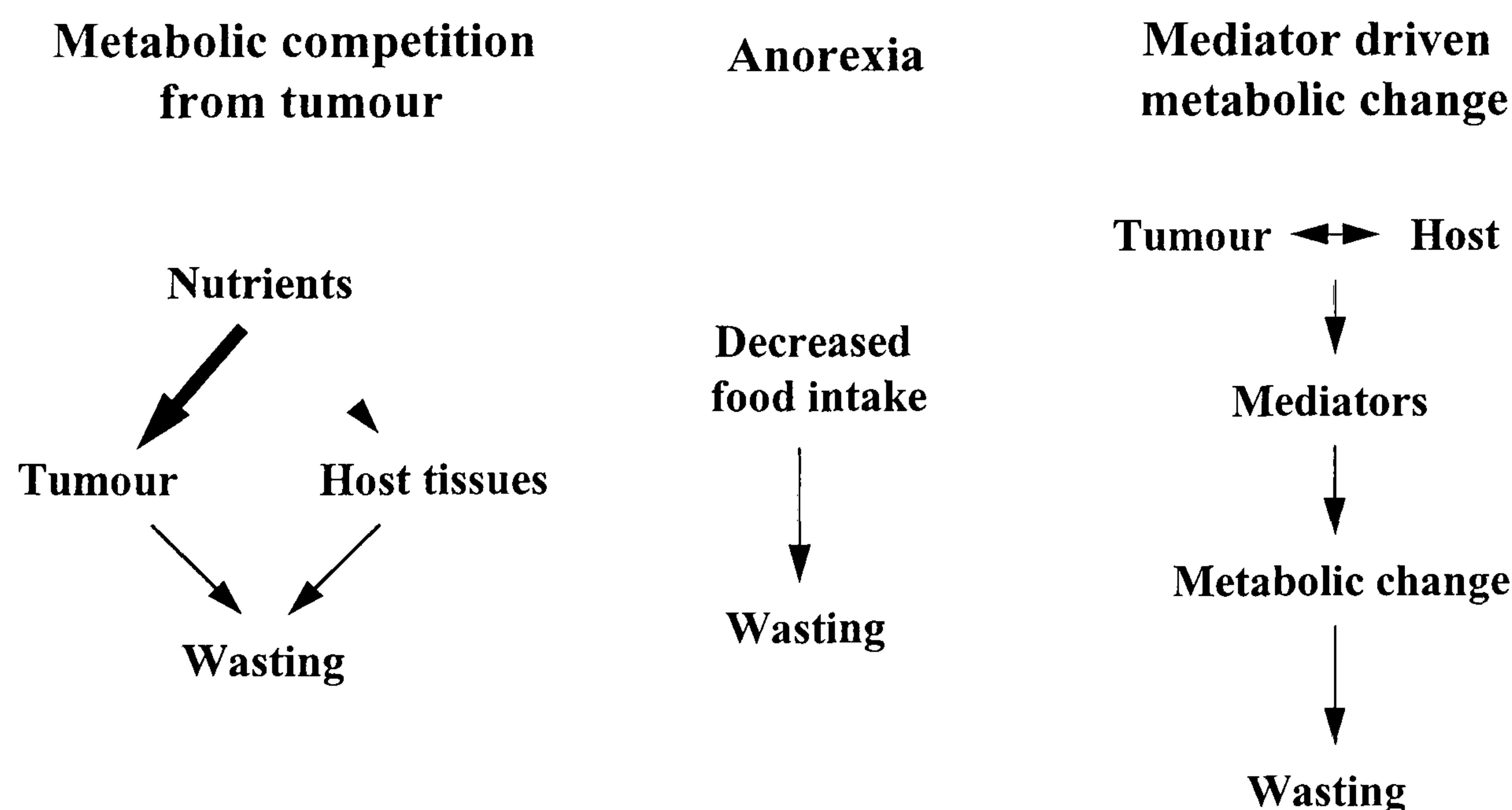


Figure 1.3. Theories of the mechanisms causing cachexia.

Metabolic competition seems unlikely as human tumours are a fraction of the weight of the host and wasting begins early in disease. Anorexia does not explain all features of disease as the metabolic changes differ from those of simple starvation and wasting is seen even with apparently adequate intake. Thus mediator induced metabolic changes seem the most likely explanation.

Neurotransmitters

Serotonergic activity in the hypothalamus suppresses appetite. Increased levels of free tryptophan, the precursor of serotonin, are found in cancer patients and correlate with reduced food intake (Cangiano et al, 1994). Increased levels of neuropeptide Y within the hypothalamus are thought to stimulate feeding, however, tumour-bearing rats are refractory to the effects of this agent when injected into this region (Chance et al, 1996). The role of such neurotransmitters in weight-loss in humans has yet to be explored.

Cytokines

Several pro-inflammatory cytokines, including tumour necrosis factor α (TNF α), interleukin-1 (IL-1), interleukin-6 (IL-6), interferon gamma (IFN γ) and ciliary neurotrophic factor (CNTF), have been implicated in cachexia. Pro-inflammatory cytokines have been shown to be produced by tumour associated macrophages and tumour cells (Erroi et al, 1989, Wigmore et al, 1994).

TNF α suppresses lipoprotein lipase activity in adipocytes so preventing fat storage (Fried & Zechner, 1989). Administration of TNF α leads to anorexia, weight loss, an acute phase protein response, protein and fat breakdown, a rise in levels of cortisol and glucagon and a fall in insulin levels, insulin resistance, anaemia, fever and elevated energy expenditure in animals (Tracey et al, 1988, Mahony and Tisdale, 1988, Moldawer et al, 1988, Charters & Grimble, 1989, Goodman, 1990, Llovera et al, 1993, Llovera et al, 1997) and humans (Starnes et al, 1988, Warren et al, 1987, Selby et al, 1987, Michie et al, 1988). However, it is rare to detect circulating TNF α in cancer patients, even those losing weight (Socher et al, 1988), and while antibodies to TNF may increase food intake in an animal model of cachexia, they do not abolish weight-loss (Gelin et al, 1991, Smith and Kluger, 1993). It has also been suggested that the pattern of weight-loss observed in experimental animals given TNF α differs from that seen in cancer cachexia (Mahony et al, 1988). Production of TNF α by isolated peripheral blood mononuclear cells has been shown to be elevated in weight-losing pancreatic cancer patients with an acute phase protein response suggesting that local rather than systemic production of this cytokine may be more important (Falconer et al, 1994a). It has been suggested that circulating TNF α concentrations exhibit circadian changes with substantially increased levels being found in cancer patients at 3am while levels may be undetectable at other times of day (Muc-Wierzgon et al, 1996). TNF α induces the release of soluble TNF receptors from cells and while circulating TNF α may not be detected, increased concentrations of these soluble TNF receptors may be found in circulation and appear to have some correlation with disease stage (Aderka et al, 1991). The incubation of muscle in the

presence of TNF α does not result in protein breakdown suggesting that other mediators are involved (Goodman, 1990).

IL-6 probably induces directly the acute phase protein response (Morrone et al, 1988, Castell et al, 1990) and is produced by some cancer cell lines (Wigmore et al, 1994). Administration of IL-6 produces weight-loss, increased energy expenditure and increased cortisol and glucagon concentrations in human subjects (Stouthard et al, 1996). Elevated circulating concentrations of IL-6 have been found to be associated with weight-loss and the acute phase protein response in lymphoma, lung and colorectal cancer patients (Fearon et al, 1991, Kurzrock et al, 1993, Scott et al, 1996). Antibodies to IL-6 will suppress the development of cachexia in animals to some extent (Strassmann et al, 1992). The administration of IL-12 to tumour bearing mice (Mori et al, 1996) or the incorporation of the IL-10 gene into an IL-6-producing mouse cancer model (Fujiki et al, 1997) both resulted in attenuated cachexia in association with reduced serum levels of IL-6. Antibodies to IL-6 given to patients with AIDS-related lymphoma produced weight-gain in those losing weight and stabilised levels of the acute phase reactant, C-reactive protein (Emilie et al, 1994). However, different clones of experimental tumours will produce very different patterns of weight-loss despite producing similar levels of IL-6 suggesting that IL-6 may work in concert with other mediators (Soda et al, 1995). Local production of IL-6 may be important in addition to circulating levels as peripheral blood mononuclear cell IL-6 production has been shown to be elevated in those pancreatic cancer patients with an acute phase protein response (Falconer et al, 1994a).

CNTF is a member of the IL-6 superfamily and will produce weight-loss, protein breakdown, fever and an acute phase protein response in animals (Henderson et al, 1994, Espat et al, 1996).

Similarly, IL-1 will produce anorexia, weight-loss, a fall in muscle protein synthesis and increased protein breakdown (Okusawa et al, 1988, Hellerstein et al, 1989, Moldawer et al, 1988, Ballmer et al, 1991). IL-1 also stimulates production of IL-6 (Yasumoto et al, 1995). The administration of anti-IL-1 antibodies to tumour bearing animals will reduce weight-loss and IL-6 production (Gelin et al, 1991, Oldenburg et al, 1993). Administration of anti-IL-1 antibodies in an inflammatory model attenuates the acute phase protein response and IL-6 production (Gershenwald et al, 1990). Mice lacking a functional IL-1 receptor exhibit an attenuated acute phase response in a model of inflammation (Solorzano et al, 1996) (those lacking a TNF receptor shown no change in the inflammatory response).

Chinese hamster ovary tumour cells genetically engineered to produce murine IFN γ produced greater cachexia when implanted in mice than the native tumour alone. However, IFN γ

injections did not produce cachexia without the presence of tumour cells (Matthys et al, 1991). Antibodies to IFN γ may prevent or attenuate cachexia in this model and other models (Matthys et al, 1991, Langstein et al, 1991). Conversely, the attenuation of cachexia produced by IL-12 causing a fall in IL-6 was attributed to increased production of IFN γ (Mori et al, 1996) and the attenuation of cachexia and tumour growth by IL-12 in sarcoma-bearing mice is not observed in IFN γ knockout mice (Cahlin et al, 1998).

Thus, a variety of pro-inflammatory cytokines appear to play a major role in aspects of cachexia. However, it is becoming increasingly apparent that individual cytokines do not work alone in the in vivo situation and that a complex network of cytokines in combination with other factors are involved. This situation is further complicated by the fact that the release of pro-inflammatory cytokines stimulates the production of antagonists which may limit the extent of their action, such as interleukin-1 receptor antagonist and soluble TNF receptors (Dinarello & Wolff, 1993, Aderka et al, 1998). Indeed, some traditionally pro-inflammatory cytokines, such as IL-6, have been described as having antiinflammatory effects (Tilg et al, 1994).

Prostaglandins

Concentrations of prostaglandin E₂ have been found to be elevated in animal models of cachexia (Tessitore et al, 1993) and some tumours produce this prostaglandin (Strelkov et al, 1989). Release of prostaglandins is a major step in the signalling pathway leading to muscle protein breakdown in normal tissues (Thomson & Palmer, 1998). It also appears that prostaglandins may mediate the actions of most pro-inflammatory cytokines (Okusawa et al, 1988, Rothwell, 1992). Specific inhibitors of prostaglandin synthesis abolish the protein catabolic effect of serum from mice with cancer cachexia (Smith & Tisdale, 1993). They will also prevent the experimental cachectic effects of TNF α (Kozak et al, 1997) and IL-1 (Hellerstein et al, 1989, Uehara et al, 1989). They will block many of the pro-inflammatory actions of TNF α in animal models (Kettelhut et al, 1987) although other studies have failed to find any effect on TNF-induced weight-loss (Mahony & Tisdale, 1989). Administration of prostaglandin inhibitors appears to prevent muscle protein breakdown in tumour-bearing rats although they do not increase food intake. However, this effect is only seen in some tumour models (Strelkov et al, 1989). It is likely that prostaglandins have a role to play in the network of mediators of cachexia. Prostaglandins also have a variety of other effects in influencing inflammation and blood clotting which are discussed below.

Hormones

Infusion of hydrocortisone or cortisol, glucagon and adrenaline in humans will produce features of cachexia such as protein loss, an acute phase protein response, increased energy expenditure and glucose intolerance (Bessey et al, 1984, Watters et al, 1986). That pro-inflammatory cytokines effect levels of classical hormones such as cortisol, insulin and glucagon has already been touched upon. Changes in hormone levels and target-organ sensitivity have been described in both animals and humans with cachexia. Profound changes are seen in hormone levels in experimental tumour-bearing animals although the patterns of change varied with the tumour cell line implanted (Besedovsky et al, 1985). In humans with cancer elevated levels of cortisol and glucagon have been observed (Schaur et al, 1979, Burt et al, 1983, Holroyde et al, 1984, Knapp et al, 1991) and these may amplify the acute phase protein response (Baumann & Gauldie, 1994).

A blunted insulin secretory response to a glucose load has been described in a group of patients with colorectal cancer (Holroyde et al, 1984). In addition, insulin resistance in terms of glucose metabolism has been noted, particularly in pancreatic cancer, although this appears to be unrelated to the loss of pancreatic tissue and seems to be due to the production of islet amyloid polypeptide (amylin) by the surrounding normal pancreatic tissue (Schwarz et al, 1978, Gullo et al, 1993, Permert et al, 1993, Permert et al, 1994). The production of amylin appears to be stimulated by the presence of pancreatic cancer cells although the signal involved has not been identified (Ding et al, 1998). Amylin will induce anorexia and weight loss when administered to rats (Arnelo et al, 1996). However, it has been suggested that the skeletal muscle anabolic effects of insulin are not affected in cancer (Newman et al, 1991). Insulin also affects the synthesis of hepatic acute phase proteins, increased concentrations causing a rise in albumin synthetic rate and a reduction in fibrinogen synthetic rate (De Feo et al, 1993).

It is increasingly being recognised in cancer patients with weight loss that small increases in circulating cortisol and falls in insulin concentration result in a marked increase in the cortisol:insulin ratio and that this may contribute to the catabolism of peripheral tissues (Fearon et al, 1998).

It has been suggested that leptin, the hormone produced by fat which suppresses appetite and increases energy expenditure to maintain weight stability (Zhang et al, 1994), may be involved in cancer cachexia as elevated leptin levels are seen in some models of inflammation (Grunfeld et al, 1996, Sarraf et al, 1997). The administration of TNF α will increase leptin concentrations (Zumbach et al, 1997) and the presence of IL-1 β also appears to be required for leptin production in inflammation (Faggioni et al, 1998b). However, initial clinical studies have

found that levels of leptin are appropriately low in weight-losing cancer patients (Simons et al, 1997).

Tumour-specific products

Additional potential mediators for cachexia have recently been described. A 24kd glycoprotein proteolysis inducing factor (PIF) has been isolated from the urine of weight-losing cancer patients but not those losing weight due to other causes (Todorov et al, 1996a, Todorov et al, 1996b). This produces protein breakdown in experimental animals and appears distinct from known cytokines (Cariuk et al, 1997). A mouse tumour-derived lipid mobilising factor (LMF) has also been described (Harai et al, 1997) and this has been recently identified in the urine of weight-losing cancer patients and appears to be associated with zinc α -2 glycoprotein (Harai et al, 1998). The failure of pro-inflammatory cytokines such as IL-6 to reliably induce cachexia in animal models has led to the suggestion that tumour-derived factors such as PIF may act as co-factors with host- or tumour-derived cytokines to produce a cachectic state (Fujiki et al, 1997).

Management of cancer cachexia

Nutritional approaches

The best way to treat cancer cachexia is to cure the cancer. Unfortunately, this remains a rare achievement among adults with advanced solid tumours. The next obvious option is to increase nutritional intake by enteral or parenteral means.

Two substantial randomised trials have examined the effect of enteral feeding in patients with advanced cancer undergoing chemotherapy (Evans et al, 1987, Ovesen et al, 1993b). Both studies included patients with a variety of cancer types who were randomised, to receive nutritional counselling (to raise their energy and protein intake) or not. Both trials included over 100 patients and over a 3 month period demonstrated a significant increase in nutritional intake in the intervention group. However, this did not produce any improvement in weight, anthropometric measures, response rate, survival or quality of life.

Parenteral nutrition is difficult to supply over long periods and has its own complications. A number of trials of parenteral nutrition were performed in cancer patients in the 1980s and, in general, showed no benefit in terms of nutritional measures and an increase in infective complications. This led the American College of Physicians to publish a position paper in 1989 (American College of Physicians, 1989) which concluded that in cancer patients “parenteral nutritional support was associated with net harm, and no conditions could be defined in which such treatment appeared to be of benefit.” Since then a number of further large trials of

parenteral nutrition in cancer, particularly in the perioperative setting, have been performed. These studies have shown a significant improvement in energy intake in patients administered parenteral nutrition but no improvement in nutritional measures or functional outcome. Once again there were increased complications and a trend to a shorter survival duration (Veterans Affairs TPN Cooperative Study Group, 1991, Sandstrom et al, 1993, Brennan et al, 1994). It has been suggested that severely malnourished patients may derive more benefit, however, these conclusions were based on very small numbers of patients within much larger studies (Veterans Affairs TPN Cooperative Study Group, 1991, De Cicco et al, 1993).

In examining the short term metabolic effects of enteral and parenteral nutrition, the majority of studies have shown a suboptimal response to feeding in cachectic cancer patients compared with those with benign causes of weight loss (Nixon et al, 1981, Shaw et al, 1987, Shaw et al, 1988, Melville et al, 1990, Shaw et al, 1991) with an apparent anabolic response only being demonstrated by one group (Bennegård et al, 1983, Lindmark et al, 1986). It has been suggested that some cancer patients without obvious cachexia exhibit some limitation of anabolism in response to feeding compared with benign depleted controls (Melville et al, 1990) and also that those with different malignancies may exhibit different responses to feeding (Shaw et al, 1988). Thus there appears to be at least a large subgroup of cachectic patients who do not benefit from the provision of apparently adequate nutrition.

Pharmacological approaches

The disappointing results of conventional nutritional supplementation in cancer patients has led to the suggestion that there is a block to weight-gain in this group (Nixon et al, 1981) perhaps related to the metabolic changes described previously. This has led to attempts to manipulate the metabolic milieu with a variety of pharmacological agents using our knowledge of the mediators thought to be involved in cachexia.

Steroids are among the most widely used agents in patients with cancer cachexia. Prednisolone at a dose of 5mg three times daily (Wilcox et al, 1984) and dexamethasone 3-6mg daily (Moertel et al, 1974) have been shown to improve appetite to a greater extent than placebo. Methylprednisolone given intravenously at a dose of 125mg daily will improve quality of life (Robustelli della Cuna et al, 1989, Popiela et al, 1989). However, steroids will not affect ongoing weight-loss, symptomatic benefits are often short lived and they are associated with a number of adverse effects including water retention, proximal myopathy and insulin resistance. Thus steroids tend to be used in the pre-terminal phases of a patients' illness and are not suitable for early intervention.

Serotonin is known to have a role in appetite control. However, a trial of the antiserotonergic cyproheptadine demonstrated no obvious benefits in weight change or symptoms (Kardinal et al, 1990).

Due to the potential role of the inflammatory state and eicosanoids in cachexia, non-steroidal anti-inflammatory drugs have been investigated in cancer patients. A randomised trial of mixed cancer patients receiving indomethacin 50mg twice daily or placebo produced a stabilisation of performance status and a near doubling of survival in the indomethacin group (Lundholm et al, 1994). Ibuprofen at a dose of 400mg three times daily has been shown to reduce levels of acute phase proteins, interleukin-6 and cortisol and to normalise whole-body protein kinetics to some extent in cachectic colorectal cancer patients (McMillan et al, 1995, Preston et al, 1995). Ibuprofen will also reduce levels of acute phase proteins and resting energy expenditure in those with pancreatic cancer (Wigmore et al, 1995).

The effects of the progestational agents megestrol acetate and medroxyprogesterone acetate on weight-loss in cancer have been studied extensively following observations of unwanted weight-gain in patients with breast cancer using these agents. Several randomised trials in mixed groups of weight-losing cancer patients have suggested that megestrol acetate will improve appetite and stabilise weight (Loprinzi et al, 1990, Bruera et al, 1990, Filiu et al, 1992, Tchekmedyan et al, 1992) although not all groups have been able to demonstrate these findings (McMillan et al, 1994a). Doses have varied from 240-1600mg per day. In one trial comparing a dose of 160 to 320mg/day, little difference was found (Gebbia et al, 1996). In another study which compared 160, 480, 800 and 1280mg/ day a marginal advantage for a dose of 800mg per day was described (Loprinzi et al, 1993a). A recent crossover study of 480mg megestrol acetate per day for 10 days in 84 patients with advanced cancer demonstrated improved appetite, activity and well being while receiving megestrol (although performance status continued to fall) but no changes in nutritional parameters and it was thus suggested that these apparent beneficial effects of megestrol are not secondary to any nutritional improvement (Bruera et al, 1998). Medroxyprogesterone acetate has similarly been shown to increase appetite and food intake with stabilisation of weight at a dose of 500mg twice daily (Simons et al, 1996, Simons et al, 1998) and to improve appetite and some negative acute phase protein concentrations at a dose of 100mg three times daily (Downer et al, 1993). These agents have a number of side effects including an increased incidence of venous thrombosis and peripheral oedema. The frequency of oedema and the fact that weight gained by patients taking megestrol acetate tended to consist of fat and water (Loprinzi et al, 1993b, Simons et al, 1998) means that it may not be a useful drug for arresting the loss of lean tissue. Of more concern is a recent trial of megestrol acetate in patients receiving

chemotherapy which found an inferior response to therapy and a trend to poorer survival (Rowland et al, 1996).

A new approach to the management of cachexia has been to combine the appetite stimulating properties of a progestational agent with the anti-inflammatory properties of the non-steroidal anti-inflammatory drugs by using megestrol acetate and ibuprofen together. Early trials suggest that this combination may stabilise quality of life and weight in advanced gastrointestinal cancer patients (McMillan et al, 1997, Wigmore et al, 1997c, McMillan et al, 1999).

Pentoxifylline has been shown to inhibit the production of $\text{TNF}\alpha$ (Strieter et al, 1988), and to suppress protein breakdown in a tumour-bearing rat model of cachexia (Combaret et al, 1998). A case report of the use of pentoxifylline in two patients with advanced cancer suggested improvements in wellbeing (Dezube et al, 1990). However, a controlled trial of pentoxifylline in patients with cancer cachexia failed to demonstrate any benefit in terms of appetite or nutritional measures compared with placebo (Goldberg et al, 1995).

Melatonin has also been suggested to influence $\text{TNF}\alpha$ production leading to a controlled but non-randomised study of 20mg melatonin daily given in the evening (Lissoni et al, 1996). In patients with a variety of metastatic solid tumours weight loss continued in both groups but melatonin appeared to produce a significant slowing of this weight loss compared with controls. There was a progressive rise in serum $\text{TNF}\alpha$ concentrations in control subjects while concentrations fell in those receiving melatonin.

Hydrazine sulphate inhibits phosphoenolpyruvate carboxykinase, an enzyme responsible for gluconeogenesis from lactate (the Cori cycle), to some degree. It was hoped that interrupting this process would normalise some aspects of carbohydrate metabolism in cachexic cancer patients and so improve nutritional status. Given orally at a dose of 60mg three times daily hydrazine sulphate has been shown to have marginal benefits over placebo in terms of appetite and maintenance of weight in a mixed group of cancer patients (Chlebowski et al, 1987). However, subsequent studies in large groups of colorectal (Loprinzi et al, 1994a) and lung (Loprinzi et al, 1994b) cancer patients have shown no effect on weight-loss with trends to worse survival and quality of life in hydrazine treated patients.

Manipulation of the hormonal environment has also been used in an attempt to promote skeletal muscle anabolism. The administration of insulin to patients with cancer may reduce protein breakdown in cachectic cancer patients, however, this requires careful control of blood glucose (Heslin et al, 1992). In addition, effects are limited by an increase in glucagon secretion (Bartlett et al, 1994) and concerns over the promotion of tumour growth by insulin (Moley et al, 1988, Beck & Tisdale, 1989). Subsequent animal studies have suggested that the combination of

insulin with somatostatin, sometimes with the addition of growth hormone, will attenuate weight-loss with an apparent gain in muscle mass without an obvious increase in tumour growth rate (Bartlett et al, 1994, Bartlett et al, 1995a). It has also been shown that the combination of insulin like growth factor-1 and its binding protein-3 (IGF-1/IGFBP-3) will stimulate muscle protein synthesis in undernourished rats. This combination will reduce the rate of weight-loss in tumour bearing mice (Svanberg et al, 1998).

Growth hormone will improve carcass weight in cachectic tumour-bearing rats (Bartlett et al, 1995b). Small clinical studies of growth hormone administration suggest it may improve nitrogen balance (Wolf et al, 1992, Tayek & Brasel, 1995) although this effect may not be seen in more malnourished patients (Tayek & Brasel, 1995). The administration of growth hormone appears not to influence the acute phase protein response in trauma patients (Petersen et al, 1997). Concerns also remain about the effects of growth hormone on promoting tumour growth (Akaza et al, 1991).

Other agents undergoing studies at the present time include agents which may modulate TNF α concentrations such as thalidomide and melatonin and β 2-adrenoceptor agonists which may impair protein breakdown (Lissoni et al, 1996, Gagnon & Bruera, 1998, Haslett, 1998).

While progress has been made in understanding the physiological changes in cancer which give rise to cachexia it remains a significant cause of morbidity and mortality in malignant disease. The metabolic alterations that occur in these patients would appear to prevent the effective use of additional calories supplied. A number of pharmacological agents have shown promise in normalising some of these metabolic changes. The n-3 polyunsaturated fatty acids, particularly eicosapentaenoic acid, have also been shown to have the potential to modulate the inflammatory process. The properties and potential of these agents are discussed below.

n-3 polyunsaturated fatty acids

Humans are unable to synthesise fatty acids with double bonds more proximal to the methyl end than the ninth carbon atom. Thus there are two families of essential fatty acids - those with the last double bond six and three atoms from this position (figure 1.4). The parent of the omega- or n-6 fatty acids is linoleic acid (18:2n-6) (see appendix 1). This is found in many plant seeds and is elongated and desaturated *in vivo* to form arachidonic acid (20:4n-6). The parent of the n-3 family is alpha-linolenic acid (18:3n-3), which is found associated with chloroplasts. Linseed, rapeseed and soybean oils contain some but the richest source, particularly of the longer chain eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids is marine algae (Leaf &

Weber, 1988, Simopoulos, 1991). These algae form the lowest level of the marine food chain and thus n-3 fatty acids are found in appreciable quantities in many marine animals where the high level of desaturation may help keep cell membranes fluid in the cold of the open ocean (Nørdoy & Dyerberg, 1989).

Interest in polyunsaturated fatty acids in human disease was initially stimulated by observations on Greenland Eskimos in the 1970's. These showed that, despite a similar fat and cholesterol intake to those in Denmark, diseases such as coronary heart disease and cancer were rare (Nielsen & Hansen, 1980, Anonymous, 1983). The major dietary difference between this population and those with a higher incidence of such diseases seemed to be in the type of fat rather than the quantity consumed. The main sources of fat in Greenland Eskimos were fish, seals and whales resulting in a higher consumption of n-3 fatty acids and a lower intake of n-6 and saturated fats (Dyerberg et al, 1975, Bang et al, 1976). In the United Kingdom n-3 fatty acids make up less than 0.5% of the fats in our diet resulting in levels of less than 1% in the body's lipid pools (Bull et al, 1983). In populations such as Eskimos with a high intake n-3 fatty acids may make up 15% of cholesterol esters and 7% of phospholipids (Dyerberg et al, 1975).

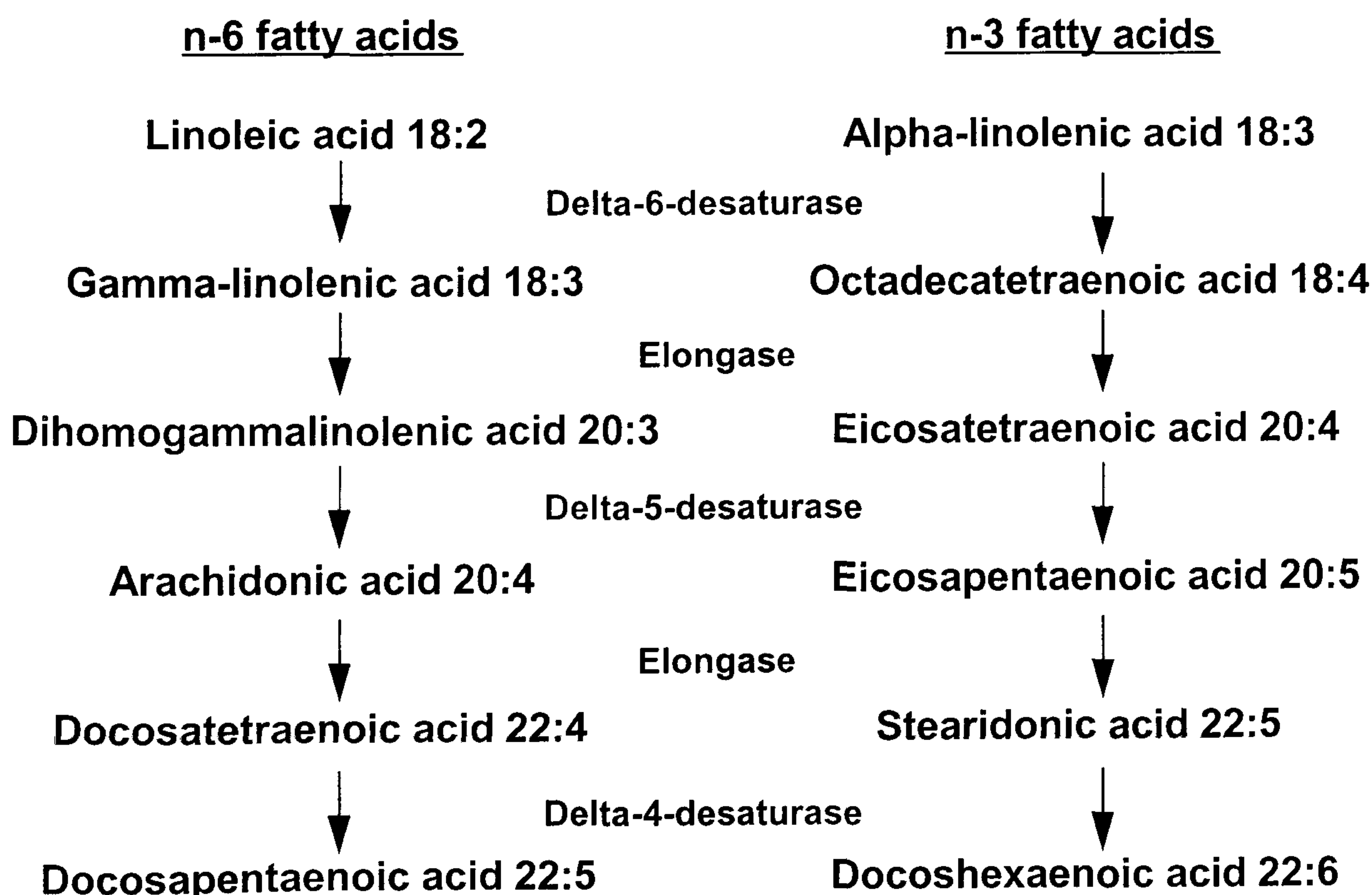


Figure 1.4. Essential fatty acid metabolism in man.

The thrust of initial research was in the cardiovascular field where n-3 fatty acids have been shown to decrease blood viscosity, improve the lipid profile and lower blood pressure (Leaf & Weber, 1988, Simopoulos, 1991). Advice to increase the amount of fish in the diet of men who had recovered from myocardial infarction led to a reduction in mortality of 29% at 2 years in a study of over 2000 patients (Burr et al, 1989). Since then research has expanded into many fields representing many increasingly prevalent diseases, especially those of an inflammatory nature. Beneficial effects for n-3 fatty acids have now been described in inflammatory bowel disease (Lorenz et al, 1989, Belluzi et al, 1996), rheumatoid arthritis (Lau et al, 1993), asthma (Arm et al, 1988), atopic dermatitis (Bjørneboe et al, 1989), psoriasis (Bittiner et al, 1988), models of sepsis (Johnson et al, 1993), organ transplantation (van der Heide et al, 1993), schizophrenia (Laugharne et al, 1996), infant problem solving (Willatts et al, 1998) and cancer. It has been suggested that during human evolution the balance of n-3/n-6 fatty acids was much higher than in the current Western diet and that the change in diet, particularly during the second half of the 20th Century has contributed to the changes in the pattern of diseases seen over this period (Simopoulos, 1991, Okuyama et al, 1997).

Biological effects of n-3 polyunsaturated fatty acids

Polyunsaturated fatty acids have been shown to have a myriad of biological effects at the tissue, cellular and molecular levels, many of which are crucial in regulating the inflammatory state. The 20 carbon polyunsaturated fatty acids are metabolised into eicosanoids - by cyclooxygenase into the prostanoids - prostaglandins and thromboxanes, and by 5-lipoxygenase into the leukotrienes. The n-6 fatty acid arachidonic acid is the major precursor for these substances in the modern diet and gives rise to the 2-series prostanoids (such as thromboxane A_2 and prostaglandin E_2 and I_2) and the 4-series leukotrienes (Leukotriene B_4 , C_4 etc). Eicosapentaenoic acid is also metabolised by these enzymes into the 3-series prostanoids and 5-series leukotrienes. Eicosanoids are a major determinant of vascular tone, the haemostatic state and the inflammatory state. Thromboxane A_2 derived from arachidonic acid is a potent vasoconstrictor and platelet aggregating agent. The actions of thromboxane A_2 are counterbalanced by those of prostaglandin I_2 (prostacyclin) which is vasodilatory and antiaggregatory. Eicosapentaenoic acid inhibits the production of thromboxane A_2 by cyclooxygenase and serves as a substrate for Thromboxane A_3 (with much weaker effects than thromboxane A_2). Eicosapentaenoic acid has little effect on the production of prostaglandin I_2 while also giving rise to prostaglandin I_3 which has similar properties (Knapp et al, 1986, Fischer & Weber, 1983). The 4-series leukotrienes derived from arachidonic acid are chemoattractive to

circulating neutrophils and monocytes and cause smooth muscle contraction and vasodilatation. The 5-series leukotrienes from eicosapentaenoic acid, particularly leukotriene B₅, are less active than their 4-series counterparts and compete with those of the 4-series for binding sites (Lee et al, 1985). Thus, with regard to eicosanoids, n-3 polyunsaturated fatty acids produce a more vasodilatory state with less platelet aggregation and a less pro-inflammatory environment. Contributing to this anticoagulatory balance fish oil supplementation has been shown to reduce plasma concentrations of fibrinogen (a positive acute phase protein) in healthy volunteers and hyperlipidaemic subjects (Høstmark et al, 1988, Radack et al, 1989).

Fish oil and eicosapentaenoic acid supplementation have been shown by a number of groups to reduce production of the pro-inflammatory cytokines interleukin-1, interleukin-6 and tumour necrosis factor by mononuclear cells in normal volunteers and this effect is maintained for some weeks after stopping supplementation (Endres et al, 1989, Meydani et al, 1991, Meydani et al, 1993, Cooper et al, 1993, Caughey et al, 1996). This effect has also been shown in cancer patients (Purasiri et al, 1994, Wigmore et al, 1997d). However, another group using apparently similar techniques and n-3 fatty acid doses have found prompt rises in pro-inflammatory cytokine production (Demols et al, 1998). Similar contradictory findings have been reported in animal studies and it remains unclear how these data can be reconciled, however, it is likely that minor differences in culture conditions, particularly the source of serum used may be important (Locniskar et al, 1983).

Fish oil and eicosapentaenoic acid have also been found to have a wide variety of effects upon leukocytes in terms of their proportions and function. Dietary supplementation with n-3 fatty acids has been reported to result in a reduction in the proportion and cytotoxicity of natural killer and lymphokine-activated killer cells (Purasiri et al, 1995), an increase in the proportion of T suppressor to T helper cells (Meydani et al, 1993), a reduction in lymphocyte proliferation in response to a T cell mitogen (Meydani et al, 1991, Meydani et al, 1993) and a reduction in the delayed hypersensitivity skin response (Meydani et al, 1993). Neutrophil adherence to endothelium is inhibited (Lee et al, 1985) and neutrophil chemotactic response is reduced (Lee et al, 1985, Endres et al, 1989). A clue to the mechanism behind some of these changes can be gained from the observation that activated human monocytes cultured in vitro both with eicosapentaenoic acid and docosahexaenoic acid show decreased expression of major histocompatibility complex II molecules HLA-DR and -DP and of intracellular adhesion molecule-1 (Hughes et al, 1996).

The relevance of these findings in the clinical situation is unclear. While beneficial effects have been seen in terms of prolonged graft survival following renal transplantation in

those given fish oil supplementation (van der Heide et al, 1993), there have been no reports of adverse effects with regard to immunosuppression. A recent study examined the immunological effects of eicosapentaenoic acid in patients receiving total parenteral nutrition after major surgery. This suggested that while circulating interleukin-6 concentrations were reduced in those receiving eicosapentaenoic acid these subjects showed increased lymphocyte proliferation and natural killer cell activity and lower glucagon concentrations than those receiving standard or fat free parenteral nutrition (Furukawa et al, 1999). Thus it seems that eicosapentaenoic acid produces a subtle immunomodulation rather than a blanket immunosuppression.

Mechanisms of n-3 fatty acid actions

The precise mechanisms by which polyunsaturated fatty acids exert specific effects remain largely unclear, however, they have been shown to influence virtually every stage of signal transduction including the activity of a number of receptors and enzymes with a fundamental role in cellular signalling (figure 1.5). When agonists stimulate receptors in the cell membrane they may activate adenylate cyclase or a phospholipase, the second messenger products of which (including lipids in the case of phospholipases) influence the actions of cAMP-dependent protein kinase and protein kinase C respectively. n-3 fatty acids have been shown to influence the effects of adenylate cyclase, probably via an inhibitory guanine nucleotide-binding protein (Alam et al, 1988, Tisdale, 1993, Price & Tisdale, 1998), phospholipase A₂ (Ballou & Cheung, 1985), cAMP-dependent protein kinase (Speizer et al, 1991) and protein kinase C (Speizer et al, 1991, Holian & Nelson, 1992). Other enzymes which have been suggested to be inhibited by n-3 fatty acids include matrix metalloproteinases involved in tumour invasion and metastasis (Suzuki et al, 1997) and farnesyl protein transferase involved in the transforming activity of *ras* oncoproteins (Singh et al, 1998). Eicosapentaenoic acid also binds to membrane voltage-sensitive sodium channels and may alter the conductance of the channel (Kang & Leaf, 1996, Xiao et al, 1998) and n-3 fatty acids bind to the cytoplasmic glucocorticoid receptor at a site different from the hormone binding site and markedly reduce its affinity for the hormone (Vallette et al, 1991, Sumida et al, 1993).

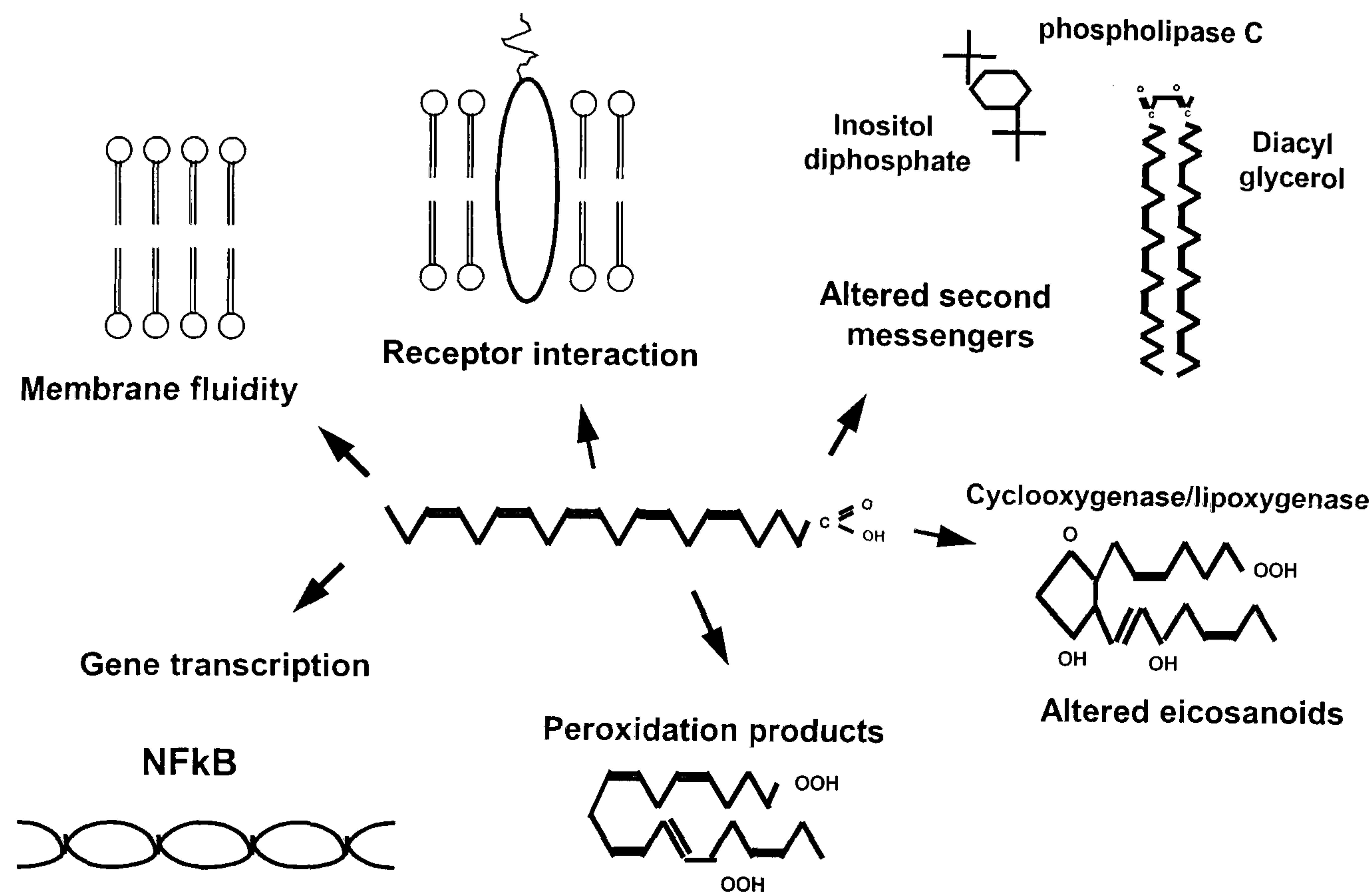


Figure 1.5. Potential mechanisms of action of fatty acids in metabolism.

In addition to eicosapentaenoic acid providing an alternative substrate for the synthesis of eicosanoids as discussed above, fish oil supplementation will reduce expression of inducible cyclooxygenase (COX-2) and so further alter eicosanoid production (Singh et al, 1997).

With n-3 fatty acid supplementation an accelerated catabolism of leukotriene B₄ has been demonstrated in addition to reduced synthesis (von Schacky et al, 1993). Peroxisome proliferator-activated receptor α is a gene transcription factor which induces the breakdown of leukotrienes and thus has a role in limiting the duration and extent of inflammation. Eicosapentaenoic acid, docosahexaenoic acid and leukotrienes themselves appear to increase the activity of this factor (Keller et al, 1993, Yu et al, 1995, Devchand et al, 1996).

The production of pro-inflammatory cytokines and acute phase proteins is controlled to some extent by the transcription factor NF- κ B (Beauparlant & Hiscott, 1996). Because of the effects of polyunsaturated fatty acids on cytokine production initial studies have been performed to investigate the effects of these molecules upon NF- κ B activation. Some polyunsaturated fatty acids appear to activate NF- κ B but so far n-3 fatty acids have not been shown to have a major effect on this pathway in vitro (Camandola et al. 1996).

Potential side effects of n-3 fatty acids

Through its effects upon eicosanoids, cytokines and leukocytes eicosapentaenoic acid and other n-3 fatty acids have broadly anti-inflammatory properties. However, few adverse effects have been noted with the administration of such agents. Fish oil supplementation has been suggested to adversely affect glucose metabolism in otherwise well patients with non-insulin-dependent diabetes mellitus (Glauber et al, 1988, Borkman et al, 1989). However, a study of patients with hypertension given 4g fish oil or placebo daily for 16 weeks specifically addressed this issue and showed no change in response to glucose tolerance test, insulin release or insulin sensitivity in those given fish oil and no difference between groups (Toft et al, 1995).

The reported effects of n-3 fatty acids in prolonging bleeding time and inhibiting platelet function have raised concerns about excessive bleeding after consuming fish oils (van Houwelingen et al, 1987). However, only one study has found this to be a clinically significant problem with an increased rate of epistaxis in hyperlipidaemic adolescents given fish oil (Clarke et al, 1990). In addition there has been concern that the immunomodulatory effects of fish oils may result in unwanted immunosuppression (Meydani et al, 1991) but there appear to have been no reports of clinical problems related to immune modulation by n-3 fatty acids. The administration of Maxepa, providing around 1.8g eicosapentaenoic acid daily, for over 7 years has not been reported to produce adverse effects (Saynor & Gillott, 1992).

n-3 polyunsaturated fatty acids and cancer

Early work using polyunsaturated fatty acids in *in vitro* studies of cancer demonstrated that eicosapentaenoic acid and other polyunsaturated fatty acids would kill or inhibit the growth of many malignant cell lines including those from human lung, breast, prostate (Bégin et al, 1986), pancreatic (Falconer et al, 1994b), melanoma (McMillan et al, 1994b) and colorectal cancers (Mengeaud et al, 1992). These effects were achieved at fatty acid concentrations of 10-300µM. The mechanism for this action has been suggested to be via cell cycle arrest and induction of apoptosis (Lai et al, 1996, Hawkins et al, 1998, Latham et al, 1998). The addition of inhibitors of prostaglandin synthesis, such as indomethacin, has been found to have no effect on this cytotoxicity in some models (Bégin et al, 1988, Mengeaud et al, 1992) while others have noted blockade of the this tumour growth suppression with cyclooxygenase and lipoxygenase inhibitors (Kumar & Das, 1997). The addition of the antioxidants vitamin E, superoxide dismutase or glutathione peroxidase and the saturated fatty acid oleic acid inhibited cell death while the pro-oxidants iron and copper enhanced it (Bégin et al, 1986, Falconer et al, 1994b, Kumar & Das, 1997, Hawkins et al, 1998). It appears likely that the cytotoxicity of

polyunsaturated fatty acids occurs via effects on peroxidation and eicosanoids. The levels of an indirect measure of lipid peroxidation have not been reliably associated with the degree of cell death suggesting other factors may be involved (Falconer et al, 1994b). Recent work using a mouse tumour model has suggested that eicosapentaenoic acid inhibits translation initiation, reducing the synthesis and expression of growth regulatory proteins, causing cell cycle arrest in G1 (Palakurthi et al, 2000). Cultured leukaemia cells with increased docosahexaenoic acid in their membranes have been shown to be restricted in their ability to passively pass through a filter and thus it may be that reduced cell deformability alters the growth of malignancies (Zerouga et al, 1997). It has also been suggested that n-3 polyunsaturated fatty acids will increase the sensitivity of tumours to cytotoxic chemotherapeutic agents, including adriamycin in cultured leukaemia cells (Guffy et al, 1984), cisplatin and doxorubicin in cultured ovarian cancer cell lines (Plumb et al, 1993) and doxorubicin in lung tumour xenografts in a mouse model (Hardman et al, 2000).

The widespread antineoplastic effects *in vitro* led to much animal work, mostly using tumours implanted into immunocompromised mice. These studies suggested that the growth rates of transplanted human prostate (Karmali et al, 1987), breast (Pritchard et al, 1989, Gonzalez et al, 1991, Gonzalez et al, 1993, Rose & Connolly, 1993, Rose et al, 1995, Hardman et al, 1995, Senzaki et al, 1998), colon (Sakaguchi et al, 1990a, Sakaguchi et al, 1990b, Calder et al, 1998) and lung (de Bravo et al, 1991, Mæhle et al, 1995) tumours could be slowed by the administration of eicosapentaenoic acid or fish oil. Reduced rates of metastases have also been shown (Rose et al, 1995, Connolly & Rose, 1996). Doses have varied from about 2 to 20g eicosapentaenoic acid/kg given with the diet but most groups started the experimental diet before the implantation of tumours. Again the anti-tumour effects seem to be inhibited by antioxidants (Gonzalez et al, 1991, Gonzalez et al, 1993) and enhanced by iron (Gonzalez et al, 1991, Hardman et al, 1995).

Other studies have shown that eicosapentaenoic acid increases the resistance of cultured cells to transformation by radiation and transfection by the Harvey *ras* oncogene *in vitro* (Takahashi et al, 1992). Studies in rats administered carcinogens have shown that those fed higher proportions of fish oil containing eicosapentaenoic acid developed fewer tumours and had decreased expression of H-*ras* (in contrast to the findings for n-6 fatty acids) (Abou-El-Ela et al, 1988, Reddy & Sugie, 1988, Karmali et al, 1989). Recent epidemiological studies have shown that increasing consumption of fish and fish oil correlates with a decreased risk of colorectal and breast cancer (Kaizer et al, 1989, Sasaki et al, 1993, Caygill et al, 1996).

It is difficult to untangle the role of docosahexaenoic acid, the other common long chain n-3 fatty acid, in these findings. This is normally found in relatively high concentrations in the

structural lipids of the brain and retina in humans (Sardesai, 1992). Many of the experiments described above were performed using mixtures of n-3 fatty acids containing both eicosapentaenoic acid and docosahexaenoic acids. Supplementation of docosahexaenoic acid alone in normal volunteers results in increased levels of both docosahexaenoic and eicosapentaenoic acids in plasma and platelet phospholipids but feeding of eicosapentaenoic acid does not result in any change in docosahexaenoic acid levels suggesting conversion in only one direction *in vivo* (von Schacky & Weber, 1985, Conquer & Holub, 1997). In studies comparing the two, docosahexaenoic acid has had similar and in some cases superior effects in inhibiting the growth or killing of tumour cell lines *in vitro* (Bégin et al, 1986, Falconer et al, 1994b, Mæhle et al, 1995), in reducing the growth rate of tumours and number of metastases in experimental tumours in mice (Rose et al, 1995) and in reducing the transformation of cultured cells by radiation and transfection (Takahashi et al, 1995). A recent study has suggested that eicosapentaenoic acid and docosahexaenoic acid will both inhibit growth of an experimental tumour in rats but by slightly different means. Eicosapentaenoic acid appears to inhibit cell proliferation while docosahexaenoic acid induces apoptosis (Calviello et al, 1998).

Work on the anti-neoplastic activity of n-3 fatty acids is extending slowly into the human clinical arena. A reduction in abnormal proliferation was noted in rectal mucosal biopsies of patients with sporadic colorectal adenomas given doses of fish oil providing between 1.4g eicosapentaenoic acid and 1.1g docosahexaenoic acid daily and 4.1g eicosapentaenoic acid and 3.6g docosahexaenoic acid daily in a controlled study (Anti et al, 1994). A study of 12 heavily pretreated patients with metastatic breast cancer given fish oil capsules providing 3.6g eicosapentaenoic acid and 2.4g docosahexaenoic acid daily found a partial regression in one patient and disease stabilisation in another (Holroyde et al, 1988). A recent controlled study of 60 patients with advanced solid tumours found that survival was significantly prolonged in those receiving fish oil capsules providing around 3g eicosapentaenoic acid and 2.1g docosahexaenoic acid daily (Gogos et al, 1998).

n-3 polyunsaturated fatty acids and cancer cachexia

The failure of conventional nutritional supplementation to affect the progress of cachexia and studies to elucidate the mechanisms of cachexia have led to the conclusion that there is a metabolic block to the accretion of lean body mass in cachectic patients (Nixon et al, 1981, Moldawer & Copeland, 1997). Modulation of a variety of metabolic mediators, such as pro-inflammatory cytokines and eicosanoids by n-3 polyunsaturated fatty acids, that have been demonstrated in healthy volunteers have led to the hypothesis that these fatty acids may down

regulate the metabolic response to cancer and so allow moderation of cachexia.

A mouse colorectal tumour model has been described in which the animals consistently lose weight with tumour progression (Bibby et al, 1987). Despite experimental diets not being commenced until the subcutaneously implanted tumours were palpable, tumour growth rate has been reduced and, in addition, weight-loss abolished with doses of about 2.5g eicosapentaenoic acid/kg given with diet or by gavage (Tisdale & Dhesi, 1990, Tisdale & Beck, 1991, Beck et al, 1991, Hudson et al, 1993, Hudson & Tisdale, 1994). It has been suggested that this effect is due to impairment of the end-organ effects of proteolysis inducing factor and lipid mobilising factor probably by an effect upon cell signalling (Smith & Tisdale, 1993, Tisdale, 1996). Similar attenuation of weight-loss with eicosapentaenoic acid has also been shown in a mouse model using lung cancer cells transfected with interleukin-6 to produce cachexia (Ohira et al, 1996).

These data have now been complemented by early studies in human cancer cachexia. A group of 18 weight-losing patients with advanced pancreatic cancer have been given an oral fish oil preparation providing around 2.2g eicosapentaenoic acid and 1.4g docosahexanoic acid daily. Before treatment all patients were losing weight at a median rate of 2.9kg per month. Following 3 months supplementation patients had a median weight gain of 0.2kg/month with less than half of the patients continuing to lose weight. There was no change in the percentage of total body water over the time of the study suggesting that patients were not simply retaining fluid. This regimen also produced a fall in the serum C-reactive protein level suggesting that some of the metabolic abnormalities of pancreatic cancer could be reversed resulting in the stabilisation of weight (Wigmore et al, 1996).

Subsequently 27 patients with unresectable pancreatic cancer were given 6g per day of 95% pure eicosapentaenoic acid orally after a 4 week dose escalation period. Patients were losing a median of 2.0kg per month at baseline. After 4 weeks patients had a median weight gain of 0.75kg and this effect remained at 3 months with a median weight gain of 0.25kg per month. Again there was no change in the percentage total body water over the course of the study confirming that the achievement of weight stability was not due to changes in hydration (Barber et al, 1997). There were no serious side effects in these studies and median survival was approximately 7 months. A subgroup of these patients underwent evaluation of the effect of the eicosapentaenoic acid preparation on pro-inflammatory cytokine production by mononuclear cells and of the acute phase protein response. Interleukin-6 and tumour necrosis factor production and C-reactive protein concentrations were found to be down-regulated (Wigmore et al, 1997d).

Summary and aims of thesis

Advanced cancer is often associated with a variety of metabolic abnormalities which result in the syndrome of cachexia causing weight-loss not responsive to conventional nutritional support. By creating an extra demand for amino acids the hepatic acute phase response may be a significant facet of such metabolic change. A more complete understanding of the mechanisms and mediators surrounding cachexia and the response to feeding in cachectic patients has the potential to advance the treatment, not only of patients with cancer but also for those with a variety of inflammatory conditions associated with wasting. A treatment for patients with cachexia is not available at present but would have the potential to improve quality and length of life in this unfortunate group. Fish oil and EPA are able to modulate some of these metabolic adaptations and may arrest weight-loss. With a degree of metabolic normalisation produced by fish oil, the supply of additional calories and protein may allow weight-gain in cachectic cancer patients (figure 1.6).

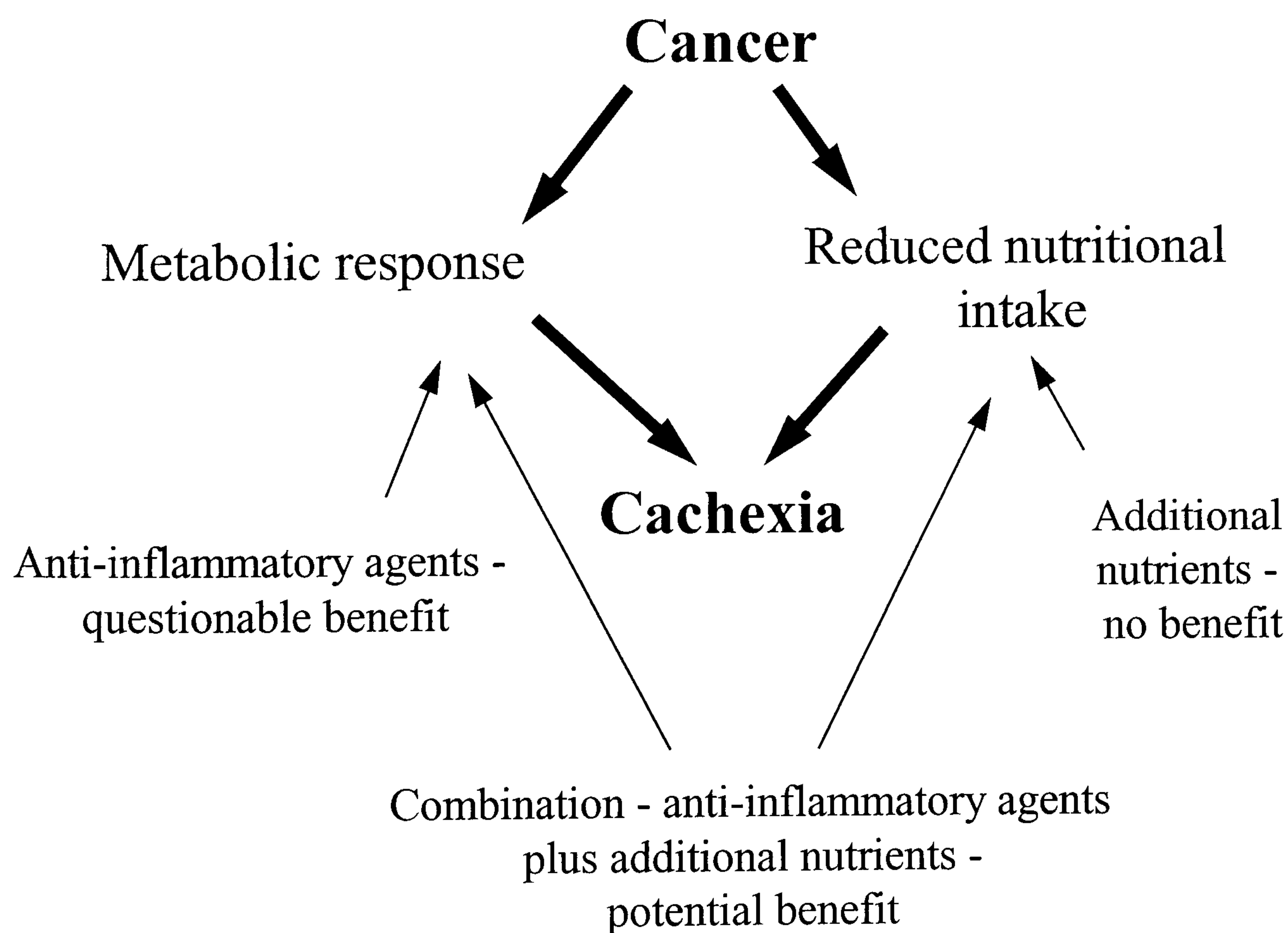


Figure 1.6. Hypothesis of cause of cancer cachexia and potential action of agents used to treat cachexia.

The work presented in this thesis will thus explore -

- 1) the role of inflammation in mediating cachexia in advanced pancreatic cancer

Specifically, the relationship of acute phase response and weight loss to performance status, the relationship of cytokines to the acute phase response and the relationship of cytokine genotype to inflammatory state and survival.

(Chapters 3, 4 and 5)

- 2) the role of other mediators such as insulin, cortisol, leptin and proteolysis-inducing factor in weight losing pancreatic cancer patients

This will be addressed by a comparison of the concentrations of neuroendocrine hormones and the excretion of proteolysis-inducing factor in addition to the concentration of acute phase proteins and cytokines and cytokine production between weight-losing pancreatic cancer patients and a group of healthy controls.

(Chapters 4, 7 and 8)

- 3) the role of acute phase protein synthesis as a cause of increased demand for amino acids in the fasted and fed state

The synthesis of albumin and fibrinogen in the fasted and fed state will be measured in weight-losing cancer patients and healthy controls. (Chapter 10)

- 4) the modulation of the metabolic alterations in cancer following the administration of a fish oil-enriched nutritional supplement

In particular the effect upon weight, body composition, dietary intake, resting energy expenditure, nitrogen balance, acute phase protein concentrations, cytokine production and concentrations, leptin, insulin and cortisol concentrations and the excretion of proteolysis inducing factor, albumin and fibrinogen synthesis and metabolic response to feeding will be examined in a group of cachectic pancreatic cancer patients. (Chapters 6, 7, 8, 9 and 10)

It is hoped that this work will clarify to some degree the relationship of the inflammatory response to cancer and its importance to the nutritional decline and poor survival of patients with pancreatic cancer. The thesis also aims to explore the potential of n-3 polyunsaturated fatty acids to modulate the inflammatory metabolic state and determine whether the supply of additional nutrients via oral dietary supplementation will result in an improvement in patients' nutritional status.

Methods

Ethical approval

Protocols for the studies described in this thesis were submitted to the Lothian Health Orthopaedic Surgery/Surgery Research Ethics Sub-Committee and received ethical approval under reference 1702/96/5/47 and 1702/97/5/32.

Clinical and nutritional assessment

Anthropometry

Height was measured to the nearest 0.5cm on a wall mounted stadiometer. Remembered pre-illness stable weight and duration of weight-loss were recorded. Subjects were weighed either on spring balance scales (Seca, Germany) or a beam scale (Avery, Birmingham, U.K.) without shoes and wearing light clothing. The same scale was used within individual studies. Body mass index was calculated by dividing the weight in kilograms by the height in metres squared. Percentage weight loss was calculated by dividing the difference between remembered stable weight and current weight by the remembered stable weight and multiplying by 100.

Mid-upper arm circumference (MUAC) was measured to the nearest 0.25cm half way between the acromion process and olecranon process. Triceps skinfold thickness (TSF) was measured using Harpenden calipers (John Bull British Indicators Ltd, UK) at the midarm over the mid point of the triceps muscle. Three measurements were performed and the mean value recorded. Midmuscle circumference (MAMC) was calculated using Jelliffe's equation (Jelliffe, 1966).

$$\text{MAMC (cm)} = \text{MUAC (cm)} - (0.3142 * \text{TSF (mm)})$$

Body composition analysis

Body composition was measured using a Xitron 4000B multiple frequency bioelectrical impedance analyser (Xitron Technologies, San Diego, CA, USA). All assessments were made with the subject supine and limbs apart. Electrodes were placed over the wrist and ankle joints and metacarpal and metatarsal heads. Repeat measurements were performed using the same pair of

limbs. Resistance was measured at 200Hz. Values for total body water (TBW) and extracellular water (ECW) were derived using equations validated in a similar patient group (Hannan et al, 1995).

$$\text{TBW (l)} = (0.2391 * (\text{height (cm)}^2 / \text{resistance at 200Hz})) + (0.1889 * \text{weight (kg)}) + (2.971 * \text{sex}) + 5.464$$

Sex - male = 1, female = 0

$$\text{ECW (l)} = (0.1782 * (\text{height}^2 / \text{resistance at 5Hz})) + (0.0688 * \text{weight}) + 3.771$$

Lean body mass was calculated from total body water assuming that lean tissue contains 73% water. Body cell mass was calculated from intracellular water (total body water minus extracellular water) assuming that cell mass contains 70% water (Shizgal, 1990). Lean body mass index was calculated by dividing the lean body mass in kilograms by the height in metres squared.

Performance status and appetite

Karnofsky performance status was noted as described by Karnofsky (Karnofsky, 1948).

- | | |
|-----|--|
| 100 | Normal, no complaints. |
| 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 80 | Normal activity with effort; some signs or symptoms of disease. |
| 70 | Cares for self. Unable to carry on normal activity or to do active work. |
| 60 | Requires occasional assistance, but is able to care for most needs. |
| 50 | Requires considerable assistance, and frequent medical care. |
| 40 | Disabled; requires special care and assistance. |
| 30 | Severely disabled; hospitalisation is indicated although death not imminent. |
| 20 | Very sick; hospitalisation necessary; active supportive treatment necessary. |
| 10 | Moribund, fatal processes progressing rapidly. |
| 0 | Dead. |

Appetite was measured on a numerical rating scale between 0 and 10, where 0 indicated absolutely no appetite and 10 indicated an extremely good appetite (Simons et al, 1996).

Nutritional intake

The daily dietary intake of subjects was measured based on the mean dietary intake recorded in a 3 day food diary calculated using CompEat 4 software (Nutrition Systems, London, UK) with the assistance of Dr Alison Hinds and Rosemary Richardson.

Blood and culture supernatant assays

Acute phase proteins

These were assessed from venous serum samples apart from fibrinogen which was assessed from a plasma sample. Serum and plasma were collected following centrifugation and stored at -70°C until analysis.

Most C-reactive protein measurements were made using an immunoturbidometric assay (Abbott Laboratories, Maidenhead, U.K.) in the clinical laboratory of the Department of Microbiology at the Royal Infirmary of Edinburgh. Reported limit of detection of this assay was 10 mg/l.

For the results described in chapter 4, C-reactive protein was measured by ELISA using rabbit anti-human-CRP antibody and peroxidase-conjugated rabbit anti-human-CRP antibody (DAKO, High Wycombe, U.K.). The lower limit of detection was 1mg/l.

For the results described in chapter 8, C-reactive protein was measured by immunoturbidometry using Tina-Quant assay kits (Boehringer Mannheim, Germany) on a Hitachi 911 analyser (Ealing, UK) in the clinical laboratory of the Department of Clinical Chemistry at the University of Liverpool. The lower limit of detection was 5mg/l.

Serum albumin was measured using an automated bromocresyl green method in the clinical laboratory of the Department of Clinical Chemistry at the Royal Infirmary of Edinburgh.

α -1-antitrypsin, α -1-acid glycoprotein, haptoglobin and ceruloplasmin, prealbumin and transferrin, were measured by immunoturbidometry using Tina-Quant assay kits (Boehringer Mannheim, Germany) on a Hitachi 911 analyser (Ealing, UK).

Fibrinogen was measured by assessing clotting time in the presence of a high thrombin concentration on a KC4A Coagulometer (Baxter Healthcare, Thetford, UK) in the clinical laboratories of the Department of Haematology at the Royal Infirmary of Edinburgh.

Coefficient of variation of all automated assays was 3% or less.

Serum cytokines

Serum interleukin-1 β (IL-1 β), interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R), soluble tumour necrosis factor receptor 55 (sTNF-R55 or sTNF-RI), soluble tumour necrosis factor receptor 75 (sTNF-R75 or sTNF-RII) were all measured by indirect ELISA from serum stored at -70°C until analysis. Samples underwent no more than one freeze/thaw cycle before analysis.

IL-1 β was measured using commercial kits (Quantikine High Sensitivity, R & D Systems, Abingdon, UK). Limit of detection was 0.2pg/ml.

For the results described in chapter 4, IL-6 was measured using a monoclonal anti-human-IL-6 antibody and peroxidase-conjugated Fab fragments of a murine monoclonal anti-human-IL-6 antibody (CLB, Amsterdam, Netherlands). The lower limit of detection was 0.25pg/ml. Otherwise IL-6 was measured by a similar method using different kits (Quantikine, R & D Systems, Abingdon, UK). Limit of detection was 0.5pg/ml.

sIL-6R was measured using a monoclonal anti-human-sIL-6R antibody and peroxidase-conjugated Fab fragments of a murine monoclonal anti-human-sIL-6R antibody (CLB, Amsterdam, Netherlands). The lower limit of detection was 4ng/ml. For the results described in chapter 4, sTNF-R55 and sTNF-R75 were measured using polyclonal and monoclonal anti-sTNF-R55 and sTNF-R75 antibodies kindly provided by Dr WA Buurman, University of Maastricht, Netherlands (Leeuwenberg et al, 1994). The lower limit of detection for sTNF-R55 was 190pg/ml and for sTNF-R75 was 2ng/ml. Otherwise, sTNF-R55 and sTNF-R75 were measured using commercial kits (Quantikine, R & D Systems, Abingdon, UK). Limits of detection were 0.156ng/ml and 0.78ng/ml respectively.

Coefficient of variation for assays were as follows across the concentration range studied-

IL-1 β less than 11.1%

IL-6 less than 6.3%

TNF receptors less than 8.8%.

Peripheral blood mononuclear cell cytokine production

Peripheral blood mononuclear cells (PBMCs) were separated from 20mls of heparin anti-coagulated blood on a hypaque gradient (Histopaque 1077, Sigma, Poole, UK) by centrifuging at 1500rpm for 30 minutes. Cells from the interface were removed and washed three times in cell culture medium (Roswell Park Memorial Institute (RPMI) medium 1640, Life Technologies, Paisley, UK) with Penicillin (50IU/ml), streptomycin (50 μ g/ml) and glutamine (2mmol/l) (Sigma)

added. Cells were resuspended, counted and incubated at 37°C in 96-well, flat-bottomed tissue culture plates (Costar, Cambridge, Massachusetts, USA) at a concentration of 2×10^5 per well in 200µl cell culture medium with 10% fetal calf serum or autologous plasma in the presence or absence of 10µg/ml *E. coli* lipopolysaccharide (Sigma). Supernatants from PBMC cultures were removed at 24 hours and stored at -70°C for subsequent analysis.

IL-1β, IL-6 and TNF concentrations from supernatants were measured by ELISA (Quantikine, R & D Systems, Abingdon, UK). Limits of detection were 195pg/ml, 240pg/ml and 13.9pg/ml respectively for stimulated cells and 19.5pg/ml, 30pg/ml and 13.9pg/ml respectively for unstimulated cells.

Coefficient of variation for assays were as follows across the concentration range studied-

IL-1β less than 7.1%

IL-6 less than 4.4%

TNF less than 8.7%.

Hormones

All samples for the measurement of baseline hormone concentration were taken at 8am after an overnight fast.

Insulin and cortisol concentrations were measured by radioimmunoassay in the clinical laboratory of the Department of Clinical Chemistry at Glasgow Royal Infirmary. Limit of detection of insulin was 0.7mU/l.

Leptin concentrations were measured by radioimmunoassay (Linco Research, St Charles, MO, USA) in the research laboratories of Zeneca Pharmaceuticals in Macclesfield. Limit of detection was <1ng/ml.

Coefficient of variation for assays were as follows across the concentration range studied-

Insulin less than 10.5%

Cortisol less than 10%

Leptin less than 8.3%.

Proteolysis inducing factor

The isolation of proteolysis inducing factor (PIF) was performed in the research laboratory of the Department of Pharmaceutical Sciences at Aston University, Birmingham by Professor Michael Tisdale and Bill Field. Urine was treated with 80% (NH₄)₂SO₄ overnight, the precipitated protein

was recovered by centrifugation, dialysed against water and concentrated. An immunoblot was performed using a monoclonal antibody to PIF secreted by a hybridoma prepared from MAC16 tumour-bearing mouse splenocytes fused with a mouse myeloma cell line (Todorov et al, 1996).

Fatty acid analysis

Fatty acid analysis was performed in the research laboratory of the Department of Pharmaceutical Sciences at Aston University, Birmingham by Professor Michael Tisdale and Bill Field.

EDTA-anticoagulated venous blood samples were centrifuged at 2500rpm for 15 minutes and the plasma removed and stored at -70°C until subsequent analysis. Lipids were isolated and purified by the method of Folch (Folch et al, 1957).

Lipids were extracted from plasma by homogenising in 20 volumes of chloroform:methanol (2:1v/v). Fatty acids were extracted from plasma phospholipids by heating at 100°C for 45-60 minutes in 2.5ml 5% sodium hydroxide in 50% methanol in an atmosphere of nitrogen. The solution contained butylated hydroxytoluene as an antioxidant and margaric acid as an internal standard. The released fatty acids were methylated by heating to 80°C for 5 minutes in the presence of boron trifluoride in methanol. The fatty acid methyl esters were extracted twice with hexane:chloroform (4:1) and analysed using a Hewlett Packard 5890 Series II gas chromatograph with a narrow bore (0.25mm) DB32 column (J&W Scientific, Fisons, UK) and a flame ionisation chamber at 250°C. The temperature programme ran for 28 minutes with an initial temperature of 180°C with a 5°C/minute increase to 220°C which was maintained for 15 minutes. The column was run with helium as the carrier gas. The fatty acids were identified by their retention times based on the use of authentic fatty acid methyl ester standards.

Routine haematology and clinical chemistry measurements

Full blood count, electrolytes, urea, glucose and liver function tests were measured using standard automated techniques in the clinical laboratories of the Departments of Haematology and Clinical Chemistry at the Royal Infirmary of Edinburgh.

Cytokine gene polymorphism genotyping

DNA extraction

DNA was extracted from 1ml samples of EDTA anticoagulated blood using a Puregene DNA isolation kit based on a simple salting out technique (Miller et al, 1988) (Gentra systems, North Carolina, USA). Red blood cells were lysed and the precipitate retained after centrifugation. White blood cells were lysed in the presence of a DNA preservative using an anionic detergent to solubilise the cellular components. RNA was removed with an RNAase and cytoplasmic and nuclear proteins removed by precipitation with a saturated NaCl solution. After centrifugation the precipitate was discarded and DNA isolated from the supernatant by precipitation with 100% isopropanol. After centrifugation the precipitate containing the genomic DNA was washed with 70% ethanol and dissolved in a buffered solution containing a DNA preservative.

Cytokine gene polymorphism genotyping was performed with the assistance of Dr Susan Lynch and Dr James Powell.

TNFB NcoI polymorphism

The polymerase chain reaction (PCR) was used to amplify a 368bp fragment of the of the TNF β genomic sequence using primers upstream 5' CCGTGCTTCGTGCTTTGGACTA 3' and downstream 5' AGAGGGGTGCATGCTTGGGTTC 3' (Genosys, Pampisford, UK) (Stuber et al, 1996). The following PCR protocol was used - 95°C for 3 minutes, 45 cycles of 95°C for 30s, 68°C for 40s, 74°C for 48s; and 74°C for 6 minutes using reagents supplied by Promega (Southampton, UK) on a Hybaid Omn-E thermal cycler (Teddington, UK). The PCR product was digested directly with 1u of NcoI restriction enzyme (Promega, Madison, Wisconsin, USA) at 37°C for 4 hours. Restriction enzyme products were analysed on 1% NuSieve agarose (FMC bioproducts, Rockland, Maine, USA) or 6% polyacrylamide gels (Biorad, Hemel Hempstead, UK). The cleaved product produced bands at 133 and 235bp representing the allele TNFB1 while the uncleaved 368bp product represented the allele TNFB2 (Figure 2.1).

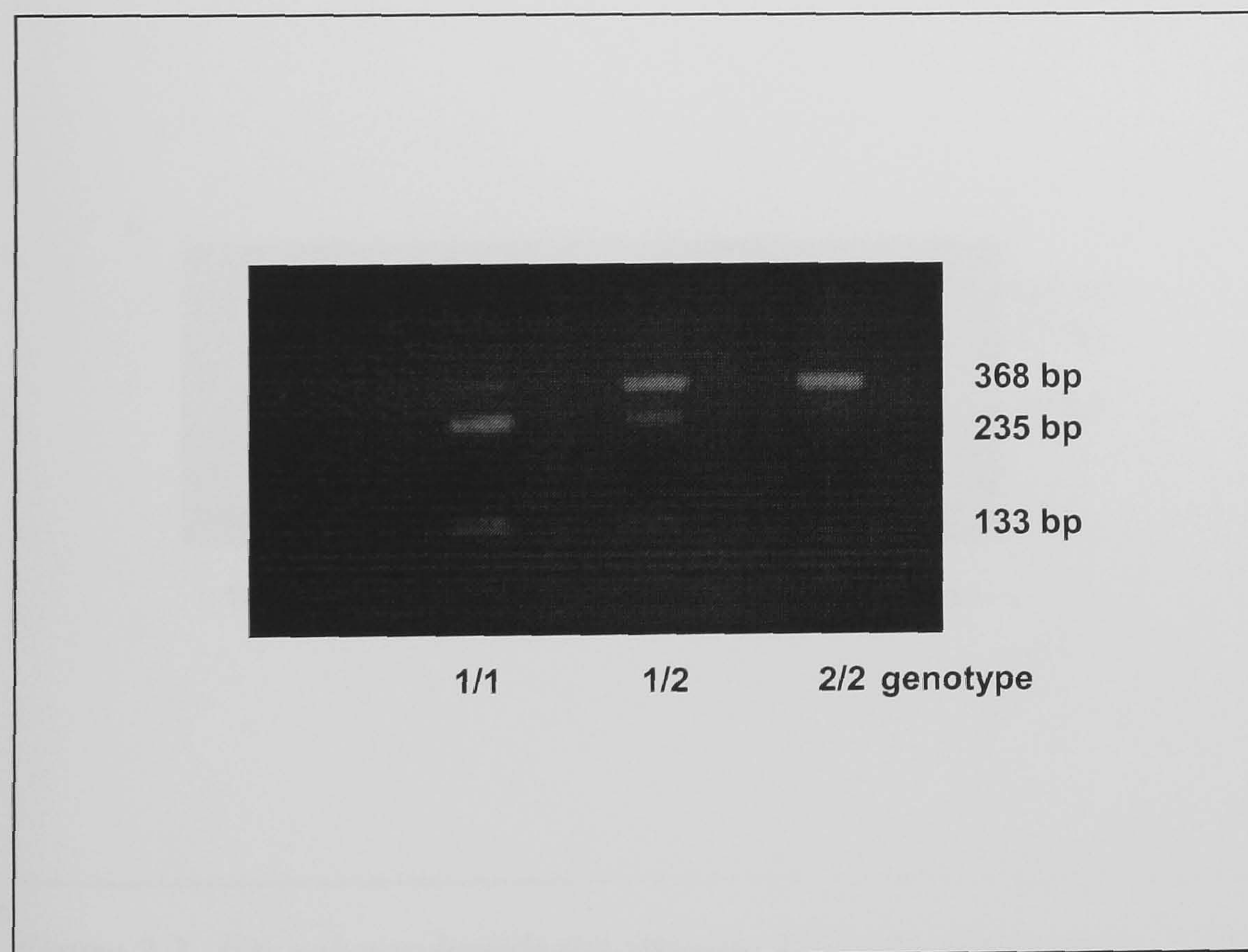


Figure 2.1. 6% polyacrylamide gel showing TNFB genotyping. 368bp fragment of TNF β gene has been amplified by PCR and digested with NcoI restriction enzyme. Allele 1 is cleaved into 133 and 235bp fragments while allele 2 remains uncleaved at 368bp.

TNF-308 G to A substitution

PCR was used to amplify a 107bp fragment of the of the TNF genomic sequence using primers upstream 5' AGGCAATAGGTTTTGAGGGCCAT 3' and downstream 5' TCCTCCCTGCTCCGATTCCG C 3' (Oswell) (Wilson et al, 1992). The following PCR protocol was used - 94°C for 3 minutes, 60°C for 1 minute, 72°C for 1 minute; 35 cycles of 94°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute; and 94°C for 1 minute, 60°C for 1 minute, 72°C for 5 minutes using reagents supplied by Promega (Southampton, UK) on a Hybaid Omn-E thermal cycler (Teddington, UK). The PCR product was digested directly with 1u of NcoI restriction enzyme (Promega) at 37°C for 4 hours. Restriction enzyme products were analysed on 9% polyacrylamide gels (Biorad, Hemel Hempstead, UK). The cleaved product produced bands at 87 and 20bp representing the TNF1 allele while the uncleaved 107bp product represented the TNF2 allele (Figure 2.2).

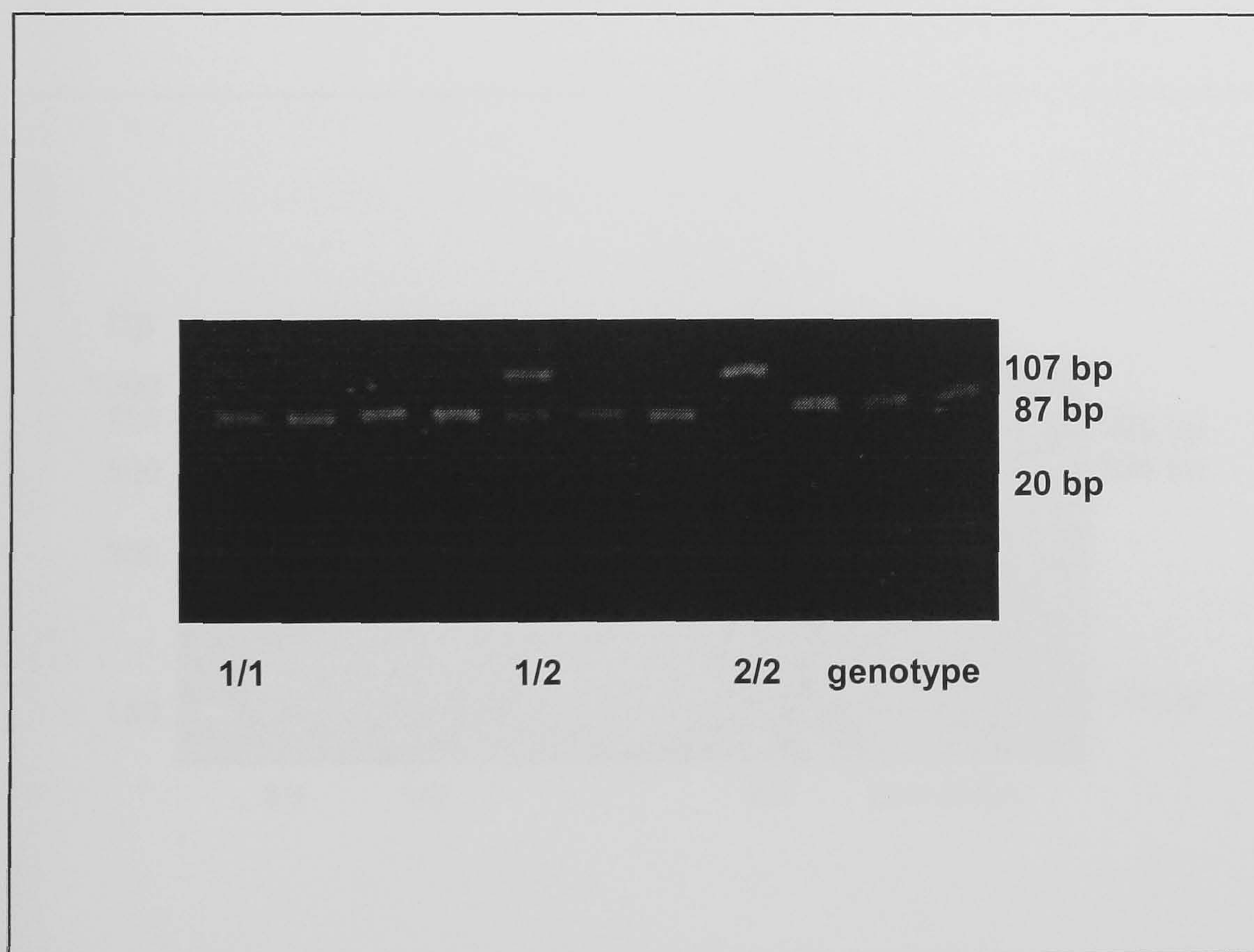


Figure 2.2. 9% polyacrylamide gel showing TNF-308 genotyping. 107bp fragment of TNF gene has been amplified by PCR and digested with NcoI restriction enzyme. Allele 1 is cleaved into 20 and 87bp fragments while allele 2 remains uncleaved at 107bp.

IL-1 β polymorphism

PCR was used to amplify a 699bp fragment of the of the IL-1 β genomic sequence using primers upstream 5' TGT TCT TAG CCA CCC CAC TC 3' and downstream 5' ATC GCT CCA GCA CTC TTG TT 3' (Genosys, Pampisford, UK) designed on the basis of the published sequence (Clark et al, 1986) using the Primer 3 program (Rozen & Skaletsky , 1996). The following PCR protocol was used - 3 cycles of 97°C for 1.5 minutes, 54°C for 1.5 minutes, 74°C for 1 minute; 32 cycles of 97°C for 0.5 minutes, 54°C for 0.5 minutes, 74°C for 1.5 minutes; and 72°C for 10 minutes using reagents supplied by Promega (Southampton, UK) on a Hybaid Omn-E thermal cycler (Teddington, UK). The PCR product was digested directly with 0.5u of TaqI restriction enzyme (Appligene, Chester-le-Street, UK) at 65°C for 4 hours. Restriction enzyme products were analysed on 1% NuSieve agarose (FMC bioproducts, Rockland, Maine, USA) or 6% polyacrylamide gels (Biorad, Hemel Hempstead, UK). The cleaved product produced bands at 524 and 175bp representing allele 1 while the uncleaved 699bp product represented allele 2 (Figure 2.3).

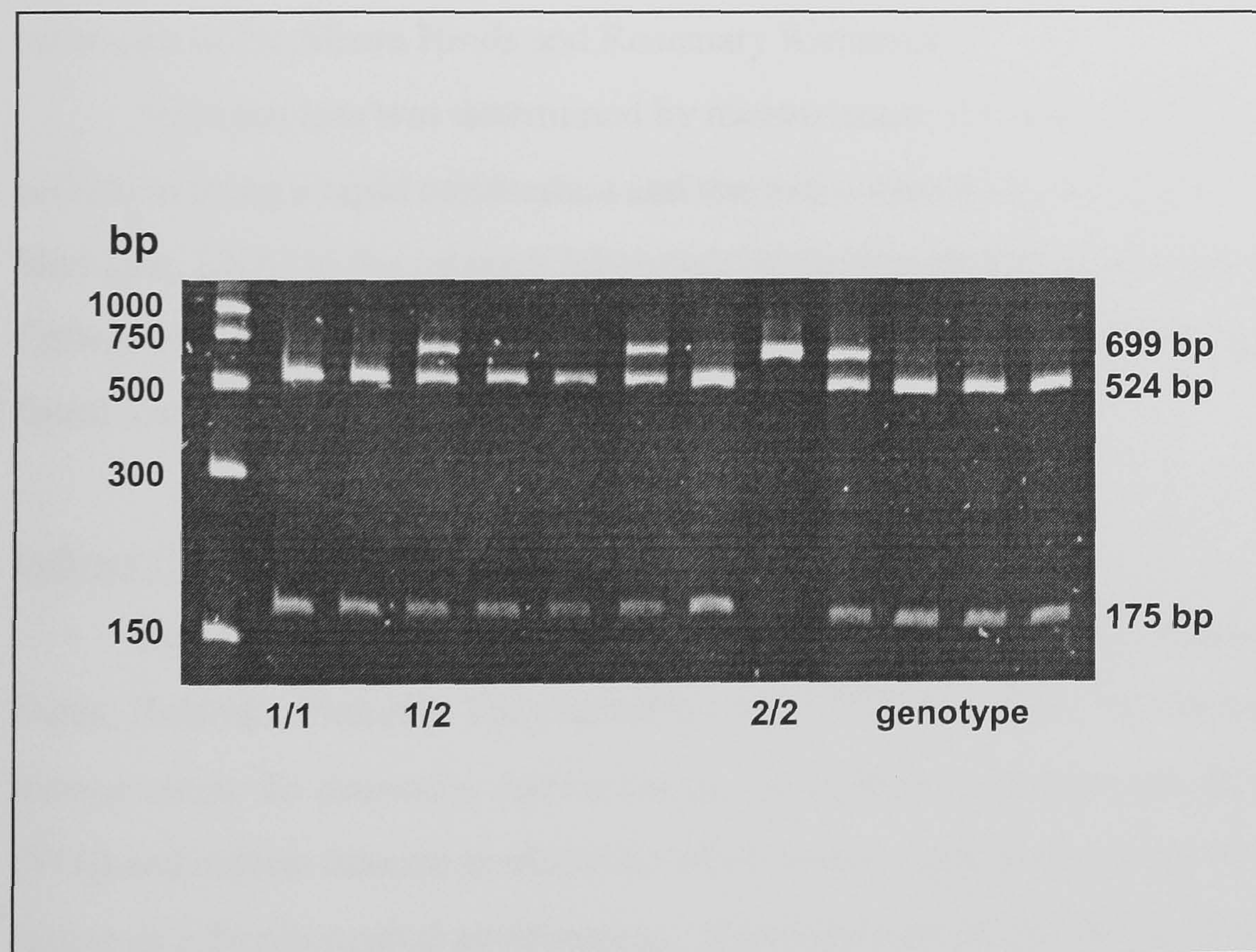


Figure 2.3. 6% polyacrylamide gel showing interleukin-1 β genotyping. 699bp fragment of IL-1 β gene has been amplified by PCR and digested with TaqI restriction enzyme. Allele 1 is cleaved into 175 and 524bp fragments while allele 2 remains uncleaved at 699bp.

Interleukin-1 receptor antagonist variable number tandem repeat polymorphism

PCR was used to amplify a fragment of the of the IL-1Ra genomic sequence using primers upstream 5' CTC AGC AAC ACT CCT AT 3' and downstream 5' TCC TGG TCT GCA GGT AA 3' (Oswell). The following PCR protocol was used - 35 cycles of 94°C for 1 minute, 60°C for 1 minute, 72°C for 1 minute; and 72°C for 5 minutes using reagents supplied by Promega (Southampton, UK) on a Hybaid Omn-E thermal cycler (Teddington, UK). The PCR products were analysed on 6% polyacrylamide gels (Biorad, Hemel Hempstead, UK). The 86 base pair variable number tandem repeat resulted in bands of around 240bp (allele 2, 2 repeats), 325bp (allele 4, 3 repeats), 410bp (allele 1, 4 repeats), 500bp (allele 3, 5 repeats) and 595bp (allele 5, 6 repeats).

Metabolic assessment

Nitrogen balance

The daily nitrogen intake of subjects was calculated using CompEat 4 software (Nutrition Systems, London, UK) based on the mean dietary intake recorded in a 3 day food diary with the assistance of Dr Alison Hinds and Rosemary Richardson.

Nitrogen loss was determined by measurement of urinary nitrogen from a 24 hour urine collection using a rapid combustion and thermal conductivity cell method (Leco FP-328, St Joseph, Michigan, USA) in the research laboratory of the Department of Nutrition at Queen Margaret College in Edinburgh with the assistance of Colin Nicolson. A further 2g per day was added for faecal losses.

Indirect Calorimetry

Subjects were studied after an overnight fast using a ventilated hood system (Deltatrac II, Datex, Helsinki, Finland). They rested for at least 30 minutes in the supine position prior to measurement. To determine resting energy expenditure measurements of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were made over at least 30 minutes with patients supine in a thermoneutral environment. Measurements for the initial 10 minutes were discarded and the remaining values averaged. The equipment was calibrated before each measurement. Precision was checked three-monthly by burning methanol.

Respiratory quotient (RQ) was calculated by dividing oxygen consumption by carbon dioxide production.

Energy expenditure (EE) was calculated using the Weir formula (Weir, 1949).

$$EE = 0.5373 * VO_2 * (3.78 + 1.16 * RQ) - 43.2$$

Substrate utilisation was calculated from oxygen consumption (and carbon dioxide production and urinary nitrogen excretion (NM) according to the equations of Cosolazio (Consolazio et al, 1963).

$$\text{Carbohydrate} = -2.91 * VO_2 + 4.12 * VCO_2 - 2.56 * NM$$

$$\text{Fat} = 1.69 * VO_2 + 1.69 * VCO_2 - 1.94 * NM$$

$$\text{Protein} = 6.25 * NM$$

Albumin and fibrinogen synthesis

Sampling protocol

Albumin and fibrinogen were measured by a flooding dose technique (Garlick et al, 1989). The study protocol is presented diagrammatically in chapter 10, figure 1. Subjects attended at 8am on two consecutive days. On the first day following an overnight fast, a venous catheter was inserted into the subject's antecubital fossa. At 10am subjects received an intravenous bolus of 2H_5 - or 2H_8 -labelled phenylalanine (3.5g 10 mole% labelled L-phenylalanine, 2% in saline, CK Gas Products, Finchampstead, UK) over approximately 5 minutes. The tracer was prepared under sterile conditions, tested for sterility and absence of pyrogens, and administered via a 0.22 μ m filter. Taking care to flush the catheter to avoid tracer contamination, 10ml blood samples were obtained before and 20, 40, 60, 80, 100 and 120 minutes after the start of the tracer infusion. The blood sample taken before the "flooding" dose was analysed for baseline albumin and fibrinogen concentrations, 2H_5 - or 2H_8 -phenylalanine enrichment in plasma albumin and fibrinogen and in the plasma free phenylalanine pool. Subsequent samples were analysed for 2H_5 - or 2H_8 -phenylalanine enrichment in plasma albumin and fibrinogen and in the plasma free phenylalanine pool. Subjects remained fasting throughout this period. On the second day, again following an overnight fast a venous catheter was inserted into the subject's antecubital fossa and subjects received a meal of a balanced whole protein liquid nutritional supplement (Fortisip, Nutricia, Zoetermeer, Holland)

providing a twelfth of the subjects estimated energy requirement (measured resting energy expenditure * 1.4) (Hunter et al, 1995). The supplement provided 13% of energy from protein, 48% from carbohydrate and 39% from fat, similar to a “typical” British diet. Subjects received such a feed on an hourly basis until the end of the study period. At 10am a similar flooding dose of phenylalanine was given as described above using the alternative label and blood samples collected similarly.

Sample preparation and isotope analysis

The study protocol required the measurement of labelled phenylalanine enrichment in the plasma free phenylalanine pool and in plasma albumin and fibrinogen (McMillan et al, 1996). For free phenylalanine analysis, 1.5ml plasma samples were diluted with 5ml deionised distilled water with 250nmol cycloleucine added as an internal standard. Diluted samples were then deproteinised by ultrafiltration (25000 molecular weight cut-off Centrifree cone, Amicon, Gloucestershire, UK), acidified and the amino acids purified by cation exchange. $^2\text{H}_5$ - or $^2\text{H}_8$ -phenylalanine enrichment was measured by gas chromatography mass spectrometry (GC-MS) as its tertbutyldimethylsilyl derivative (Slater et al, 1995). $^2\text{H}_8$ -phenylalanine was found to form $^2\text{H}_7$ -phenylalanine over time (around 53% $^2\text{H}_7$ -phenylalanine by 120 minutes). Both isotopes were measured in the appropriate samples.

Albumin was extracted from 1ml of serum by differential solubility in absolute ethanol from trichloroacetic acid (10% weight/weight) -precipitated protein. To remove traces of free phenylalanine, the ethanolic albumin solution was washed three times with 5ml deionised distilled water using ultrafiltration. Purified albumin was then hydrolysed and labelled phenylalanine enrichment measured by GC-MS (Slater et al, 1995).

Following washing of the plasma three times in an ultrafiltration cone as described above, fibrinogen was removed as a fibrin clot. Clotting was performed by diluting 1.5ml of plasma to 20ml with saline and 0.5ml calcium chloride (0.5mol/l). Fifteen units of human albumin-free thrombin (Sigma, Poole, UK) was then added and after 10 minutes the fibrin was collected on an etched glass rod. The fibrin was then hydrolysed under vacuum at 145°C for 4 hours with 6 mol/l HCl and its labelled phenylalanine enrichment measured as described above.

Sample preparation was performed with the assistance of Dr Donald McMillan and Jason Donnelly. GC-MS was performed by Dr Tom Preston.

Calculations

Plasma volume was predicted from body weight and height (Retzlaff et al, 1969).

Men - Plasma volume (ml) = (23.7 * height (cm)) + (9.0 * weight (kg)) - 1709

Women - Plasma Volume = (40.5 * height) + (8.4 * weight) - 4811

Fractional synthesis rates of albumin and fibrinogen were calculated by dividing the rate of change of labelled phenylalanine enrichment of albumin or fibrinogen by the area under the curve of precursor enrichment versus time (Ballmer et al, 1990). Calculations were performed with the assistance of Dr Tom Preston.

Statistical analysis

Statistical analysis was carried out using Statview (Abacus Concepts, Inc, Berkeley, California, U.S.A.). Data were largely nonparametric in distribution and thus nonparametric statistics were used.

Data are presented as median (interquartile range) unless otherwise stated.

Paired comparisons between individuals at two time points were made using the Wilcoxon signed rank test.

Comparisons between groups involving more than two variables were made using the Kruskal Wallis test.

Comparisons between control and patient groups were made using the Mann-Whitney *U* test.

Categorical variables were compared by the Chi-squared test.

Correlation was assessed using Spearman's rank correlation coefficient.

Survival was analysed using the Kaplan-Meier technique and groups compared by the log rank test.

Results were considered to be statistically significant with a p value of <0.05.

Relationship of weight loss to the acute phase response and performance status

Summary

Weight-loss and the acute phase response have been associated with poor quality of life and survival in advanced pancreatic cancer, however, little information is available on changes in these factors over time and their inter-relationships. This chapter examined changes in weight, Karnofsky performance status, C-reactive protein (CRP) and serum albumin in 25 patients with advanced pancreatic cancer given supportive symptomatic treatment only. Patients were assessed at approximately monthly intervals on a total of 70 occasions allowing assessment of changes over 37 intervals. Changes were assumed to occur in a linear pattern and were averaged to 28 days.

Overall, patients had a median weight-loss of 2.3kg/28 days. Median CRP levels rose by 15mg/l and serum albumin fell by 1g/l on average over 28 days. Karnofsky performance status fell by 4 points every 28 days.

The nine patients assessed close to death were compared with the 13 assessed closer to diagnosis. The increase in CRP level and fall in weight and performance status was significantly greater within eight weeks of death than within eight weeks of diagnosis. Only 13% of patients had an elevated CRP level close to diagnosis compared with 100% of those close to death. In multivariate analysis Karnofsky performance status was significantly associated with percentage weight loss and CRP levels.

These data further implicate the acute phase protein response as being associated with the progressive weight-loss seen in patients with advanced pancreatic cancer. Changes appear to accelerate close to death. Moreover, the development of cachexia is associated with a reduction in patients' functional capacity.

Introduction

Greater knowledge of the extent and relationship of changes in nutritional, inflammatory and functional factors is important for understanding the natural history of advanced pancreatic cancer and so for assessing the effects of any nutritional therapy and thus whether therapy directed at reversing the weight-loss observed in these patients might have the potential to influence quality of life.

No attempt has been made previously to quantify changes in nutritional and inflammatory markers such as weight, albumin and C-reactive protein (CRP) from month to month in patients with advanced pancreatic cancer and to relate these changes to one another or to consider the effects such changes may have on the functional status of patients.

It has been shown previously that the presence of the acute phase protein response (APPR) is a major predictor of survival in those with advanced pancreatic cancer and that between the time of diagnosis and death there is a significant rise in the number of patients exhibiting an APPR (as defined by a CRP level $>10\text{mg/l}$) and a fall in serum albumin concentration (Falconer et al, 1995). Although CRP and albumin are respectively the major positive and negative acute phase proteins in man, their levels also vary with nutritional status and vascular permeability (Fleck et al, 1985, Steel & Whitehead, 1994). It has been shown that 85% of patients with advanced pancreatic cancer exhibit weight-loss at diagnosis and that this progresses inexorably until death (Wigmore et al, 1997). The functional decline of patients with advanced cancer has an important effect on quality of life in these patients and it is not clear how this deterioration relates to the ongoing cachectic state (Ovesen et al, 1993a).

Patients and Methods

Twenty five patients with unresectable pancreatic cancer diagnosed by histology or unequivocal radiological findings were studied. Patients received full symptomatic supportive care over the period of the study. None of the patients received chemotherapy or radiotherapy and were examined at least 4 weeks after surgical intervention. Patients were examined on a total of 70 occasions allowing changes over 37 individual periods to be assessed. Patients were reviewed at approximately monthly intervals (median 28 days, interquartile range 16-35 days). Changes in nutrition-related variables were transformed to give a calculated change over 28 days. At the time of assessment no patient had clinical evidence of infection. Two patients developed significant ascites between assessments and their weight change was excluded from analysis thereafter.

At each review patients were weighed on a beam scale and Karnofsky performance status was noted. A venous blood sample was taken for determination of CRP and albumin. Total body water was estimated by bioelectric impedance analysis.

Further details of methods are found in chapter 2.

Results

Patient characteristics and changes in weight, CRP, albumin and Karnofsky performance status are presented in table 3.1. Patients had a median age of 64 (range 29-76). 10 patients had UICC stage II disease, 4 patients stage III disease and 11 patients stage 4 disease.

Overall, patients had a median weight change of -2.3kg (-4.5--0.6) per 28 day period with gains in weight only being observed on three of 35 occasions (9%). CRP rose by a median of 15mg/l (0-48) while albumin fell by a median of 1g/l (-3-+1) over the average 28 day period. CRP fell on only three of 37 occasions (8%). Karnofsky performance status fell by a median of 4 points (-19-0) over 28 days. An improvement in performance status was not observed in this group of patients.

Table 3.1. Characteristics of 25 patients with advanced pancreatic cancer and of 13 patients assessed close to diagnosis and 9 patients assessed close to death and changes in weight, C-reactive protein and albumin over intervals of 28 days.

| | Number of intervals | Age | UICC Stage | | | Change in weight | Change in CRP | Change in Albumin | Change in KPS |
|-----------------------------|---------------------|-----------------|------------|---|-----|-----------------------|------------------|-------------------|--------------------|
| | | | 2 | 3 | 4 | | | | |
| All patients | 37 | 64 (29-76) | 10 | 4 | 11 | -2.3 (-4.5--0.6) | 15 (0-48) | -1 (-3-+1) | -4 (-19-0) |
| Patients close to diagnosis | 13 | 70 (51-76) | 4 | 2 | 7 | -2.5 (-4.6--1.1) | 0 (0-28) | -1 (-3-+2) | 0 (-4-0) |
| Patients close to death | 9 | 55 a (29-76) | 2 | 3 | 4 b | -5.6 c (-7.2--2.6) | 69 d (39-128) | -5 e (-7-0) | -20 f (-42--16) |

Values are median with interquartile range in parentheses except for age where values are median and range.

CRP - C-reactive protein

KPS - Karnofsky performance status

UICC - Union Internationale Contre le Cancer

a - Age of patients close to death compared with those close to diagnosis p=0.08

b - Disease stage of patients close to death compared with those close to diagnosis p=0.91

c - Change in weight of patients close to death compared with those close to diagnosis p=0.047

d - Change in CRP of patients close to death compared with those close to diagnosis p=0.0021

e - Change in albumin of patients close to death compared with those close to diagnosis p=0.27

f - Change in KPS of patients close to death compared with those close to diagnosis p=0.0045

Nine patients were assessed within 8 weeks of death and 13 patients were assessed within eight weeks of diagnosis. In patients close to death, rate of weight-loss, rise in CRP and fall in Karnofsky performance status were significantly greater than the rates close to the time of diagnosis ($p=0.047$, 0.0021 and 0.0045 respectively). Although albumin fell faster close to death this did not reach statistical significance ($p=0.27$). All nine patients close to death had an elevated CRP while only two of 13 patients close to diagnosis (15%) did so ($p<0.001$).

There was a significant correlation between Karnofsky performance status and both percentage weight loss and lean body mass index ($p=0.0001$ and 0.016 respectively) (Figures 3.1 and 3.2) but there was no significant correlation between Karnofsky performance status and body mass index ($p=0.45$) (Figure 3.3). There were also significant correlations between Karnofsky performance status and both CRP and albumin (both $p=0.0001$) (Figure 3.4 and 3.5). There was a significant correlation between percentage weight loss and CRP ($p=0.038$) and lean body mass index and CRP ($p=0.019$) (Figure 3.6). However, there was no significant correlation between body mass index and CRP ($p=0.29$). Serum albumin concentration correlated with CRP ($p=0.0001$) and with percentage weight loss, body mass index and lean body mass index ($p=0.0006$, 0.022 and 0.01 respectively).

Multiple regression analysis involving the factors found to correlate significantly revealed associations between Karnofsky performance status and CRP and percentage weight loss ($p=0.013$ and 0.0002 respectively). CRP concentration was otherwise related only to albumin ($p=0.013$).

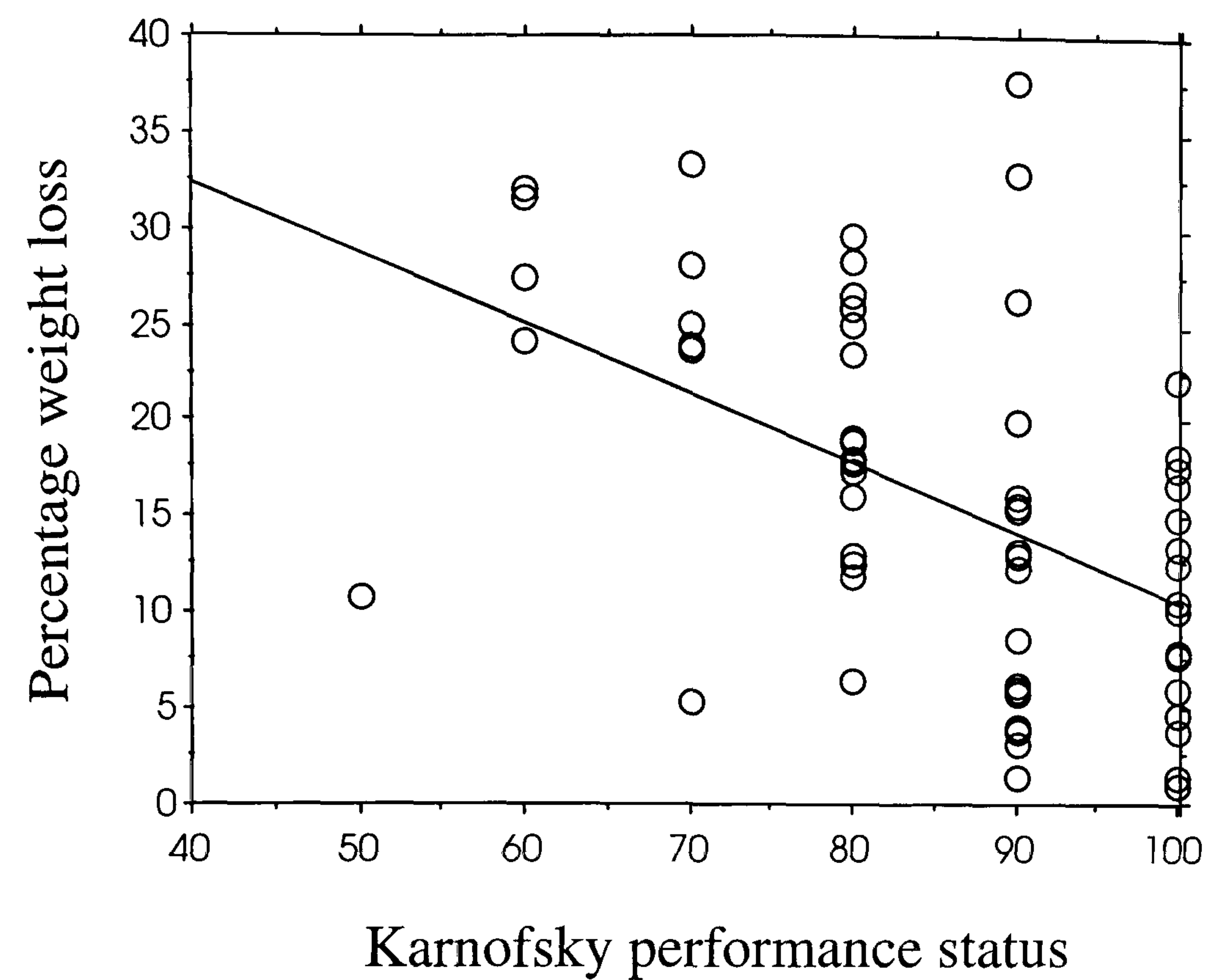


Figure 3.1. Significant correlation between Karnofsky performance status and percentage weight loss ($r=-0.50$, $p=0.0001$).

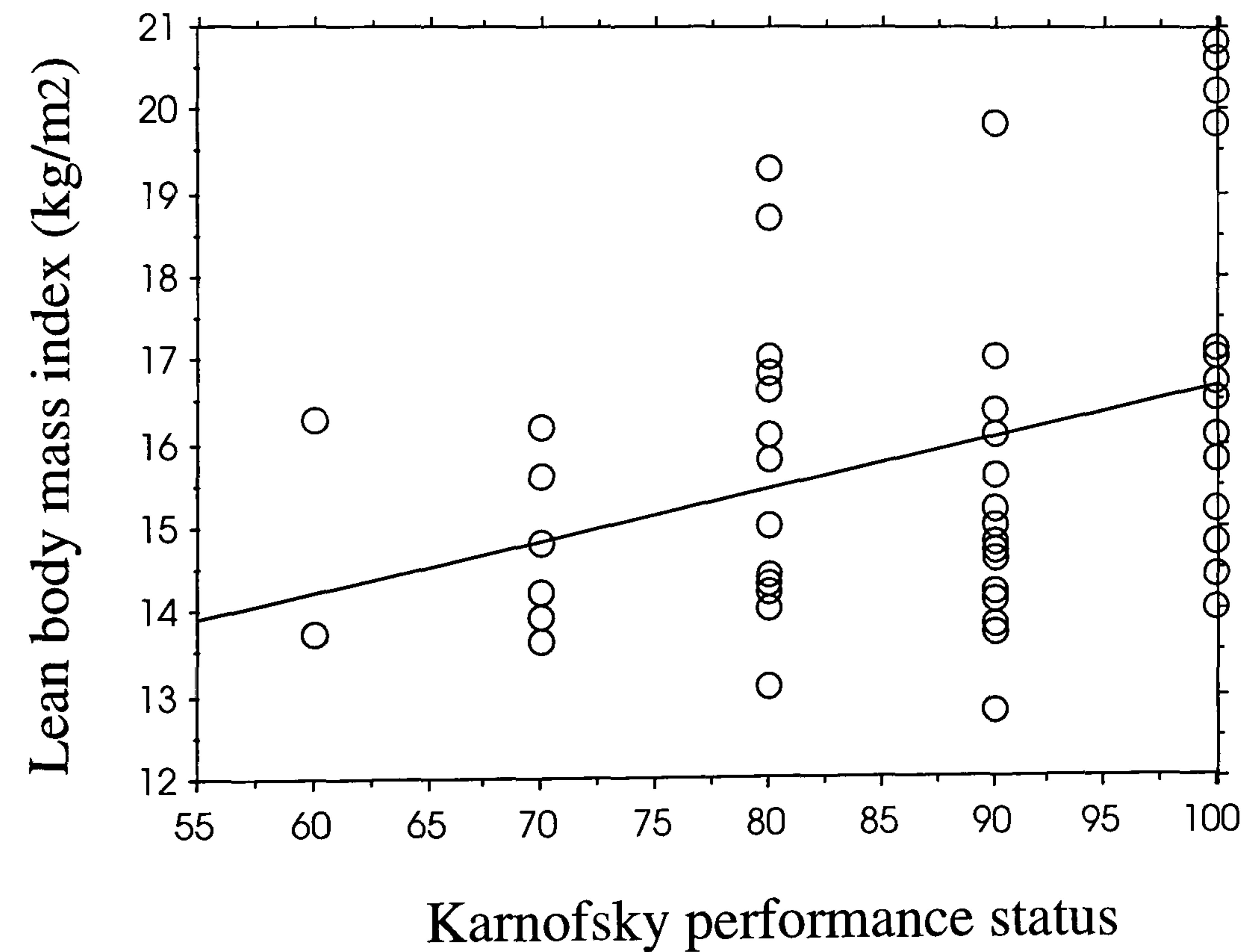


Figure 3.2. Significant correlation between Karnofsky performance status and lean body mass index ($r=0.33$, $p=0.016$).

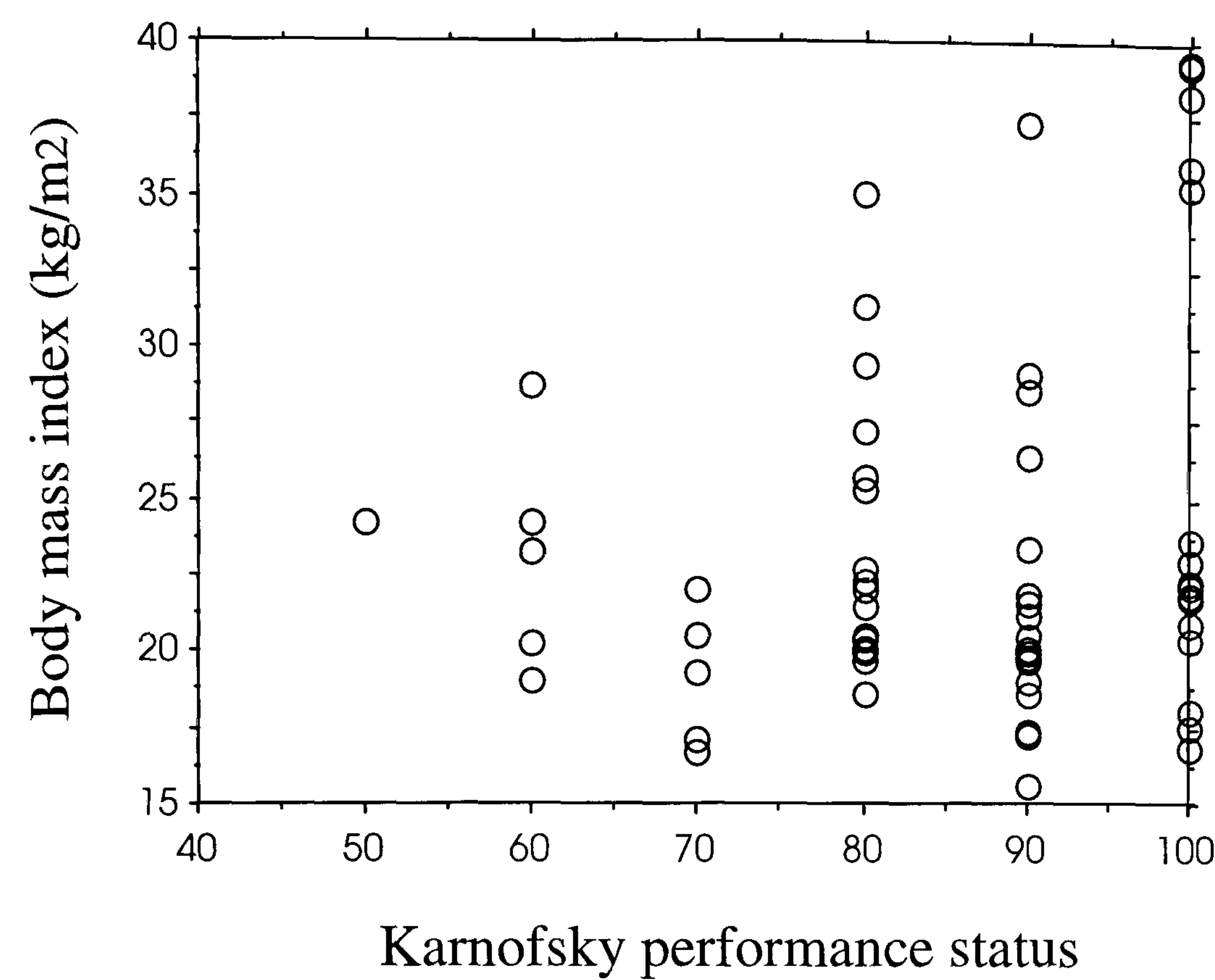


Figure 3.3. Lack of significant correlation between Karnofsky performance status and body mass index ($r=0.09$, $p=0.45$).

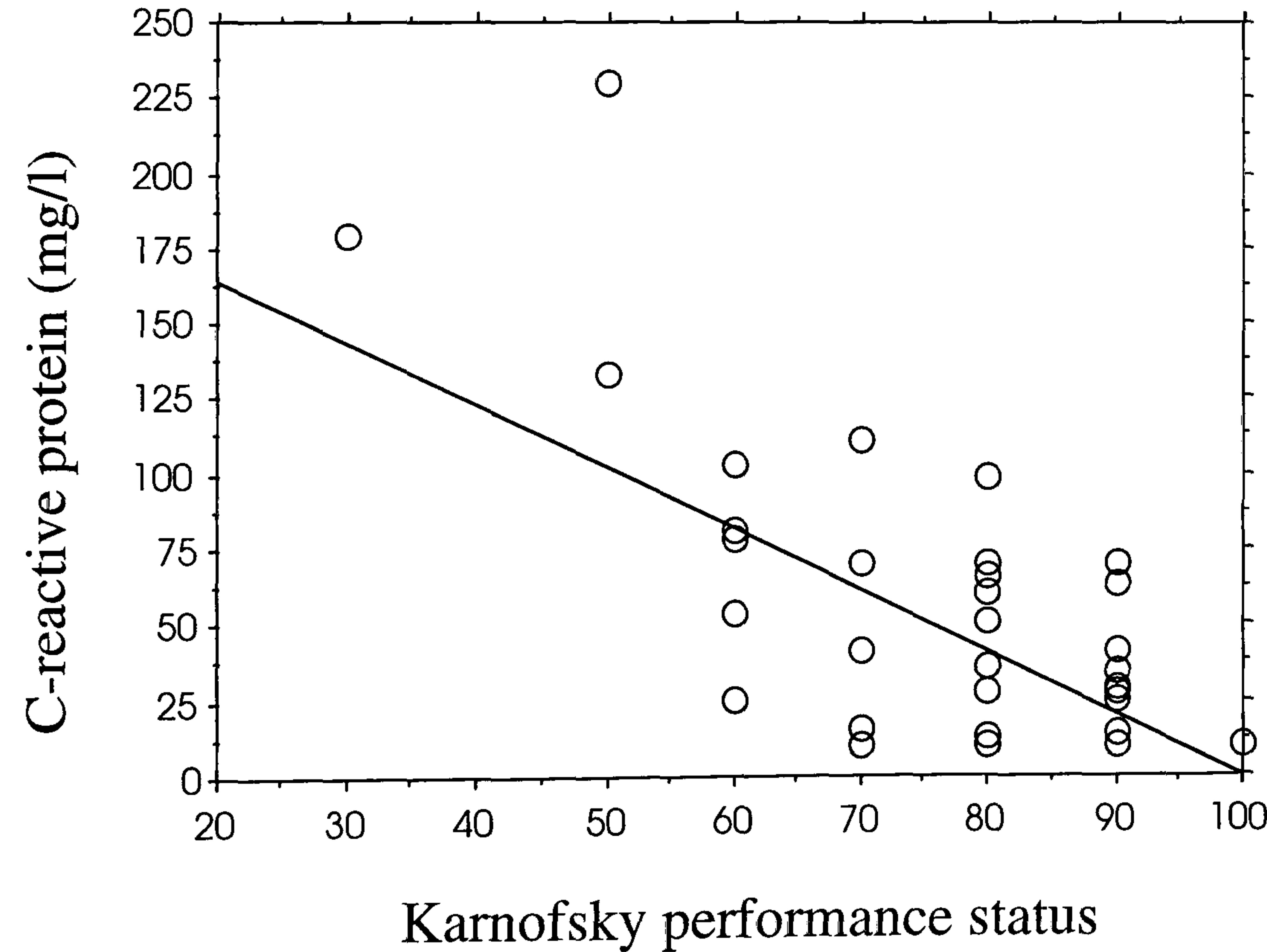


Figure 3.4. Significant correlation between Karnofsky performance status and C-reactive protein ($r=-0.62$, $p=0.0001$).

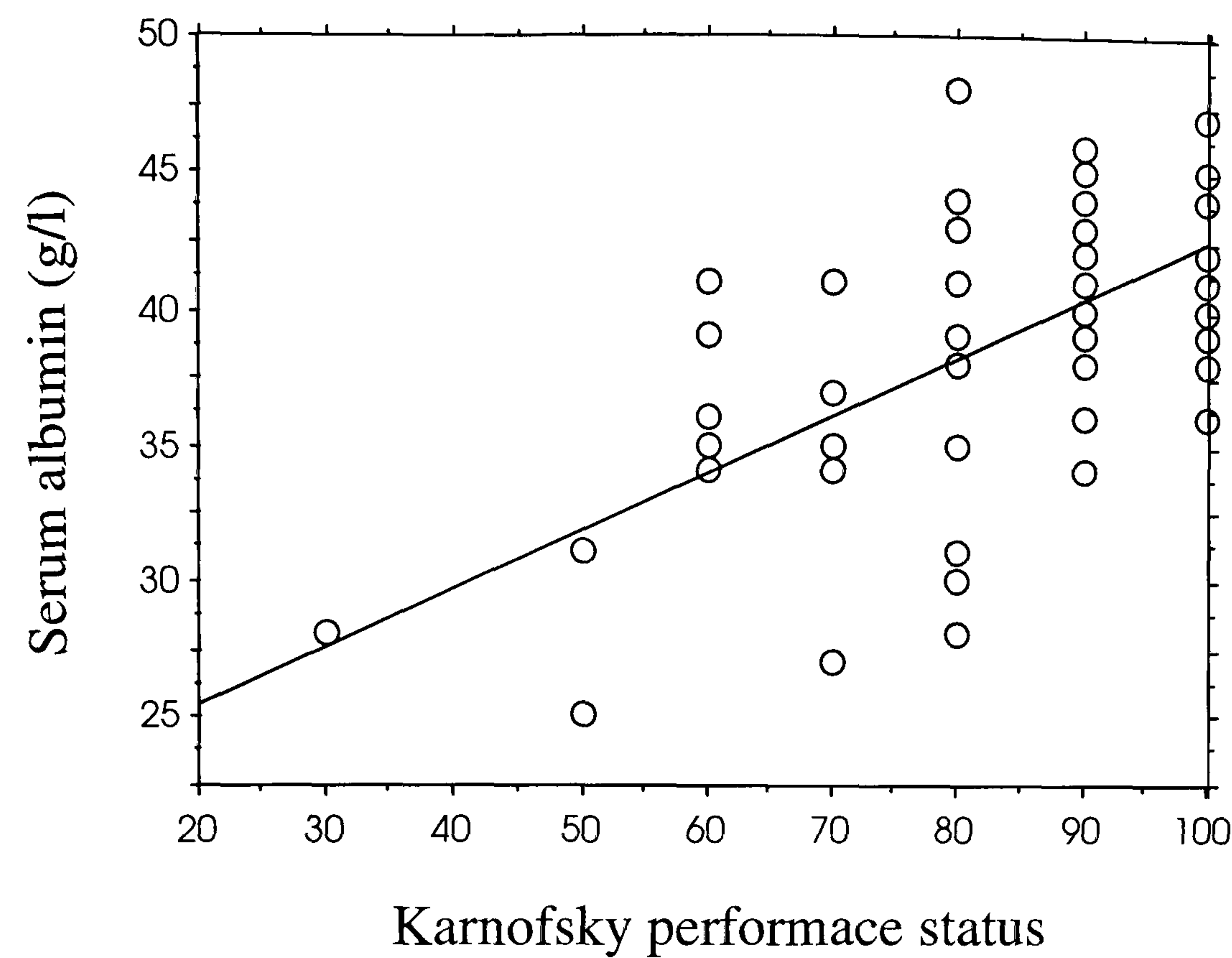


Figure 3.5. Significant correlation between Karnofsky performance status and serum albumin ($r=0.54$, $p=0.0001$).

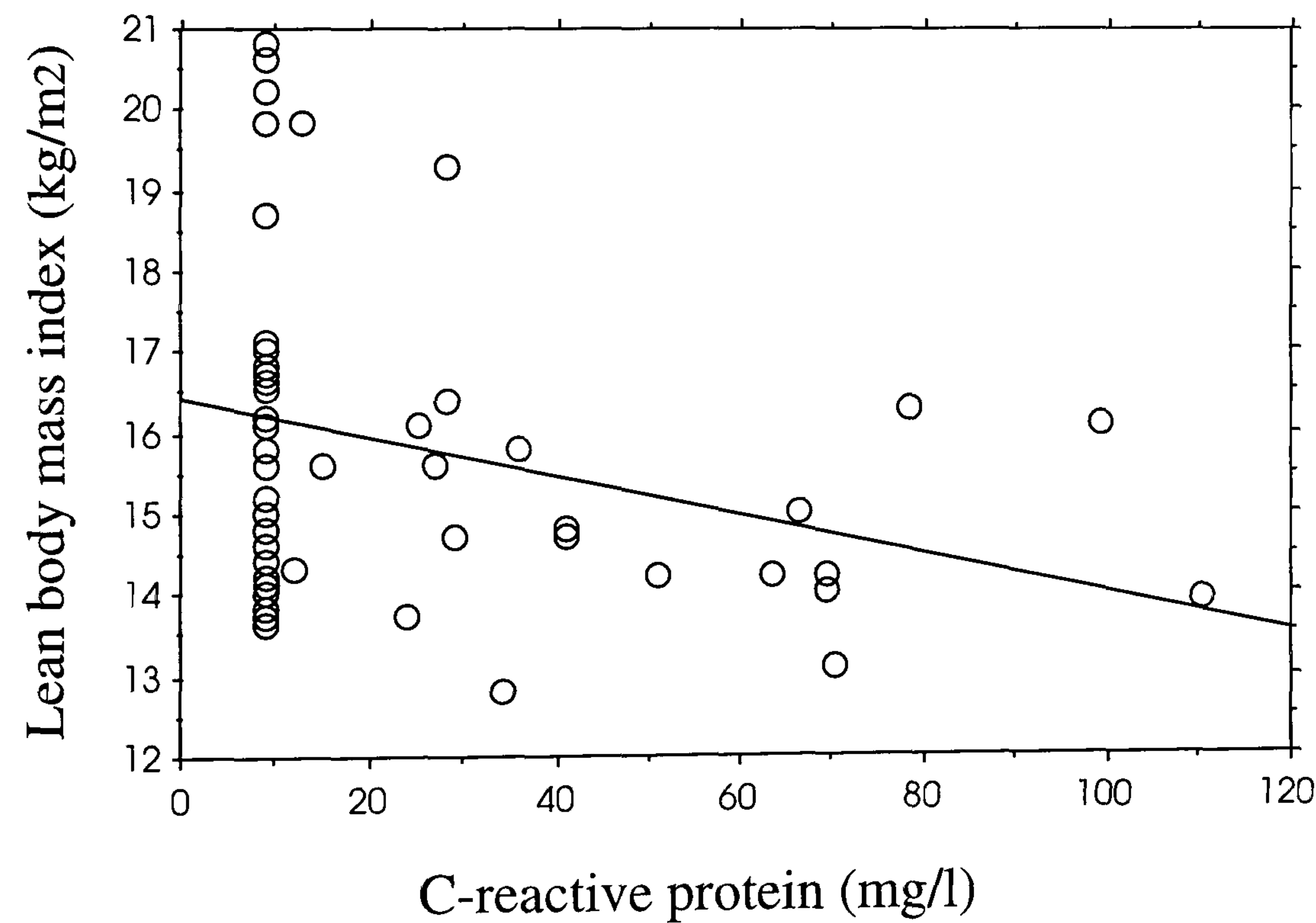


Figure 3.6. Significant correlation between C-reactive protein and lean body mass index ($r=-0.32$, $p=0.019$).

Discussion

In the present study patients with advanced pancreatic cancer lost weight at a median rate of 2.3kg over each 28 day period. This value is very similar to that reported in previous studies of pancreatic cancer patients prior to nutritional intervention who had been losing weight at a rate of 2.9kg/month (Wigmore et al, 1996) and 2.2kg/month (Barber et al, 1997) respectively. In a previous study in which the natural history of the pattern of weight-loss was examined in pancreatic cancer patients, it had appeared that weight-loss occurred in a relatively linear pattern (Wigmore et al, 1997). The current data suggests that near to death weight-loss may be accelerated. This may reflect a pre-terminal worsening of inanition and anorexia.

The progressive increase in CRP levels and fall in weight over time is similar to the pattern observed in previous studies of patients with pancreatic (Falconer et al, 1995) and lung cancer (Staal-van den Brekel et al, 1995). Only 2 of 13 of patients (15%) in the present chapter had an elevation of CRP around the time of diagnosis (between four and eight weeks after surgical intervention or bile duct stenting) whereas all nine patients sampled within 8 weeks of death had an elevated CRP concentration. Moreover, the rate at which CRP increased seemed to accelerate with time. The reason for the progressive increase in the prevalence and intensity of the acute phase response in patients with advancing disease is not clear. It may relate to changes in tumour production of pro-inflammatory cytokines (Wigmore et al, 1994) or progressive inflammation around the enlarging tumour may stimulate increased host cytokine production.

The relationship between high serum CRP levels and weight-loss may support the view that in the face of reduced food intake, the demand for amino acids to produce acute phase proteins results in ongoing skeletal muscle catabolism and thus contributes to progressive weight-loss (Reeds et al, 1994). Alternatively, both the APPR and muscle breakdown may be driven simultaneously by pro-inflammatory cytokines and other proteolytic factors (Todorov et al, 1996, Fujiki et al, 1997). If the latter hypothesis is correct then upstream targetting of increased pro-inflammatory cytokine release would be more likely to result in therapeutic benefit rather than targetting the acute phase response itself.

An association between the APPR and weight-loss has also been shown in non-small-cell lung cancer (Staal-van der Brekel et al, 1995) and between APPR and disease stage in oesophageal cancer (Wayman et al, 1997). Interestingly, the present chapter found a relationship between the level of the acute phase response and lean body mass index but not with the body mass index suggesting that the APPR may be preferentially driving loss of lean tissue rather than fat.

Low albumin concentrations were significantly associated with weight-loss and with increased CRP perhaps reflecting its role as a marker of nutritional status and as a negative acute phase protein. A low serum albumin concentration was also associated with reduced performance status which may explain the previously noted association between hypoalbuminaemia and reduced survival in pancreatic and colorectal cancer patients (Falconer 1995, Heys et al, 1998).

The functional ability of patients as measured by Karnofsky performance status was, like CRP, significantly related to weight loss and lean body mass index but not overall body mass index, suggesting that it is lean tissue that is important in determining function. However, in multivariate analysis, performance status was related to CRP and percentage weight-loss alone. Thus individual patients may adapt to a particular weight and when this falls (particularly with loss of lean tissue) functional ability deteriorates despite the fact that patients may remain relatively overweight in terms of body mass index.

This chapter attempted to quantify the changes over time in weight, CRP, albumin and Karnofsky performance status in patients with advanced pancreatic cancer and has shown that the rate of weight-loss, rise in CRP and deterioration in functional ability accelerate close to death. It has also shown that CRP levels rise and performance status falls with loss of lean tissue. These findings may provide a benchmark with which to compare changes in these factors following nutritional or anticancer treatment and further implicate pro-inflammatory events in exacerbating weight-loss and affecting functional ability in these patients.

The next chapter will examine the relationship of the acute phase protein response to concentrations of pro-inflammatory cytokines in cachectic cancer patients.

Relationship of pro-inflammatory cytokines to the acute phase protein response

Summary

The level of the acute phase response is a major predictor of survival in patients with advanced pancreatic cancer. Moreover, as shown in Chapter 3, the acute phase response also relates with weight loss and functional ability. This chapter examines the association between the acute phase protein response, as determined by serum C-reactive protein, and serum levels of interleukin-6, soluble interleukin-6 receptor and the soluble tumour necrosis factor receptors in pancreatic cancer patients.

Thirty-four blood samples were collected from 13 patients with advanced pancreatic cancer. Samples were also collected from 6 healthy subjects. Levels of C-reactive protein, interleukin-6, soluble interleukin-6 receptor, soluble tumour necrosis factor receptor 55 and soluble tumour necrosis factor receptor 75 were measured by indirect ELISA.

Serum levels of C-reactive protein, interleukin-6 and soluble tumour necrosis factor receptor 55 and 75 were significantly higher in cancer patients than in controls. Serum soluble interleukin-6 receptor levels were not significantly different between the two groups.

In cancer patients, a significant positive association was found between the level of the acute phase protein response and serum concentrations of interleukin-6, soluble tumour necrosis factor receptor 55 and soluble tumour necrosis factor receptor 75. No association was found between levels of soluble interleukin-6 receptor and any other factor.

In vitro work has suggested that pro-inflammatory cytokines stimulate the acute phase protein response. This chapter suggests that in vivo there is a direct correlation between pro-inflammatory cytokine levels and concentrations of C-reactive protein. This confirms the value of the acute phase response as a marker of the inflammatory state in general and of in vivo pro-inflammatory cytokine activity in particular in advanced pancreatic cancer patients.

Introduction

The previous chapter has shown that the weight-loss and deterioration in functional ability in patients with advanced pancreatic cancer patients is associated with a hepatic acute phase protein response (APPR). The APPR involves a reprioritisation of protein synthesis within the liver in response to trauma and inflammation. The roles of the APPR include limitation of tissue damage, isolation and destruction of infective organisms and the promotion of repair (Baumann & Gauldie, 1994). However, the presence of an APPR as measured by an elevated serum C-reactive protein (CRP) level is strongly associated with a shorter duration of survival in patients with advanced pancreatic cancer (Falconer et al, 1995). Thus, in patients with cancer cachexia, the reprioritisation of protein synthesis associated with the APPR may lead to persisting muscle catabolism and wasting which is detrimental to survival.

Interleukin-6 (IL-6) appears to have a major role in inducing the APPR (Heinrich et al, 1990) but previously a relationship between serum IL-6 levels and the APPR in pancreatic cancer patients as measured by an elevated C-reactive protein (CRP) has not been demonstrated (Falconer et al, 1994). Tumour necrosis factor α (TNF α) will also produce many of the features of cachexia when administered to humans (Warren et al, 1987) but significant serum levels are rarely detected in cancer (Falconer et al, 1994a). However, elevated production of IL-6 and TNF α by peripheral blood mononuclear cells (PBMC) isolated from patients with an APPR suggests that local production of these cytokines may be more important to regulation of the APPR than serum levels (Falconer et al, 1994a).

Of additional interest in the context of the APPR and cachexia are the receptors for TNF α and IL-6. Two receptors for TNF of 55 and 75kD have been described (Hohman et al, 1989). Soluble forms of these receptors - sTNF-R55 and sTNF-R75 - appear to be shed from cells in response to TNF release, possibly to limit the activity of this cytokine (Olsson et al, 1993). TNF receptor levels are associated with disease severity in a number of inflammatory conditions Goldie et al, 1995, Nakayama et al, 1996, Yoshida et al, 1996). In contrast to many other soluble receptors, it has been shown that the IL-6 signal may be delivered via binding to soluble IL-6 receptor (sIL-6R) and subsequent interaction of this complex with gp130 on the cell surface whether or not membrane-bound IL-6R is present (Taga et al, 1989). The capacity of the cell to respond to IL-6 may depend not only on cell surface IL-6R, and gp130 expression but also the level of sIL-6R, at least in vitro (Mackiewicz et al, 1992, Tamura et al, 1993, Modur et al, 1997). Serum levels of sIL-6R may therefore be a more important determinant of the response to IL-6 than serum levels of IL-6.

The aim of this chapter is to assess the relationship between serum levels of IL-6, sIL-6R, sTNF-R55 and sTNF-R75 and the APPR in patients with advanced pancreatic cancer.

Patients and Methods

After local ethical committee approval 13 patients gave written, informed consent for the collection of a total of 34 venous blood samples. All had unequivocal diagnosis of unresectable pancreatic cancer based on histological or operative findings. All samples were taken at least 14 days after surgery or biliary drainage. No patient had clinical evidence of current infection. All repeated samples were taken at least eight days apart. After gaining consent, samples were also obtained from 6 healthy control subjects with no active medical conditions.

Serum was stored at -70°C until batch analysis by indirect ELISA for IL-6, sIL-6R, sTNF-R55, sTNF-R75 and CRP.

Further details of methods are found in Chapter 2.

Results

Cancer patients had a median age of 67 years (range 51-76). Healthy control subjects had a median age of 54 years (range 50-62) ($p=0.043$ vs patients). Seven cancer patients had stage II disease, one stage III and five stage IV (UICC system). The data for CRP, IL-6, sTNF-R55, sTNF-R75 and sIL-6R are summarised in figure 4.1.

Cancer patients had significantly higher serum levels of CRP (healthy control - $<1\text{mg/ml}$ versus cancer patients 5.4mg/l ($2.6\text{-}22.2$), $p=0.001$), IL-6 (0.5 ($<0.25\text{-}1.0$) vs 5.2pg/ml ($<0.25\text{-}11.5$), $p=0.041$), sTNF-R55 (1.88ng/ml ($1.59\text{-}2.03$) vs 2.69ng/ml ($1.91\text{-}3.78$), $p=0.022$) and sTNF-R75 (4.62ng/ml ($3.17\text{-}5.40$) vs 6.62 ($4.12\text{-}8.94$), $p=0.021$), however, sIL-6R levels were not significantly different (33.0ng/ml ($17.5\text{-}39.5$) vs 40.0ng/ml ($30.2\text{-}49.6$), $p=0.093$).

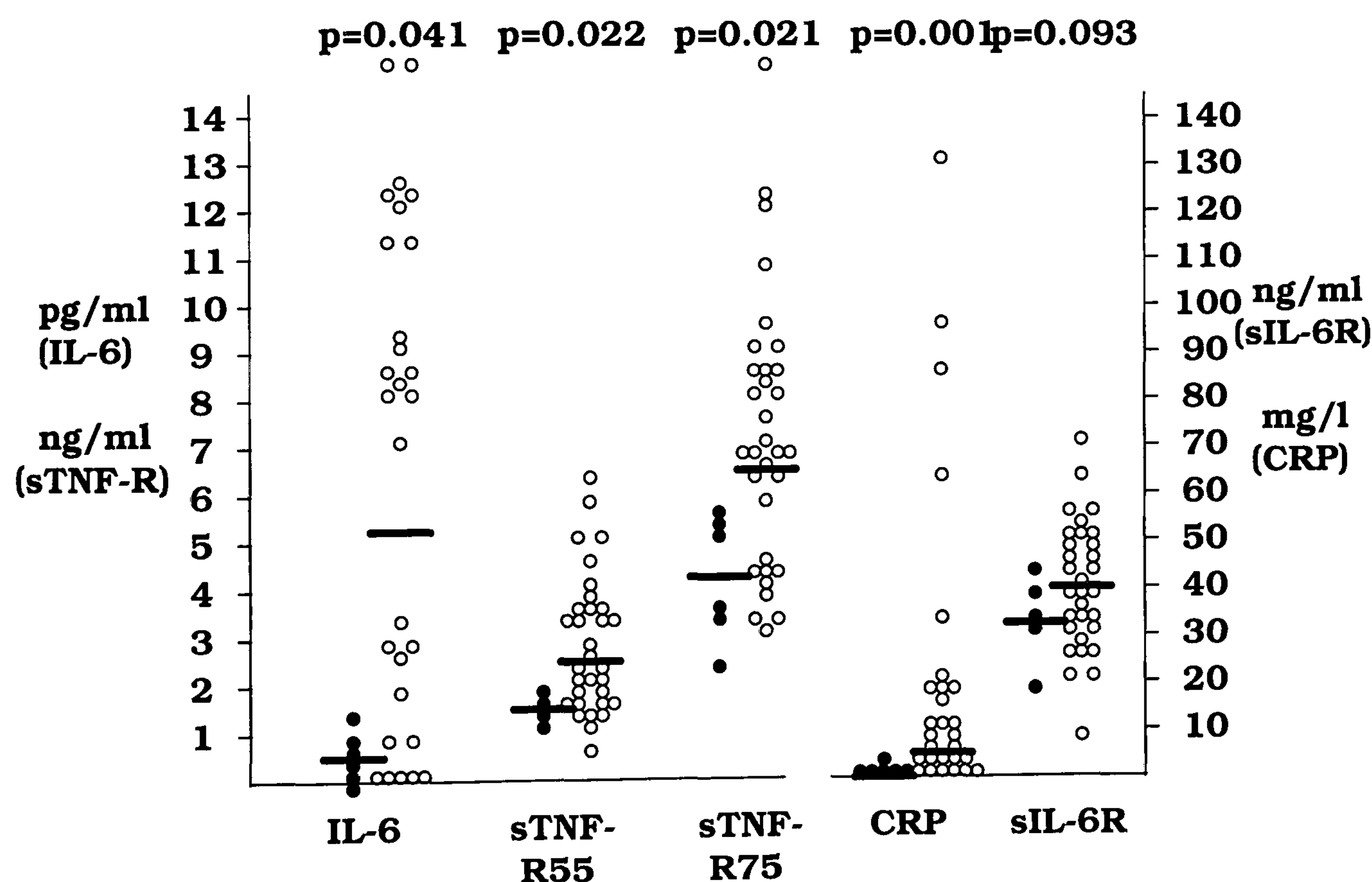


Figure 4.1. Serum levels of interleukin-6 (IL-6), soluble tumour necrosis factor 55 (sTNF-R55) and 75 (sTNF-R75), C-reactive protein (CRP) and soluble interleukin-6 receptor (sIL-6R) in cancer patients (open circles) and healthy controls (closed circles). Medians are shown by a horizontal line.

There was no significant relationship between any parameter and patient disease stage. There was no significant relationship between IL-6, sIL-6R or sTNF-R55 and age. sTNF-R75 significantly increased with age ($p=0.03$).

There was a significant positive correlation between serum CRP and IL-6 ($p=0.0004$), sTNF-R55 ($p=0.0008$), and sTNF-R75 ($p=0.0024$). There was, however, no significant correlation between CRP and sIL-6R ($p=0.34$) (Figure 4.2), indeed there was no correlation between sIL-6R and any other factor assessed. There was a positive correlation between IL-6 and sTNF-R55 and sTNF-R75 ($p=0.038$ and 0.0074 respectively) and a strong positive correlation between the two TNF receptors ($p=0.0003$) (Figure 4.3).

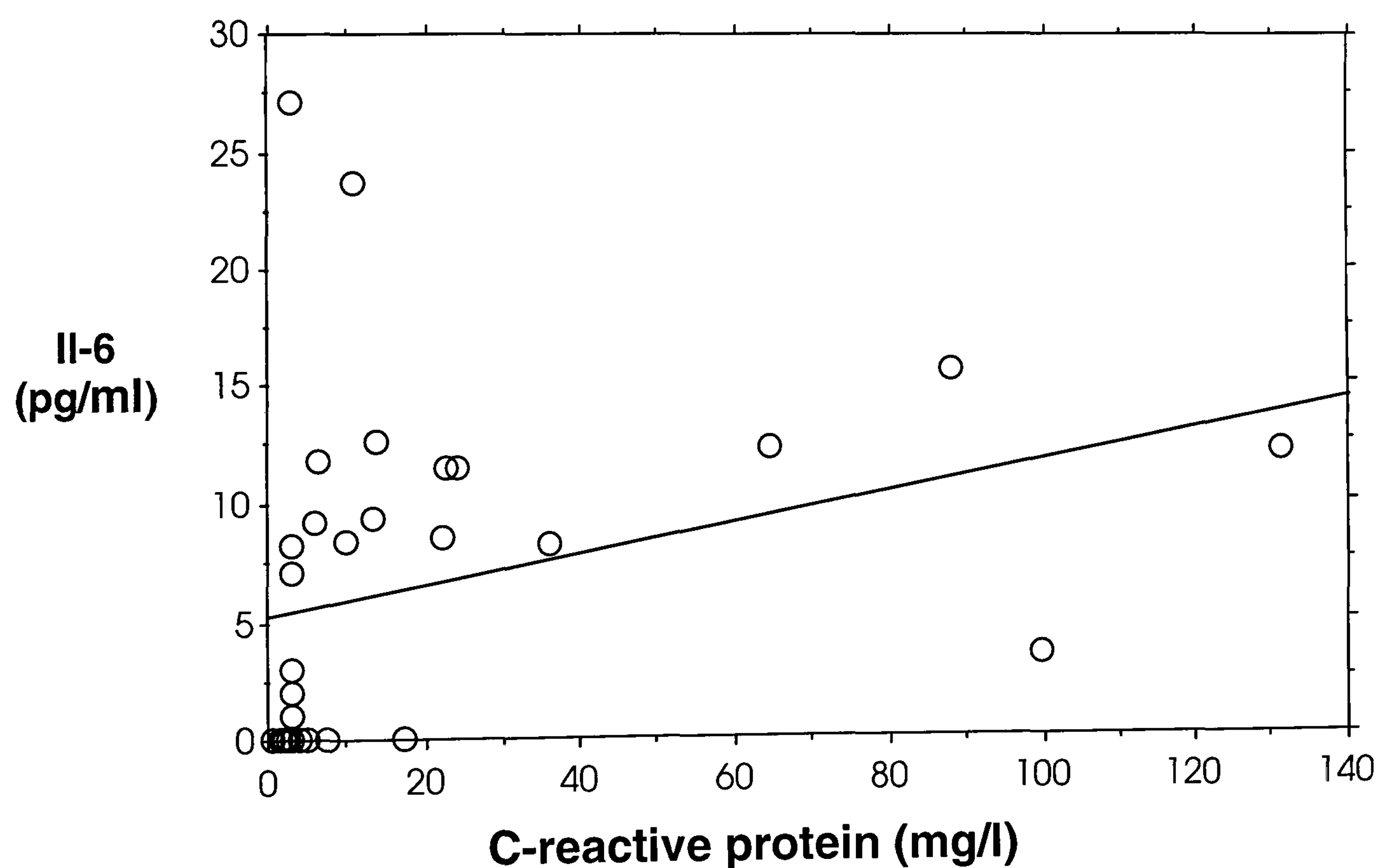


Figure 4.2a. Correlation between serum CRP and IL-6 levels in pancreatic cancer patients ($n=34$) ($\rho=0.62$, $p=0.0004$).

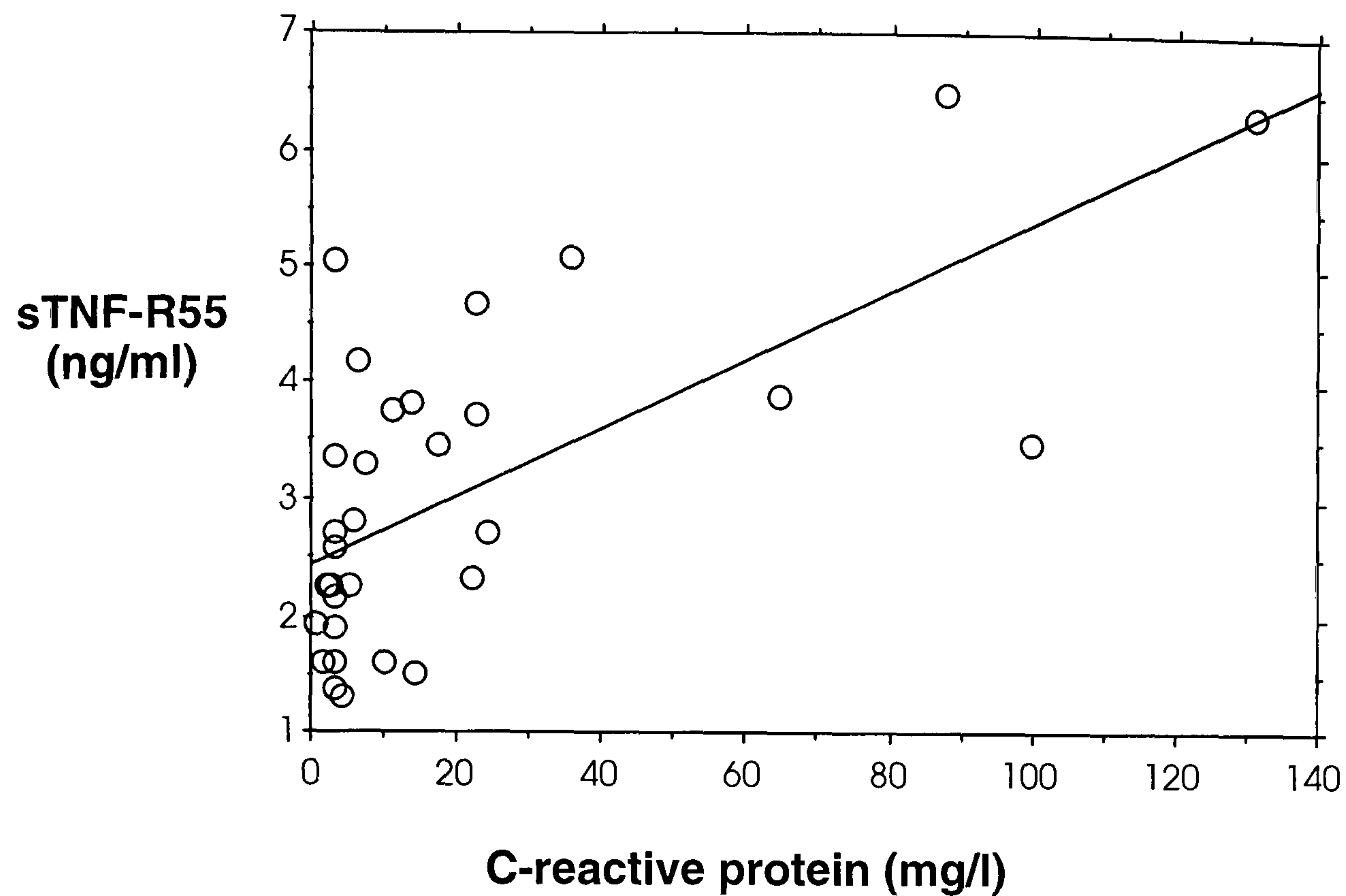


Figure 4.2b. Correlation between serum CRP and sTNF-R55 levels in pancreatic cancer patients ($n=34$) ($\rho=0.59$, $p=0.0008$).

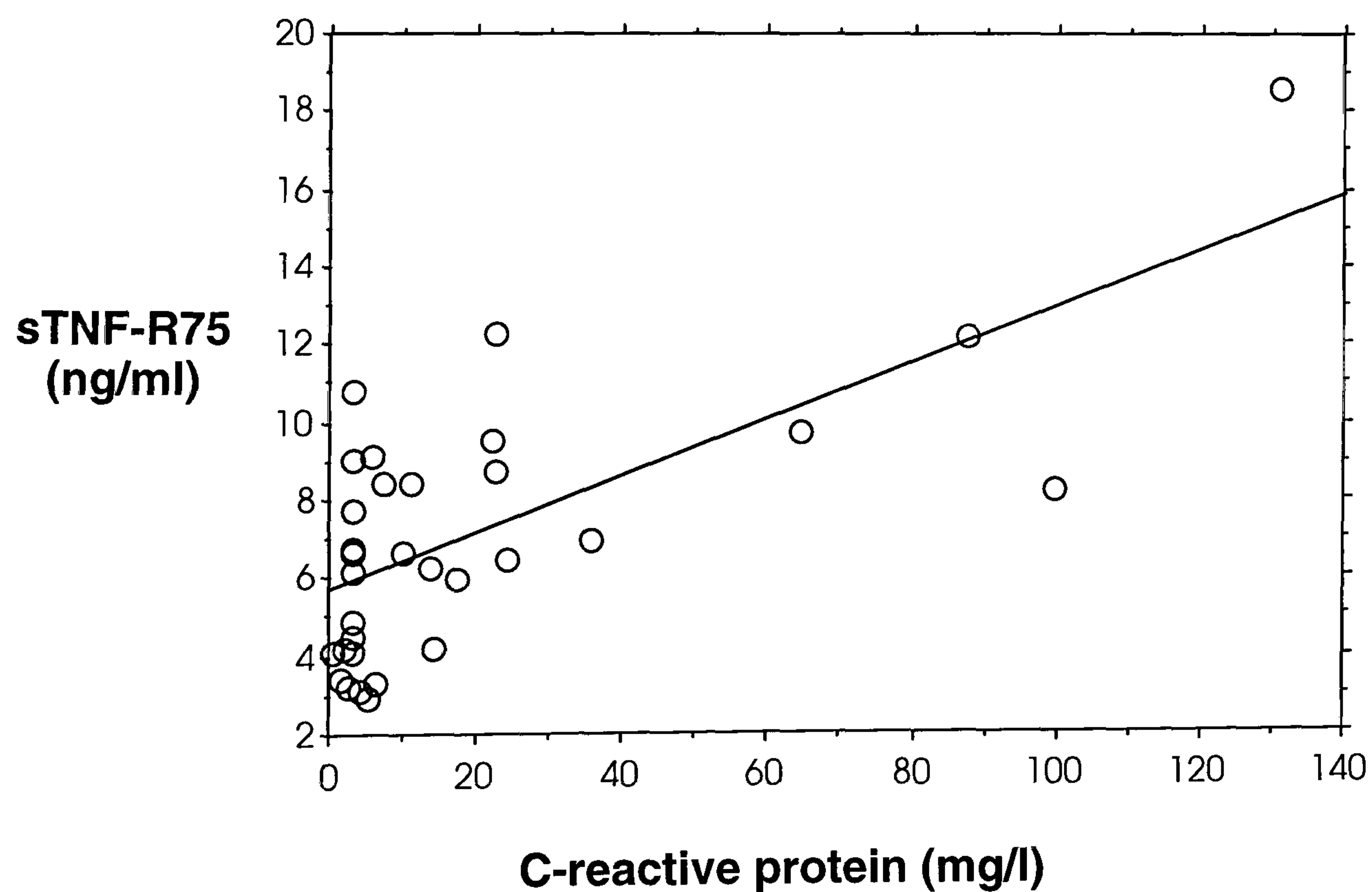


Figure 4.2c. Correlation between serum CRP and sTNF-R75 levels in pancreatic cancer patients ($n=34$) ($\rho=0.53$, $p=0.0024$).

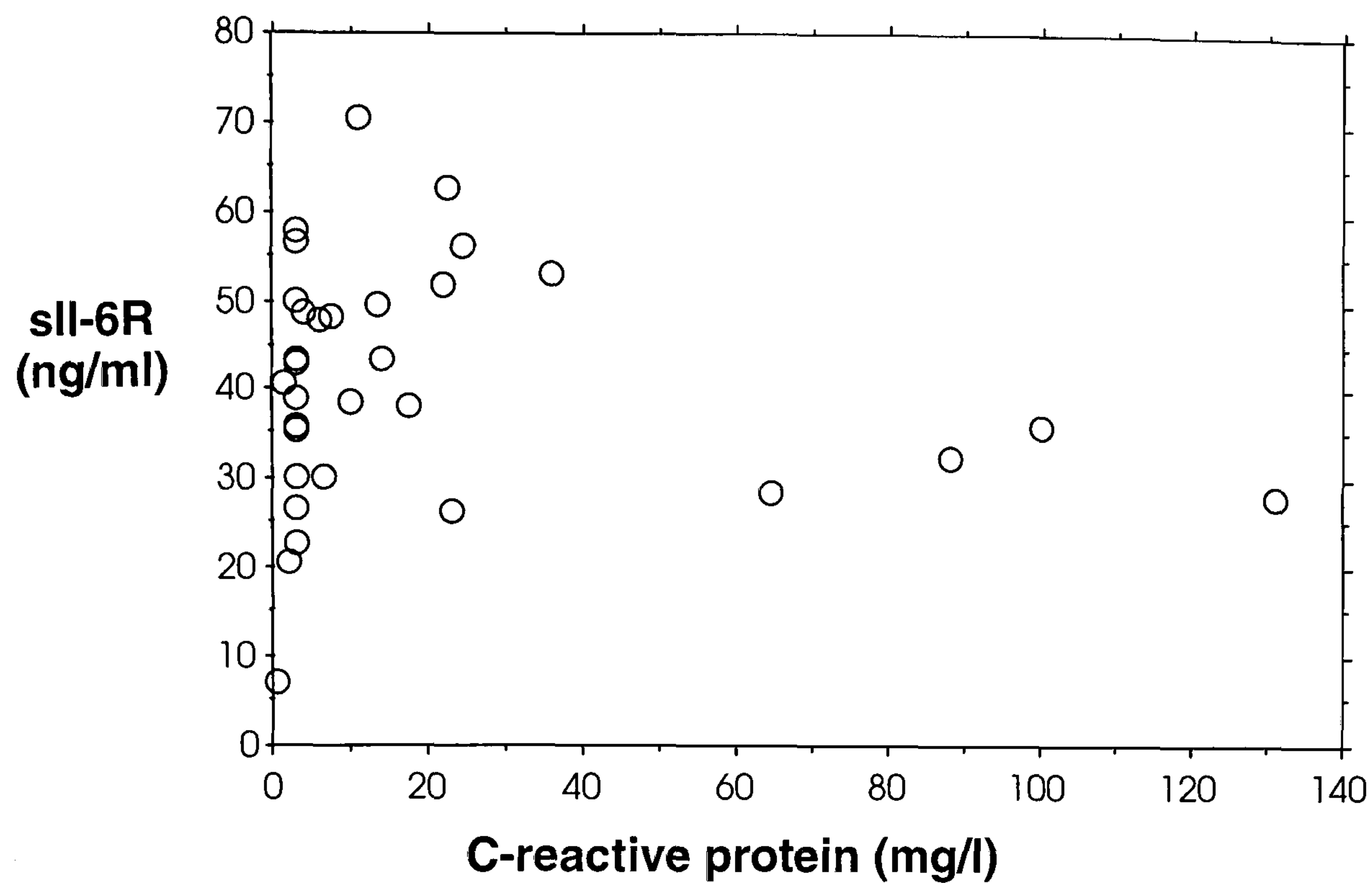


Figure 4.2d. Lack of significant correlation between serum CRP and sII-6R levels in pancreatic cancer patients ($n=32$) ($\rho=0.17$, $p=0.34$).

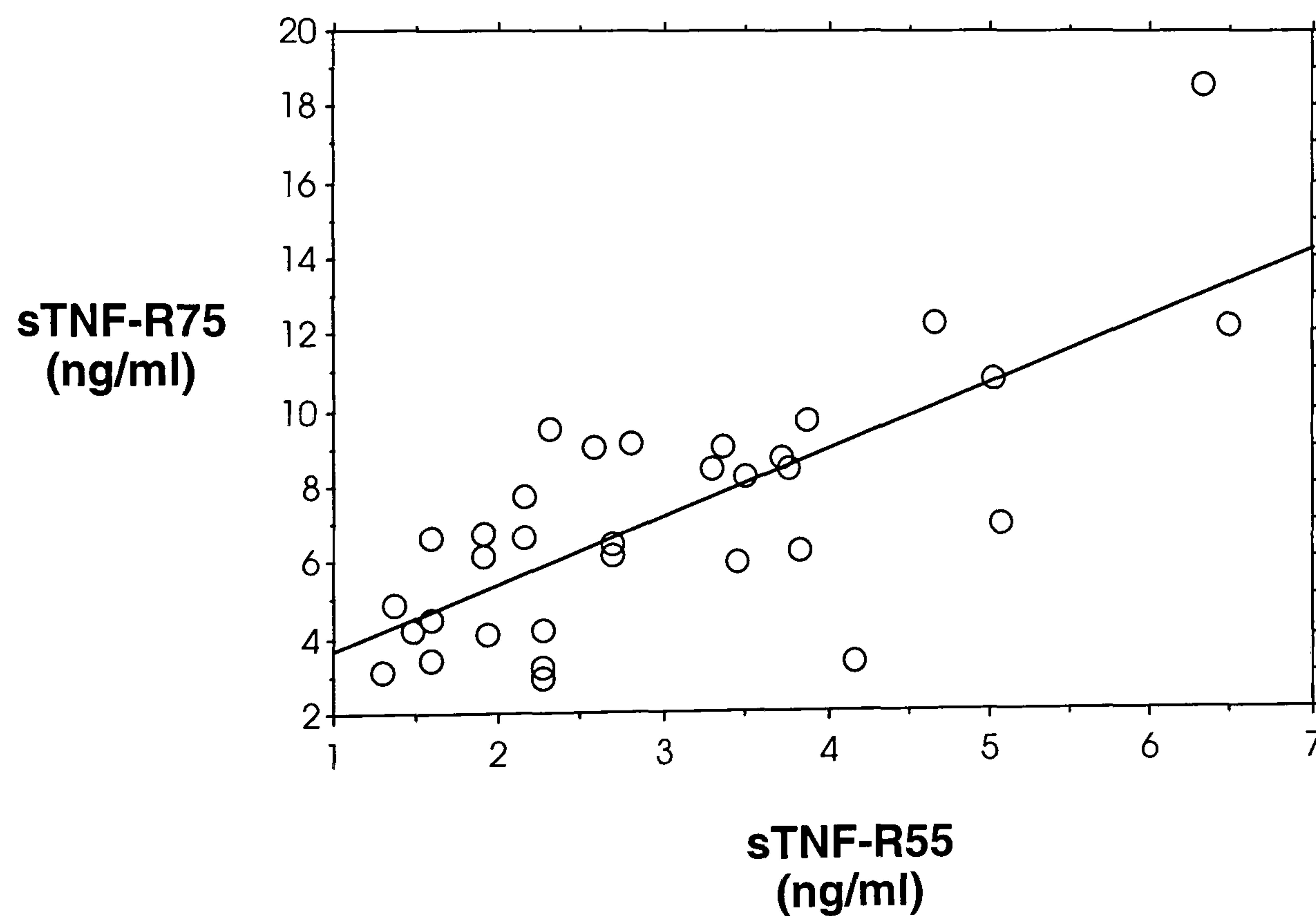


Figure 4.3. Correlation between serum sTNF-R55 and sTNF-R75 levels in pancreatic cancer patients ($n=34$) ($\rho=0.64$, $p=0.0003$).

Discussion

This chapter has demonstrated that patients with advanced pancreatic cancer have an acute phase protein response, as shown by elevated levels of CRP, and elevated pro-inflammatory cytokine levels with elevated levels of IL-6 and the TNF receptors compared with healthy controls. sIL-6R levels, however, were not significantly elevated. Previously, CRP and IL-6 levels have been shown to be elevated in a similar group of patients (Falconer et al, 1994a) but concentrations of receptors of TNF and IL-6 have not previously been examined. sIL-6R have been found to be elevated compared with controls in patients with haematological malignancy (Lavabre-Bertrand et al, 1995) and interstitial lung disease (Yokoyama et al, 1995).

The present chapter also examined the relationship between the acute phase protein response and serum IL-6, sIL-6R, sTNF-R55 and sTNF-R75 levels in patients with advanced pancreatic cancer and has shown that while IL-6, sTNF-R55 and sTNF-R75 levels are significantly positively associated with the level of the acute phase response, sIL-6R is not. This would suggest that, in advanced pancreatic cancer, serum sIL-6R may not be important in conducting the IL-6 signal. The IL-6 bound to sIL-6R may be unavailable for the induction of signalling via gp130 or hepatocytes may be refractory to this mechanism in vivo. In addition, serum levels of sIL-6R may not accurately reflect relevant tissue levels. However, the results of the present study suggest that sIL-6R would appear to be a poor indicator of the inflammatory response in pancreatic cancer. Studies of an animal model of autoimmune disease have suggested the presence of elevated sIL-6R levels in association with increasing levels of IL-6 (Suzuki et al, 1993) and a weak but significant association was shown between sIL-6R and disease stage in human subjects with haematological malignancy (Lavabre-Bertrand et al, 1995). However, a lack of association between serum sIL-6R and CRP was seen in patients with interstitial lung disease (Yokoyama et al, 1995) as in the present study.

A close association between levels of sTNF-R55 and sTNF-R75 has previously been shown in sarcoidosis (Nakayama et al, 1996) and asthma (Yoshida et al, 1996). An association between TNF receptor levels and disease severity was found in these conditions and also in cancer (Aderka et al, 1991) and severe sepsis (Goldie et al, 1995) although no such association was found in a small group of patients with mild psoriasis (Bonifati et al, 1995). Whether the high levels of TNF receptors in this variety of inflammatory conditions represents an attempt by the body to limit cytokine effects by binding TNF α or if this binding serves to hold TNF α in the circulation resulting in sustained, controlled release remains to be elucidated. It also remains to be seen whether assessment of the levels of TNF receptors may provide a more accurate representation of TNF α activity than TNF α itself.

While the present study has shown significant correlations between CRP concentration and IL-6 and TNF receptors using methods appropriate to the nonparametric distribution of the data there were outliers, particularly among the patients with a relatively low serum CRP. Clearly the acute phase protein response is a heterogeneous process involving many proteins in addition to CRP. It may be that the concentration of another protein or an index of values for several proteins will provide a more accurate assessment of the acute phase response and, consequently, a better correlation with other inflammatory mediators.

An association between the acute phase response and serum levels of IL-6 in pancreatic cancer patients has not been shown in the past although increased PBMC IL-6 production has been seen in those with an APPR (Falconer et al, 1994a). Others have found a relationship between serum IL-6 levels and survival in lymphoma (Kurzrock et al, 1993) and weight loss in colon cancer (Preston et al, 1995). Previous studies showing the presence of an APPR to be a strong predictor of poor survival in pancreatic cancer have used a serum CRP level of 10mg/l or more to define those patients with an APPR (Falconer et al, 1995). The use of ELISA techniques in the current study rather than a turbidometric assay has allowed us to examine the relationship of inflammatory mediators to CRP levels below 10mg/l. This data indicates a clear association between IL-6 and the APPR. It remains a matter of conjecture whether local IL-6 production in the liver is more important in stimulating the APPR than circulating serum levels. It would appear that the IL-6, sTNF-R55 and sTNF-R75 are associated with changes in CRP even at relatively low levels and may have a role in determining the level of the APPR in vivo in these patients.

The use of multiple samples from individual patients may have artificially strengthened the correlation between the acute phase response and the measured cytokines. This method was used to maximise the data available. Similar findings are noted in an overlapping group of patients studied in Chapter 7 using different ELISA kits.

In conclusion, this chapter has shown that the level of the APPR is significantly associated with serum levels of IL-6, sTNF-R55 and sTNF-R75 in patients with pancreatic cancer but there is no such association with sIL-6R. This suggests that sIL-6R does not have a significant influence on the magnitude of the APPR in pancreatic cancer. In vitro work has shown that pro-inflammatory cytokines stimulate the acute phase protein response. This chapter suggests that, in vivo, with the direct correlation between pro-inflammatory cytokine levels (particularly IL-6) and concentrations of C-reactive protein, the acute phase protein response is an indicator of the wider inflammatory state.

The next chapter examines the possible influence of the patient's genotype on cytokine production, the APPR and survival.

Relationship of cytokine genotype to inflammatory state and survival

Summary

Polymorphisms of cytokine genes have been suggested to influence the severity and outcome of a number of conditions. Pro-inflammatory cytokines contribute to the cachexia associated with pancreatic cancer and also stimulate the acute phase response which has been found to be related to shortened survival in such patients. This chapter examines the effect of polymorphisms of the interleukin (IL)-1 β , IL-1 receptor antagonist (IL-1Ra) and tumour necrosis factor (TNF) genes upon survival of patients with pancreatic cancer.

Genomic DNA was obtained from 64 patients with pancreatic cancer and 101 healthy controls. Using the polymerase chain reaction and subsequent endonuclease digestion the subject's genotype for a diallelic polymorphism of the interleukin-1 β gene, a variable repeat polymorphism of the IL-1Ra gene and two diallelic polymorphisms of the TNF gene (TNFB and TNF-308) were determined.

IL-1 β production by peripheral blood mononuclear cells was measured in 22 patients and serum concentrations of the two tumour necrosis factor receptors and C-reactive protein were measured in 45 of the cancer patients with no evidence of infection or jaundice, one month after surgical intervention and survival noted.

Patients homozygous for allele 2 of the IL-1 β gene had significantly shorter survival than other groups ($p=0.0001$). These patients also exhibited substantially higher IL-1 β production ($p=0.022$) (although numbers of patients were small). Possession of allele 2 was also associated with significantly shorter survival (median 144 vs 256 days, $p=0.034$) and significantly higher CRP level ($p=0.0003$).

No association was found between IL-1Ra genotype and the inflammatory state or survival.

There was no association between either TNF genotype and concentrations of any of the measured inflammatory mediators. While those with an elevated C-reactive protein concentration had significantly poorer survival, there was no association between either TNF genotype and survival.

The possession of a genotype resulting in increased IL-1 β production appears to be related to shortened survival and increased serum CRP level. However, no association was found between IL-1Ra genotype or two TNF genotypes and the inflammatory state or survival

in advanced pancreatic cancer. This may reflect the role of IL-1 β in the genesis of local inflammation as a cofactor in inducing an acute phase protein response and cachexia in cancer. It is also possible that this polymorphism may reflect a particularly aggressive tumour phenotype resulting in shortened survival.

Introduction

Previous chapters have explored the relationship between the inflammatory state and the acute phase protein response, and between the acute phase response and the functional deterioration of patients with advanced pancreatic cancer. As discussed previously, the acute phase protein response is modulated, at least in part, by pro-inflammatory cytokines. Many pro-inflammatory cytokines, including interleukin (IL) -1, IL-6, interferon- γ and tumour necrosis factor (TNF), will give rise to aspects of cachexia when administered in animal models (Mahony & Tisdale, 1988, Gelin et al, 1991, Hellerstein et al, 1989, Matthys et al, 1991, Strassmann et al, 1992). Interleukin-1 receptor antagonist (IL-1Ra) has broadly antiinflammatory actions in antagonising the action of IL-1 (Dinarello & Wolff, 1993). Although production of these cytokines depends on a variety of clinical factors, there is increasing evidence that genetic factors may be involved. Variations in the genes coding for IL-1 β , IL-1Ra and TNF have been found to affect production of the relevant cytokines (Pociot et al, 1992, Danis et al, 1995, Stüber et al, 1996). Coincidentally, in each case allele 2 has been associated with increased production of the relevant cytokine. If these polymorphisms were relevant to the survival of patients it would reinforce the relationship between the inflammatory state and survival in pancreatic cancer.

It remains difficult to predict survival in patients with advanced pancreatic cancer. It has previously been demonstrated that nutritional and inflammatory factors such as albumin and C-reactive protein (CRP) are of major importance in determining survival (Falconer et al, 1995). This appears to be due to their relationship to the progressive nutritional decline seen in the majority of patients with advanced pancreatic cancer (as discussed in Chapter 3).

Despite progress in diagnosis and staging pancreatic cancer still has a dismal prognosis (Ahlgren, 1996). Surgical resection with intent to cure is not possible in 80-90% of patients thus, for the vast majority of patients, palliation of biliary obstruction is the main goal of surgery (Rosewicz & Weidenmann, 1997).

Operative bypass or endoscopic stenting are the two main options for palliating biliary obstruction in pancreatic cancer. It has been suggested that surgical bypass is associated with longer initial hospital stay and early complications while late blockage of endoscopic stents results in late complications and hospital readmissions (van den Bosch et al, 1994). Thus, surgical intervention may be more appropriate for those with a longer anticipated survival whilst endoscopic stenting may be more suitable for those with shorter survival. Albumin and CRP are sensitive to clinical events often experienced by patients around the time of diagnosis,

such as jaundice, cholangitis and surgery, and thus they are of limited use in determining the form of palliative therapy for these patients. If polymorphisms of cytokine genes were related to survival they would offer a window on the inflammatory state of the patient independent of their clinical condition and therefore may also be useful in guiding palliative intervention.

This chapter examines a biallelic TaqI polymorphism of the IL-1 β gene, a variable repeat polymorphism of the IL-1Ra gene and two polymorphisms of the TNF gene in patients with advanced pancreatic cancer and related this to CRP concentration, IL-1 β production or TNF receptor concentrations and survival.

Methods

Subjects

After informed consent, venous blood was collected for IL-1 β and TNF genotyping from 64 patients with a diagnosis of unresectable pancreatic cancer based on histological or unequivocal radiological or operative findings. Blood was also collected from 101 healthy volunteers attending the Blood Transfusion Service Plasmapheresis Centre in Edinburgh.

For cancer patients survival was noted from time of histological confirmation of pancreatic adenocarcinoma (80%) or of unequivocal radiological or operative findings in those for whom histological confirmation was not obtained.

DNA was extracted from plasma samples and genotyping performed for the IL-1 β TaqI polymorphism (Pociot et al, 1992), the interleukin-1 receptor antagonist variable number tandem repeat polymorphism (Danis et al, 1995), the TNFB NcoI polymorphism (Shimura et al, 1994) and the TNF-308 G to A substitution polymorphism (Stüber et al, 1996).

The inflammatory state was assessed in patients without evidence of infection or jaundice approximately one month after diagnosis, surgery or endobiliary stenting. It was possible to obtain venous blood from 45 such patients for the measurement of serum CRP and TNF receptor concentrations and 22 such patients for measurement of peripheral blood mononuclear cell (PBMC) IL-1 β production.

Further details of methods are found in Chapter 2.

Results

Subject characteristics and genotype

Subject characteristics and prevalence of genotypes are presented in table 5.1. Thirty patients had UICC stage II disease, 8 patients stage III and 26 patients stage IV. No subject underwent resectional surgery or received chemotherapy or radiotherapy. There was no significant difference in the distribution of genotypes between pancreatic cancer patients and healthy controls.

Table 5.1. Characteristics and genotype of 64 patients with pancreatic cancer and 101 healthy subjects.

| | | Cancer patients | Healthy volunteers |
|--|-----|-----------------|--------------------|
| Age | | 64 (57-72) | 42 (36-47) |
| Gender | | F:30 M:34 | F:32 M:69 |
| IL-1 β genotype ^a | 1/1 | 68% | 55% |
| | 1/2 | 25% | 40% |
| | 2/2 | 6% | 5% |
| Frequency of IL-1Ra alleles ^b | 1 | 66% | 67% |
| | 2 | 30% | 30% |
| | 3 | 3% | 2% |
| | 4 | 1% | 1% |
| TNFB genotype ^c | 1/1 | 9% | 16% |
| | 1/2 | 56% | 47% |
| | 2/2 | 34% | 38% |
| TNF308 genotype ^d | 1/1 | 56% | 64% |
| | 1/2 | 38% | 31% |
| | 2/2 | 6% | 5% |

m - male

f - female

Comparison by Chi squared test

a - p=0.18

b - p=0.97

c - p=0.35

d - p=0.89

Acute phase protein response and survival

Survival of patients with advanced pancreatic cancer stratified by the presence or absence of an elevated CRP concentration is shown in figure 5.1. Those with an elevated CRP concentration had a significantly shorter survival (Median 137 (interquartile range 88-251) days versus 243 (152-320) days, $p=0.0073$). Disease stage was not a significant predictor of survival in this group of patients ($p=0.65$).

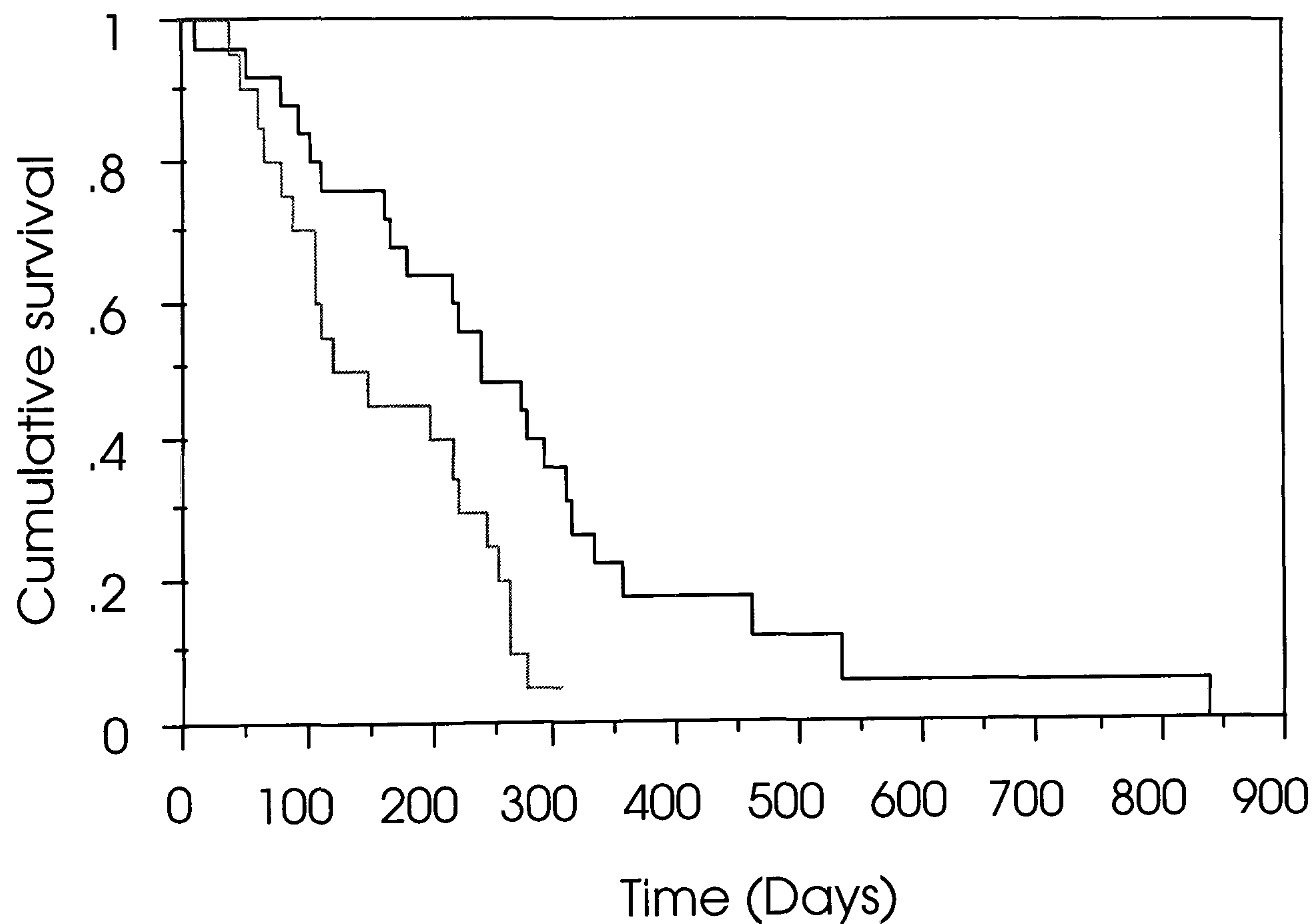


Figure 5.1. Kaplan Meier survival curve (from diagnosis) of 45 patients with advanced pancreatic cancer stratified for C-reactive protein. C-reactive protein <10mg/l - solid line, C-reactive protein >10mg/l - dotted line. Comparison by log rank test $p=0.0073$.

IL-1 β polymorphism, cytokine levels, acute phase protein response and survival

IL-1 β was only detectable in serum from only two patients of the 45 patients in whom it was measured. One patient of genotype 2/2 had a serum level of 7.24pg/ml while a patient of 1/2 genotype had a level of 1.16pg/ml. Both patients had substantially elevated serum CRP levels at 151 and 53mg/l respectively.

IL-1 β production by PBMCs from the 22 cancer patients in whom it was measured according to their IL-1 β genotype is presented in figure 5.2. PBMC IL-1 β production by those with a 2/2 genotype (median 9.7ng/ml (range 7.9-11.5)) was significantly higher than those with 1/2 (2.2 (interquartile range 1.5-2.7), $p=0.046$) or 1/1 (1.9 (1.4-2.6), $p=0.027$) genotype. There was no significant difference between PBMC IL-1 β production between 1/2 and 1/1 genotype ($p=0.72$). There was no significant relationship between PBMC IL-1 β production and CRP level ($r=0.28$, $p=0.21$).

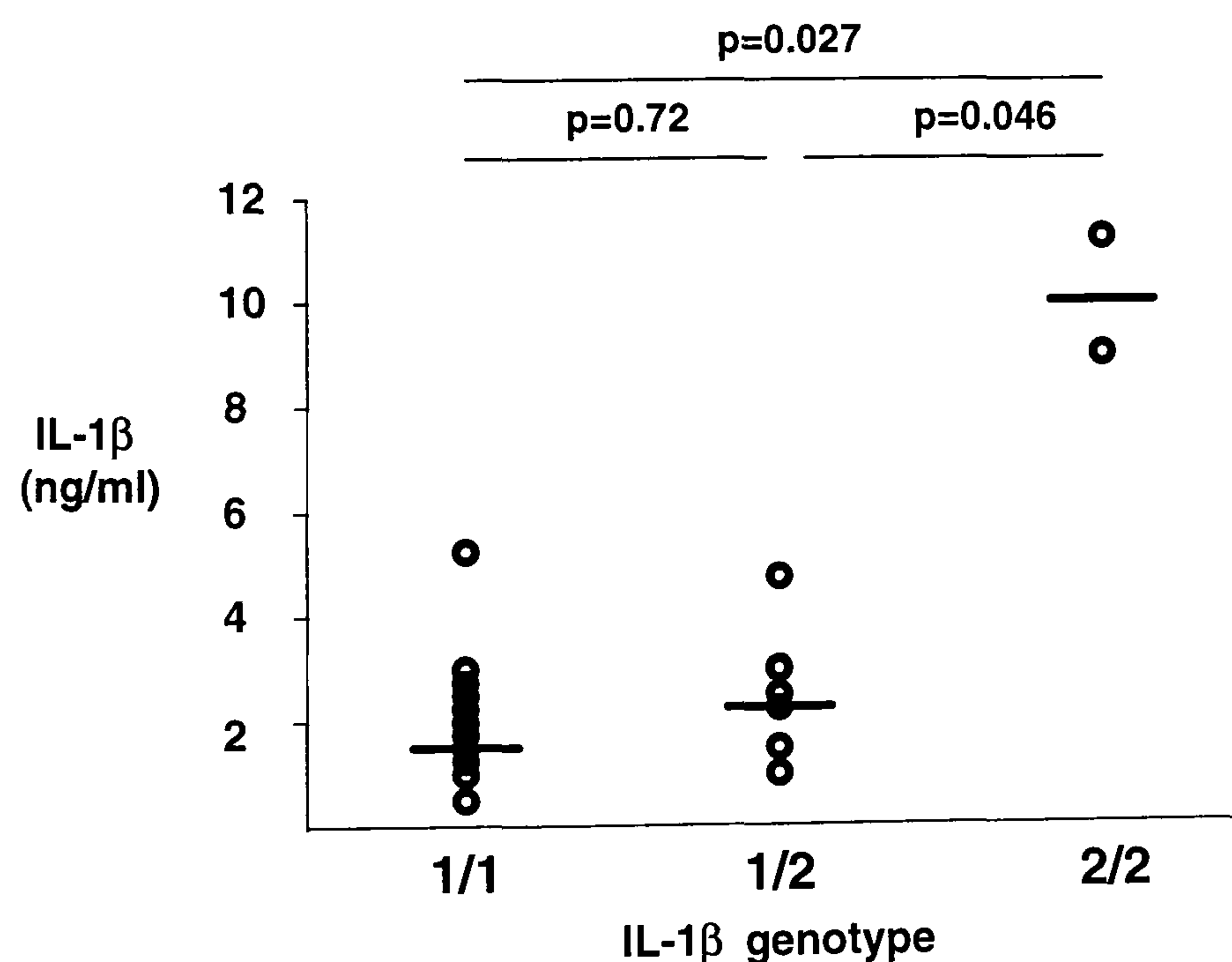


Figure 5.2. Endotoxin-stimulated interleukin-1 β production by peripheral blood mononuclear cells from 22 patients with advanced pancreatic cancer presented by IL-1 β genotype. Horizontal bar represents median value. Comparisons between groups by Mann whitney U test.

Serum CRP concentration in relation to IL-1 β genotype of cancer patients is presented in figure 5.3. CRP levels from those with the 2/2 (28mg/l (20-130)) or 1/2 genotype (31mg/l (13-72)) were significantly higher than those with the 1/1 genotype (0mg/l (0-10)) ($p=0.015$ and 0.0042 respectively). There was no significant difference between CRP levels from those with 2/2 and 1/2 genotypes ($p=0.42$).

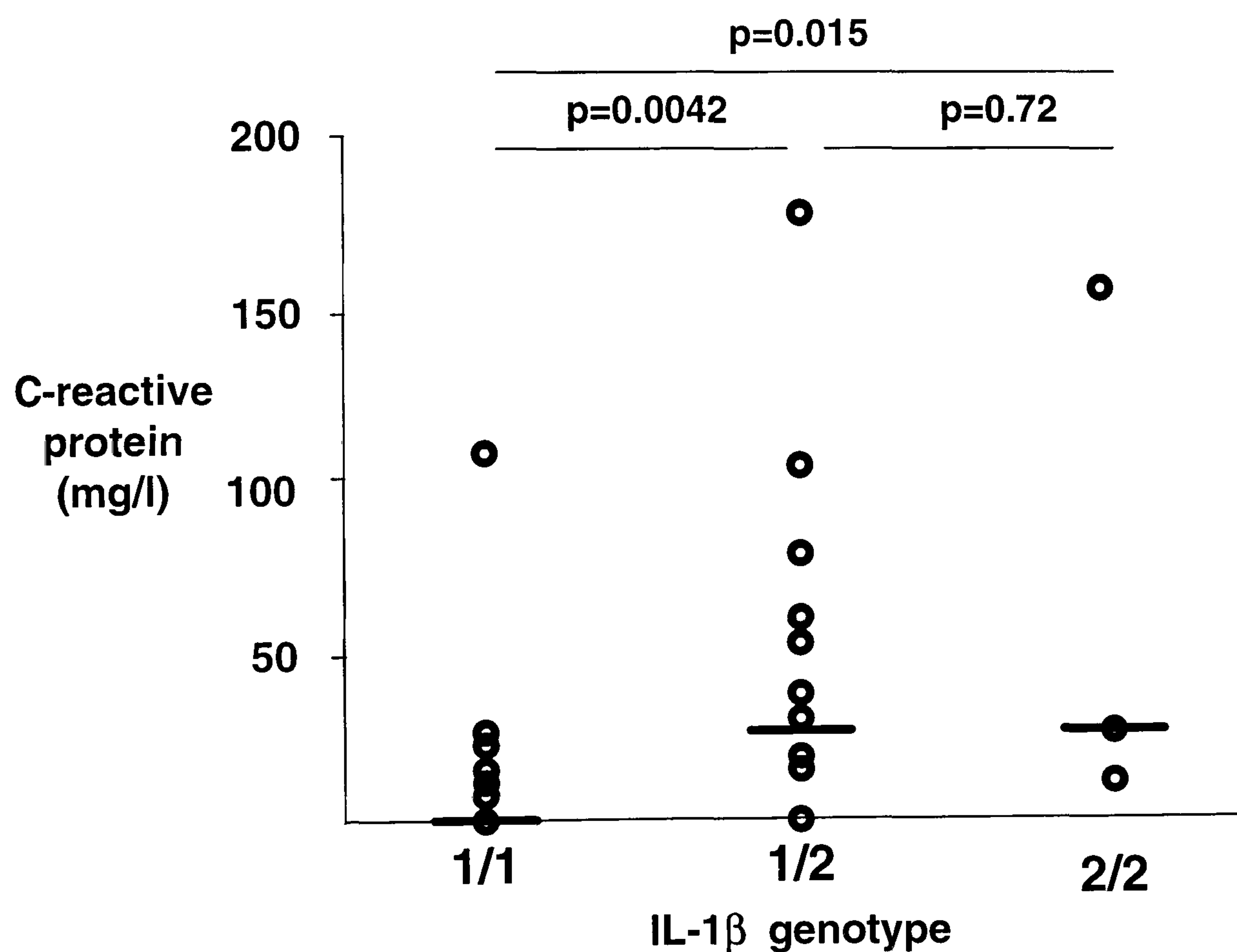


Figure 5.3. Serum C-reactive protein concentrations in 43 patients with advanced pancreatic cancer presented by IL-1 β genotype. Horizontal bar represents median value. Comparisons between groups by Mann whitney U test.

Survival of patients by IL-1 β genotype is presented in figure 5.4. Those with 2/2 genotype (88days (69-108)) had significantly shorter survival than those with 1/2 (173 days (107-269)) or 1/1 (243 days (126-333)) genotype ($p=0.012$ and 0.0001 respectively). The difference between survival of those with 1/1 and 1/2 genotype is not statistically significant ($p=0.10$). All 4 censored individuals possess the 1/1 genotype.

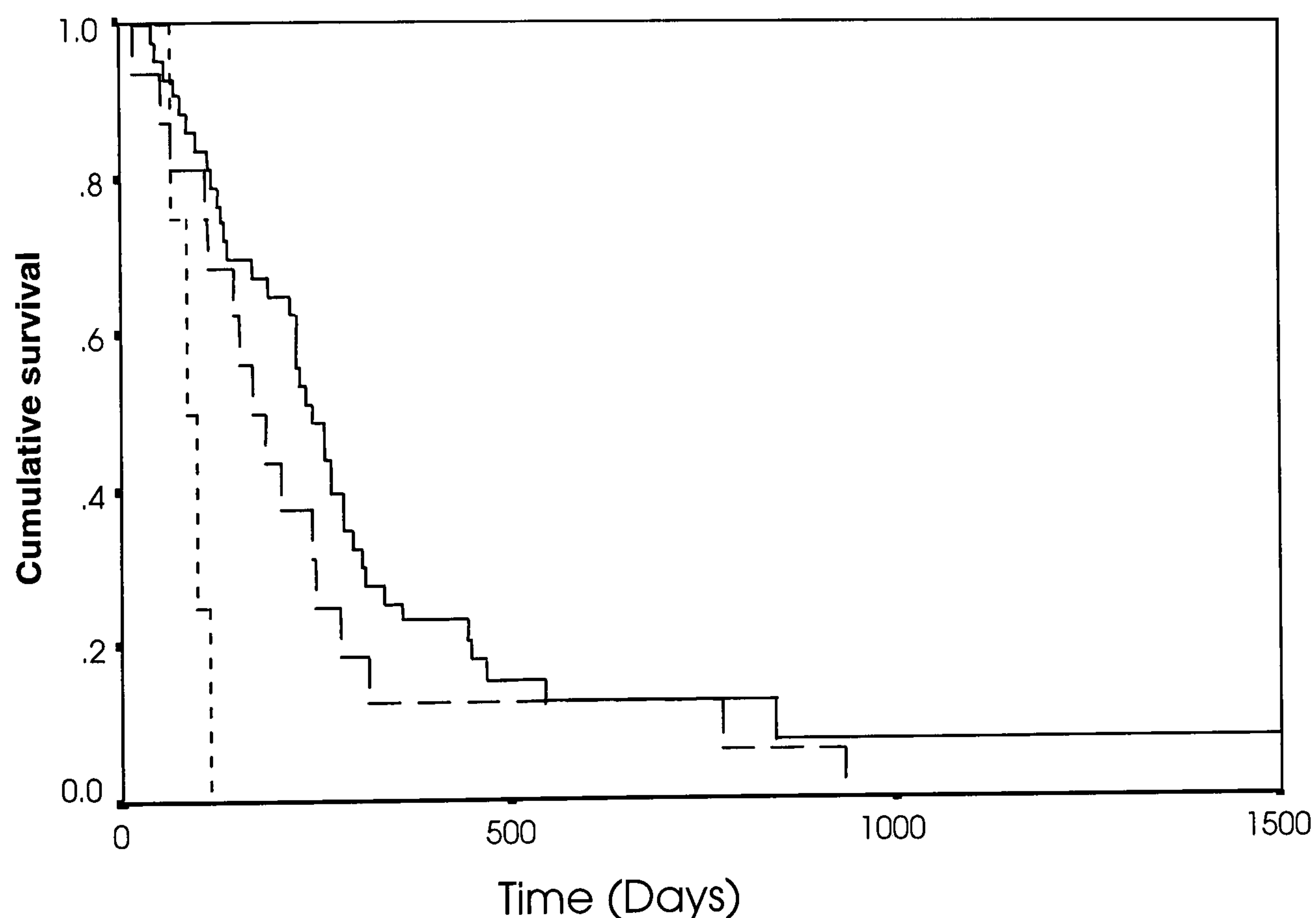


Figure 5.4. Kaplan-Meier survival curve (from diagnosis) of 64 patients with advanced pancreatic cancer presented by IL-1 β genotype. Median survivals - 1/1 genotype 243 days (solid line), 1/2 173 days (broken line), 2/2 88 days (dotted line). Comparison by log rank test - Overall $p=0.0005$, 1/1 vs 1/2 $p=0.010$, 1/1 vs 2/2 $p=0.0001$, 1/2 vs 2/2 $p=0.012$.

Those possessing allele 2 had significantly shorter survival (148 days (85-245)) than those who did not (243 days (126-333)) ($p=0.043$) (figure 5.5).

When survival by IL-1 β genotype was split by disease stage genotype remained a significant predictor of survival in those with stage II and IV disease ($p<0.03$) (patient numbers were insufficient for stage III disease).

For predicting survival at six months the possession of allele 2 has a sensitivity of 48%, a specificity of 81%, a positive predictive value of 65%, a negative predictive value of 67% and an accuracy of 67%.

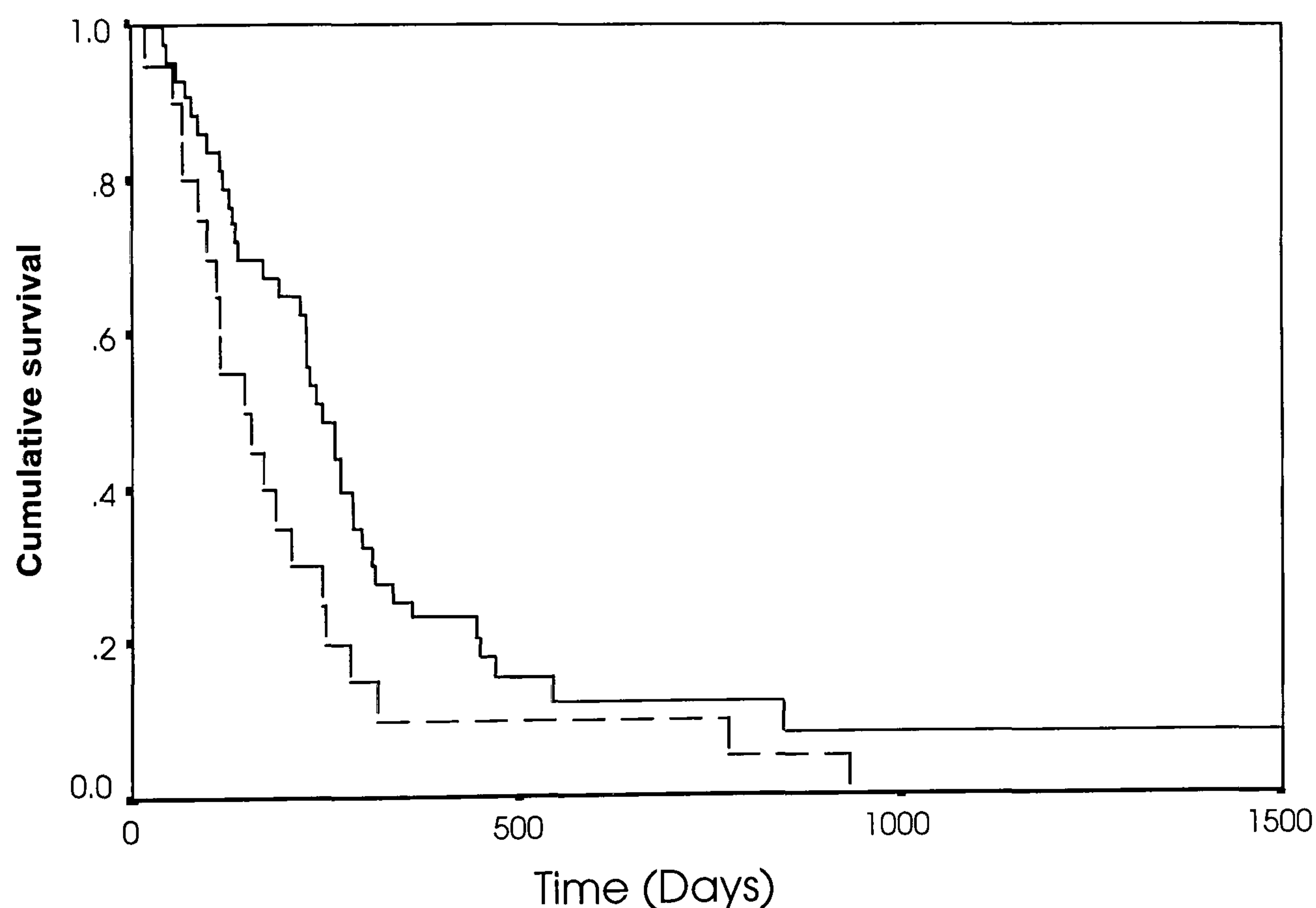


Figure 5.5. Kaplan-Meier survival curve (from diagnosis) of 64 patients with advanced pancreatic cancer stratified for possession of IL-1 β allele 2. Median survivals - possession of allele 2 - 148 days (broken line), no allele 2 - 243 days (solid line). Comparison by log rank test - $p=0.043$.

IL-1Ra polymorphism, cytokine levels, acute phase protein response and survival

There was no obvious relation between IL-1Ra genotype and the inflammatory response. In particular, there was no difference between CRP, TNF-R 55 and TNF-R75 concentrations in patients with or without IL-1Ra allele 2 (Figure 5.6). There was no relationship between IL-1Ra genotype and survival ($p=0.17$, log rank test). Similarly, the possession of IL-1Ra allele 2 did not appear to influence survival (allele 2 present median survival 265 (135-333) days, allele 2 absent 181 (85-254) days, $p=0.24$, log rank test) (Figure 5.7).

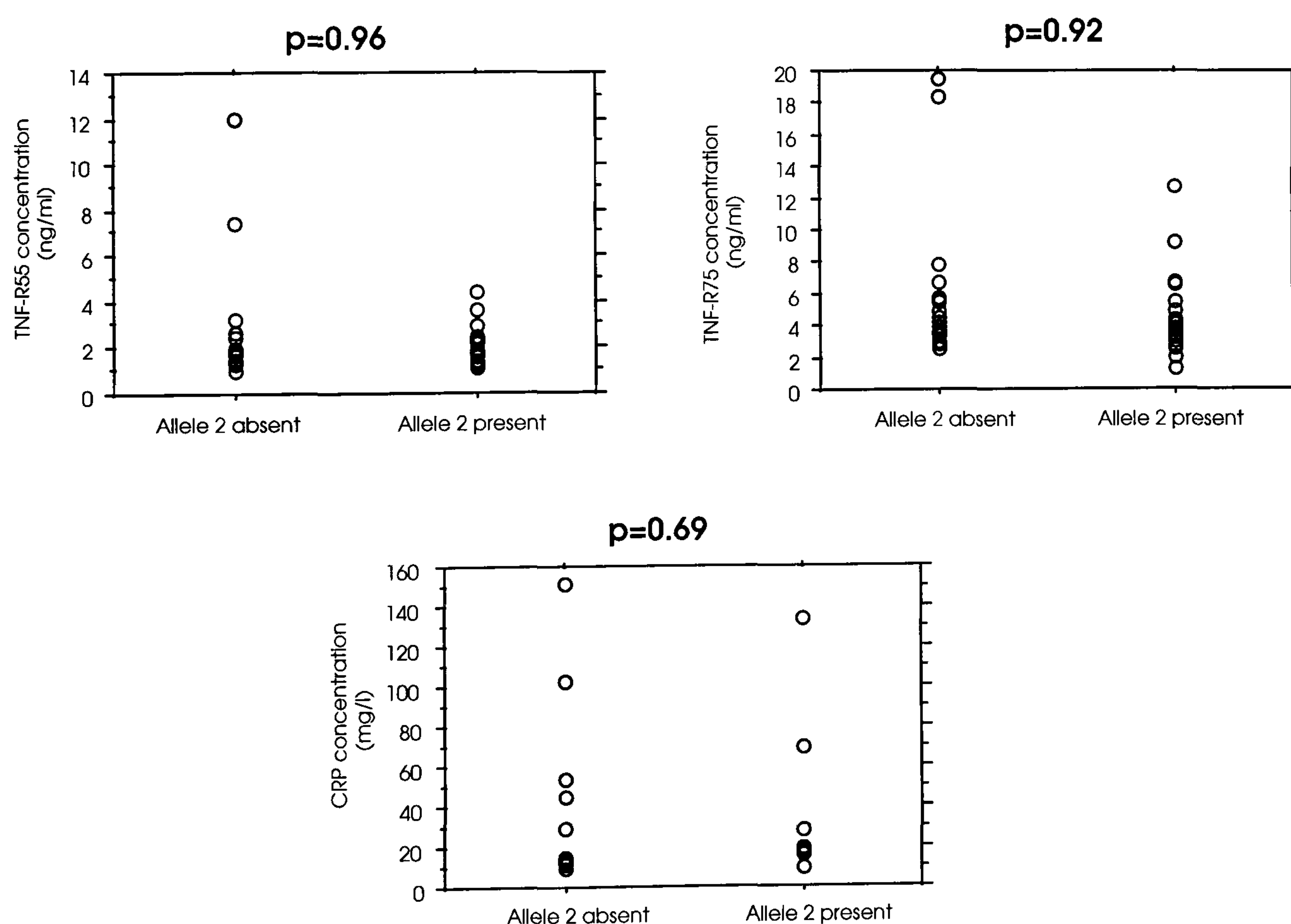


Figure 5.6. Serum concentrations of soluble tumour necrosis factor receptor (sTNF-R) 55 and 75 and C-reactive protein (CRP) from 45 patients with advanced pancreatic cancer presented by IL-1Ra genotype. Statistical analysis by Mann Whitney U test.

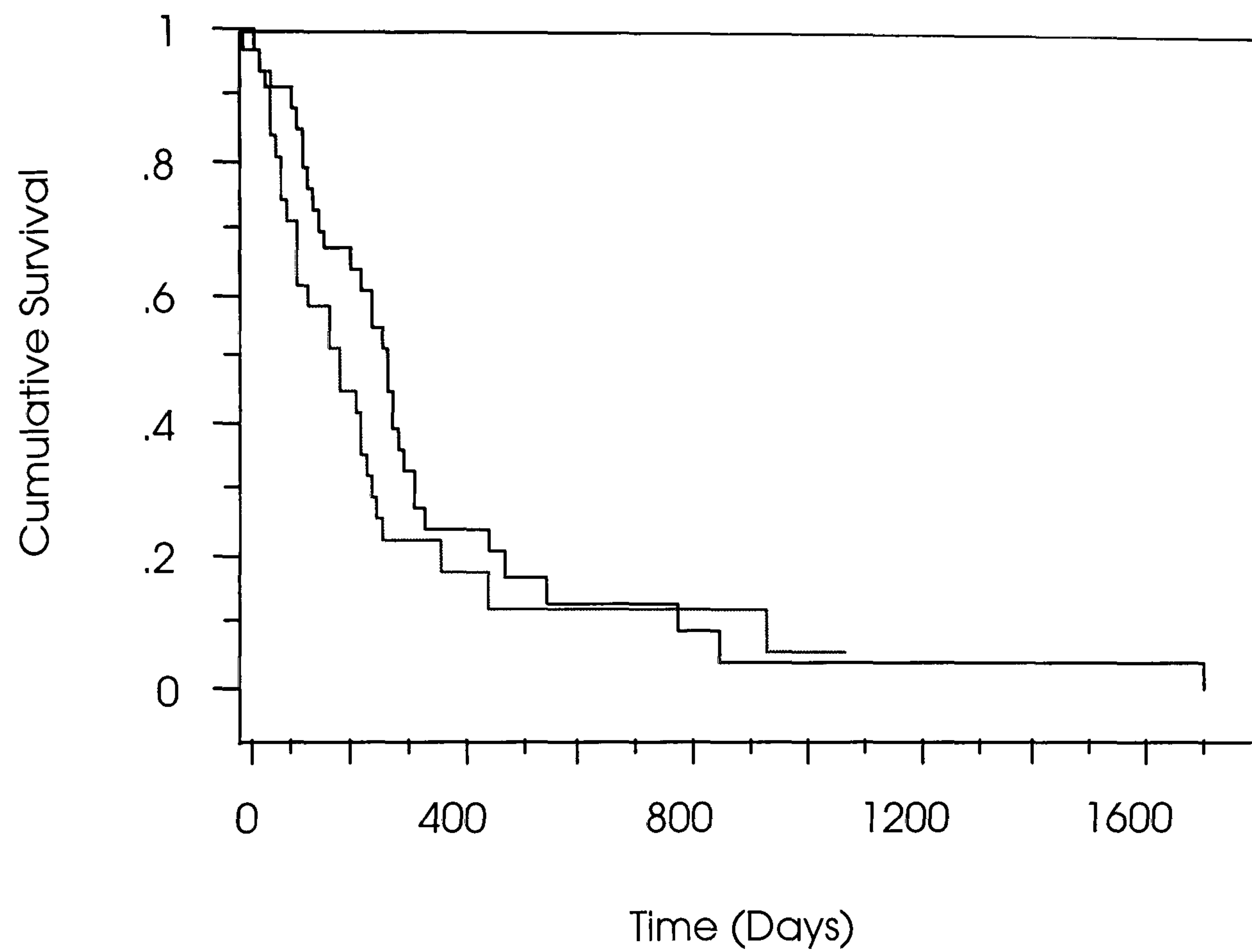


Figure 5.7. Kaplan Meier survival curve (from diagnosis) of 64 patients with advanced pancreatic cancer by IL-1Ra genotype. Allele 2 present - solid line, Allele 2 absent - dashed line. Comparison by log rank test $p=0.24$.

TNF polymorphisms, cytokine levels, acute phase protein response and survival

Serum concentrations of TNF-R 55, TNF-R75 and CRP are shown in relation to TNF genotype in figure 5.8. There were no significant differences in serum concentrations of either TNF receptor or CRP between TNFB or TNF308 genotypes. In addition, there was no significant difference between serum concentrations of TNF-R55, TNF-R75 and CRP between those bearing allele 2 of either polymorphism and those with allele 1.

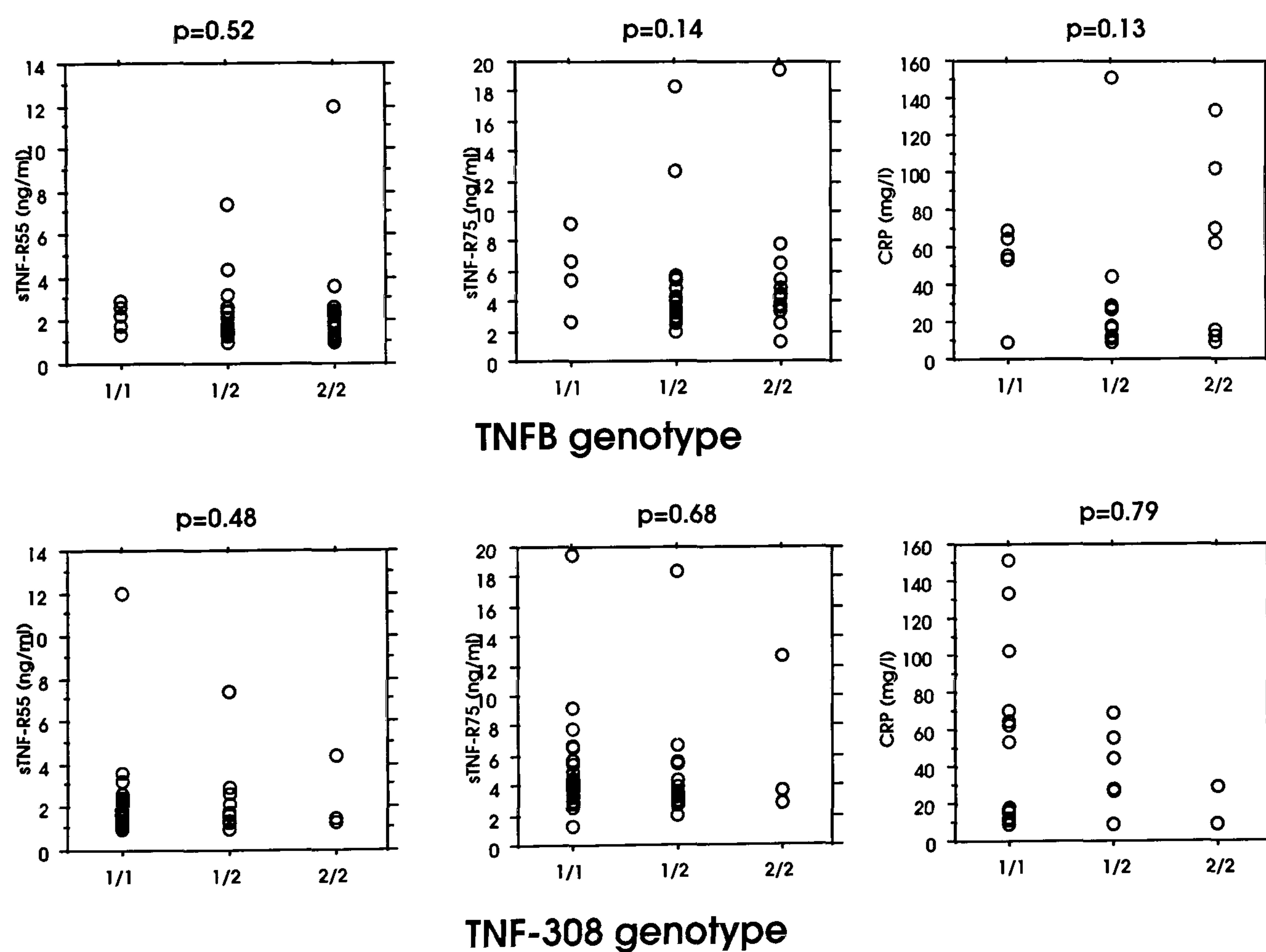


Figure 5.8. Serum concentrations of soluble tumour necrosis factor receptor (sTNF-R) 55 and 75 and C-reactive protein (CRP) from 45 patients with advanced pancreatic cancer presented by TNFB and TNF-308 genotype. Statistical analysis by Kruskal Wallis test.

Survival of the 64 pancreatic cancer patients stratified by TNF genotype is shown in figures 5.9 and 5.10. For the TNFB polymorphism, those with 1/1 genotype had a median survival of 235 (122-266) days, 1/2 - 202 (112-357) days and 2/2 - 256 (76-333) days. For the TNF-308 polymorphism, those with 1/1 genotype had a median survival of 202 (97-333) days, 1/2 - 227 (136-294) days and 2/2 - 82 (57-185) days. There was no significant relationship between either genotype and survival. In addition, the possession of the TNFB2 or TNF2 genotype made no difference to survival ($p=0.51$ and 0.77 respectively).

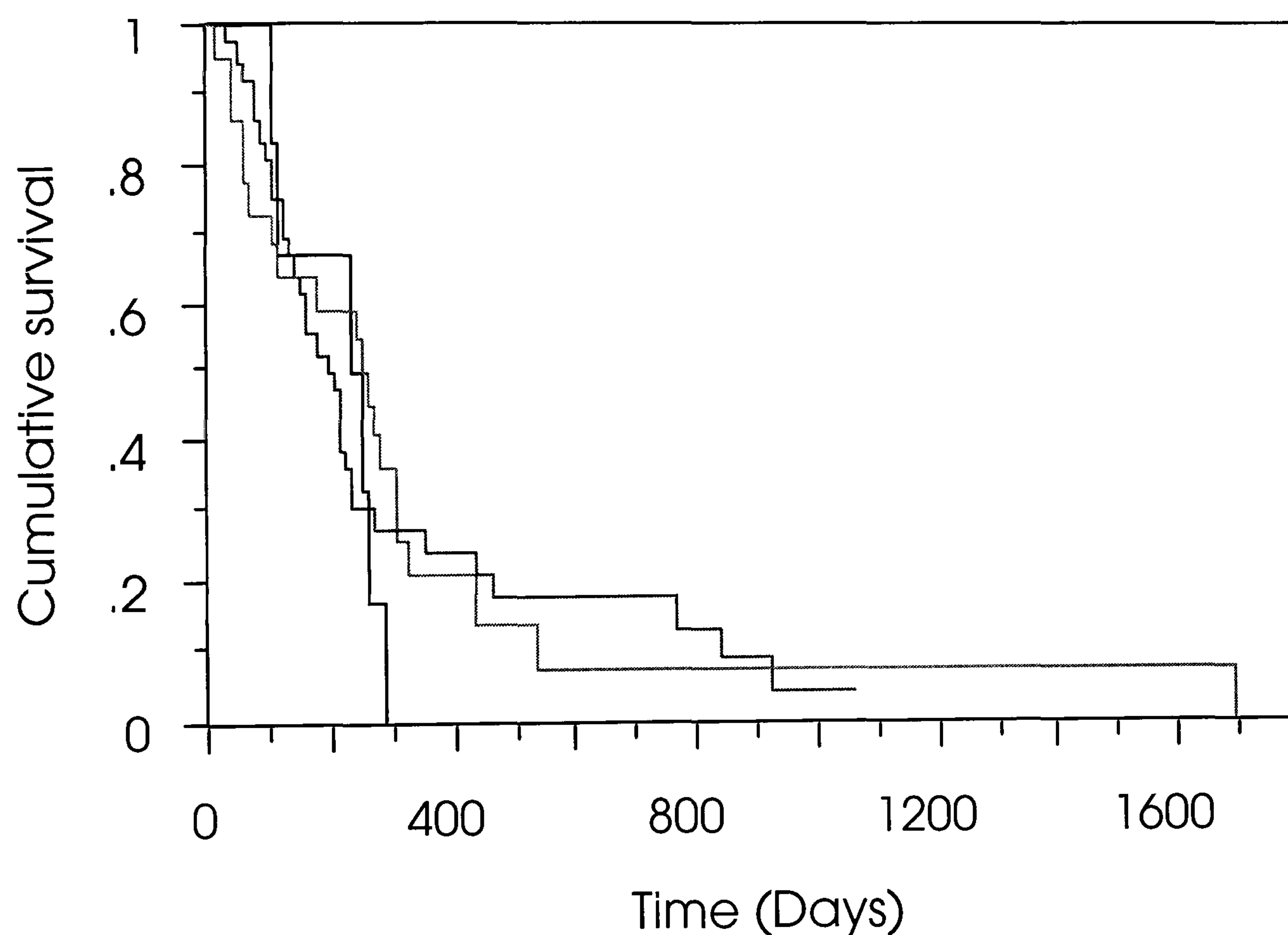


Figure 5.9. Kaplan Meier survival curve (from diagnosis) of 64 patients with advanced pancreatic cancer by TNFB genotype. 1/1 - solid line, 1/2 - dashed line, 2/2 - dotted line. Comparison by log rank test $p=0.78$.

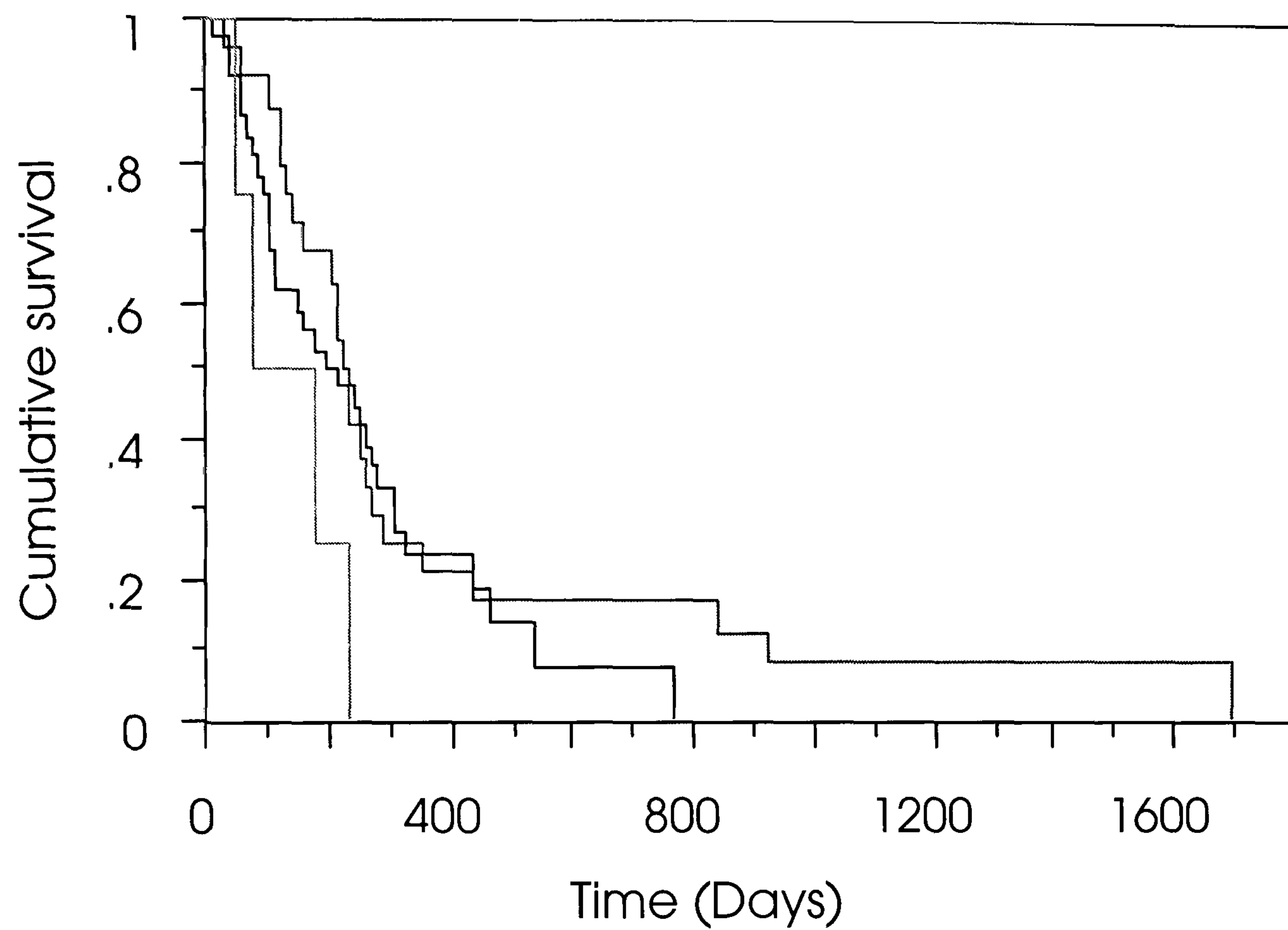


Figure 5.10. Kaplan Meier survival curve (from diagnosis) of 64 patients with advanced pancreatic cancer by TNF-308 genotype. 1/1 - solid line, 1/2 - dashed line, 2/2 - dotted line. Comparison by log rank test $p=0.13$.

Discussion

In this chapter it has been shown that IL-1 β genotype appears to be important in determining IL-1 β production, serum CRP level and survival from diagnosis in patients with advanced pancreatic cancer. However, neither of the TNF genotypes studied or the IL-1Ra genotype significantly influenced the inflammatory state or survival.

There was no difference in the frequency distribution of IL-1 β genotypes between normal subjects and cancer patients. The distribution found in normal subjects is very similar to that described in other European populations (Pociot et al, 1992, Bioque et al, 1995, Heresbach et al, 1997). There was, however, a trend towards fewer cancer patients possessing allele 2. If possession of allele 2 shortens survival it is possible that some patients who died were missed before assessment, thus our estimate of the importance of allele 2 may be a conservative one.

Distribution of the IL-1Ra genotypes was almost identical between patients and controls and was similar to that reported in other studies (Blakemore et al, 1994, Mansfield et al, 1994, Crusius et al, 1995, Danis et al, 1995). However, a number of studies have suggested an increased frequency of allele 2 in those with conditions in which inflammation may play a role such as systemic lupus erythematosus, ulcerative colitis and multiple sclerosis (Blakemore et al, 1994, Mansfield et al, 1994, Crusius et al, 1995). No relationship was found between IL-1Ra genotype and the inflammatory state in the present study.

Distribution of the TNF genotypes was also similar between pancreatic cancer patients and controls. Frequencies of the genotypes were also similar to those described by other groups in healthy subjects and in patients with inflammatory and malignant diseases (Shimura et al, 1994, O'Mahony et al, 1998, Wilson et al, 1992, Stuber et al, 1996, Westendorp et al, 1997). It has been suggested that the TNFB2 heterozygote is less common in patients with lung cancer than in the control population and may thus protect against this disease (Shimura et al, 1994). The present study provides no evidence to support this hypothesis in pancreatic cancer.

It is rare to detect IL-1 in the serum of cancer patients, thus measures of production are used for gauging levels that may occur in the relevant tissue compartments (Moldawer & Copeland, 1997). Only one group has previously studied the relationship between the IL-1 β polymorphism examined in the present study and production of IL-1 β by leukocytes (Pociot et al, 1992). Examining a group of 45 healthy individuals they were able to show a stepwise increase in IL-1 β production from 1/1 to 1/2 to 2/2 genotype. While numbers in the present study were insufficient to confirm this relationship, differences between the 2/2 genotype and the other groups were significant and there was a trend in agreement with this previous study ($p=0.072$ - Kruskal-Wallis test).

The relationship between IL-1 β genotype and the acute phase protein response has not previously been examined. It is known that the administration of IL-1 α or β will induce an acute phase response in mice (Moldawer et al, 1988) and that the administration of antibodies to the IL-1 receptor will attenuate the acute phase response to turpentine abscess in mice (Gerschenwald et al, 1990). It is thought that the acute phase response is primarily activated by IL-6 (Castell et al, 1990) but it has been suggested that blockade of IL-1 action will reduce IL-6 production (Yasumoto et al, 1995). It is thus possible that with increased IL-1 β production there is increased IL-6 production and so increased stimulation of the acute phase protein response. In the present study we failed to identify any simple relationship between serum CRP levels and IL-1 β production although numbers were small and IL-1 β genotype again seemed to be important with those possessing allele 2 having significantly higher CRP levels than those without.

TNF α is also rarely detected in serum samples from patients with cancer (Falconer et al, 1994). However, the two TNF receptors are shed from cells in response to TNF release and therefore the serum concentration of soluble TNF receptors may provide an indirect measure of TNF α release (Olsson et al, 1993). In the previous chapter it was demonstrated that the serum concentrations of soluble TNF receptors correlate with the level of the acute phase protein response in advanced pancreatic cancer. However, in the present study we were unable to show any relationship between either TNF genotype and concentrations of the two TNF receptors. In addition the present study was unable to show a relationship between either TNF genotype and the acute phase response as measured by serum CRP concentration. A relationship between the TNFB genotype and serum TNF α concentration was found in septic patients (Stuber et al, 1996) and between the TNF-308 genotype and TNF α production by lymphoid cell lines (Abraham et al, 1993). However, others have found no relationship between TNF-308 genotype and TNF α production by cultured blood cells (Westendorp et al, 1997). It may be that serum concentrations of the TNF receptors and CRP are an inadequate measure of TNF α production or that the polymorphisms studied are not sufficiently discriminatory of the propensity to produce this cytokine.

The possession of IL-1Ra allele 2 has been associated with increased production of IL-1Ra and decreased production of IL-1 α (Danis et al, 1995). However, it has also been associated with an increased susceptibility to a variety of inflammatory conditions (Blakemore et al, 1994, Mansfield et al, 1994, Crusius et al, 1995) with a suggestion of increased disease severity in systemic lupus erythematosus (Blakemore et al, 1994). The present study found no association between this genotype and the inflammatory state as measured by CRP or the TNF

receptors. The production of IL-1Ra itself was not measured. The role of IL-1Ra in mediating the action of IL-1 in cancer remains unclear but it would appear that the possession of allele 2 is not an adverse factor in pancreatic cancer.

The administration of pro-inflammatory cytokines, including IL-1, IL-6, interferon- α and TNF, produces features of cachexia in experimental models (Mahony & Tisdale, 1988, Gelin et al, 1991, Hellerstein et al, 1989, Matthys et al, 1991, Strassmann et al, 1992). These agents all induce an acute phase response and it has been suggested that the need for amino acids to manufacture acute phase reactants in the absence of adequate nutritional intake contributes to breakdown of skeletal muscle (Reeds et al, 1994) and so the weight loss of cachexia. It has previously been shown that the presence of an acute phase response is associated with nutritional decline and poor survival in pancreatic cancer (Falconer et al, 1994, Falconer et al, 1995, Wigmore et al, 1997 and preceding chapters). In this chapter it has been shown that IL-1 β genotype is related to survival. The stepwise decrease in median survival between the 1/1, 1/2 and 2/2 genotypes may be related to the apparent increase in IL-1 β production associated with these genotypes and would therefore be compatible with the hypothesis that a more marked pro-inflammatory state is detrimental to survival in pancreatic cancer, perhaps due to cytokine-mediated nutritional decline. Alternatively, it has been suggested that pro-inflammatory cytokines will promote tumour growth in animal models (Gelin et al, 1991). Blockade of the IL-1 receptor has been shown to reduce liver metastasis in a melanoma model (Vidal-Vanaclocha et al, 1994) perhaps due to an IL-1 induced increase in endothelial receptor expression (Vidal-Vanaclocha et al, 1996). Therefore, IL-1 may accelerate tumour progression accounting for the shortened survival of the 2/2 genotype group.

The work presented in this chapter has demonstrated that advanced pancreatic cancer patients with an elevated CRP concentration, despite having no evidence of infection or jaundice, have a significantly shorter survival than those without. However, despite the apparent importance of the inflammatory state, the present study found no association between survival and either TNF polymorphism. Those with the 2/2 genotype for the TNF-308 polymorphism had an almost significantly shorter survival than the other two groups ($p=0.054$) but this genotype was only seen in 6% of patients making it of little practical value. Previous studies of the TNFB polymorphism in cancer have suggested that two different genotypes, 1/2 and 1/1, are associated with poor survival in lung and oesophageal cancer respectively (Shimura et al, 1994, O'Mahony et al, 1998). Although no measurement of the inflammatory state was made in the cancer patient groups in these studies, these genotypes have been associated with significantly lower TNF α concentrations in septic patients (Stüber et al, 1996). This would

appear to conflict with the observation that elevated pro-inflammatory cytokine levels are associated with a worse outcome in cancer (Falconer et al, 1994a, Falconer et al, 1995). It may be that pro-inflammatory cytokines other than TNF α are of more importance in determining outcome in cancer patients, that the cytokines of importance vary between different malignancies or that the polymorphisms studied are of limited importance in this patient group.

The very poor survival of patients with IL-1 β 2/2 genotype is clinically of limited value due to the low prevalence of this genotype. However, the possession of allele 2 was also a significant predictor of survival. This was present in a third of the cancer patients in the present study and almost a half of the population as a whole thus it may be a valuable clinical tool for predicting survival. It has been suggested that endoscopic palliation of jaundice is appropriate in those patients with a life expectancy of 6 months or less (Rosewicz & Weidenmann, 1997, van den Bosch et al, 1994). Existing measures of the inflammatory state such as CRP are sensitive to surgery, infection and jaundice while IL-1 β genotype is not. Using this method to predict 6 month survival had an accuracy of 67% in the current study, suggesting that it may be useful in guiding palliative biliary drainage in these patients. It may also be useful in predicting patients who may benefit from therapy for cachexia targeted at the inflammatory state.

In conclusion, this chapter confirms the association between the inflammatory state and shortened survival in pancreatic cancer. The IL-1 β genotype is related to IL-1 β production and the level of the acute phase protein response (although numbers of patients were small) and also affects survival in patients with advanced pancreatic cancer. However, genotypes resulting from a polymorphism of the IL-1Ra gene and two polymorphisms of the TNF gene were not found to be associated with outcome or the inflammatory state in such patients.

The next chapters examine the potential of eicosapentaenoic acid to influence the inflammatory state and associated metabolic abnormalities associated with cachexia in patients with advanced pancreatic cancer.

Nutritional effects of an oral nutritional supplement enriched with fish oil in pancreatic cancer patients

Summary

Previous studies have suggested that administration of oral eicosapentaenoic acid (EPA) will down-regulate inflammatory mediators of weight loss and thus may stabilise weight in patients with advanced pancreatic cancer. The aim of this chapter is to determine if a combination of EPA with a conventional oral nutritional supplement could produce weight gain in these patients.

20 patients with unresectable pancreatic adenocarcinoma were asked to consume 2 cans of a fish oil-enriched nutritional supplement per day in addition to their normal food intake. Each can contained 310kcal, 16.1g protein and 1.09g EPA. Patients were assessed for weight, body composition, dietary intake, resting energy expenditure (REE) and performance status.

Patients consumed a median of 1.9 cans per day. All patients were losing weight at baseline at a median rate of 2.9kg per month. After administration of the fish oil-enriched supplement, patients had significant weight-gain at both three (median 1kg, $p=0.024$) and seven weeks (median 2kg, $p=0.033$). Dietary intake increased significantly by almost 400kcal per day ($p=0.002$). REE per kg body weight and per kg lean body mass fell significantly. Performance status and appetite were significantly improved at 3 weeks.

In contrast to previous studies of oral conventional nutritional supplements in weight-losing cancer patients, this study suggests that an EPA-enriched supplement may reverse cachexia in advanced pancreatic cancer.

Introduction

As described in Chapter 3, pancreatic cancer is almost inevitably associated with progressive nutritional decline. Weight-loss in patients with gastrointestinal cancer is often refractory to therapeutic intervention and is associated with a shorter survival time and a reduced quality of life (DeWys et al, 1980, Ovesen et al, 1993a). The provision of conventional oral nutritional supplements may increase overall dietary intake but this does not generally lead to any benefit in terms of nutritional status (Evans et al, 1987, Ovesen et al, 1993b). Consequently it has been suggested that the metabolic processes which contribute to weight-loss in patients with cancer may also block the accretion of lean tissue (Moldawer & Copeland, 1997).

The association between pro-inflammatory cytokine production, the acute phase protein response, weight loss and survival explored in the preceding chapters permits speculation that agents capable of down-regulating pro-inflammatory cytokine production or the acute phase response may affect the progress of cachexia. Attempts to manipulate the inflammatory response in cancer patients using non-steroidal anti-inflammatory drugs with the intention of improving nutritional status have been made previously with promising results (McMillan et al, 1997, McMillan et al, 1999).

The n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are immunomodulatory and have been shown to suppress endotoxin-induced production of pro-inflammatory cytokines such as IL-1 and tumor necrosis factor (TNF) by peripheral blood mononuclear cells (PBMC) from healthy volunteers (Meydani et al, 1993). Studies of weight-losing pancreatic cancer patients receiving high-purity EPA have demonstrated suppression of PBMC IL-6 production (Wigmore et al, 1997d). EPA has also been shown to have inhibitory effects on the growth of human pancreatic cancer cell lines in vitro (Falconer et al, 1994b) and to have anti-tumour and anti-cachectic effects in the chemoresistant murine MAC16 colon adenocarcinoma model (Beck et al, 1991). The cachexia seen in this animal model has been attributed to the production of a proteolysis inducing factor by tumour cells and such a factor is also found in tumour-bearing humans with cachexia (Todorov et al, 1996). It has been suggested that EPA may act by inhibiting the end organ effects of this factor (Tisdale, 1996).

It has previously been reported that administration of a mixed fish oil preparation (providing around 2.2g EPA and 1.4g DHA daily) and a pure EPA preparation (providing 6g EPA daily) will stabilise weight in patients with unresectable pancreatic cancer (Wigmore et al, 1996, Barber et al, 1997). Clearly, in order to lay down new tissue and thereby increase body weight additional macronutrients need to be consumed.

The present study aimed to assess whether the provision of additional oral nutrients together with fish oil could reverse weight-loss in patients with advanced pancreatic cancer.

Materials and methods

Patients

Patients enrolled were men or non-pregnant, non-lactating women between 18 and 80 years with histological confirmation or unequivocal operative or radiological diagnosis of unresectable adenocarcinoma of the pancreas with evidence of ongoing weight-loss. Patients had a life expectancy of over two months and a WHO performance status •2 at enrolment. Written, informed consent was obtained from all patients. Patients were excluded if they had received surgery or endoscopic stenting during the previous four weeks, had other active medical conditions, another malignancy or were receiving medication which could profoundly modulate metabolism or weight. No patients had received radiotherapy or chemotherapy. A total of twenty patients were recruited to the study. Confirmation of unresectable pancreatic adenocarcinoma was by histology (16/20) or unequivocal operative or radiological findings (4/20). 17 patients had cancers of the head of pancreas and three of the body. On enrolment none of the patients were jaundiced, pyrexial, ascitic, severely anaemic or had clinical or radiological evidence of infection and none were taking steroid drugs. All patients had adequate pain control at the time of study. Pancreatic enzyme supplements were administered if patients had or developed clinical evidence of steatorrhoea.

Patients were studied formally at baseline, 3 and 7 weeks although patients were assessed approximately monthly until death, withdrawal from the study or until their condition deteriorated such that further assessment was not possible. Trial results were monitored independently.

Fish oil-enriched nutritional supplement

The fish oil-enriched nutritional supplement was provided by Ross Products Division, Abbott Laboratories, Columbus, Ohio, USA. The composition of this product is shown in appendix 2. Patients were requested to store product in the refrigerator.

Patients were asked to consume two cans per day (providing 610kcal, 32.2g protein, 2.2g EPA and 0.96g DHA). Compliance was assessed by a diary of consumption, return of labels from empty cans and by plasma fatty acid analysis at baseline and after 3 weeks consumption of the supplement.

Toxicity assessment

Blood was drawn for full blood count, electrolytes, urea, glucose and liver function tests. These were measured using standard automated techniques in the haematology and clinical chemistry laboratories of the Royal Infirmary, Edinburgh, UK. A history and full clinical examination was performed at each of these visits. Toxicity reported to general practitioners or observed on clinical examination was documented.

Nutritional and metabolic assessment

At the initial assessment, height, pre-illness stable weight and duration of weight-loss were recorded. At each review subjects were weighed, and mid-arm muscle circumference (MAMC) and triceps skinfold thickness (TSF) measured. Body composition was measured by bioelectrical impedance.

The daily caloric intake and resting energy expenditure of patients were measured at baseline and after three weeks of supplement administration.

The daily nitrogen intake of patients was measured at baseline and after three weeks of supplement administration. Values were based on the mean dietary intake.

Nitrogen loss was determined by measurement of urinary nitrogen. A further 2g per day was added for faecal losses.

Acute phase protein response

The APPR was documented by assaying serum C-reactive protein.

Fatty acid analysis

Plasma phospholipid fatty acid analysis was performed before commencement of the supplement and 3 weeks thereafter.

Performance status and appetite

Karnofsky performance status was noted before commencement of the supplement, at three weeks and at monthly intervals thereafter.

Appetite was measured on a numerical rating scale between 0 and 10, where 0 indicated absolutely no appetite and 10 indicated an extremely good appetite (Simons et al, 1996).

Survival

Survival was recorded from the time of diagnosis and study baseline to the time of death. Diagnosis was defined as the date of confirmation of adenocarcinoma of the pancreas by histological examination or of unequivocal operative or radiological findings in patients in whom definitive pathological confirmation of diagnosis was lacking.

Further details of methods are found in Chapter 2.

Results

Patient characteristics

Patients comprised 10 males and 10 females of median age 62 years (range 51-75). Tumour stages were as follows: stage 2, 8 patients; stage 3, 3 patients and stage 4, 9 patients. No patients underwent prior chemotherapy or radiotherapy. Eight patients underwent non-resectional palliative surgical procedures and eight patients had endobiliary stenting prior to the study. Patients had tumours of the pancreatic head in 17 cases and the pancreatic body in the remainder. Baseline characteristics of patients is shown in table 6.1. No patients on whom data was available over the 7 week study period developed clinically detectable ascites or oedema.

Table 6.1. Baseline characteristics of study patients with advanced pancreatic cancer (n=20).

| | |
|---|------------------|
| Weight (kg) | 55.2 (48.8-61.2) |
| Body mass index (kg/m ²) | 19.8 (17.8-21.8) |
| Rate of weight change per month (kg) | -2.9 (-4.4--2.2) |
| Percentage weight loss | 17.9 (15.9-22.8) |
| Mid arm muscle circumference (cm) | 20.8 (18.4-22.4) |
| Triceps skinfold thickness (mm) | 11.1 (7.8-15.9) |
| Percentage total body water | 52.9 (51.2-56.1) |
| Lean body mass (kg) | 41.5 (38.1-44.8) |
| Fat mass (kg) | 14.5 (12.3-16.7) |
| C-reactive protein (mg/l) | 10 (<10-27) |
| Karnofsky performance status (10 (moribund) to 100 (normal)) | 85 (80-90) |
| Appetite (0 (none) to 10 (excellent)) | 5 (3-7) |

Figures are median (interquartile range)

Tolerance/toxicity

Patients consumed a median of 1.9 cans of the fish oil-enriched supplement per day (range 1.2-2).

Full blood count, electrolytes, urea, glucose and liver function tests were assessed at each review. Values did not change significantly over the course of the study.

Two patients developed and one patient had worsening of pre-existing steatorrhoea with administration of the supplement. This was successfully treated by pancreatic enzyme supplementation. Otherwise no patient developed an adverse event outwith the expected progression of advanced pancreatic cancer.

At 3 weeks, two patients were unavailable for analysis due to disease progression. At 7 weeks a further 5 patients were unavailable due to disease progression. Reasons for stopping the supplement during the 7 week study period were temporary interruption in supply in one patient (after 3 weeks), excessive weight gain in one patient (after 3 weeks), dislike of taste in two patients (after 3 weeks) and progression of disease in the remainder. Data were only available for one patient not taking the supplement at 7 weeks. Thus all 18 patients assessed at 3 weeks and 12 of 13 patients assessed at 7 weeks were consuming the supplement.

Body weight

Changes in body weight for individual patients are depicted graphically in figure 6.1. Weight change at 3 (n=18) and 7 (n=13) weeks is shown in table 6.2. Patients had statistically significant weight gain at 3 and 7 weeks of median 1 and 2kg respectively. The patient who had discontinued the supplement at 3 weeks but on whom data was available at 7 weeks gained 0.5kg over the first 3 weeks on the supplement and lost 0.9kg in the subsequent 4 weeks off the supplement. Excluding this patient, median weight gain at 7 weeks was 2.5kg (0.2-4.6).

Anthropometry and body composition analysis

Values for MAMC and TSF recorded before commencement of the supplement and changes after 3 and 7 weeks are shown in tables 6.1 and 6.2. There was no significant change in either MAMC or TSF over this period.

Values for total body water expressed as a percentage of total body weight are also presented in tables 6.1 and 6.2. There was no significant change in this factor in patients remaining in the trial at 3 and 7 weeks.

Values for calculated lean body mass and fat mass are presented in tables 6.1 and 6.2. There was a statistically significant rise in calculated lean body mass at three and seven weeks (of median 1.0kg and 1.9kg respectively) but no change in fat mass at either time point.

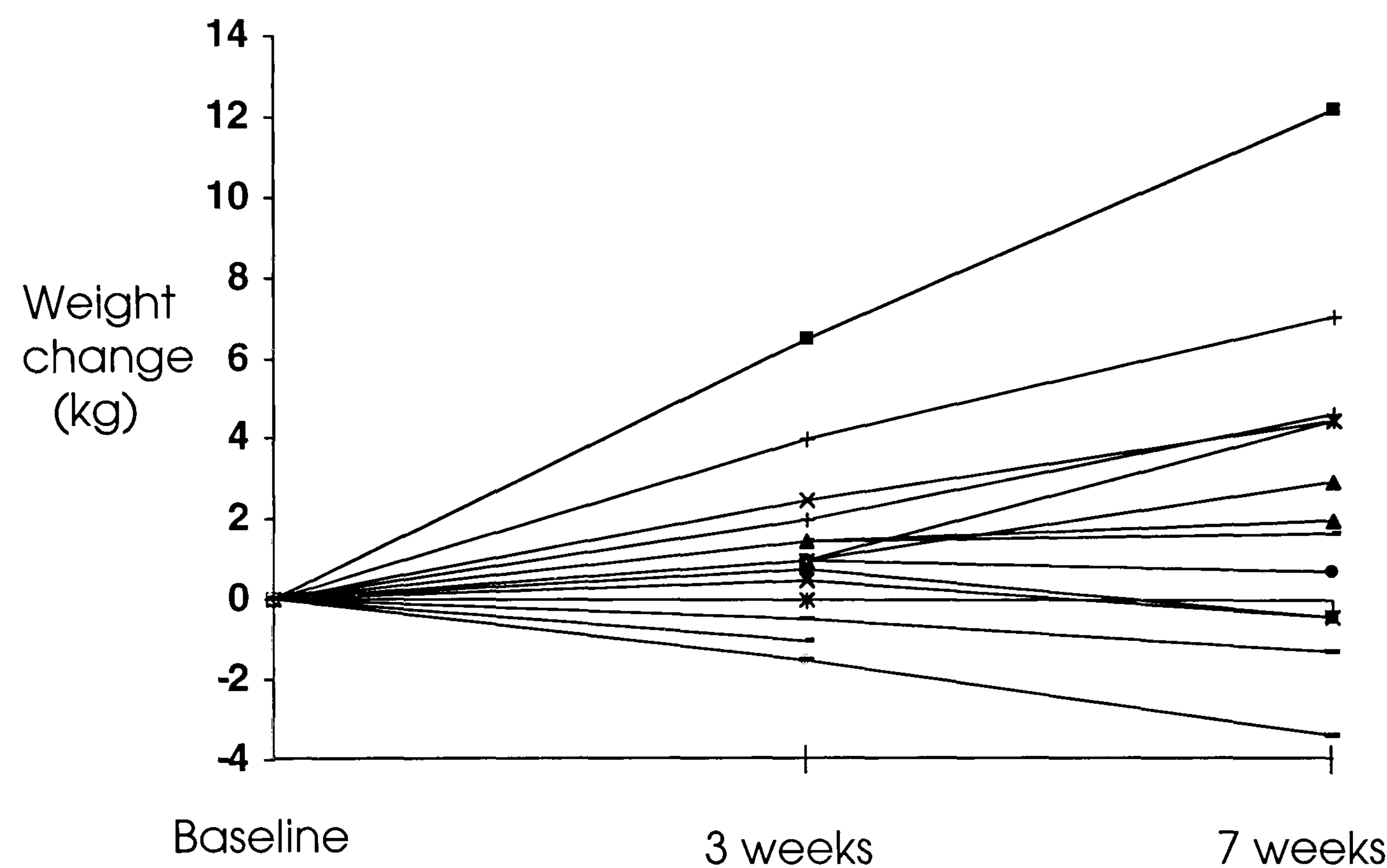


Figure 6.1. Weight change of 20 patients with advanced pancreatic cancer administered a fish oil-enriched nutritional supplement after 3 and 7 weeks. Median rate of weight loss prior to intervention was 2.9kg per month. Median weight gain at 3 weeks was 1.0kg ($p=0.028$ vs baseline Wilcoxon signed rank test). Median weight gain at 7 weeks was 2.0kg ($p=0.033$).

Table 6.2. Change in characteristics of patients with advanced pancreatic cancer after 3 and 7 weeks administration of a fish oil-enriched nutritional supplement.

| Duration of supplement administration | 3 weeks | | 7 weeks | |
|---|---------------------|--------|---------------------|--------|
| Number of patients | 18 | | 13 | |
| | | p | | p |
| Weight change (kg) | +1.0 (-0.1-+2.0) | 0.024 | +2.0 (-0.4-+4.6) | 0.033 |
| Change in mid arm muscle circumference (cm) | 0 (-0.2-+0.5) | NS | +0.4 (-0.7-+1.1) | NS |
| Change in triceps skinfold thickness (mm) | 0 (-0.2-+0.4) | NS | +0.2 (-0.5-+0.6) | NS |
| Change in percentage total body water | +0.1 (-0.3-+1.9) | NS | -0.1 (-0.4-+1.5) | NS |
| Change in lean body mass (kg) | +1.0 (+0.6-+1.4) | 0.0064 | +1.9 (+1.0-+3.0) | 0.0047 |
| Change in fat mass (kg) | -0.2 (-0.8-+0.9) | NS | +0.2 (-0.8-+2) | NS |
| Change in C-reactive protein (mg/l) | 0 (0-+3) | NS | 0 (-5-+22) | NS |
| Change in Karnofsky performance status (10 (moribund) to 100 (normal)) | +10 (0-+10) | 0.0047 | +10 (0-+10) | 0.046 |
| Change in appetite (0 (none) to 10 (excellent)) | +1 (0-+1) | 0.001 | +1 (0-+1) | NS |

Figures are median (interquartile range).

Paired comparison with baseline by Wilcoxon signed rank test

Nutritional intake

The daily caloric intake of patients before commencement of supplement and after 3 weeks (n=18) are presented in table 6.3. There was a statistically significant increase in caloric intake after 3 weeks (p=0.0016). The median increase for each patient was 372kcal per day.

Energy expenditure

REE and REE expressed per kg body weight and per kg lean body mass as measured at baseline and after 3 weeks are presented in table 6.3. While there was no change in overall REE (p=0.18), REE kg per body weight and REE kg per lean body mass both fell significantly (p=0.025 and 0.018 respectively).

Table 6.3. Caloric intake and resting energy expenditure of 18 patients with advanced pancreatic cancer at baseline and after 3 weeks administration of a fish oil-enriched nutritional supplement.

| Duration of supplement administration | Caloric intake (kcal/day) | REE (kcal/day) | REE/kg body weight (kcal/kg/day) | REE/kg lean body mass (kcal/kg/day) | Nitrogen balance (g) |
|---------------------------------------|---------------------------|-----------------------|----------------------------------|-------------------------------------|-----------------------|
| 0 | 1450 (1048-2043) | 1339 (1159-1448) | 24.2 (23.1-27.7) | 34.0 (29.6-35.6) | -0.8 (-4.9-+1.3) |
| 3 weeks | 1798 a (1355-2474) | 1303 b (1186-1470) | 24.0 c (20.2-25.8) | 31.8 d (27.7-33.9) | +1.9 e (-1.1-+2.8) |

Figures are median (interquartile range).

Paired comparison of value at 3 weeks versus baseline using Wilcoxon signed rank test

REE - Resting energy expenditure.

a p=0.0016

d p=0.018

b p=0.18

e p=0.016

c p=0.025

Nitrogen balance

Patients had a significant improvement in their nitrogen balance from -0.8g/day at baseline to +1.9g/day after 3 weeks (p=0.016) (table 6.3).

Acute phase protein response

Values for serum CRP concentration before commencement of the supplement and changes after 3 and 7 weeks are shown in tables 6.1 and 6.2. There was also no change in the percentage of patients exhibiting an APPR as defined by a CRP •10mg/l throughout the study period.

Fatty acid analysis

Fatty acid content of plasma phospholipids of patients before commencement of supplement and after 3 weeks are presented in table 6.4. There was a statistically significant increase in both EPA and DHA in plasma phospholipids after 3 weeks.

Performance status and appetite

Scores for Karnofsky performance status before administration of the supplement and change after 3 and 7 weeks are shown in tables 6.1 and 6.2. There was a statistically significant improvement in Karnofsky performance status compared with baseline after 3 and 7 weeks administration of the supplement (p=0.0047 and 0.046 respectively).

Appetite scores before administration of the supplement and change after 3 and 7 weeks are shown in tables 6.1 and 6.2. There was a statistically significant improvement in appetite at 3 weeks (p=0.0095).

Table 6.4. Percentage of fatty acids in plasma phospholipid profile of 18 patients with advanced pancreatic cancer at baseline and after 3 weeks administration a fish oil-enriched nutritional supplement.

| Fatty acid | Percentage at baseline | Percentage after 3 weeks supplement | p value |
|-------------|---------------------------|--|---------|
| EPA 20:5n-3 | 0.5 (0.2-1.6) | 5.2 (2.6-6.7) | 0.0003 |
| DHA 22:6n-3 | 2.9 (1.6-3.9) | 4.8 (3.3-5.3) | 0.0086 |
| ALA 18:3n-3 | 0.7 (0-1.1) | 1.1 (0.6-1.8) | 0.11 |
| AA 20:4n-6 | 6.4 (4.1-7.5) | 5.1 (4.3-6.4) | 0.33 |
| LA 18:2n-6 | 18.6 (16.2-20.2) | 15.8 (12.5-18.3) | 0.040 |
| OA 18:1n-9 | 25.8 (19.8-30.7) | 21.8 (19.1-24.4) | 0.11 |
| POA16:1n-7 | 3.7 (2.9-4.6) | 3.1 (2.1-3.9) | 0.28 |
| SA 18:0 | 9.4 (8.5-11.3) | 10.6 (9.4-11.7) | 0.62 |
| PA 16:0 | 32.0 (29.3-33.2) | 33.2 (29.8-35.0) | 0.79 |

Figures are median (interquartile range).
Paired comparison of value at 3 weeks versus baseline using Wilcoxon signed rank test

- EPA - eicosapentaenoic acid
- DHA - docosahexaenoic acid
- ALA - alpha linolenic acid
- AA - arachidonic acid
- LA - linoleic acid
- OA - Oleic acid
- POA - Palmitoleic acid
- SA - Stearic acid
- PA - Palmitic acid
- See appendix 1

Survival

Nineteen of the patients have died. One patient remained alive at 14 months from the time of diagnosis. The survivor has histological confirmation of the diagnosis of pancreatic adenocarcinoma. The censored median survival from the time of diagnosis for all patients was 254 days (124-311). The censored median survival from the time of commencing the supplement was 170 days (90-270). Survival is shown in figure 6.2.

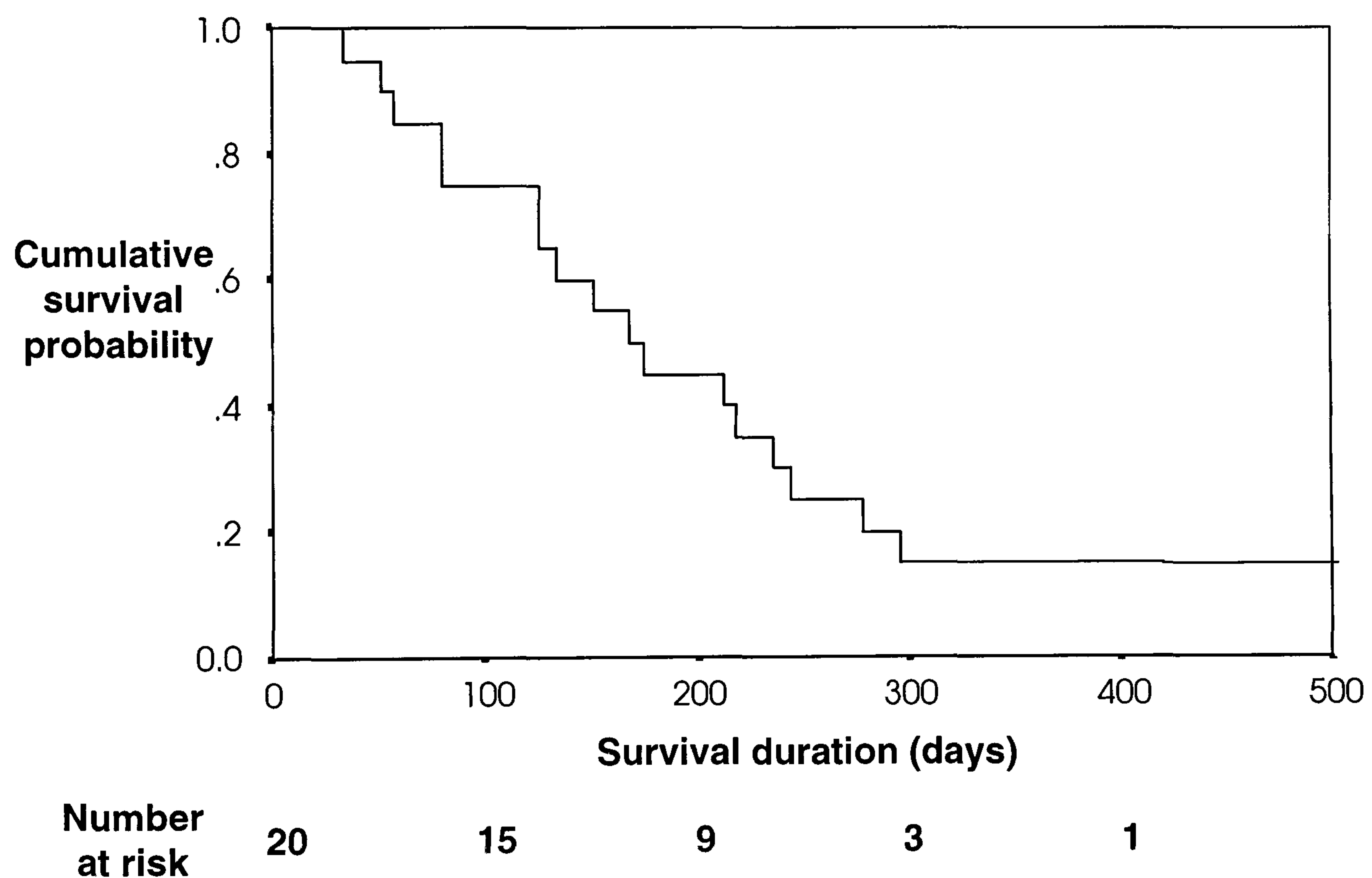


Figure 6.2. Duration of survival of 20 patients with advanced pancreatic cancer from time of commencement of fish oil-enriched nutritional supplement.

Discussion

In the present chapter, the administration of a nutritional supplement enriched with fish oil resulted in weight gain in a group of patients with advanced pancreatic cancer who had previously been losing weight at a median rate of 2.9kg per month.

Not all of the patients who entered the present study completed the 7 week study period. This is a reflection of the advanced stage at presentation and subsequent poor survival of patients with pancreatic cancer. By observing weight change in those survivors who remained on study at 3 (n=18/20) and 7 (n=13/20) weeks there might be a bias to overestimate the overall efficacy of EPA as an anti-cachectic agent. Nevertheless, the finding of weight gain in the majority of patients at both time points contrasts markedly with previous study of patients receiving supportive care alone which demonstrated near uniform progressive weight loss in patients with this disease (Wigmore et al, 1997a and Chapter 3). In addition, previous controlled trials of oral nutritional supplementation in patients with advanced cancer have succeeded in increasing nutritional intake but have not been able to show any change in weight change compared with controls (Evans et al, 1987, Ovesen et al, 1993b). There was a variation in response of patients in the present study with some continuing to lose weight. The factors determining response are not clear as there was no relationship between weight change and disease stage, previous surgery or the acute phase response. Such analysis is complicated by the fact that even patients who continued to lose weight experienced a slowing of their rate of weight loss with the administration of the trial supplement.

It has been reported that increased body weight following parenteral and enteral nutritional support in cancer patients is largely due to the accumulation of body water (Cohn et al, 1982). However, in the present study there was no change in hydration as total body water as a percentage of body weight remained stable. Values for lean body mass and fat mass were derived from bioimpedence measurements. Overall there was a small but statistically significant increase in lean body mass with administration of the supplement while fat mass remained stable. The coefficient of variation for the estimate of lean body mass has been estimated to be around 2kg (Jensen et al, 1997). Most of the changes documented in this study were around this value and therefore the interpretation of data on individual patients is difficult. Nevertheless, the changes for the group as a whole suggest that patients were laying down lean tissue rather than fat to account for weight gain and this may have contributed to the observed rise in Karnofsky performance status. This contrasts with studies of synthetic progestogens such as medroxyprogesterone acetate and megestrol acetate in similar patients in whom weight gain is due to the accumulation of fat (Loprinzi et al, 1993, Simons et al, 1998). Measurements of MAMC and TSF demonstrated no significant change from baseline levels but this is not

surprising given the small changes in bioimpedence derived body composition seen. However, they do provide supporting evidence that protein and fat reserves were at least stabilised with administration of the fish oil-enriched supplement. These findings are in contrast to the continuing decline in MAMC and TSF (Wigmore et al, 1997a) and Karnofsky performance status (Chapter 3) seen in similar patients who undergo no specific intervention.

Patients in this study achieved a significant increase in nutritional intake and a relative fall in resting energy expenditure. Patients did not simply replace normal food intake with the supplement provided. Overall, patients had an improvement in energy balance of around 500kcal per day. This is entirely compatible with the weight gain seen and may have provided additional energy for activities of daily living, reflected in the observed improvements in Karnofsky performance status. Appetite appeared to increase over the early part of the study period and may account for the overall increase in food intake. The improvement in nitrogen balance of around 2g per day is also compatible with the gain in lean body mass suggested by body composition analysis.

The fish oil-enriched nutritional supplement was well tolerated with all patients able to consume over one can (237ml) per day and 15 of 18 managing over 1.75 cans per day. The only adverse event attributable to the trial preparation was steatorrhoea in two patients. A further patient described worsening of their pre-existing steatorrhoea. Steatorrhoea is common in patients with advanced pancreatic cancer (Perez et al, 1983).

There was a marked rise in levels of EPA and DHA in plasma phospholipids representing 5.2% and 4.8% respectively of fatty acids after 3 weeks of receiving the supplement. This also provided an objective measure of patient compliance. Such a pattern of change is similar to that previously reported following fish oil supplementation (Leaf and Weber, 1988, Wigmore et al, 1996). The percentage of EPA incorporation achieved is similar to that seen after supplementation with crude fish oil capsules in pancreatic cancer patients given a similar final dose equivalent to 2g EPA per day after a dose escalation period (Wigmore et al, 1996).

Down-regulation of pro-inflammatory cytokine release and C-reactive protein concentrations have been demonstrated in pancreatic cancer patients following the administration of EPA for one month (Wigmore et al, 1997d). CRP may be used as a marker for pro-inflammatory cytokine activity in vivo. In the present cohort of patients, the proportion with an elevated CRP remained stable throughout the study period. In patients with advanced pancreatic cancer receiving no specific intervention, CRP tends to rise with disease progression (Falconer et al, 1994a and Chapter 3). The provision of fish oil supplementation provided by the trial supplement in the current study may have prevented this progressive rise in CRP. A more detailed analysis of the full spectrum of acute phase proteins and their modulation in this group of patients is presented in Chapter 8.

Polyunsaturated fatty acids such as EPA have been shown to have an inhibitory effect on human pancreatic carcinoma cell lines in vitro (Falconer et al, 1994b). These effects may occur via cell cycle arrest and the induction of apoptosis (Lai et al, 1996). EPA will also slow the growth of experimental tumors in mice (Beck et al, 1991) and it is possible that part of the effect upon cachexia seen in the present study was secondary to inhibition of tumor growth. However, serial tumor imaging in pancreatic cancer is difficult to interpret and expensive and was thus not performed.

Overall survival of patients with pancreatic cancer is very poor with a median of 4.1 months (Ahlgren, 1996). Median survival from diagnosis in the present study was over 8 months although a condition of enrolment was that survival was expected to be over 2 months. Similar conditions apply to most chemotherapy trials where median survival of untreated patients has been noted to be between 63 and 122 days and overall median survival of treated patients to be between 160-170 days (Fearon et al, 1996). Clearly, overall survival in this study is at the upper end of that seen in chemotherapy trials but without the side-effects associated with chemotherapy. A recent randomised, controlled study of a mixed fish oil preparation (providing around 3g of EPA and 2g of DHA daily) in a group of patients with advanced cancer has suggested a modest survival benefit for those patients receiving fish oil (Gogos et al, 1998).

The study presented in this chapter suggests that a fish oil-enriched nutritional supplement has the potential to be a safe, effective anti-cachectic agent, in contrast to conventional nutritional supplements. A randomised, controlled trial would be required to confirm the observed anti-cachectic effect, evaluate any effect on survival and reveal any as yet undetected side-effects. Such a study is currently under way.

The next chapter examines the effects of the fish oil-enriched nutritional supplement on potential mediators of cachexia.

Appendix to Chapter 6

Changes in functional and quality of life measurements in weight losing pancreatic cancer patients after the provision of fish oil-enriched nutritional supplement

As a prelude to a planned randomised trial a number of functional and quality of life measures were studied in subgroups of the patients in this study.

Methods

Patients and interventions were as previously described.

Fatigue score

Subjects were asked to describe their fatigue on a 10 point linear analogue scale from 10 (most fatigued) to 0 (least fatigued) (Christensen et al, 1982). This was assessed in 7 patients.

Grip strength

Subjects' grip strength was measured in the seated position with the nondominant hand using a hand grip dynamometer (Grip-A, Takai, Japan). The best of three attempt was taken with a rest of around 2 minutes between attempts (Schroeder & Hill, 1991). This was assessed in 7 patients.

Peak expiratory flow rate

Peak expiratory flow rate was measured with a peak flow meter (Vitalograph). The best of three attempt was taken with a rest of around 2 minutes between attempts. This was assessed in 7 patients.

Quality of life measures

Four different questionnaires were used to assess quality of life in various patients. The Hospital Anxiety Depression scale (Zigmond & Snaith, 1983) and the Rotterdam Symptom Checklist (de Haes et al, 1990, Watson et al, 1992) were assessed in 12 patients and the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire C30 (Aaronson et al, 1993) and the EuroQOL group 5D questionnaire were assessed in 7 patients. Copies of the questionnaires are included in appendix 3.

Statistics

Results are presented as median and range.

Results and discussion

The performance of patients in the functional and quality of life assessments are presented in table 6.5. The presentation of quality of life data is controversial but in this instance we have chosen to present data by domain. Numbers were too small to make meaningful comparisons with changes in weight or performance status. In general, however, the majority of patients showed stable or improved function and quality of life. The EQ-5D score and Rotterdam Symptom Checklist appeared less able to demonstrate a change with many patients remaining unchanged or with small changes in absolute values.

Key for Table 6.5

Fatigue scale - 0-10 - higher score = worse function

PEFR - peak expiratory flow rate

HAD - Hospital Anxiety Depression scale - Score of 8-11 or more suggests high likelihood of anxiety or depression

RSC - Rotterdam Symptom Checklist -

Physical, psychological - high score = more symptoms

Activity - high score = poor activity

Overall - high score = poor quality of life

QLQ-C30 - function - high score = good function

global - high score = good quality of life

symptoms - high score = more symptoms

EQ-5D - Score and thermometer - higher score = better quality of life

Table 6.5. Performance of patients in the functional and quality of life assessments at baseline and after 3 weeks of fish oil-enriched nutritional supplement.

| | Baseline | 3 weeks | Proportion improving or stable | p value |
|--------------------------|---------------------|--------------------|--------------------------------------|---------|
| Fatigue (0-10) | 4 (2-6) | 4 (2-5) | 6/7 | 0.085 |
| Grip strength (kg) | 36 (25-45) | 37 (26-48) | 6/7 | 0.047 |
| PEFR (l/min) | 360 (270-510) | 380 (310-520) | 5/7 | 0.67 |
| HAD Anxiety (0-21) | 5 (0-13) | 4 (0-17) | 5/12 | 0.93 |
| HAD Depression | 4 (1-10) | 6 (1-11) | 6/12 | 0.39 |
| RSC Physical (%) | 25 (12-65) | 27 (6-56) | 6/12 | 0.93 |
| RSC Psychological | 26 (0-62) | 25 (0-71) | 6/12 | 0.89 |
| RSC Activity | 12 (0-62) | 9 (0-62) | 11/12 | 0.18 |
| RSC Overall | 38 (0-75) | 38 (0-75) | 9/12 | 0.74 |
| QLQ-C30 Function (%) | 77 (44-86) | 83 (53-97) | 6/7 | 0.075 |
| QLQ-C30 Symptom | 24 (13-53) | 18 (8-47) | 5/7 | 0.67 |
| QLQ-C30 Global | 58 (25-67) | 67 (33-75) | 5/7 | 0.29 |
| EQ-5D Score (0-1) | 0.76 (0.36-0.80) | 0.73 (0.29-1.0) | 5/7 | 1.0 |
| EQ-5D Thermometer (%) | 60 (34-70) | 70 (28-80) | 6/7 | 0.074 |

Figures presented are median (range).
Comparison by Wilcoxon signed rank test.

Metabolic mediators in cancer patients and the effects of a fish oil-enriched nutritional supplement.

Summary

Weight loss in cancer is often refractory to conventional nutritional supplementation perhaps due to a variety of metabolic changes. Such metabolic abnormalities may be mediated in part by pro-inflammatory cytokines, hormones and tumour derived products. In Chapter 6 it was demonstrated that a fish oil-enriched nutritional supplement could reverse the loss of lean tissue in cachectic cancer patients and thus may overcome the aforementioned metabolic changes. This chapter examines a number of potential mediators of cachexia in the same group of advanced pancreatic cancer patients before and after receiving the fish oil-enriched supplement and compares them with a group of healthy controls.

Serum concentration of interleukin-6 and its soluble receptor, tumour necrosis factor receptor 55 and 75 and leptin, peripheral blood mononuclear cell production of interleukin-1 β , interleukin-6 and tumour necrosis factor and urinary excretion of proteolysis inducing factor (PIF) were measured in 20 weight-losing patients with pancreatic cancer and 6 healthy subjects. Leptin was only measured in the cancer patients. Cancer patients were asked to consume 2 cans per day of a nutritional supplement providing a total of over 600kcal and 2g eicosapentaenoic acid per day. Samples were repeated after 3 weeks of consuming the supplement.

Serum concentrations of IL-6, TNF-R55 and TNF-R75 were significantly higher in cancer patients than controls. There was a significant correlation between concentrations of these cytokines and the level of the acute phase response measured as the C-reactive protein concentration. There was no significant difference in production of the measured cytokines between cancer patients and controls. However, there was a significant correlation between IL-6 production and the acute phase response. PIF was excreted by 89% of the weight losing cancer patients and none of the controls ($p=0.0002$).

After 3 weeks of consumption of the fish oil-enriched nutritional supplement there was no change in serum cytokine or leptin concentrations. However, there was a significant fall in the production of interleukin-6 and a fall in the proportion of patients excreting PIF.

Modulation of production of IL-6 and other mediators of cachexia by the fish oil-enriched nutritional supplement examined in the present study may explain the beneficial nutritional effects seen in Chapter 6.

Introduction

Chapter 6 described the reversal of weight loss in a group of patients with advanced pancreatic cancer given a nutritional supplement enriched with fish oil. A significant increase in nutritional intake was also noted. However, previous studies of nutritional intervention in patients with advanced cancer have failed to show any nutritional benefit despite an increase in caloric intake (Evans et al, 1987, Ovesen et al, 1993b). This thesis has been exploring the hypothesis that the failure of nutritional intervention alone to affect weight loss in advanced cancer is due to a combination of metabolic changes which prevent the efficient use of these nutrients. Mediators of these changes are thought to include pro-inflammatory cytokines (as described in Chapters 4 and 5), hormones and tumour specific products. Weight losing patients with cancer have been observed to have upregulation of interleukin (IL)-6 and tumour necrosis factor (TNF) production (Falconer et al, 1994a), to have an elevated cortisol/insulin ratio (Fearon et al, 1998) and to excrete in their urine proteolysis inducing factor (PIF), a newly described glycoprotein which causes muscle protein breakdown when administered to animals (Todorov et al, 1996a). Leptin is a hormone produced by fat which affects energy expenditure and appetite (Zhang et al, 1994). It has been suggested that in inflammatory models leptin concentrations are inappropriately high, perhaps contributing to hypermetabolism (Grunfeld et al, 1996a)

Fish oil is rich in the n-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA). EPA and fish oil have been shown to reduce production of pro-inflammatory cytokines by isolated peripheral blood mononuclear cells in both healthy volunteers (Endres et al, 1989, Meydani et al, 1993) and pancreatic cancer patients (Wigmore et al, 1997). It has also been suggested that EPA may modulate the activity of PIF on muscle in vitro (Tisdale, 1996).

The present chapter examines the effects of a fish oil-enriched nutritional supplement upon a variety of cytokine and hormonal mediators and other metabolic indicators in an attempt to explain the mechanisms whereby addition of fish oil to a conventional nutritional supplement may alter the metabolic milieu and allow some restoration of body composition.

Methods

Patients and fish oil-enriched nutritional supplement

Enrolment criteria and patients studied and the fish oil-enriched nutritional supplement administered are described in Chapter 6.

6 weight stable healthy controls were also studied.

Venous blood samples were collected at 8am after an overnight fast. After baseline sampling cancer patients were administered 2 cans per day of the fish oil-enriched nutritional supplement and underwent repeat blood sampling after 3 weeks.

Blood samples were assessed for concentrations of IL-6, soluble IL-6 receptor (sIL-6R), soluble TNF receptor (sTNF-R) 55 and 75, C-reactive protein and leptin. Peripheral blood mononuclear cells (PBMC) isolated from blood samples were assessed for their production of IL-1, TNF α and IL-1 β .

24 hour urine collections from before and after intervention were assessed for the presence of PIF.

Further details of methods are found in Chapter 2.

Results

Baseline mediator status

Serum concentrations of IL-6, sIL-6R, TNF-R55 and TNF-R75 are shown in table 7.1. Concentrations of IL-6 and the TNF α receptors were significantly higher in the cancer patients than controls ($p < 0.006$). sIL-6R concentrations were not significantly different between the two groups ($p = 0.10$).

There was no significant difference in PBMC production of IL-1 β , IL-6 or TNF α between cancer patients and controls ($p > 0.33$) (table 7.1).

17 of 19 weight losing cancer patients had detectable PIF in urine at baseline while it was detected in the urine of none of the 6 control subjects ($p = 0.0002$) (figure 7.4).

Table 7.1. Serum cytokine concentration and peripheral blood mononuclear cell cytokine (PBMC) production of 18 weight losing pancreatic cancer patients and 6 healthy controls.

| | Cancer patients | Healthy controls | p value |
|------------------------------|-----------------|------------------|---------|
| Serum IL-6 | 6.0 | 1.75 | 0.0055 |
| (pg/ml) | (2.5-7.9) | (1.5-2.75) | |
| Serum sIL-6R | 107.6 | 85.5 | 0.10 |
| (ng/ml) | (80.0-127.8) | (48.8-100.0) | |
| Serum TNF-R55 | 1.71 | 1.09 | 0.0051 |
| (ng/ml) | (1.23-1.88) | (0.92-1.17) | |
| Serum TNF-R75 | 3.46 | 2.22 | 0.0051 |
| (ng/ml) | (2.78-4.26) | (1.52-2.59) | |
| PBMC IL-1 β production | 2.33 | 3.80 | 0.33 |
| (ng/ml) | (1.64-3.60) | (2.19-4.93) | |
| PBMC IL-6 production | 16.5 | 12.5 | 0.44 |
| (ng/ml) | (10.5-28.4) | (9.4-21.8) | |
| PBMC TNF α production | 0.77 | 0.84 | 0.67 |
| (ng/ml) | (0.62-1.69) | (0.43-1.25) | |

Figures are median and interquartile range.

Comparison by Mann Whitney U test.

Mediator status after 3 weeks fish oil-enriched nutritional supplement

As described previously, patients tolerated the supplement well, consuming a median of 1.9 cans/day (range 1.25-2.0). The 18 evaluable patients had a median prestudy weight loss of 17.9% (interquartile range 15.9-20.7) and a rate of weight loss of 2.9kg/month (-3.9--2.1). After 3 weeks of consumption of the fish oil-enriched supplement patients had a median weight gain of 1.0kg (interquartile range -0.1-+2.0) ($p=0.024$ versus baseline).

There was no significant change in the serum concentration of IL-6, sTNF-RI, sTNF-RII or sIL-6R over the 3 week supplementation period (figure 7.1).

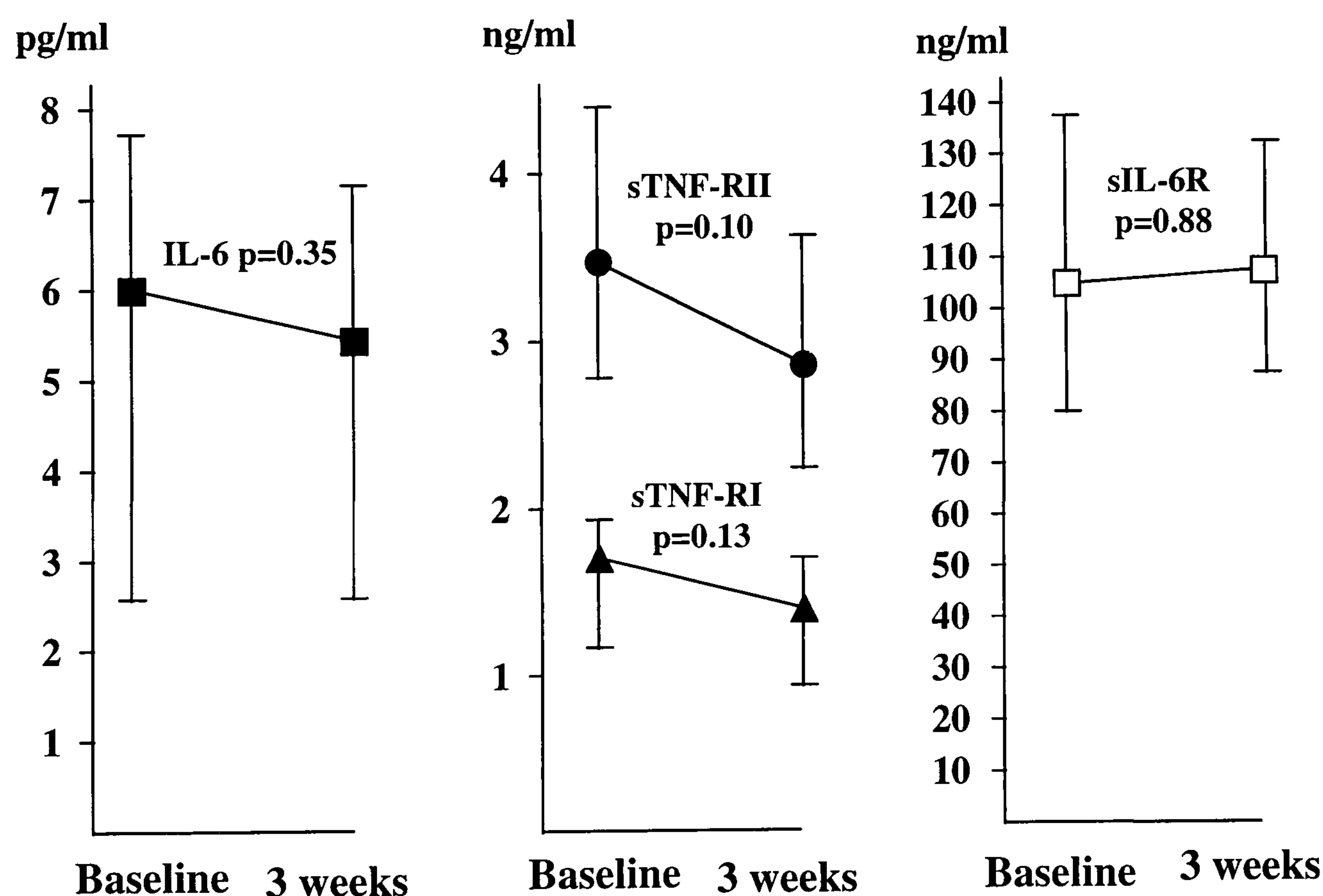


Figure 7.1. Serum concentrations of interleukin-6 (IL-6) (filled square), soluble tumour necrosis factor I (sTNF-RI) (triangle) and II (sTNF-RII) (circle) and soluble interleukin-6 receptor (sIL-6R) (open square) in 18 patients with advanced pancreatic cancer at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Graphs show median and interquartile range. Comparison by Wilcoxon signed rank test.

There was a significant fall in IL-6 production by peripheral blood mononuclear cells stimulated with lipopolysaccharide and cultured in fetal calf serum ($p=0.015$) after 3 weeks of the trial supplement. There was a trend to reduced production of IL-1 β in cells cultured under these conditions ($p=0.068$) but no change in TNF production ($p=0.55$) (figure 7.2). There was no significant change in PBMC production of the measured cytokines when cells were cultured without lipopolysaccharide or in autologous plasma.

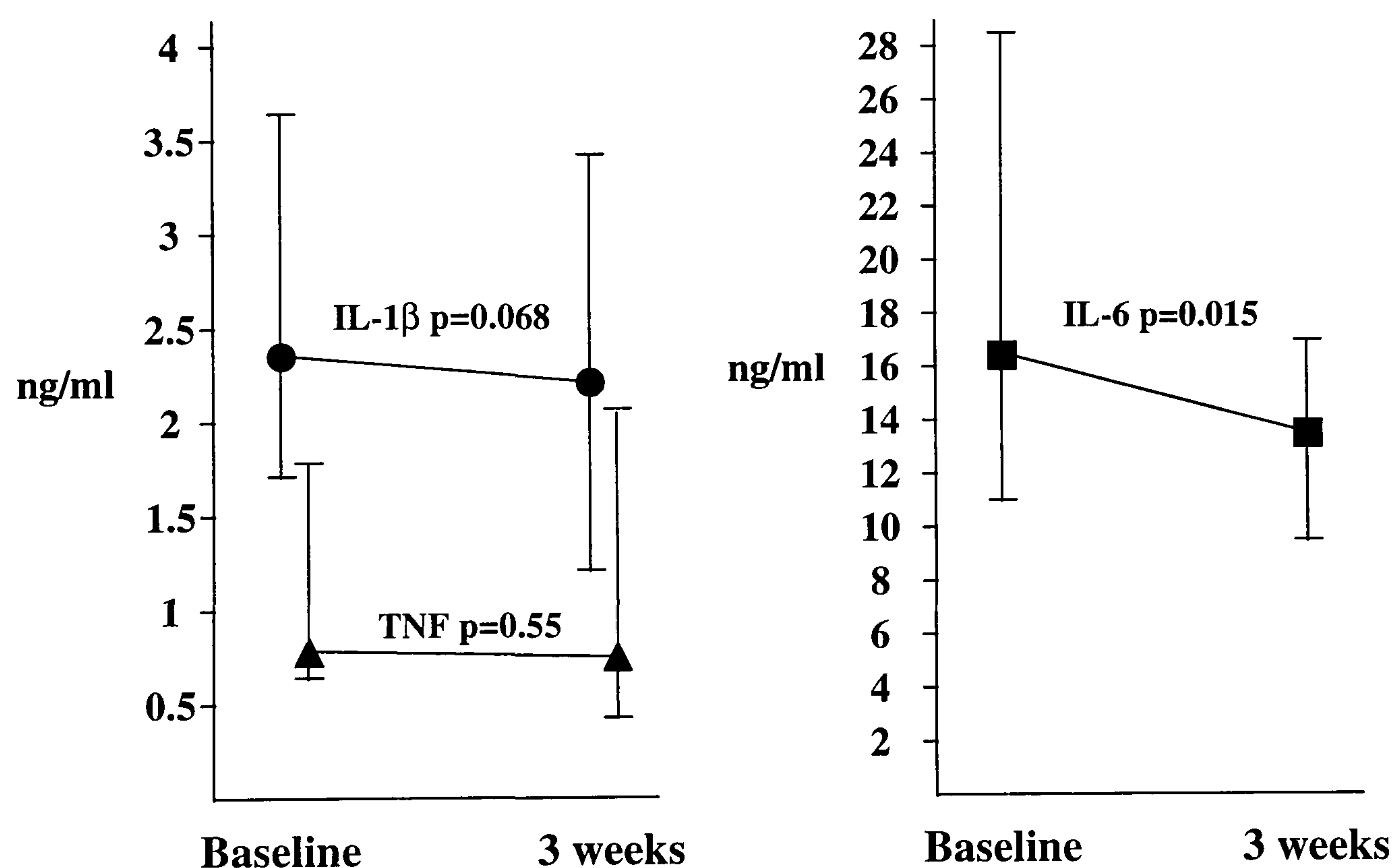


Figure 7.2. Peripheral blood mononuclear cell production of interleukin-6 (IL-6) (square), tumour necrosis factor (TNF α) (triangle) and interleukin-1 β (IL-1 β) (circle) in 18 patients with advanced pancreatic cancer at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Graphs show median and interquartile range. Comparison by Wilcoxon signed rank test.

There was no significant change in the serum concentration of leptin over the supplementation period ($p=0.38$) (figure 7.3).

There was a significant fall in the proportion of patients with detectable excretion of PIF in urine from 89% at baseline to 40% after 3 weeks of the fish oil-enriched nutritional supplement ($p=0.0022$) (figure 7.4).

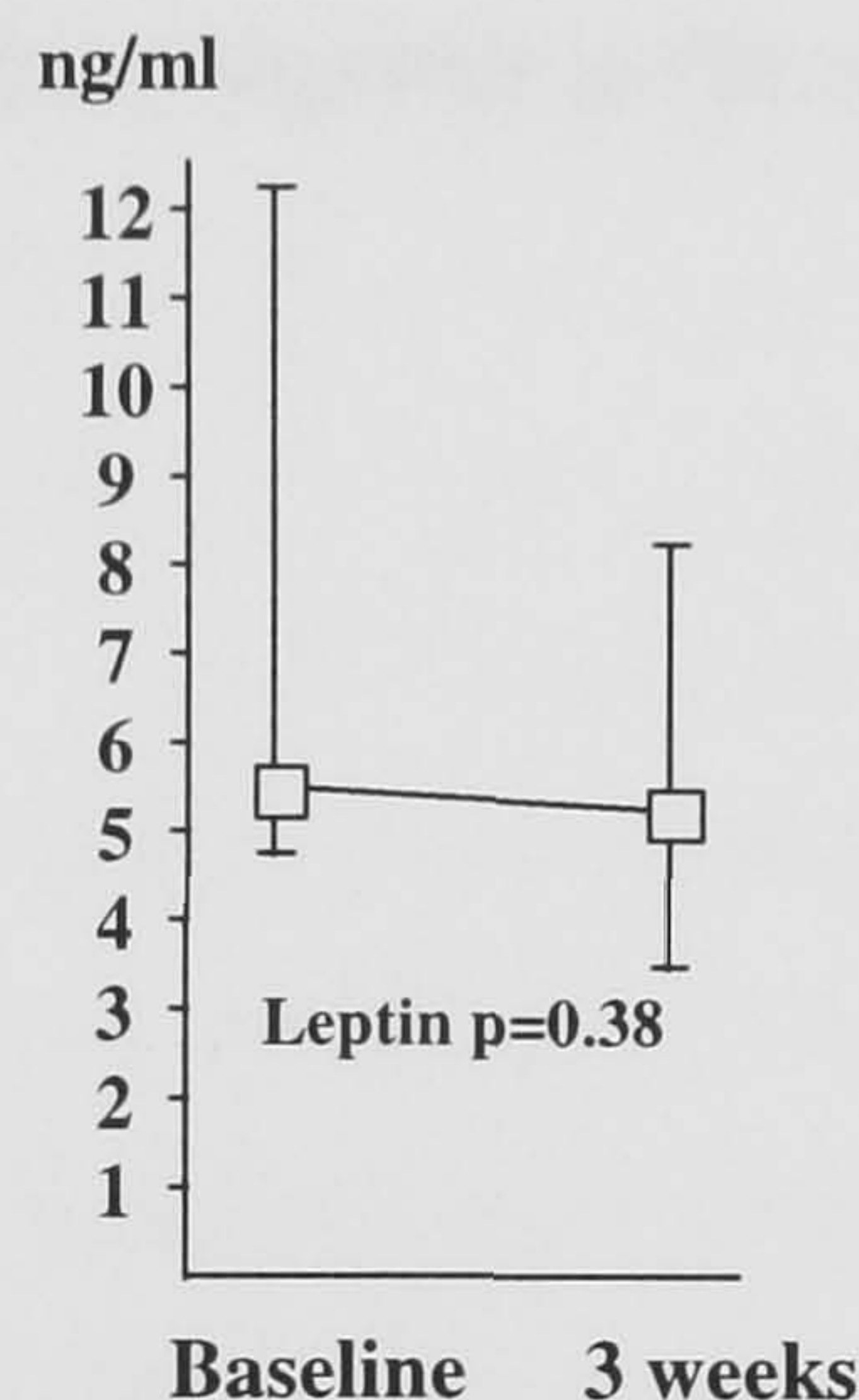


Figure 7.3. Serum concentration of leptin in 18 patients with advanced pancreatic cancer at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Graph shows median and interquartile range. Comparison by Wilcoxon signed rank test.

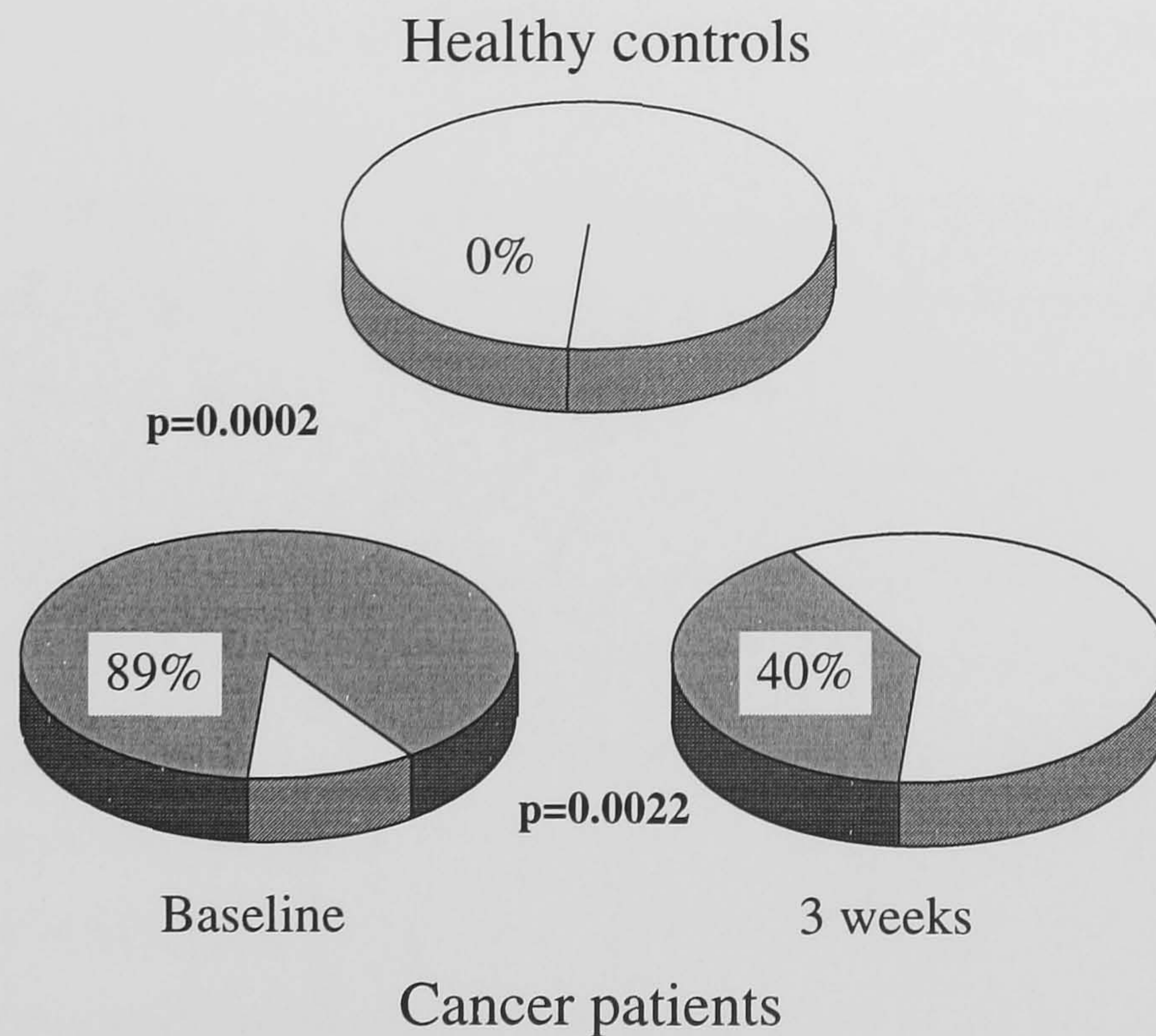


Figure 7.4. Proportion of healthy control subjects and patients with advanced pancreatic cancer excreting proteolysis inducing factor in urine at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Comparison by Chi squared test.

Relationship between serum cytokines or cytokine production and the acute phase response

There was a statistically significant relationship between the concentration of a number of cytokines and the concentration of C-reactive protein in the cancer patients (IL-6 - $r=0.50$, $p=0.0023$, TNF-R55 - $r=0.59$, $p=0.0004$, TNF-R75 - $r=0.57$, $p=0.0005$). There was also a marginal but statistically significant relationship between PBMC IL-6 production and C-reactive protein ($r=0.39$, $p=0.025$).

Further details of the acute phase response in the control and patient groups are found in Chapter 8.

Discussion

The study presented in this chapter has demonstrated that there are increases in serum pro-inflammatory cytokine concentrations and PIF excretion in weight losing pancreatic cancer patients when compared with healthy controls. Similar differences in the concentrations of IL-6 and the TNF receptors were described in an overlapping group of pancreatic cancer patients using different ELISA kits described in Chapter 4. However, the present chapter did not demonstrate any upregulation of cytokine production by PBMCs isolated from the cancer patients compared with controls (despite increased circulating concentrations of IL-6 and TNF receptors in the cancer patients' serum). It has previously been assumed that ex vivo cytokine production is a better measure of the true in vivo situation than circulating serum concentrations (Falconer et al, 1994a) and higher cytokine production has previously been shown in pancreatic cancer patients (Falconer et al, 1994a, Wigmore et al, 1997d). Cytokine production in the healthy controls was variable in the present study and it may be that this, combined with the small number of control subjects obscured any difference. The use of different batches of fetal calf serum or endotoxin may also have affected culture conditions and therefore cytokine production. Patients in the present study were recruited at a relatively early stage in their disease in an attempt to ensure survival for follow up after intervention and this may also have contributed to the difference between the present study and previous studies of apparently similar patients.

PIF was initially found to be produced by an experimental murine cancer cell line and shown to induce protein breakdown in vitro (Todorov et al, 1996a). An identical glycoprotein has been isolated from the urine of weight-losing cancer patients but not those losing weight for other reasons (Todorov et al, 1996b, Cariuk et al, 1997). In the present study no PIF excretion was observed in healthy controls while the majority of the weight-losing cancer patients did have detectable PIF in urine (in line with previous observations).

With regard to the changes observed after the administration of a fish oil-enriched nutritional supplement the present study has shown that in a group of cachectic pancreatic cancer patients the weight gain described in Chapter 6 is associated with a significant fall in peripheral blood mononuclear cell IL-6 production and a fall in the proportion of patients excreting PIF.

Fish oil and EPA have been shown to reduce production of IL-1, IL-6 and TNF α from stimulated peripheral blood mononuclear cells isolated from healthy volunteers after consuming 1-3g EPA per day for 16-24 weeks (Endres et al, 1989, Meydani et al, 1993) and of

IL-6 in pancreatic cancer patients given 6g EPA daily for 4 weeks (Wigmore et al, 1997d). Although not observed in the present study, perhaps due to reasons discussed above, production of IL-6 and TNF α may be elevated in weight-losing patients with pancreatic cancer (Falconer et al, 1994a, Wigmore et al, 1997d). The present study has shown a significant reduction in IL-6 production and a trend to a reduction in IL-1 β after 3 weeks of fish oil supplementation in pancreatic cancer patients receiving the equivalent of 2g EPA per day. There was no change in TNF α production. The significance of these findings is unclear in the light of the similarity in cytokine production between patients and controls. However, the observation that there was a weak correlation between the ex vivo production of IL-6 and serum CRP concentration from the cancer patients suggests that this mediator is of importance in determining the APPR. In addition, Chapter 3 demonstrated a tendency for progression of the acute phase response (stimulated by pro-inflammatory cytokines) with time. This stability of acute phase protein production and circulating concentrations may represent a change from the progressive upregulation in patients without intervention.

TNF α is frequently not detected in the serum of cachectic cancer patients (Socher et al, 1988) and it has been suggested that circulating concentrations of the soluble TNF receptors may give a better estimate of tissue TNF α production (Olsson et al, 1993). The role of soluble IL-6 receptors in the inflammatory state remains unclear, although it has been suggested that it may help to mediate the IL-6 signal in cells lacking membrane receptors (Taga et al, 1989). However, the present study did not reveal any reduction in the concentrations of IL-6, sIL-6R or TNF receptors in pancreatic cancer patients administered a fish oil-enriched nutritional supplement despite these cytokines being found in elevated concentrations in the cancer patients. IL-1 β was only detectable in the serum of two of the patients in the present study. The relationship of serum IL-6 and the TNF receptors with CRP concentration observed in the present study is similar to that described in Chapter 4.

The present study found no change in the concentration of leptin after 3 weeks receiving the trial supplement. Leptin is a hormone produced by fat which decreases food intake and increases energy expenditure (Zhang et al, 1994). It has been suggested that leptin concentrations are increased in inflammatory states, thus contributing to weight loss in animal models (Grunfeld et al, 1996, Sarraf et al, 1997). However, leptin concentrations would appear to be appropriately low in human cancer cachexia (Simons et al, 1997). Body composition analysis of patients in the present study suggested that the weight gained was largely lean tissue with no change observed in fat mass (see chapter 6). Thus no great change in leptin concentrations would have been expected.

EPA has been shown to inhibit muscle protein breakdown by PIF in vitro (Tisdale et al, 1996). The present study has shown that the administration of a fish oil-enriched nutritional supplement results in a reduction in the proportion of patients excreting PIF in the urine perhaps suggesting that EPA will not only inhibit the end-organ effects of PIF but also reduce its production.

The present chapter has demonstrated that the positive changes in weight and body composition produced by the fish oil-enriched nutritional supplement in cachectic cancer patients are associated with down-regulation of some mediators of the metabolic response to cancer which have previously been suggested to prevent weight gain in such patients. Modulation of these mediators by the fish oil component of the supplement may explain this beneficial effect.

The next chapter describes details of the acute phase protein response in pancreatic cancer patients without intervention and after receiving the fish oil-enriched nutritional supplement.

Progression of the acute phase response in cancer patients and the effects of a fish oil-enriched nutritional supplement

Summary

The presence of an acute phase protein response (APPR) has been suggested to shorten survival and contribute to weight-loss in patients with pancreatic cancer. Fatty acids derived from fish oil have been shown to alter pro-inflammatory cytokine production and acute phase protein synthesis in vitro. The study presented in this chapter was designed to determine the effects of a fish oil-enriched nutritional supplement upon the concentrations of a range of individual acute phase proteins in patients with advanced pancreatic cancer. 18 patients with pancreatic cancer received the supplement (providing 2 g eicosapentaenoic acid and 1 g docosahexaenoic acid per day) for 3 weeks while another 18 received full supportive care alone. Six healthy subjects served as additional controls. Acute phase proteins were measured before and after the 3 week intervention period in cancer patients. At baseline albumin, transferrin and pre-albumin were significantly reduced and fibrinogen, haptoglobin, α -1-acid glycoprotein, α -1-antitrypsin, ceruloplasmin and C-reactive protein (CRP) were significantly elevated in the cancer patients compared with healthy controls (reflecting their roles as negative and positive acute phase proteins respectively). In the supplemented cancer group the only significant change in acute phase protein concentrations over the 3 week study period was an increase in transferrin. In the control cancer group there were further significant reductions in albumin, transferrin and pre-albumin and a significant increase in CRP concentration. These results suggest that many positive and negative acute phase proteins are altered in advanced pancreatic cancer. The APPR tends to progress in untreated patients but may be stabilized by the administration of a fish oil-enriched nutritional supplement. This may have implications upon the progress of wasting in such patients and may help explain the nutritional benefits of the fish oil-enriched supplement described in Chapter 6.

Introduction

The previous chapter described a reduction in IL-6 production (known to stimulate the acute phase protein response) with the administration of a fish oil-enriched nutritional supplement. However, in Chapter 6, although a reversal of weight loss with this supplement was demonstrated, there was no significant change in the concentration of the positive acute phase protein, C-reactive protein (CRP). This would appear counter to the hypothesis that if the inflammatory state and acute phase protein response were key metabolic processes preventing weight gain with conventional nutrition in cancer then the arrest of weight loss would be associated with an attenuation of the acute phase response. However, Chapter 3 suggested that there is a progressive rise in CRP in untreated cachectic cancer patients. This progressive rise was not observed in the Chapter 6 and thus may have reflected a change from the expected progression of the APPR, modulated by the supplement.

The acute phase protein response (APPR) is seen following a wide variety of insults to the body including trauma, infection, inflammation and cancer. C-reactive protein (CRP) is the archetypal positive acute phase protein in humans (Baumann & Gauldie, 1994) and is widely used as a marker for the acute phase response in clinical practice. Previous studies have shown that at diagnosis about 40% of patients with pancreatic cancer exhibit an elevated serum level of C-reactive protein. This proportion increases until about 80% have an elevated CRP level close to the time of death (Falconer et al, 1994a). Similar findings were described in Chapter 3. An elevated CRP level has been found to be an independent and robust predictor of poor survival in pancreatic cancer (Falconer et al, 1995) and this was confirmed in Chapter 5.

In the human hepatocyte the production of acute phase proteins is regulated by interleukin (IL)-6 and other pro-inflammatory cytokines (Castell et al, 1990). Thus the level of the acute phase response in vivo may be taken as indirect evidence of pro-inflammatory cytokine production. The work presented in Chapter 4 supports this assumption. From studies in animal models a variety of pro-inflammatory cytokines have been strongly implicated in both the anorexia and altered metabolism thought to contribute to cancer cachexia (Tracey et al, 1988, Yasumoto et al, 1995). However, it is not known whether the loss of lean tissue in cachexia is due to a direct effect of cytokines at the level of skeletal muscle or whether net loss of muscle protein is due to indirect effects of altered metabolism elsewhere or a combination of these two.

It has been suggested that with the induction of the acute phase response in the face of an inadequate dietary intake, the imbalance in amino acid composition between skeletal muscle and acute phase proteins may contribute to increased loss of muscle (Reeds et al, 1994). Clearly for this mechanism to be important there would have to be a uniform upregulation of the positive acute phase proteins whose serum concentrations are measured in gram quantities and that have a high turnover rate. However, there is little information on the range of acute phase proteins that are upregulated in cachectic cancer patients.

Cancer cachexia has proven resistant to intervention with supplemental enteral or parenteral nutrition (Nixon et al, 1981, Ovesen et al, 1993). This may be due to metabolic changes, including the APPR, which are, at least in part, stimulated by increased tumour or host production of pro-inflammatory cytokines (Castell et al, 1990, Falconer et al, 1994, Strassmann et al, 1993, Yasumoto et al, 1995). We have previously shown that fish oil or eicosapentaenoic acid (EPA), a polyunsaturated fatty acid derived from fish oil, administered to patients with advanced pancreatic cancer may attenuate weight-loss whilst down-regulating production of IL-6 and serum levels of CRP (Wigmore et al, 1996, Wigmore et al, 1997d). In vitro work has suggested that EPA may have differential effects on a spectrum of acute phase proteins produced in response to IL-6 (Wigmore et al, 1997e).

The present chapter was designed to explore the effect of the administration of a nutritional supplement enriched with fish oil, rich in EPA, upon progress of the acute phase protein response in patients with advanced pancreatic cancer in comparison with patients receiving full supportive care alone, looking at the levels of several individual acute phase proteins.

Methods

Subjects

After informed consent and local ethical committee approval, 36 weight-losing patients with a diagnosis of advanced pancreatic cancer based on histological or unequivocal radiological or operative findings were studied and compared with 6 healthy individuals. Weight was measured with subject in light clothing without shoes using a beam scale. No subjects had clinically evident oedema or ascites.

All subjects underwent venous blood sampling at least three weeks after surgery or bile duct stenting. The initial 18 patients received full supportive care alone and underwent a further venous blood sample after a median of 28 days. The subsequent 18 patients received 2 cans per day of a nutritional supplement enriched with fish oil providing a total of 2.6 MJ, 32.2 g protein, 99.4 g carbohydrate, 13 g fat, 2.18 g EPA and 0.92 g docosahexaenoic acid (DHA) in a volume of 480 mL as described previously. After a median of 24 days a second venous blood sample was taken. (The two patients who were unable to be reassessed after consumption of the supplement as described in Chapter 6 were not considered in the current chapter).

Samples were assessed for the concentration of the positive APP C-reactive protein, α -1-antitrypsin, α -1-acid glycoprotein, haptoglobin and ceruloplasmin and the negative APP, albumin, prealbumin and transferrin, at both timepoints. The positive APP fibrinogen was measured at baseline only.

Results

Subject characteristics

Characteristics of the patient groups and controls are shown in table 8.1. Patients were well matched for age and disease stage. Cancer patients were slightly older than healthy controls.

Table 8.1. Characteristics of cancer patients and healthy controls.

| | n | Age | Prestudy weight loss (%) | UICC disease stage | | |
|---|----|----------------|--------------------------------|-----------------------|---|---|
| | | | | 2 | 3 | 4 |
| Cancer patients receiving fish oil-enriched supplement | 18 | 64 (56-66) | 17.9 (15.9-20.7) | 8 | 2 | 8 |
| Cancer patients receiving supportive care | 18 | 60 (54-70) | 11.8 (5.6-23.5) | 10 | 2 | 6 |
| Healthy controls | 6 | 54* (50-56) | 0 | — | | |

Values are median (interquartile range)

*p=0.017 between all cancer patients and healthy controls by Mann Whitney U test

Acute phase protein concentrations in cancer patients compared with healthy controls

Serum concentrations of the negative acute phase proteins, albumin, prealbumin and transferrin were significantly lower in cancer patients than healthy controls (figure 8.1).

Concentrations of the positive acute phase proteins, CRP, fibrinogen, α -1-antitrypsin, α -1-acid glycoprotein, haptoglobin and ceruloplasmin were significantly higher in cancer patients than healthy controls.

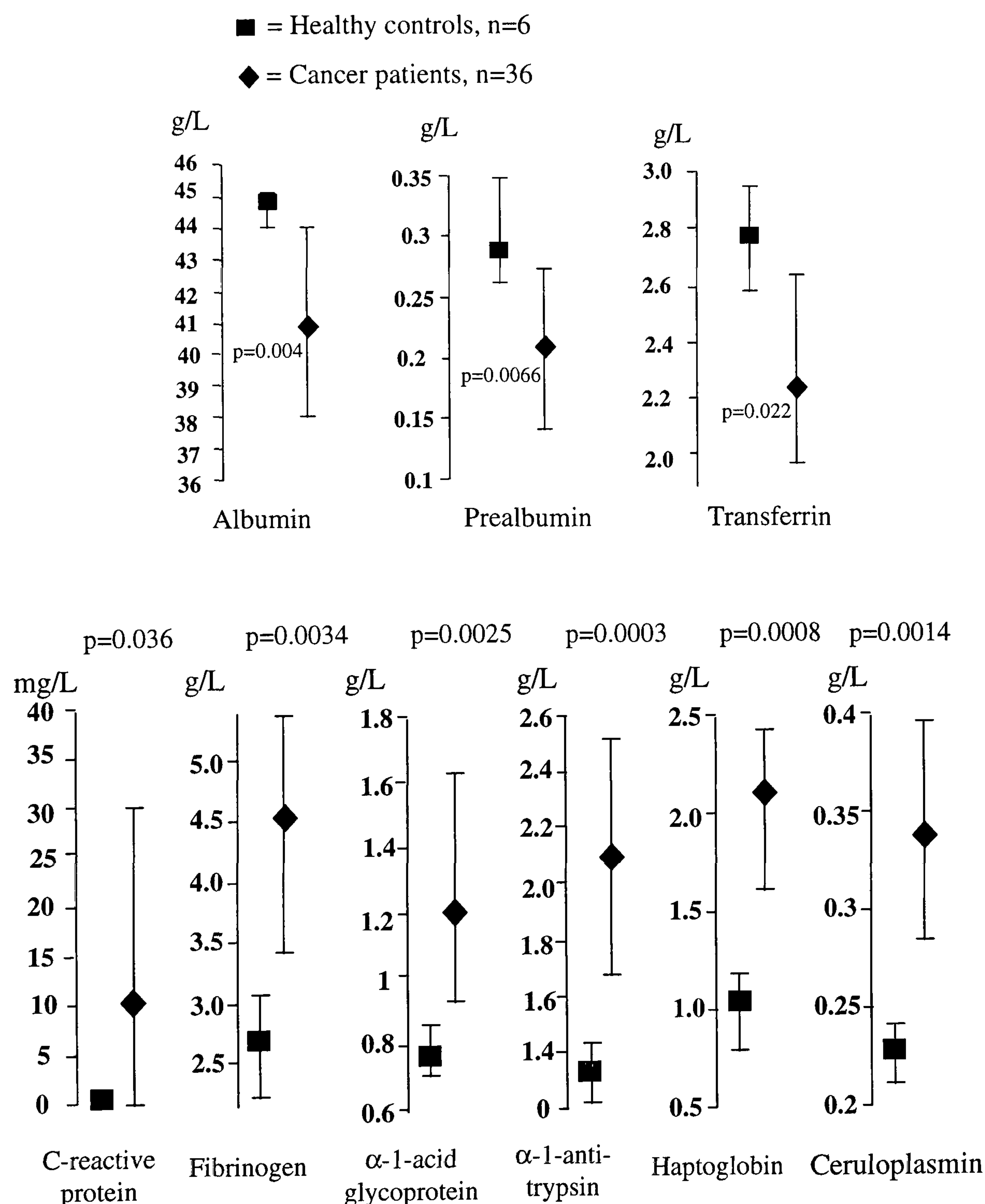


Figure 8.1. Concentrations of acute phase proteins in 36 patients with advanced pancreatic cancer and 6 healthy controls. Graphs show median and interquartile range. Comparison by Mann-Whitney U test.

Effect of feeding nutritional supplement enriched with fish oil on acute phase protein concentrations and nutritional status in cancer patients

Patient groups were well matched for baseline serum levels of acute phase proteins although ceruloplasmin concentration was significantly higher in patients receiving supportive care alone (table 8.2).

Table 8.2. Baseline serum levels of acute phase proteins from patients with advanced pancreatic cancer who subsequently received nutritional supplement enriched with fish oil and those who went on to receive full supportive care alone.

| | Cancer patients receiving supplement | Cancer patients receiving supportive care |
|--|---|--|
| C-reactive protein (mg/L) | <5 | 11 |
| [<5] | (<5-21) | (<5-30) |
| α -1-acid glycoprotein (g/L) | 1.12 (0.89-1.39) | 1.33 (0.94-2.10) |
| [0.55-1.4] | | |
| α -1-antitrypsin (g/L) | 2.07 (1.62-2.33) | 2.22 (1.76-2.73) |
| [1.1-2.3] | | |
| Haptoglobin (g/L) | 2.01 (1.45-2.21) | 2.26 (1.75-2.63) |
| [0.5-2.0] | | |
| Ceruloplasmin (g/L) | 0.30 (0.27-0.35) | 0.38* (0.32-0.44) |
| [0.15-0.3] | | |
| Albumin (g/L) | 41 (38-43) | 41 (35-44) |
| [36-47] | | |
| Prealbumin (g/L) | 0.20 (0.14-0.22) | 0.22 (0.13-0.30) |
| [0.15-0.4] | | |
| Transferrin (g/L) | 2.17 (1.89-2.61) | 2.38 (2.01-2.75) |
| [2.2-4.0] | | |
| Total positive APP (g/L) | 5.53 (4.74-6.38) | 6.28 (5.10-7.40) |
| Total negative APP (g/L) | 43.6 (40.2-45.4) | 43.7 (36.4-46.8) |

Normal range in squared brackets Values are median (interquartile range)

*p=0.035 comparison between patient groups by Mann Whitney U test

There was a significant difference in the pattern of weight loss between the two groups with those receiving the supplement gaining a median of 1kg (as shown in chapter 6) while those receiving supportive care lost a median of 2.8kg (table 8.3).

Table 8.3. Baseline weight and change in weight in patients with advanced pancreatic cancer receiving nutritional supplement enriched with fish oil and those receiving full supportive care only.

| | Initial weight (kg) | Change in weight (kg) |
|---|------------------------|--------------------------|
| Cancer patients receiving supplement | 55.0 (46.5-60.5) | +1.0 (-0.1-+2.0) |
| Cancer patients receiving supportive care | 58.5 (47.8-70.7) | -2.8* (-3.7--1.7) |

Values are median (interquartile range)

*p=0.0001 comparison between patient groups by Mann Whitney U test

There were significant differences in the changes observed in the serum concentrations of CRP, albumin, prealbumin and transferrin between supplemented and unsupplemented cancer patients over the study period (figure 8.2).

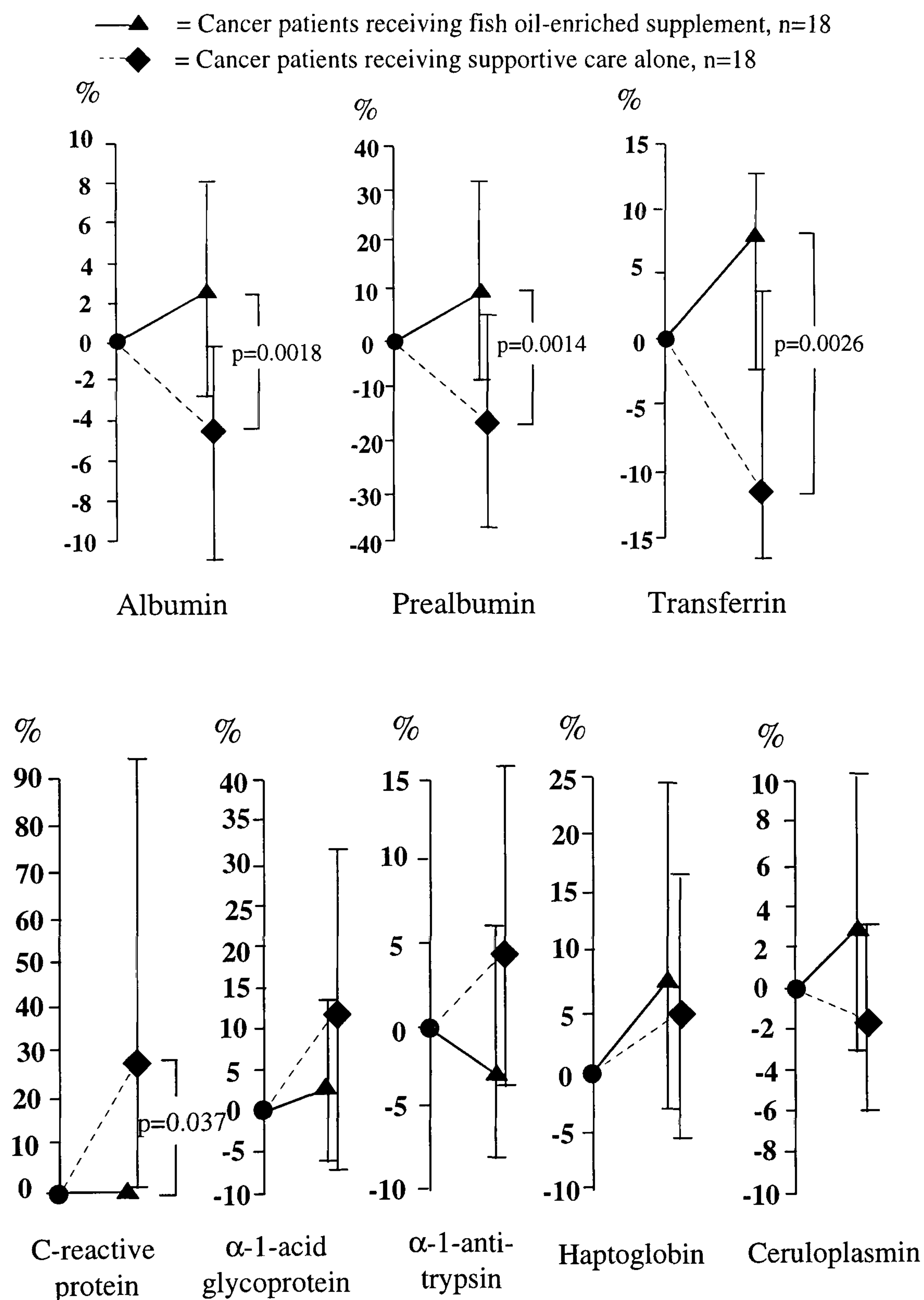


Figure 8.2. Percentage changes in concentrations of acute phase proteins in weight losing patients with advanced pancreatic cancer given either nutritional supplement enriched with fish oil or full supportive care. Graphs show median and interquartile range. Comparison between groups by Mann-Whitney U test.

In patients receiving the nutritional supplement enriched with fish oil, there were no significant changes from baseline in serum acute phase protein levels over the assessment period apart from a rise in the concentration of the negative acute phase protein transferrin ($p=0.031$). In patients receiving full supportive care, there was a further increase in the concentration of the positive acute phase protein CRP ($p=0.0013$) and a further reduction in the concentration of the negative acute phase proteins albumin, prealbumin and transferrin ($p=0.012$, 0.0048 and 0.038 respectively).

Overall changes in concentration of acute phase proteins in cancer patients

Total measured positive acute phase proteins did not change significantly in each group although there was a trend towards an increase in the cancer patients who received supportive care alone ($p=0.07$). However, there was a marked difference in the pattern of total production of negative acute phase proteins between the two patient groups with a significant increase in those receiving the nutritional supplement enriched with fish oil ($p=0.048$) and a significant decrease in those receiving supportive treatment ($p=0.016$). Thus, the difference between the groups was highly significant ($p=0.0012$) (table 8.4).

Table 8.4. Changes in total production of positive and negative acute phase proteins in patients with advanced pancreatic cancer receiving nutritional supplement enriched with fish oil and those receiving full supportive care only over the 4 week study period.

| | Change in total positive acute phase proteins (g/L) | Change in total negative acute phase proteins (g/L) |
|--|--|--|
| Cancer patients receiving supplement | -0.02 (-0.19-+0.53) | +1.32 (-1.07-+2.95) |
| Cancer patients receiving supportive care | +0.42 (-0.30-+1.24) | -2.44* (-5.72-+0.28) |

Measured positive acute phase proteins were C-reactive protein, α -1-antitrypsin, α -1-acid glycoprotein, haptoglobin and ceruloplasmin. Measured negative acute phase proteins were albumin, prealbumin and transferrin.

Values are median (interquartile range)

* $p=0.0012$ comparison between patient groups by Mann Whitney U test

Discussion

In the present study the concentrations of CRP, fibrinogen, α -1-antitrypsin, α -1-acid glycoprotein, haptoglobin and ceruloplasmin were significantly higher in cancer patients than healthy controls whilst albumin, prealbumin and transferrin were significantly lower. This finding reflects the role of these proteins as positive and negative acute phase proteins respectively (Baumann & Gauldie, 1994) and confirms the tendency for patients with advanced cancer to exhibit an APPR as observed previously in pancreatic and other malignancies (Falconer et al, 1994, Staal-van den Brekel et al, 1995, Wayman et al, 1997).

It is possible that the so-called negative acute phase reactants (Albumin, prealbumin and transferrin) might also have been reduced as a result of the relative protein-calorie malnutrition observed in the cancer patients. Indeed, following administration of the fish oil enriched-supplement the weight of patients increased as did transferrin concentration and this may reflect the role of the latter as a fast-turnover hepatic protein sensitive to short-term improvements in nutritional status.

Although hepatocyte albumin secretion may be reduced in vitro by pro-inflammatory cytokines (Castell et al, 1990) it has been demonstrated recently that in cachectic cancer patients with hypoalbuminaemia and an ongoing APPR, albumin synthesis rates are unaltered (Fearon et al, 1998 and see also chapter 10). Thus the low serum albumin concentration observed in the present study is likely to reflect either an increased transcapillary escape rate, an increased degradation rate or a combination of the two. Transcapillary escape has been shown to occur at an elevated rate in weight-losing cancer patients and has been suggested to contribute to the hypoalbuminemia of the acute phase response (Fleck et al, 1985). However, lymphatic return must be similarly increased as there is no relationship between transcapillary escape and albumin concentration (Ballmer et al, 1994) and no change in the intravascular albumin pool with elevated transcapillary escape (Ballmer-Weber et al, 1995).

The demand for specific amino acids to manufacture positive acute phase proteins in patients with an inadequate nutritional intake has been suggested to contribute to ongoing muscle protein breakdown (Reeds et al, 1994) and in a prolonged APPR this may lead to accelerated weight-loss and a shortened survival. Although the present study was not a formal randomized trial, cancer patients who received the fish oil-enriched nutritional supplement had no change in the concentration of total positive acute phase proteins but total negative acute phase proteins increased compared with patients receiving supportive care alone. This suggests either an attenuation of the APPR, an improvement in nutritional status or, more

likely, a combination of the two. The patients receiving supportive care alone had no significant change in total positive acute phase proteins but sustained a further fall in negative acute phase proteins. This suggests either progression of the APPR, a further reduction in nutritional status or, again, a combination of the two. It has been suggested, however, that the negative acute phase proteins are not good indicators of nutritional status in the presence of an acute phase response (Fleck, 1989).

The supplement used in this study contained appreciable amounts of the DHA in addition to EPA. DHA is a closely related polyunsaturated fatty acid, also of the n-3 class, which is metabolised to EPA *in vivo* (von Schacky & Weber, 1985). The relative contribution of these two agents to the effects observed in the present study is not clear. In addition, it is likely that the patients receiving the supplement consumed more calories and protein than those receiving supportive care so it is not possible to discriminate an effect of EPA on the APPR and hence nutritional status from a direct effect of the calories and protein supplied. However, previous studies of conventional oral supplements in cancer patients have demonstrated no effect on either serum albumin concentration or body weight (Evans et al, 1987, Ovesen et al, 1993) suggesting that the effects observed in the present study are at least in part due to the fish oil component of the supplement. EPA at a dose of 6g daily has been shown to reduce CRP concentrations in pancreatic cancer patients over 4 weeks (Wigmore et al, 1997). Fish oil preparations providing between 1 and 4g EPA daily have also been shown to reduce plasma fibrinogen concentrations in healthy and hyperlipidaemic subjects after as little as 3 weeks (Høstmark et al, 1988, Radack et al, 1989, Saynor & Gillott, 1992).

The link between the concentration of various plasma proteins and changes in whole body nitrogen kinetics is complex. Under normal circumstances hepatic protein synthesis is thought to contribute about 15-20% of whole body protein turnover. During an APPR the contribution of hepatic protein synthesis is thought to rise, with the synthesis of individual acute phase reactants such as fibrinogen increasing markedly (Preston et al, 1998 and see also chapter 10). The measured concentration of a plasma protein is the end result of a variety of processes including synthesis, degradation and distribution between the extravascular and intravascular pools. Thus although synthesis might increase, if degradation also increases the net effect may be no change in the plasma concentration but a significant alteration in overall protein turnover. If the latter process is not 100% efficient, this would result in net protein loss from the body and accelerated wasting. It has been previously demonstrated that in fasting cancer patients with an ongoing APPR, fibrinogen synthesis is increased by around 200% while the plasma concentration is increased by some 50% (Preston et al, 1998). However,

while the rate of albumin synthesis is unchanged plasma concentrations are may be decreased by around 25% (Fearon et al, 1998). Thus further interpretation of the changes in acute phase protein concentrations in the present study requires further evaluation with kinetic studies in order to determine their true relation to nutritional status in general and nitrogen economy in particular. Unfortunately, examination of synthesis and, more so, degradation of many acute phase proteins is methodologically extremely difficult due to their relatively low circulating concentrations. Further studies of the synthesis of albumin and fibrinogen in cancer patients are found in Chapter 10.

Several explanations exist for the mechanism of the apparent stabilisation of the APPR in patients receiving the fish oil-enriched supplement observed in the present study. In vitro, the APPR is largely regulated by IL-6 (Castell et al, 1990). It has been shown previously that patients with advanced pancreatic cancer exhibiting an APPR have elevated production of IL-6 by isolated peripheral blood mononuclear cells (Falconer et al, 1994a) (although this was not observed in chapter 7) and that the administration of a pure preparation of EPA will reduce this production of IL-6 to control levels (Wigmore et al, 1997d). The previous chapter demonstrated a decrease in IL-6 production in the intervention group in the present study. The production of pro-inflammatory cytokines and APPs is controlled to some extent by the transcription factor NF- κ B (Beauparlant and Hiscott 1996). Polyunsaturated fatty acids have been shown to activate NF- κ B but initial work suggests that EPA has little effect on this pathway in vitro (Camandola et al, 1996). The administration of polyunsaturated fatty acids to hepatocytes in culture has suggested that EPA may have direct effects on the modulation of APP production (Wigmore et al, 1997e). Additional elucidation of the mechanism of action of the fish oil-enriched supplement used in the present study and the relative contributions of its components requires further study.

A randomised study would have been preferable. A sequential design was used as the trial preparation was not available initially, thus patients who would have been recruited for interventional studies were studied with best supportive care in the meantime.

In summary, the present study suggests that the improvements in nutritional status and the modulation of metabolic mediators produced by a fish oil-enriched nutritional supplement in weight losing patients with advanced cancer (described in Chapters 6 and 7), occurs in association with the prevention of progression of the APPR.

The next chapter examines the metabolic response to feeding in cachectic cancer patients and the effects of the fish oil-enriched supplement on the response to feeding.

The metabolic response to feeding in cancer patients and the effects of a fish oil-enriched nutritional supplement

Summary

Cachectic patients with advanced cancer often fail to gain weight even when additional nutrients are supplied. Increased energy expenditure has been reported in cancer patients and an increased metabolic response to feeding may prevent the effective use of additional nutrients supplied. However, the work presented in Chapters 6, 7 and 8 has suggested that eicosapentaenoic acid (EPA) -based preparations may elements of the metabolic changes of cancer cachexia. This hypothesis is examined further in the present chapter.

Sixteen weight-losing, non-diabetic patients with unresectable pancreatic adenocarcinoma were compared with six healthy, weight-stable controls. After baseline indirect calorimetry, subjects received four hourly meals of a liquid feed, each providing a twelfth of the estimated total daily energy requirement. Indirect calorimetry and measurement of serum insulin and glucose concentrations were performed regularly over the four hour feeding period. Body composition was estimated by bioimpedence analysis and urine collection allowed calculation of urinary nitrogen excretion. Cancer patients were then given a fish oil-enriched nutritional supplement providing 2g of EPA and 600kcal daily and underwent repeat metabolic study after 3 weeks.

At baseline, overall resting energy expenditure was not significantly different between patients and controls. However, energy expenditure per kg body weight, lean body mass or body cell mass was significantly greater in the cancer patients. Changes in insulin and glucose concentrations in the cancer patients over the feeding period suggested relative glucose intolerance. The percentage change in the area under the curve of energy expenditure was significantly lower in the cancer patients (median 7.9% (interquartile range 3.4-9.0)) than controls (12.6% (9.9-15.1), $p=0.0051$). Fat oxidation was significantly higher in the fasting state in the cancer patients (median 1.26g/kg/min (interquartile range 0.95-1.38) than controls (0.76g/kg/min (0.62-0.92), $p=0.020$).

After 3 weeks of consuming the fish oil-enriched supplement the weight of the cancer patients increased and there was a statistically significant increase in the energy expenditure in

response to feeding (9.6% (6.3-12.4)) such that it was no longer different from baseline values in healthy controls ($p=0.073$). Similarly, fasting fat oxidation fell to 1.02g/kg/min (0.8-1.18), no longer significantly different from baseline healthy control values ($p=0.1$).

Thus, while it would appear that weight-losing patients with advanced pancreatic cancer have an increased resting energy expenditure they do not exhibit any increase in the metabolic cost of feeding (to account for their lack of anabolic response to feeding) but in fact show a reduction appropriate to their wasted state. In addition, provision of a fish oil-enriched nutritional supplement resulted in some normalisation of the metabolic state. This work also emphasises the importance of considering the fed state when examining metabolic changes in disease.

Introduction

As discussed in Chapters 1 and 3, weight loss is a major cause of morbidity and mortality in patients with advanced cancer (Warren et al, 1935, Ovesen et al, 1993a). Such cancer patients often have a reduced dietary intake. However, while it is possible to increase energy intake by enteral or parenteral means, this seems to have no impact on patients' progressive weight loss (Nixon et al, 1981, Evans et al, 1987, American College of Physicians, 1989, Veterans Administration, 1991, Ovesen et al, 1993b). This has led to the suggestion that there is a metabolic block to the accretion of lean tissue in patients with cancer (Nixon et al, 1981, Moldawer and Copeland, 1997). The role of mediators of this metabolic block such as pro-inflammatory cytokines, alterations in the balance of neuroendocrine hormones and specific, tumour-derived proteolytic and lipid mobilising factors have been discussed in Chapters 1 and 7. It has been reported that weight-losing patients with advanced cancer may have an increased resting energy expenditure, particularly those with an acute phase protein response (Falconer et al, 1994, Staal-van den Brekel et al, 1994). This has been suggested to be due to an increase in futile metabolic cycles such as the Cori cycle (Edén et al, 1984) or mitochondrial uncoupling proteins which produce heat and are stimulated in inflammatory models with similar mediators to cancer (Faggioni et al, 1998). However, another component of energy expenditure is that associated with the digestion of food and to date there has been little attempt to study the metabolic response to feeding in cachectic patients. An exaggerated response to feeding may to some extent explain the lack of an anabolic response of such patients to the provision of additional food.

In Chapters 6, 7 and 8 it was shown that a nutritional supplement containing eicosapentaenoic acid (EPA), an n-3 polyunsaturated fatty derived from fish oil, will reverse weight loss and reduce energy expenditure in a group of pancreatic cancer patients and will affect the production and action of a number of mediators of cachexia such as interleukin-6 and proteolysis inducing factor, and also stabilise the acute phase protein response. It is possible, therefore, that such a preparation will modulate the metabolic response to feeding.

The present chapter examines the metabolic response to feeding in cachectic cancer patients (excluding those with diabetes) compared with healthy controls and also determines the effect on the response to feeding of the administration of a nutritional supplement containing eicosapentaenoic acid for three weeks in the same cancer patients.

Materials and methods

Subjects

Sixteen patients with an unequivocal diagnosis of pancreatic cancer who were losing weight with no clinical evidence of ascites, peripheral oedema, diabetes mellitus or malabsorption were examined in the present study (two patients with diabetes mellitus studied in previous chapters were excluded). Six weight-stable healthy individuals served as controls. None of the patients received chemotherapy or radiotherapy and none had undergone surgery in the preceding 4 weeks. No patients had clinical or radiological evidence of infection, were jaundiced or severely anemic, or were receiving steroids. The study was approved by the local ethical committee and all subjects gave written informed consent.

Metabolic study protocol

The study protocol is presented diagrammatically in figure 9.1. Subjects attended at 8am on two consecutive days. On the first day, following an overnight fast, the subject rested in a supine position for at least 30 minutes and underwent indirect calorimetry using a ventilated hood technique to allow measurement of energy expenditure and substrate utilisation. A venous catheter was inserted into the subject's antecubital fossa and baseline blood samples were collected for assessment of insulin, cortisol and glucose. C-reactive protein was also measured to determine the presence or absence of an acute phase protein response which was taken to be present if C-reactive protein concentration was 10mg/l or greater.

Subjects were weighed on spring balance scales without shoes and wearing light clothing. Body composition was measured by bioelectrical impedance analysis.

Energy expenditure was measured by indirect calorimetry every 40 minutes over the subsequent 4 hours for 20 minutes. The second 10 minute period of each measurement was used to calculate energy expenditure. Subjects underwent blood sampling for measurement of serum insulin and glucose every 60 minutes during this 4 hour period.

On the second day, following an overnight fast subjects a venous catheter was again inserted into the antecubital fossa for blood sampling. Subjects then received hourly meals for four hours of a balanced whole protein liquid nutritional supplement (Fortisip, Nutricia, Zoetermeer, Holland) providing a twelfth of the subjects estimated energy requirement (measured resting energy expenditure * 1.4) (Hunter et al, 1995). The supplement provided 13% of energy from protein, 48% from carbohydrate and 39% from fat, similar to a "typical" British diet. The

subject continued to rest in the supine position during the feeding period. Energy expenditure was measured by indirect calorimetry every 40 minutes as on the previous day and subjects underwent blood sampling for measurement of serum insulin and glucose every 30 minutes during the feeding period.

Patients collected urine for the 24 hours prior to attendance. After emptying their bladders on arrival, subjects provided a urine sample over the four hour study period on each day to provide estimation of fasting and fed urinary nitrogen excretion.

The daily dietary intake of subjects was measured based on the mean dietary intake recorded in a 3 day food diary.

Fish oil-enriched nutritional supplement

After baseline metabolic study, patients were asked to consume a nutritional supplement enriched with fish oil (providing 610kcal, 32.2g protein, 2.2g EPA and 0.96g docosahexaenoic acid (DHA) per day) for 3 weeks. The composition of this product is shown in appendix 2. After the 3 week supplementation period patients attended for repeat metabolic assessment.

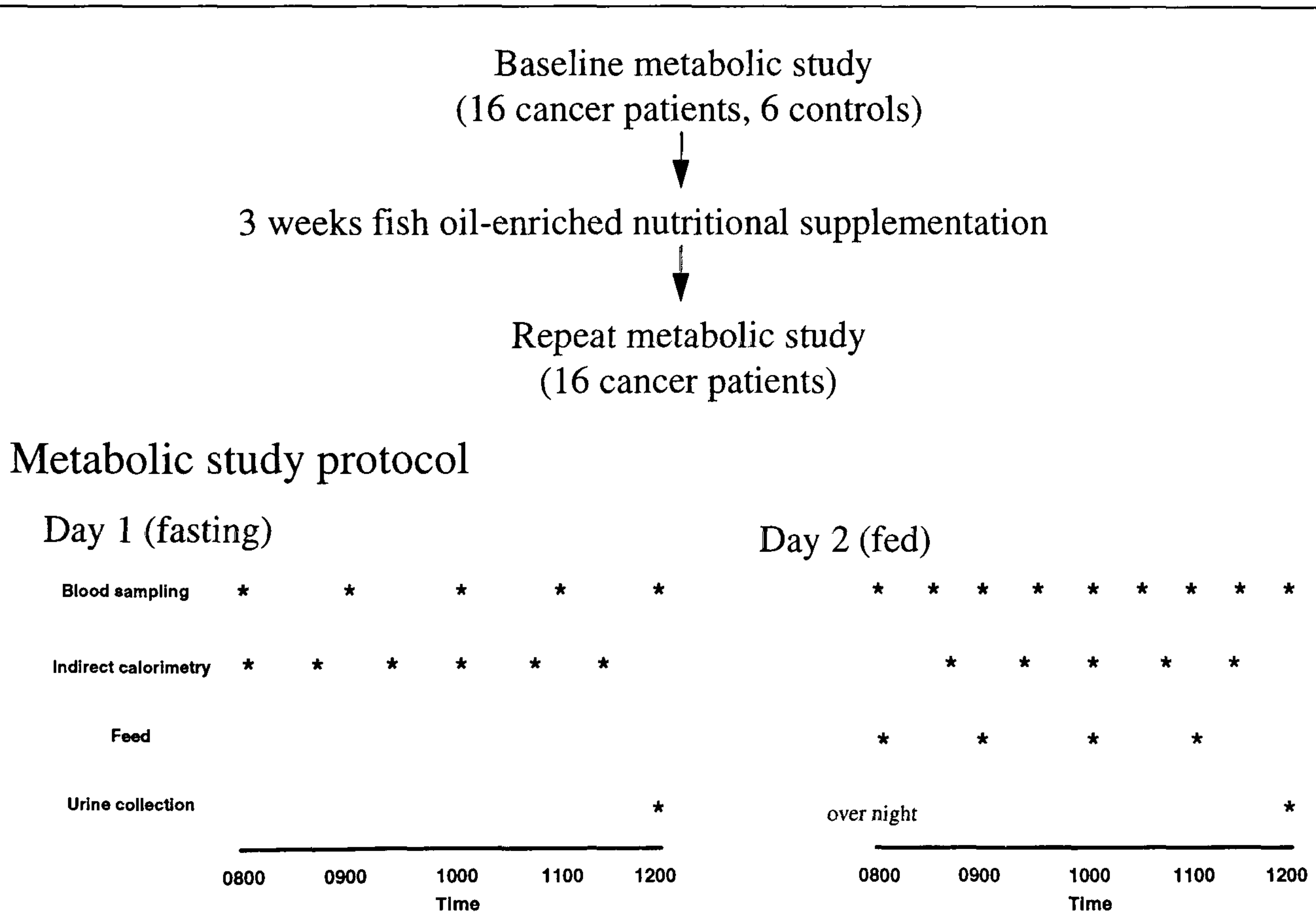


Figure 9.1. Study protocol.

Results

Subject characteristics are shown in table 9.1. Cancer patients were older than the controls but groups were of equal sex distribution. Cancer patients were significantly lighter than controls having lost around 18% of their preillness stable weight.

Table 9.1. Characteristics of weight losing cancer patients and healthy controls.

| | Cancer patients (n=16) | Healthy controls (n=6) | p |
|------------------------|------------------------|------------------------|--------|
| Age | 63 | 54 | 0.014 |
| (years) | (56-66) | (50-62) | |
| Sex | 7 male | 3 male | 1.0 |
| | 9 female | 3 female | |
| Weight | 55.2 | 77.2 | 0.0015 |
| (kg) | (48.8-61.2) | (70.3-96.0) | |
| Percentage weight loss | 17.7 | 0 | 0.0002 |
| | (15.3-21.4) | | |

Median (interquartile range).

Comparison by Mann Whitney U test or Chi squared test.

Baseline metabolic study

Baseline fasting metabolic characteristics are shown in table 9.2. There was no difference in overall resting energy expenditure between the cancer and control groups. However, as the cancer patients were significantly lighter, energy expenditure expressed per kilogram body weight, lean body mass or body cell mass was significantly greater in cancer patients than controls. There was no difference in energy expenditure between those cancer patients with or without an acute phase protein response. However, dietary intake expressed as a proportion of resting energy expenditure was significantly lower in patients with an acute phase protein response as defined by an elevated C-reactive protein concentration (C-reactive protein elevated 1.0 (0.87-1.27) versus C-reactive protein normal 1.42 (1.11-1.62), $p=0.049$).

Fasting serum insulin was significantly lower in cancer patients than controls. There was no significant difference between fasting cortisol concentrations between the two groups. However, the cortisol : insulin ratio was significantly higher in cancer patients. There was no difference in fasting glucose concentrations between the two groups.

Table 9.2. Baseline fasting metabolic features of weight losing cancer patients and healthy controls and of patients after 3 weeks consumption of a fish oil-enriched nutritional supplement.

| | Cancer patients at baseline (n=16) | Healthy controls (n=6) | p (patients vs controls) | Cancer patients after 3 week supplementation period | p (vs baseline) | p (vs controls) |
|--|---------------------------------------|---------------------------|-----------------------------|---|--------------------|--------------------|
| Resting energy expenditure (kcal/day) | 1360 (1170-1450) | 1475 (1270-1750) | 0.18 | 1300 (1190-1470) | 0.12 | 0.042 |
| Resting energy expenditure (kcal/kg body weight/day) | 24.0 (22.3-27.0) | 18.8 (17.6-21.1) | 0.0023 | 23.6 (20.2-25.8) | 0.025 | 0.039 |
| Resting energy expenditure (kcal/kg lean body mass/day) | 33.8 (28.9-35.3) | 28.7 (26.4-29.8) | 0.031 | 31.8 (27.7-33.9) | 0.018 | 0.46 |
| Resting energy expenditure (kcal/kg body cell mass/day) | 62.7 (57.1-70.3) | 54.8 (47.6-57.2) | 0.028 | 61.6 (58.1-69.8) | 0.030 | 0.062 |
| Insulin (mU/l) | 3.3 (2.4-5.4) | 6.2 (4.7-11.9) | 0.022 | 5.0 (3.4-6.9) | 0.0064 | 0.13 |
| Glucose (mmol/l) | 5.5 (4.9-6.0) | 5.1 (4.8-5.6) | 0.42 | 5.7 (4.8-6.9) | 0.29 | 0.23 |
| Cortisol (nmol/l) | 552 (478-751) | 520 (417-588) | 0.34 | 551 (476-602) | 0.063 | 0.61 |
| Cortisol : insulin ratio | 153 (92-200) | 84 (53-89) | 0.0096 | 105 (63-177) | 0.0084 | 0.14 |

Median (interquartile range).
Comparison between patients and controls by Mann Whitney U test and between patients over time by Wilcoxon signed rank test.

In the fasting state there was little change in metabolic measurements over a 4 hour period. The area under the curve of energy expenditure increased from baseline by 2.1% (1.0-3.5) in cancer patients and by 1.1% (0.7-1.3) in controls. This difference between these groups was, however, not statistically significant ($p=0.09$). There was a decline in insulin concentration as the fasting period continued such that the area under the curve decreased from baseline by 13.9% (2.9-40.7) in cancer patients and 27.1% (20.2-33.0) in controls. Again there was no significant difference between the two groups ($p=0.81$).

The change in serum insulin concentrations of the cancer and control subjects over the study feeding period are shown in figure 9.2. There was a prompt and substantial increase in insulin concentration in control subjects. Cancer patients had a slower increase in insulin concentrations. Insulin concentrations were significantly lower in cancer patients at 0, 30, 60 and 90 minutes ($p<0.05$, Mann Whitney U test). Thereafter there was no significant difference between insulin concentrations between cancer patients and controls. In absolute terms, the change in insulin concentration over the study period in cancer patients was about two thirds of that in healthy controls although this difference did not reach statistical significance (cancer patients 2195u/240mins (954-3823) versus controls 3234u/240mins (2948-5810), $p=0.11$). Due to the lower fasting concentration in cancer patients the percentage change in the area under the curve of insulin concentration from baseline was very similar between the two groups (cancer patients 256% (114-371) versus control subjects 216% (163-261), $p=0.80$).

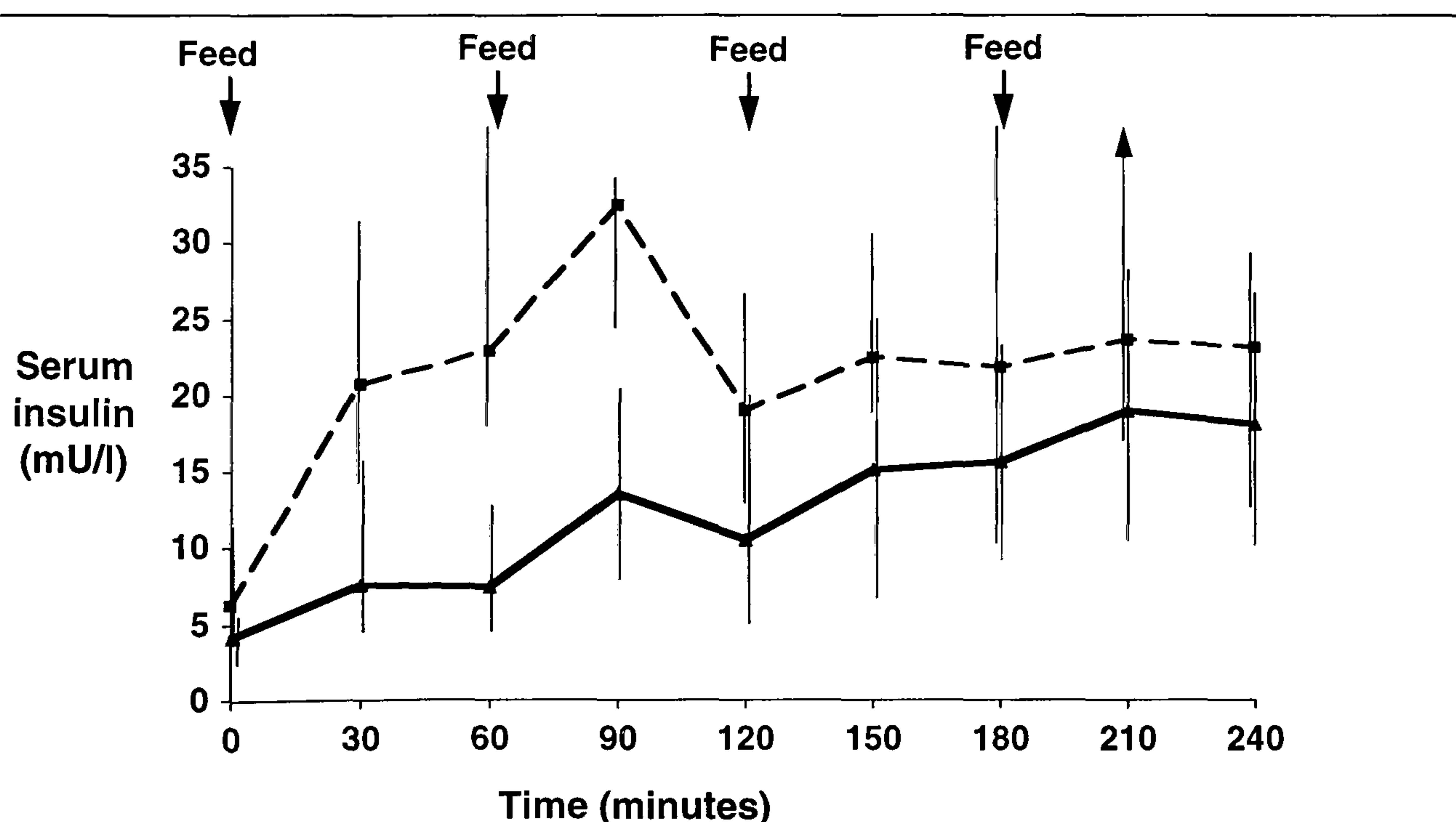


Figure 9.2. Change in serum insulin concentration over 4 hour feeding period in weight losing cancer patients (solid line) and healthy controls (dotted line).

The change in glucose concentrations of cancer and control subjects over the feeding period are shown in figure 9.3. There was no change in glucose concentration in control subjects over the feeding period. Cancer patients exhibited an increase in glucose concentrations over the study period and had a significantly greater area under the curve of glucose concentration (cancer patients 30.7% (24.4-48.9) versus control subjects 3.4% (-11.7-+11.6), $p=0.0064$).

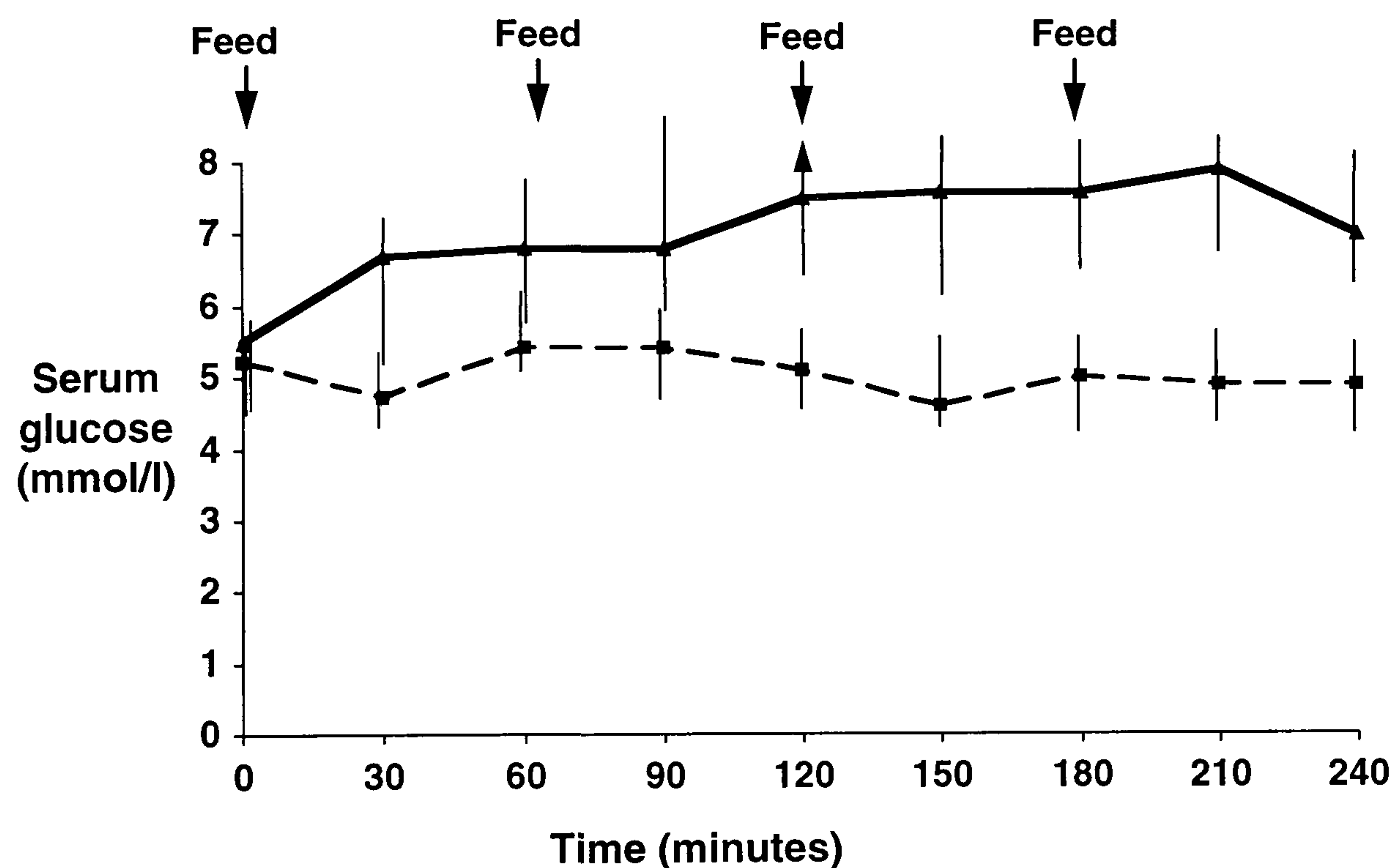


Figure 9.3. Change in serum glucose concentration over 4 hour feeding period in weight losing cancer patients (solid line) and healthy controls (dotted line). Median and interquartile range are shown. Absolute change - cancer patients 426 mmol/240mins (260-598) versus control subjects 38mmol/240mins (-156-+140), $p=0.0040$ Mann Whitney U test.

The change in energy expenditure of cancer and control subjects over the study feeding period are shown in figure 9.4. Both groups had a rise in energy expenditure with feeding. This increase was significantly greater in control subjects than cancer patients (cancer patients 7.9% (3.4-9.0) versus control subjects 12.6% (9.9-15.1), $p=0.0051$). In absolute terms this difference was more marked (cancer patients 14.0kcal/200mins (6.2-18.8) versus control subjects 25.2kcal/200mins (21.5-33.1), $p=0.0025$).

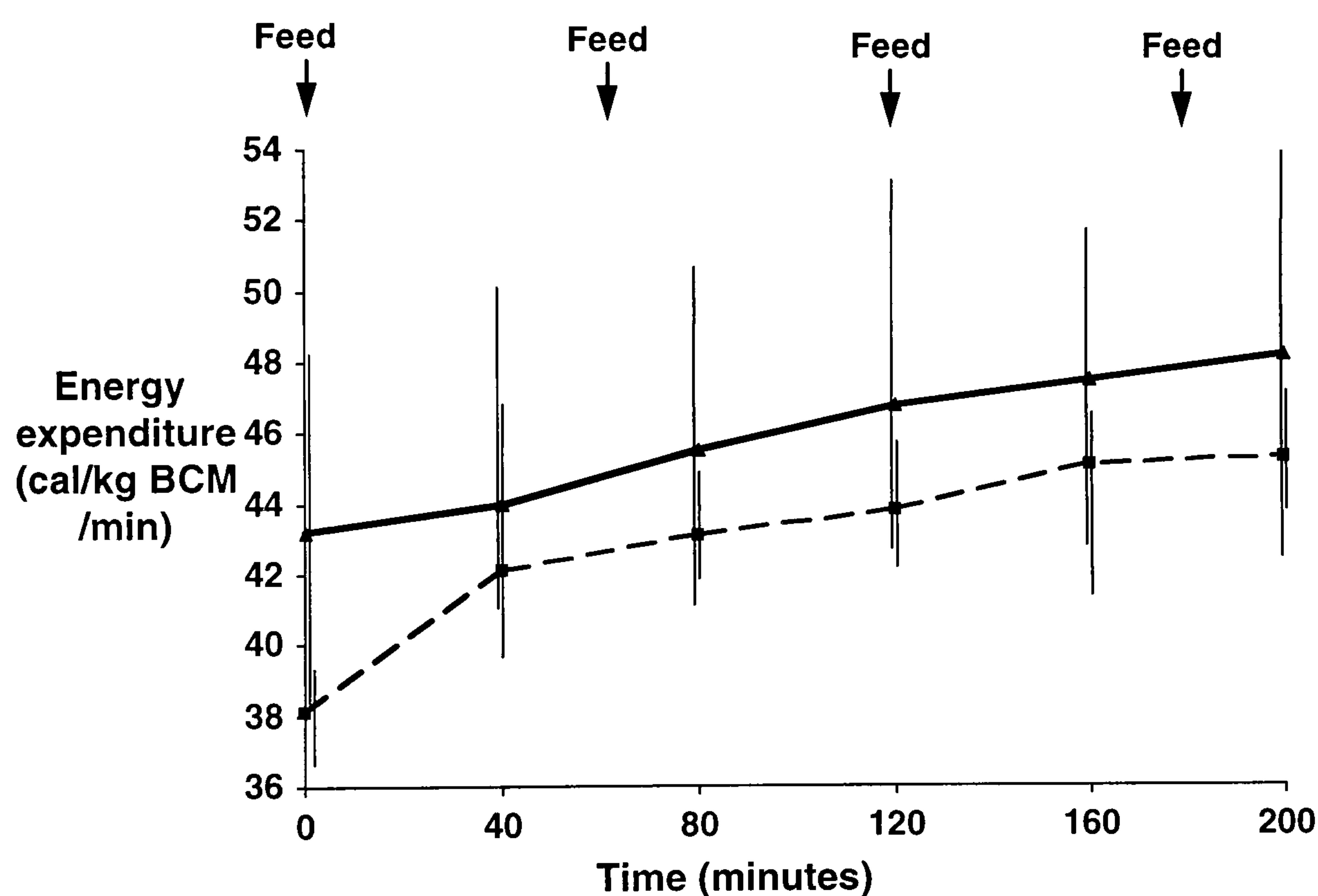


Figure 9.4. Change in energy expenditure over 4 hour feeding period in weight losing cancer patients (solid line) and healthy controls (dotted line). Median and interquartile range are shown.

Substrate utilisation rates are shown in table 9.3. Rates of protein oxidation were similar in both groups in the fasting state. There was a small and non-significant rise in protein oxidation with feeding in both cancer patients and controls. Carbohydrate oxidation doubled in controls on feeding and tripled in cancer patients, a statistically significant rise in both groups. Levels of carbohydrate oxidation tended to be higher in controls compared with cancer patients but this difference was not statistically significant (Figure 9.5). Fat oxidation was significantly higher in the fasting state in cancer patients (median 1.26g/kg/min (interquartile range 0.95-1.38) than controls (0.76g/kg/min (0.62-0.92), $p=0.020$) (Figure 9.6). This fell by 26% in cancer patients ($P=0.001$) and by 22% in controls ($p=0.17$) on feeding. In the fed state the difference in fat oxidation rates was no longer significantly different between patients and controls.

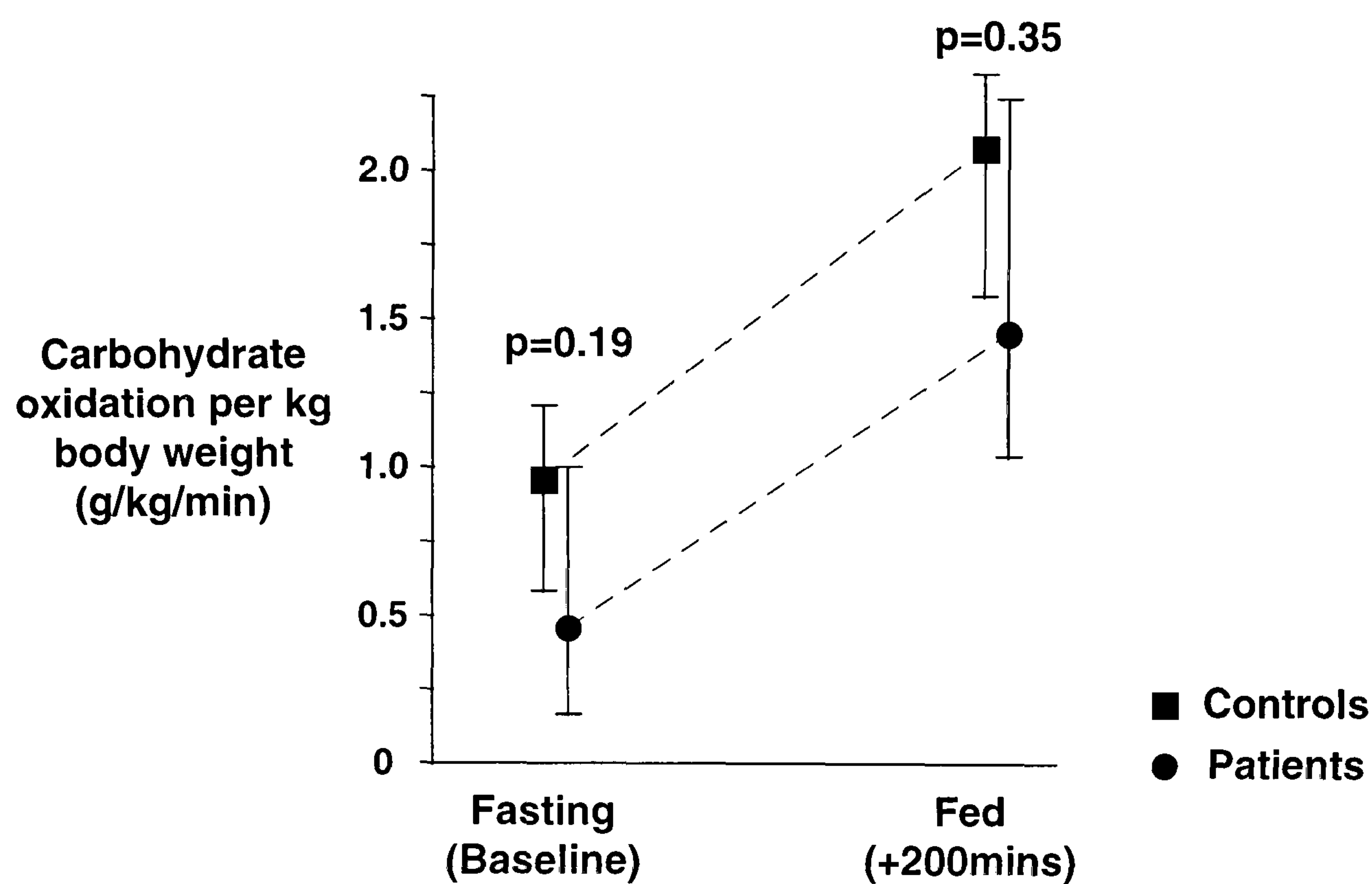


Figure 9.5. Carbohydrate oxidation rate in the fed and fasting state in weight losing cancer patients and healthy controls. Comparison between fasting and fed values by Wilcoxon signed rank test - Cancer patients $p=0.0010$, controls - $p=0.046$.

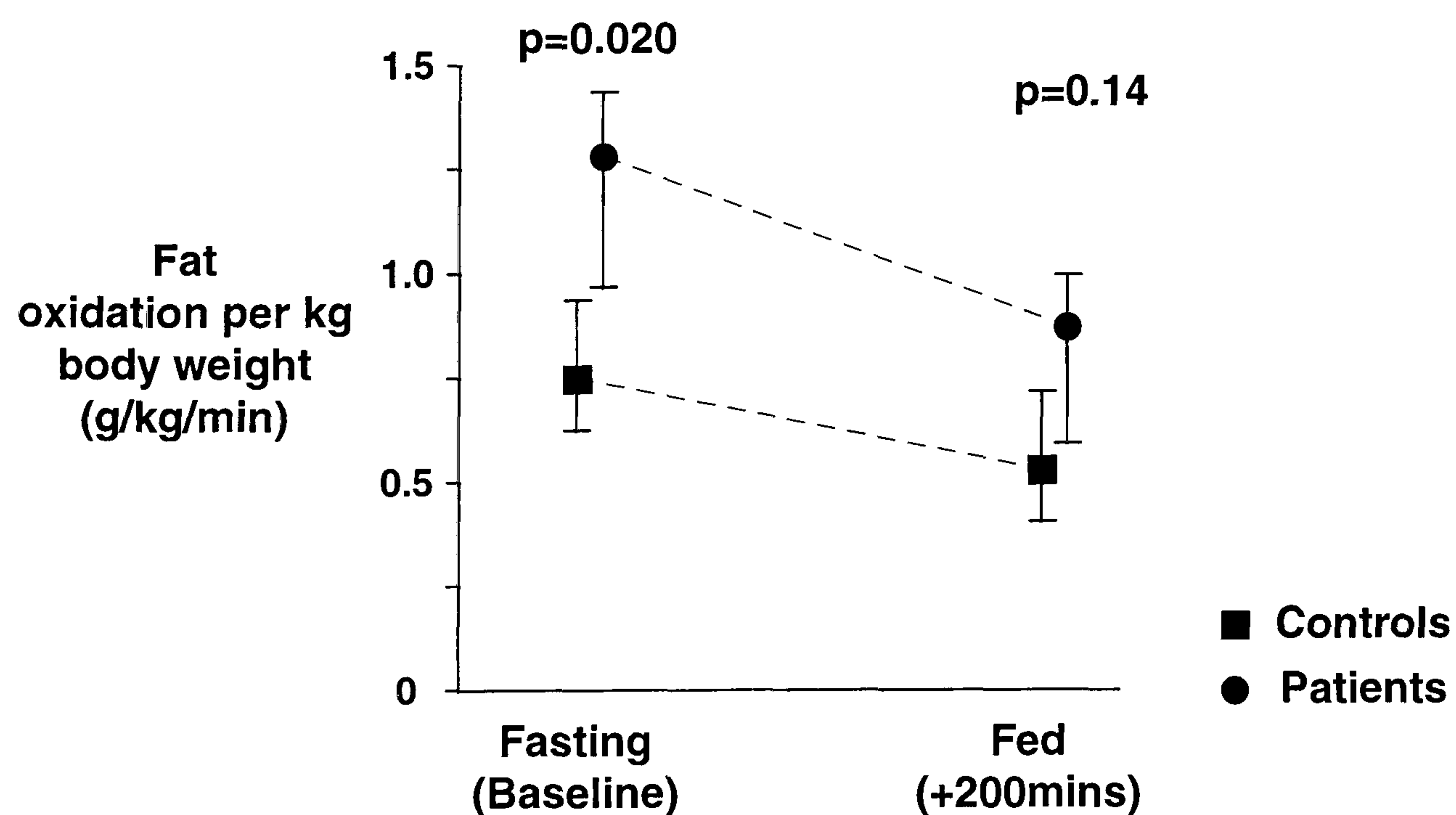


Figure 9.6. Fat oxidation rate in the fed and fasting state in weight losing cancer patients and healthy controls. Comparison between fasting and fed values by Wilcoxon signed rank test - Cancer patients $p=0.0010$, controls - $p=0.17$.

Table 9.3. Baseline fasting and fed substrate utilisation of weight losing cancer patients and healthy controls and of patients after 3 weeks consumption of a fish oil-enriched nutritional supplement.

| | Cancer patients at baseline (n=16) | Healthy controls (n=6) | p (patients vs controls) | Cancer patients after 3 week supplementation period | p (vs baseline) | p (vs controls) |
|---|------------------------------------|------------------------|--------------------------|---|-----------------|-----------------|
| Fasting protein oxidation (mg/kg/min) | 0.49 (0.36-0.64) | 0.58 (0.46-0.69) | 0.24 | 0.62 (0.42-0.81) | 0.092 | 1.0 |
| Fed protein oxidation | 0.73 (0.55-0.86) | 0.68 (0.57-0.86) | 0.76 | 0.92 (0.62-1.14) | 0.31 | 0.13 |
| p (fasting vs fed protein oxidation) | 0.052 | 0.46 | | 0.023 | | |
| Fasting carbohydrate oxidation | 0.44 (0.15-1.03) | 0.95 (0.57-1.17) | 0.19 | 0.96 (0.56-1.19) | 0.24 | 1.0 |
| Fed carbohydrate oxidation | 1.47 (1.03-2.28) | 2.08 (1.5-2.33) | 0.35 | 1.93 (1.24-2.69) | 0.14 | 0.76 |
| p (fasting vs fed carbohydrate oxidation) | 0.0010 | 0.046 | | 0.0010 | | |
| Fasting fat oxidation | 1.26 (0.95-1.38) | 0.76 (0.62-0.92) | 0.020 | 1.02 (0.80-1.78) | 0.16 | 0.10 |
| Fed fat oxidation | 0.89 (0.59-1.00) | 0.58 (0.38-0.71) | 0.14 | 0.66 (0.59-0.90) | 0.24 | 0.28 |
| p (fasting vs fed fat oxidation) | 0.0010 | 0.17 | | 0.0045 | | |

Median (interquartile range). Comparison between patients and controls by Mann Whitney U test and between patients over time by Wilcoxon signed rank test.

Metabolic study after 3 weeks fish oil-enriched nutritional supplement

After 3 weeks of the fish oil-enriched nutritional supplement patients had a median weight gain of 1.0kg (interquartile range -0.25-+1.75) ($p=0.049$ versus baseline). Prior to enrolment patients had a rate of weight loss of 2.9kg/month (-3.8--1.9). Patients tolerated the supplement well, consuming a median of 1.9 cans/day (range 1.25-2.0).

Changes in baseline fasting metabolic characteristics are shown in table 9.2. Resting energy expenditure did not change after the 3 week supplementation period ($p=0.12$). However, because of the weight gain observed, resting energy expenditure per kg body weight and per kg lean body mass both fell by a small but statistically significant amount ($p=0.025$ and 0.018 respectively).

Serum fasting insulin concentrations rose significantly ($p=0.0064$) over the 3 week supplementation period and were no longer significantly different from controls. Fasting serum concentrations of cortisol did not change after the supplementation period. However, there was a significant fall in the cortisol:insulin ratio ($p=0.0084$). There was no change in fasting glucose concentration ($p=0.29$).

The change in insulin concentrations with feeding after the 3 week supplementation period is shown in figure 9.7. The pattern observed was similar to that before intervention with significantly lower insulin concentrations at 30, 60 and 90 minutes but no difference in values thereafter. Absolute and percentage change from baseline were again not significantly different between patients and controls studied at baseline. Change in glucose concentration with feeding was no different after the 3 week supplementation period, with an increase of 31.8% (23.7-43.9) being observed in the area under the curve from baseline.

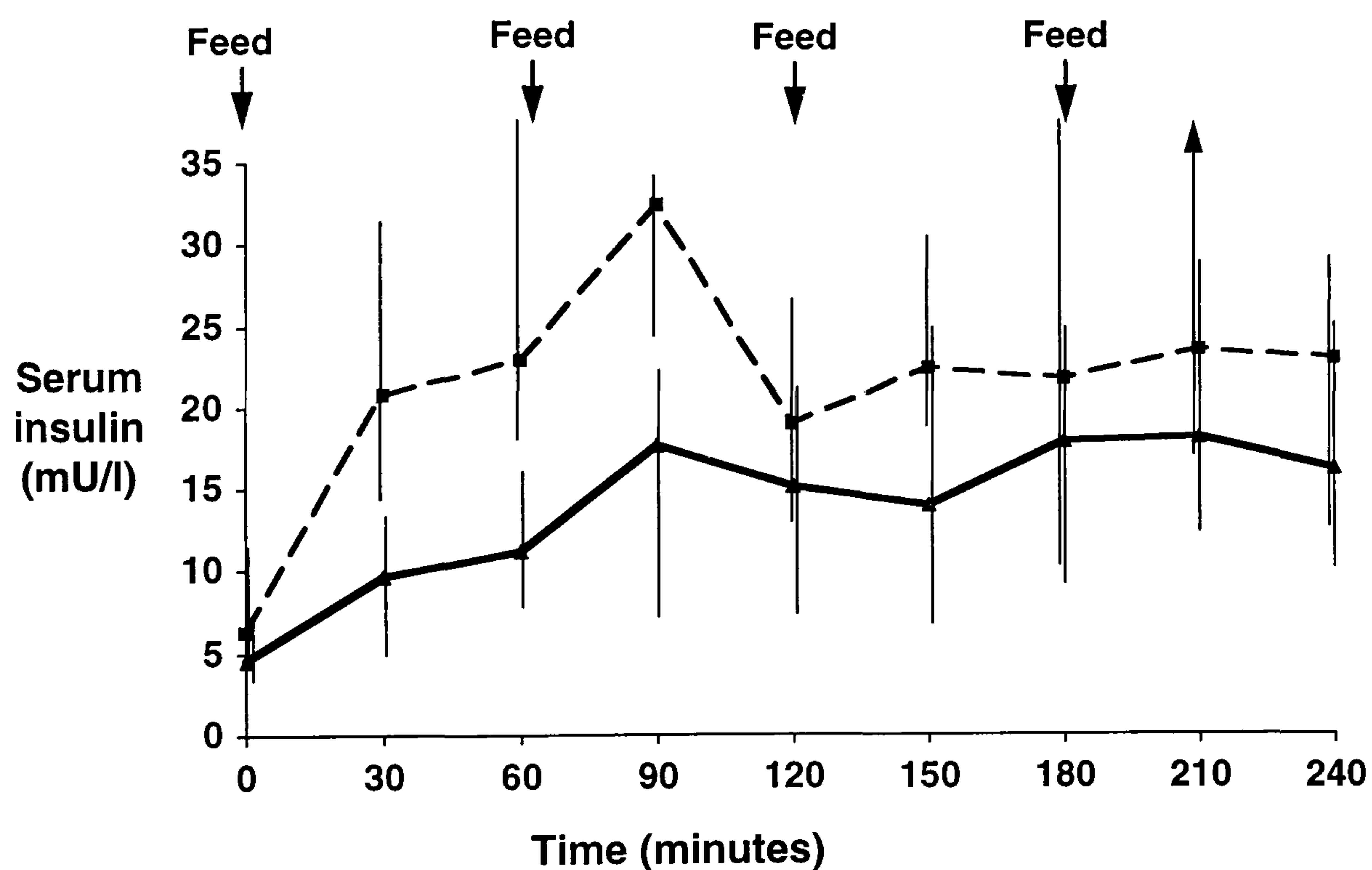


Figure 9.7. Change in serum insulin concentration over 4 hour feeding period in weight losing cancer patients (solid line) after 3 weeks fish oil-enriched nutritional supplementation. Values for healthy controls are also shown (dotted line). Percentage change in area under curve - cancer patients 202% (135-343) versus control subjects 216% (163-261), $p=0.97$ Mann Whitney U test. Paired comparison with baseline by Wilcoxon signed rank test, $p=0.90$. Absolute change - 1694u/240mins (1160-3964) - $p=0.18$ versus baseline, $p=0.18$ versus control.

The percentage change in the area under the curve of energy expenditure from baseline was 9.6% (6.3-12.4) in cancer patients after the 3 week supplementation period (figure 9.8). This was significantly greater than for the same subjects at baseline ($p=0.041$) and was no longer significantly different from healthy controls ($p=0.073$). The absolute change in energy expenditure also increased significantly in cancer patients to 18.8kcal/200mins (12.3-23.0) ($p<0.033$).

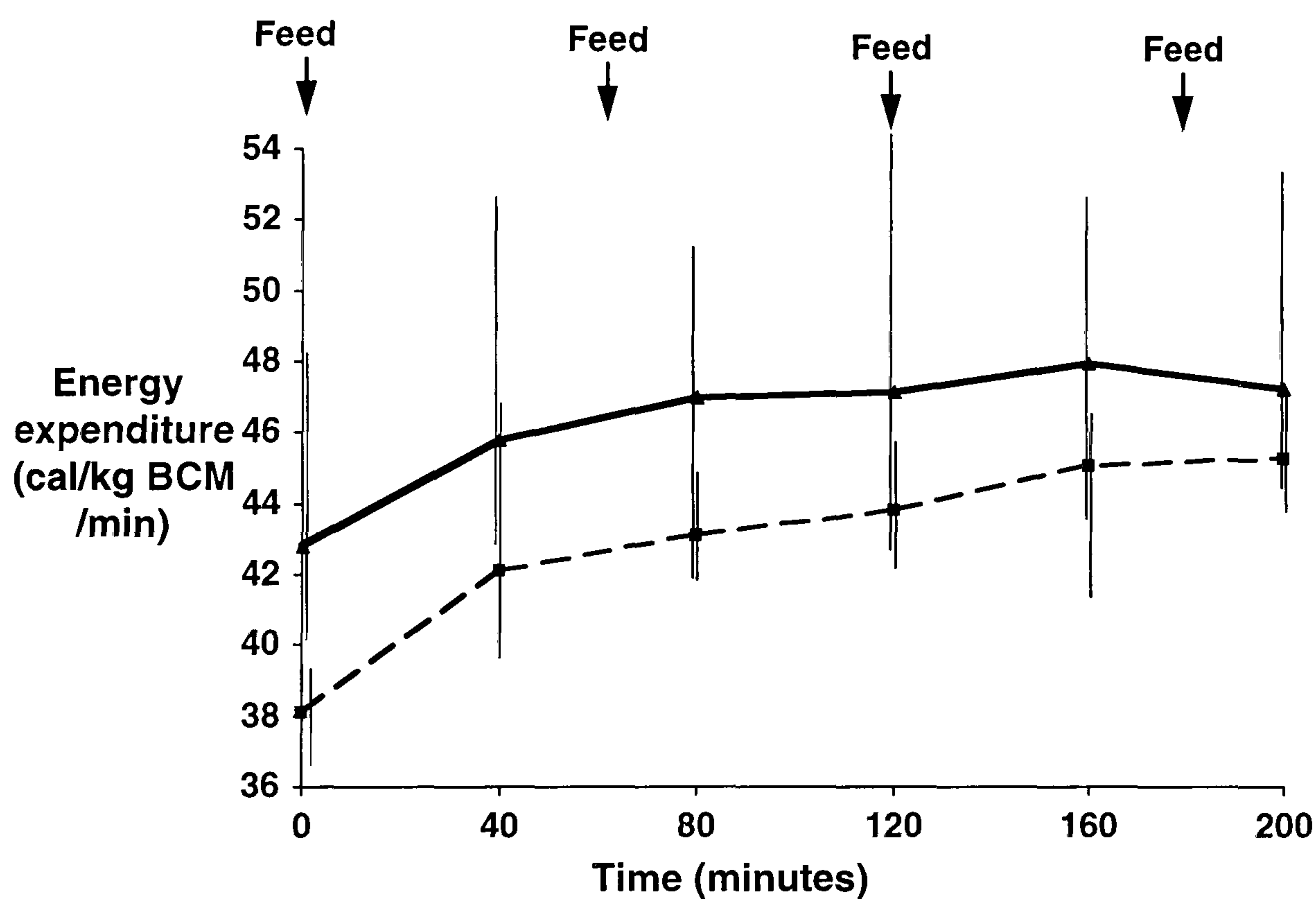


Figure 9.8. Change in energy expenditure over 4 hour feeding period in weight losing cancer patients (solid line) after 3 weeks fish oil-enriched nutritional supplementation. Values for healthy controls are also shown (dotted line). Median and interquartile range are shown. Percentage change in area under curve - cancer patients 9.6% (6.3-12.4) versus control subjects 12.6% (9.9-15.1), $p=0.073$ Mann Whitney U test. Paired comparison with baseline by Wilcoxon signed rank test, $p=0.041$. Absolute change - 18.8 kcal/200mins (12.3-23.0) - $p=0.033$ versus baseline, $p=0.018$ versus control.

Changes in substrate utilisation after 3 weeks administration of the fish oil-enriched nutritional supplement are shown in table 9.3. Rates of protein and carbohydrate oxidation both rose slightly in the fed and fasting state in cancer patients becoming similar to values observed in control subjects. Fasting fat oxidation fell to 1.02g/kg/min (0.8-1.18), no longer significantly different from baseline healthy control values ($p=0.10$) (Figure 9.9). This again fell promptly on feeding as seen in control subjects. Median percentage substrate utilisation is shown in figure 9.10. At baseline there was a significant difference in fat oxidation rate in the fasting state between patients and controls (figure 9.10). However, after 3 weeks of fish oil-enriched nutritional supplement consumption there were no significant differences between the percentage nutrient utilisation of patients and baseline control values (figure 9.10).

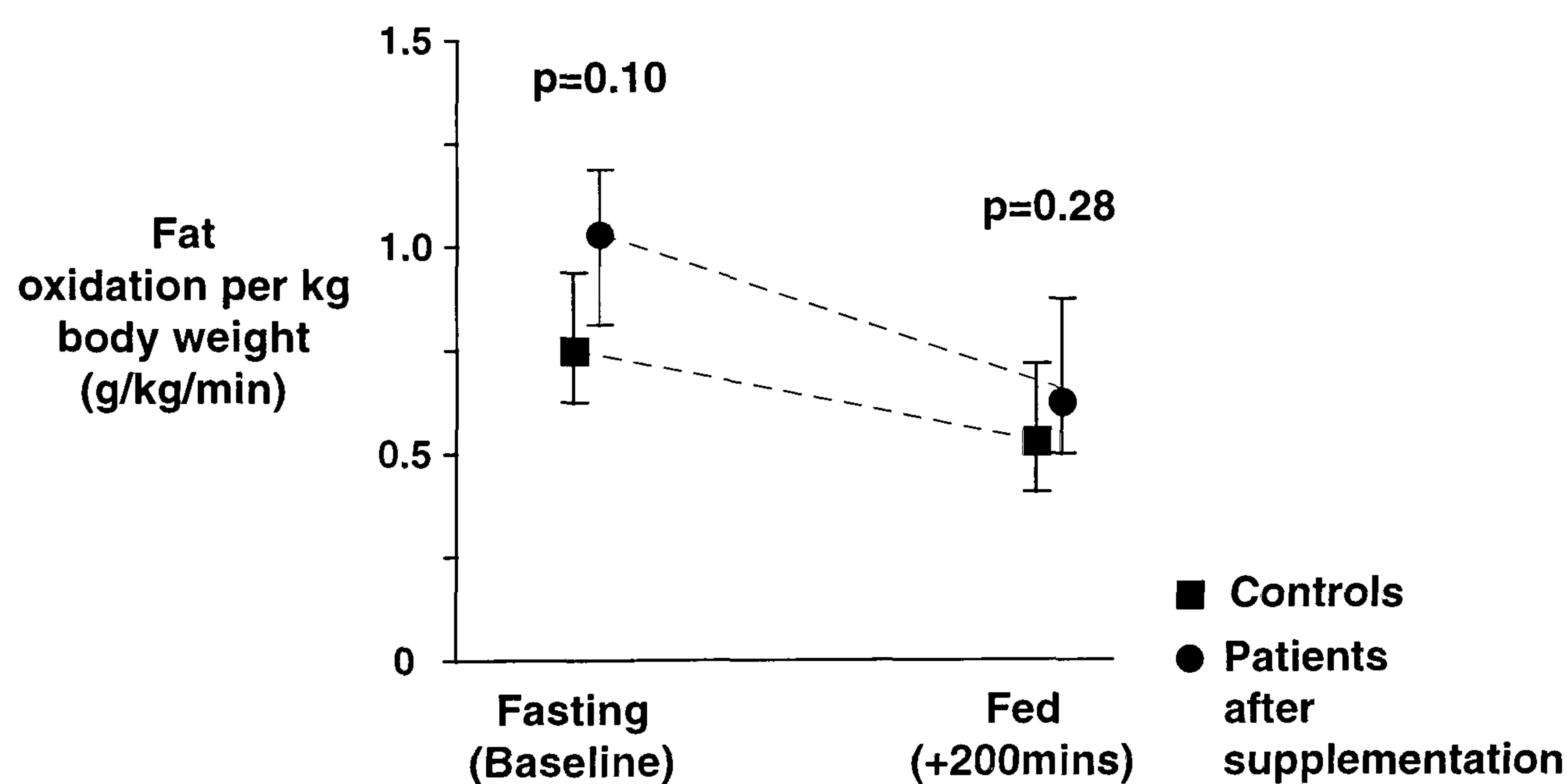


Figure 9.9. Fat oxidation rate in the fed and fasting state in weight losing cancer patients after 3 weeks fish oil-enriched nutritional supplementation and healthy controls. Comparison between fasting and fed values by Wilcoxon signed rank test - Cancer patients $p=0.0045$. Paired comparison with before supplementation - fasting $p=0.16$, fed $p=0.24$.

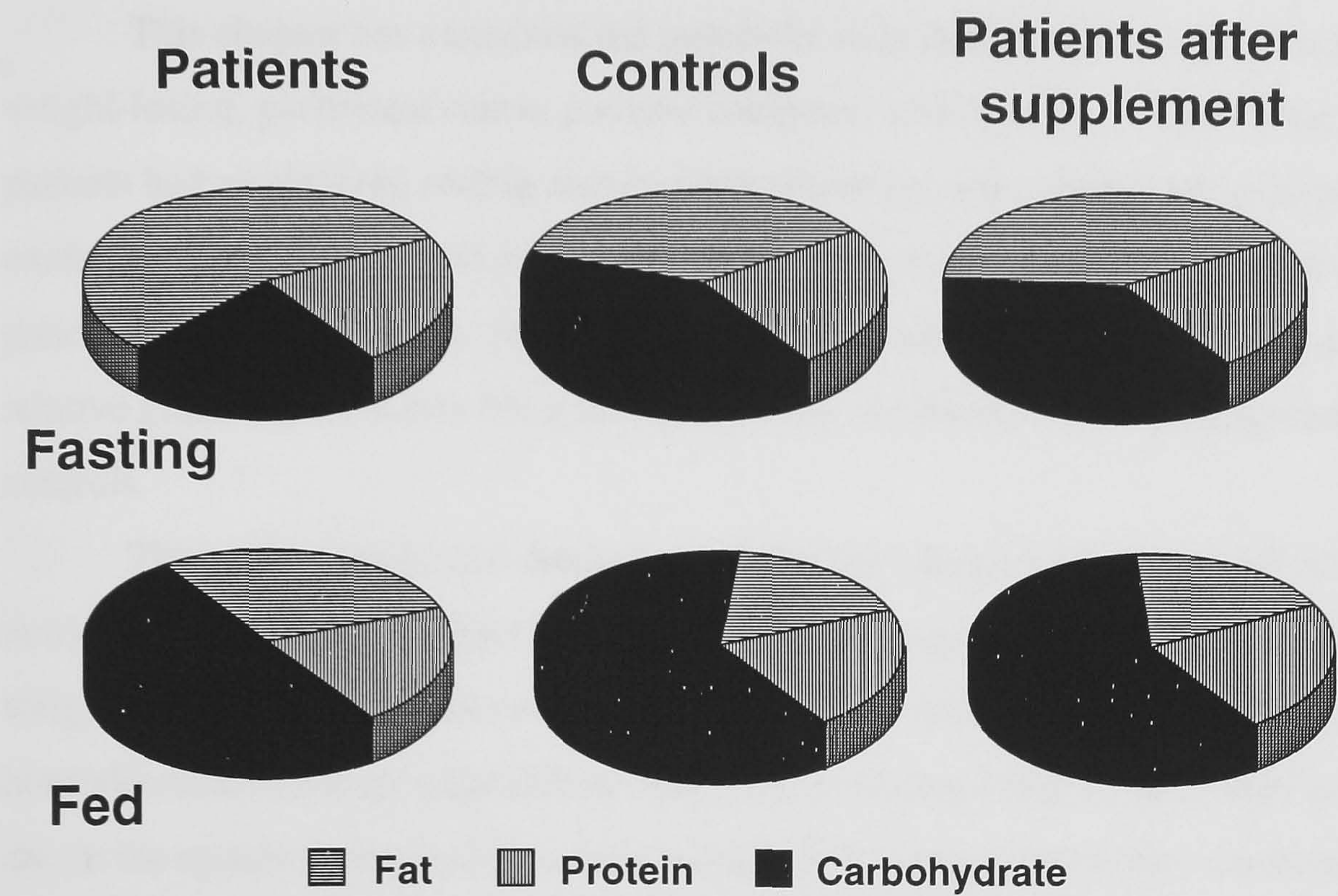


Figure 9.10. Percentage substrate oxidation in weight losing pancreatic cancer patients (n=16) and healthy controls (n=6) and the same cancer patient group after 3 weeks fish oil-enriched nutritional supplementation. Median is shown. Fasting fat oxidation - patients versus controls $p=0.032$, Mann Whitney U test. Otherwise $p>0.1$.

Discussion

This chapter has examined the metabolic state during fasting and in response to feeding in weight-losing, pancreatic cancer patients compared with healthy, weight stable controls. Cancer patients had an elevated resting energy expenditure per unit weight and an increased rate of fat oxidation in the fasting state and a lower serum insulin concentration. Dietary intake was lower in patients with an acute phase protein response. In response to feeding cancer patients exhibited relative glucose intolerance but a reduction in the metabolic cost of feeding compared with controls.

The present study also demonstrated that the administration of a fish oil-enriched nutritional supplement containing EPA for 3 weeks resulted in weight gain in this previously weight-losing group (as described previously), an increase in fasting insulin concentrations and a normalisation of energy expenditure with a fall in resting energy expenditure per unit weight and a rise in the apparent metabolic cost of feeding. Substrate utilisation also normalised.

Elevated resting energy expenditure has been demonstrated in the fasting state in weight-losing patients with advanced pancreatic cancer (Falconer et al, 1994) and lung cancer (Staal-van den Brekel et al, 1994). These studies were also able to demonstrate a relationship between the acute phase protein response and energy expenditure. No such clear relationship was seen in the present study, however, energy intake was lower in patients with an acute phase protein response. A similar relative reduction in energy intake in association with an acute phase protein response has been reported previously in patients with advanced pancreatic cancer (Wigmore et al, 1997b). The pro-inflammatory cytokines which stimulate the acute phase protein response will also produce anorexia in animal models (Moldawer et al, 1988). It is not clear why no relationship between resting energy expenditure and the acute phase protein response was seen in the present study although it may be that C-reactive protein alone may be an imprecise measure and that other proteins or a combination may give a better impression of the inflammatory state as discussed in chapter 8. In addition, the patients examined in the present study may have been at an earlier stage in their disease than those examined previously.

An elevated cortisol : insulin ratio compared to controls has previously been noted in patients with advanced pancreatic cancer (Fearon et al, 1998). This would imply a catabolic hormonal environment consistent with the continuing weight loss seen in these patients.

Relative glucose intolerance has been noted in cancer patients for many years (Rohdenberg et al, 1919). A blunted insulin secretory response to oral glucose administration in association

with delayed clearance of glucose has been reported in cachectic colorectal cancer patients (although fasting insulin concentrations did not differ between patients and controls in this study) (Holroyde et al, 1984). Another study of colorectal cancer patients suggested normal insulin sensitivity but impaired insulin responsiveness implying a postreceptor defect in insulin action (Copeland et al, 1987). In the present study there was a relative blunting of the insulin response in the cancer patients with lower insulin concentrations over the early part of the feeding period. Over the whole time course there was, however, no statistically significant difference between absolute change in insulin concentration or percentage change between cancer patients and controls. This may reflect relatively small patient numbers combined with substantial inter-individual variation. If anything, the present results only suggest a delay in the insulin response rather than a major difference in its overall magnitude. It has been suggested that islet amyloid polypeptide (amylin) produced by normal pancreatic tissue in patients with pancreatic cancer may contribute to insulin resistance (Permert et al, 1993, Permert et al, 1994).

Although non-cancer-bearing subjects who have lost weight have been reported to have lower resting energy expenditure in the fasted state, there is little evidence in normal subjects of energy conservation through a change in the energy cost of feeding (Piers et al, 1992, Leibel et al, 1995). This chapter has examined the hypothesis that in cancer patients in whom there appears to be an increase in resting energy expenditure and a failure to gain weight there may be an increased cost of feeding. However, in the present study we found that the increase in energy expenditure with feeding was less than that seen in controls. Moreover, the size of the feeding stimulus provided was based on resting energy expenditure and this was relatively larger in cancer patients. Thus the observed reduction in the metabolic response to oral feeding in the cancer patients was even greater in relation to the quantity of feed provided.

It has previously been shown that non-weight losing cancer patients have no difference in their response to feeding compared with healthy controls (Melville et al, 1990). Intravenous nutrition has been shown to produce a similar metabolic response in malnourished cancer patients and malnourished controls (Lindmark et al, 1986). Although no normally nourished control group was examined in the latter study, it was suggested that while cachectic cancer patients may exhibit metabolic changes in the fasting state, these patients were able to conserve energy normally upon intravenous feeding. The present study suggests this may also be true to some degree with oral feeding, although the absolute magnitude of the reduction in the cost of feeding observed in cancer patients in the present study is small.

Due to a lack of pancreatic lipase, pancreatic cancer patients frequently have problems with malabsorption, particularly of fat, (Perez et al, 1983). None of the subjects in the present study had symptomatic malabsorption but clearly if there was a reduction in the proportion of feed absorbed or the rate of absorption this may have accounted for some of the observed differences. However, the prompt rise in blood glucose concentrations and the rise in insulin concentration of equal proportion to controls seen in the cancer patients does not suggest a substantial difference in absorption between the two groups. Moreover, the cancer patients were able to gain weight over the three week study period again suggesting reasonable absorption.

As in the present study, an increase in the proportion of fat oxidation has previously been observed in fasting gastrointestinal cancer patients when compared with controls (Hansell et al, 1986, Chen & Chung, 1994). A corresponding significant reduction in carbohydrate oxidation has also been reported in weight-losing cancer patients (Hansell et al, 1986). In the latter study these changes were more pronounced in advanced disease. Although there was a trend towards a reduction in carbohydrate oxidation in the present study this was not statistically significant. A similar pattern of nutrient oxidation changes has been observed in septic patients (Askenazi et al, 1980). However, no such differences were seen in a small group of lung cancer patients who had lost little weight (Melville et al, 1990). The equations used for the calculation of substrate utilisation are not ideal in the fed state (Garlick, 1986). Within these limitations, the cancer patients in the present study demonstrated a marked increase in carbohydrate oxidation in the fed state. Such a change in substrate oxidation is observed in healthy individuals and has been documented in other groups of cancer patients (Lindmark et al, 1986, Melville et al, 1990). Thus, it appears that in the fasting state weight-losing cancer patients preferentially oxidise fat, perhaps due to depletion of glycogen stores. In the fed state a switch to normal substrate oxidation patterns is seen.

The administration of an EPA containing nutritional supplement to patients with pancreatic cancer resulted in an increase in weight, an increase in fasting insulin concentration, a decrease in resting energy expenditure and a normalisation of the metabolic cost of feeding and substrate utilisation. It has previously been shown that EPA will reduce pro-inflammatory cytokine production in cancer patients (Wigmore et al, 1997d and Chapter 7). These cytokines increase energy expenditure, reduce insulin levels, increase cortisol concentrations and induce insulin resistance, stimulate the acute phase response and cause protein and fat breakdown (McNamara et al, 1992). Modulation of these and other factors by the EPA containing supplement may explain

the relative normalisation of the metabolic state observed. EPA also modulates the activity of other factors implicated in weight loss in cancer patients such as proteolysis inducing factor (Tisdale, 1986 and Chapter 7) and the present study has also shown changes in the neuroendocrine state with a rise in fasting insulin concentrations.

Fish oil supplementation has been suggested to adversely affect glucose metabolism in otherwise well patients with non-insulin-dependent diabetes mellitus (Glauber et al, 1988, Borkman et al, 1989). However, a study of patients with hypertension given 4g fish oil or placebo daily for 16 weeks specifically addressed this issue and showed no change in response to glucose tolerance test, insulin release or insulin sensitivity in those given fish oil and no difference between groups (Toft et al, 1995). The present study did not demonstrate any adverse effect on glucose tolerance with the administration of the fish oil-enriched supplement.

The apparent improvement in the nutritional state of the cancer patients in the present study may have contributed to the metabolic changes observed over the three week study period. Patient's caloric intake increased by around 400kcal. Previous studies of conventional nutritional supplements in cancer patients in which a similar increment in protein and energy intake was achieved failed to produce nutritional benefit (Evans et al, 1987, Ovesen et al, 1993b). Thus it is conceivable that it was the EPA component of the supplement which resulted in modulation of the weight-losing state and allowed the protein and calories supplied to be used with more benefit. To compare the metabolic changes associated with the fish oil-enriched supplement we performed a paired comparison with subjects prior to consumption of the supplement and a comparison with the data for the patients who were only studied at baseline and who did not receive the fish oil-enriched supplement. The ideal study design would have been to also observe the effects of the supplement on control subjects. However, the present study design avoided the ethical issue of administering an unnecessary intervention to a healthy group and allowed the assessment of whether the supplement restored metabolic function towards that observed in health.

The present chapter suggests that the energy cost of feeding contributes little to the loss of weight in cancer patients, however, with their reduced food intake the increased resting energy expenditure in the fasting state may become more important. The increase in the cost of feeding after EPA supplementation in association with weight gain implies a normalisation of the metabolic state and less need to conserve energy in the presence of weight gain.

The next chapter examines further the metabolic effects of the fish oil-enriched supplement in terms of the protein kinetics associated with the acute phase protein response.

Acute phase protein synthesis in the fasted and fed state in cancer patients and the effects of a fish oil-enriched nutritional supplement

Summary

The presence of an acute phase protein response may be an important determinant of survival in advanced cancer perhaps because it may accelerate breakdown of lean tissue to provide amino acids for acute phase protein synthesis. In the fasting state albumin synthesis rates are not different in cancer patients compared with healthy controls despite lower circulating concentrations in cancer patients. Fibrinogen concentrations are elevated in the fasting state in cancer patients in association with higher synthesis rates. The present chapter examines the effect of feeding on albumin and fibrinogen synthesis to determine whether an abnormal response to feeding would explain the lower albumin concentrations of cancer patients. A fish oil-enriched nutritional supplement has been shown to reverse aspects of cachexia in cancer patients (Chapters 6-9). The present study also examined the effect of this supplement on hepatic export protein synthesis .

Albumin and fibrinogen synthesis were measured in the fed and fasting state in 8 weight-losing patients with advanced pancreatic cancer. 6 healthy controls were also studied at baseline. Protein synthesis was measured by an intravenous flooding dose of labelled phenylalanine. Tracer incorporation into albumin was measured by gas chromatography/mass spectrometry. Nitrogen balance was also measured. Patients were then studied again after the provision of a nutritional supplement enriched with fish oil providing 600kcal and 2g eicosapentaenoic acid per day for three weeks.

At baseline all patients were losing weight at a median rate of 2.4kg per month and had lost a median of 19% of their preillness stable weight. Cancer patients had a significantly lower serum albumin concentration and a significantly higher plasma fibrinogen concentration than controls ($p<0.01$). Cancer patients exhibited a 29% rise in albumin synthesis on feeding from a median of 11.3g/day to 14.2g/day. Healthy controls had a very similar ($p=0.7$) increase of 24% on feeding from 13.5g/day to 16.4g/day. Fasting fibrinogen total synthetic rate was significantly higher in cancer patients than controls at 3.3g/day versus 1.0g/day ($p<0.005$). In cancer patients in the fed state fibrinogen synthetic rate rose significantly by

38% to 4.5g/day while in controls there was no significant change in fibrinogen synthesis.

After 3 weeks of consumption of the fish oil-enriched nutritional supplement patients had a significant stabilisation of weight ($p=0.012$) with a median weight gain of 1kg (range +6--1.5). Albumin concentration was stable with a median change of 0g/l (range +3--3) ($p=0.67$). Fibrinogen concentration was also stable with a median change of -0.4g/l (range -1.78-+0.81). Nitrogen balance significantly improved with a median change of +2.6g/day (range +8.3--0.7) ($p=0.025$). Fasting albumin synthesis rate was unchanged at 10.5g/day but with feeding there was no significant change (median increase 3% ($p=0.29$)). Fasting fibrinogen synthesis was also unchanged (median 2.9g/day) and this increased by only 17% on feeding to 3.7g/day.

Thus cancer patients went from an statistically significant initial overall 33% rise in albumin and fibrinogen synthesis (from 14.7 to 18.7g/day on feeding at baseline) to a situation where the change was not significant (from 12.3 to 14.6g/day after intervention). This was associated with a stabilisation in weight and acute phase protein concentrations and a reversal of nitrogen balance suggesting that modulation of liver export protein synthesis may have a clinically significant effect on protein distribution. Thus the targeting of the inflammatory response and its associated metabolic changes (including the acute phase protein response) may be an avenue for further therapeutic intervention in cancer cachexia.

Introduction

Chapter 3 demonstrated a progressive deterioration in nutritional and functional status in association with exacerbation of the acute phase protein response (APPR) in patients with cancer cachexia. The APPR is thought to be stimulated by pro-inflammatory cytokines such as interleukin-6 (IL-6) (Castell et al, 1990). Chapter 4 described an association between concentrations of IL-6 and the acute phase protein, C-reactive protein. In Chapter 5 an association between both increased pro-inflammatory cytokine production and the presence of an APPR and poor survival was demonstrated. One mechanism by which the APPR may influence survival may be that, with inadequate nutritional intake, the demand for amino acids to manufacture acute phase proteins accelerates loss of lean tissue resulting in cachexia.

It has previously been shown that in the fasting state the total albumin synthesis rate is unchanged in patients with advanced cancer compared with controls despite much lower albumin concentrations (Fearon et al, 1998). By contrast, the total synthesis rate of fibrinogen is significantly increased in the fasting state in cancer patients accompanied by higher circulating concentrations compared with controls (Preston et al, 1998). This would suggest that in the fasting state for albumin at least, a reduced synthesis rate does not explain the hypoalbuminemia observed. In contrast, plasma fibrinogen concentrations reflect, at least in part, an increased rate of synthesis by the liver.

It has been suggested that synthesis of albumin is stimulated by feeding in healthy subjects (de Feo et al, 1992, Hunter et al, 1995) while fibrinogen synthesis remains unchanged (de Feo et al, 1992). If there was a reduced albumin synthetic response to feeding in cancer this might contribute to hypoalbuminaemia. Alternatively, if there was an increased fibrinogen synthetic response to feeding in cancer (and this process affected other positive acute phase proteins) then this might contribute to the metabolic demands on the nitrogen economy of such patients and might ultimately contribute to loss of lean tissue, particularly if dietary protein intake was restricted.

The failure of conventional nutritional supplementation to prevent weight loss in advanced cancer patients has been attributed to metabolic changes including the APPR (Nixon et al, 1981, Evans et al, 1987, Ovesen et al, 1993b). Chapters 6, 7, 8 and 9 have demonstrated improvement in nutritional status, modulation of metabolic mediators and stabilisation of acute phase protein concentrations with the administration of a fish oil-enriched nutritional supplement in cachectic pancreatic cancer patients.

The aim of the present study was to quantify the level of hepatic export protein synthesis in cachectic cancer patients in the fasted and fed state in comparison with healthy controls. This study also examined the effect of the fish oil-enriched nutritional supplement on albumin and fibrinogen synthesis.

Methods

Subjects

Eight patients with an unequivocal diagnosis of pancreatic cancer who were losing weight with no clinical evidence of ascites or peripheral oedema were examined. Six weight-stable healthy individuals served as controls. None of the patients received chemotherapy or radiotherapy and none had undergone surgery in the preceding 4 weeks. No patients had clinical or radiological evidence of infection, were jaundiced or severely anaemic, or were receiving steroids. The study was approved by the local ethical committee and all subjects gave written informed consent.

Study protocol

The study protocol is presented diagrammatically in figure 10.1. Subjects attended at 8am on two consecutive days. On the first day following an overnight fast, a venous catheter was inserted into the subject's antecubital fossa. The patient rested in a supine position for at least 30 minutes and underwent measurement of resting energy expenditure by indirect calorimetry. At 10am subjects received an intravenous bolus labelled phenylalanine. Blood samples were obtained over 120 minutes after the tracer infusion. The blood samples were analysed for baseline albumin and fibrinogen concentrations, labelled phenylalanine enrichment in plasma albumin and fibrinogen and in the plasma free phenylalanine pool. Subjects remained fasting throughout this period.

On the second day, again following an overnight fast a venous catheter was inserted into the subject's antecubital fossa and subjects received a meal of a balanced whole protein liquid nutritional supplement (Fortisip, Nutricia, Zoetermeer, Holland) providing a twelfth of the subjects estimated energy requirement (measured resting energy expenditure * 1.4) (Hunter et al, 1995). The supplement provided 13% of energy from protein, 48% from carbohydrate and 39% from fat, similar to a "typical" British diet. Subjects received such a feed on an hourly basis until the end of the study period. At 10am a similar flooding dose of phenylalanine was given as described above and blood samples collected similarly.

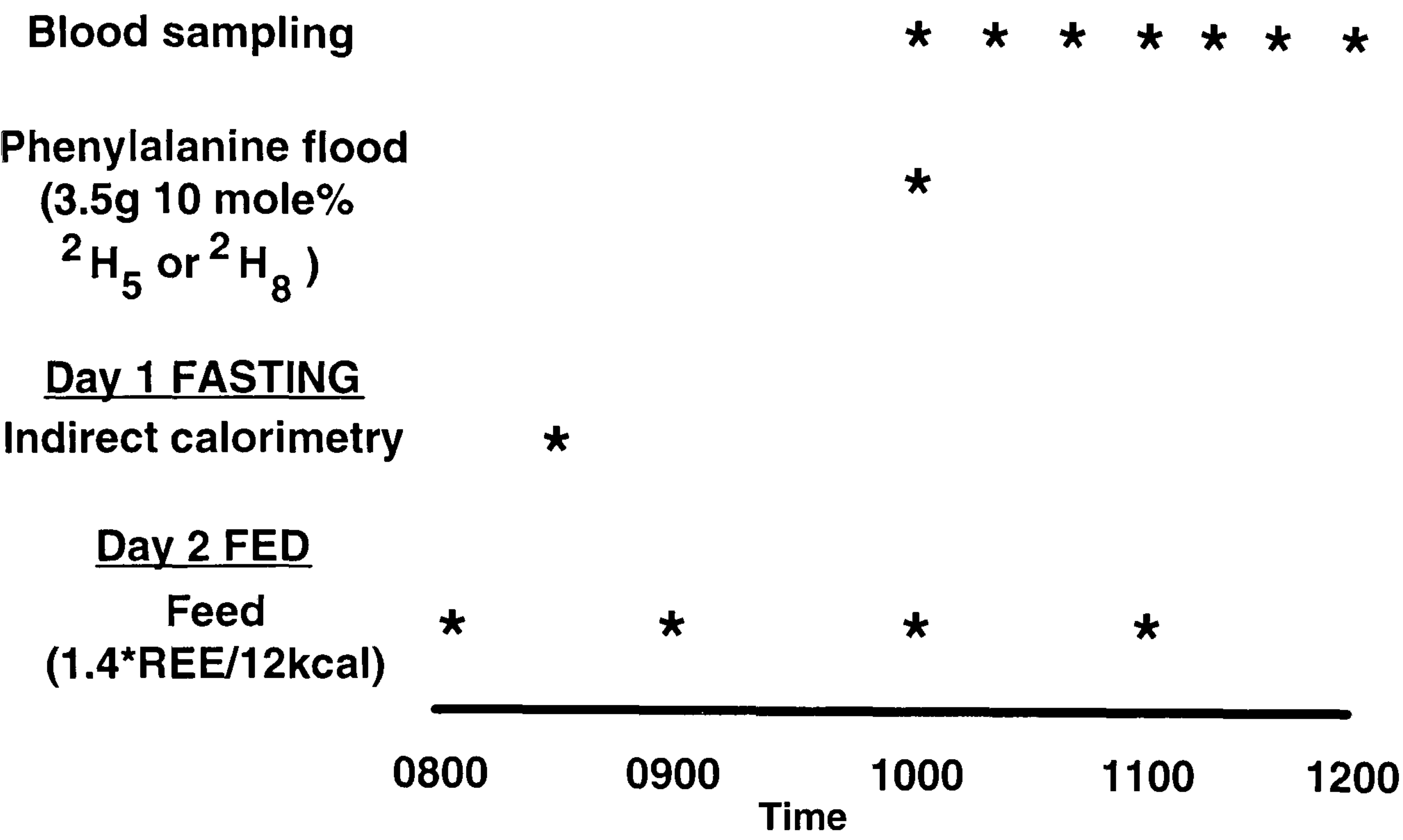


Figure 10.1. Study protocol.

Fish oil-enriched nutritional supplement

The cancer patients were then asked to consume 2 cans per day of a fish oil-enriched nutritional supplement providing 600kcal, 32g protein and 2g eicosapentaenoic acid per day in a volume of 480ml. After 3 weeks the cancer patients underwent repeat measurement of protein synthesis as described above.

Statistics

Data are presented as median and range.

Results

Baseline study

Subject characteristics are shown in table 10.1. Cancer patients weighed substantially less than controls having lost around 20% of their pre-illness stable weight. Cancer patients had significantly lower serum albumin concentrations and significantly higher plasma fibrinogen concentrations compared with controls.

Table 10.1. Characteristics of cancer patients and healthy controls examined.

| | Cancer patients | Healthy controls | p value |
|-----------------------------------|---------------------|----------------------|---------|
| Age (years) | 65 (56-70) | 54 (50-62) | 0.0054 |
| Sex | 2 female 6 male | 3 male 3 female | 0.33 |
| Weight (kg) | 56.2 (44.0-70.5) | 77.2 (58.0-100.0) | 0.0098 |
| Percentage weight loss | 18.9 (12.7-37.5) | 0 | 0.0013 |
| Rate of weight loss (kg/month) | 2.4 (0.8-4.9) | 0 | 0.0013 |
| Albumin (g/l) | 42 (37-44) | 45 (43-47) | 0.0033 |
| Fibrinogen (g/l) | 4.86 (3.72-6.02) | 2.72 (1.87-3.19) | 0.0019 |

Values presented are median and range

Comparison by Mann Whitney U test or Chi squared test

Insulin concentration change with time is shown in while fasting in figure 10.2 and while feeding in figure 10.3. There was no obvious change in insulin concentration attributable to the flooding dose of phenylalanine.

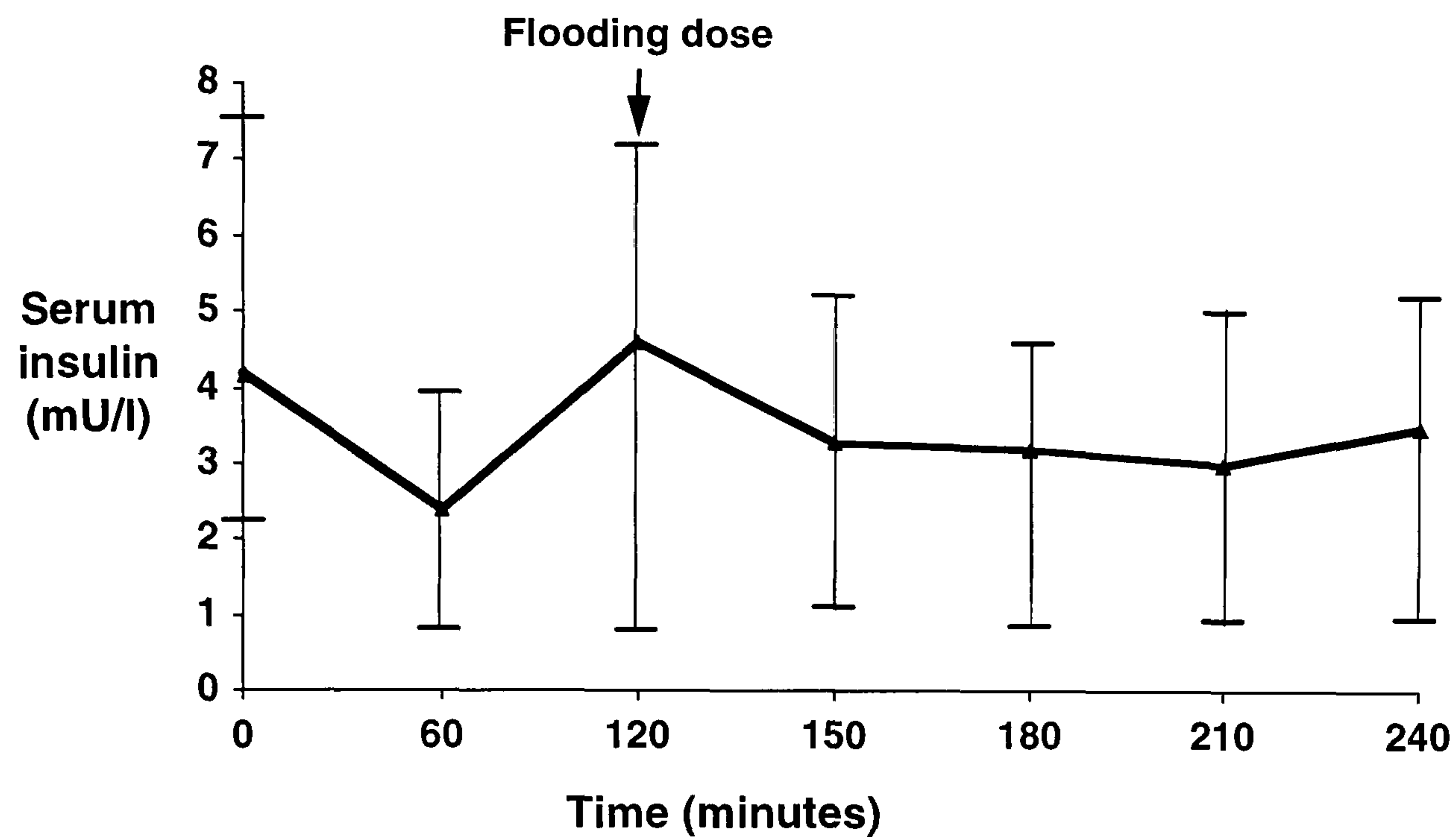


Figure 10.2. Insulin concentration in 8 patients with advanced pancreatic cancer in the fasted state. Median and range presented.

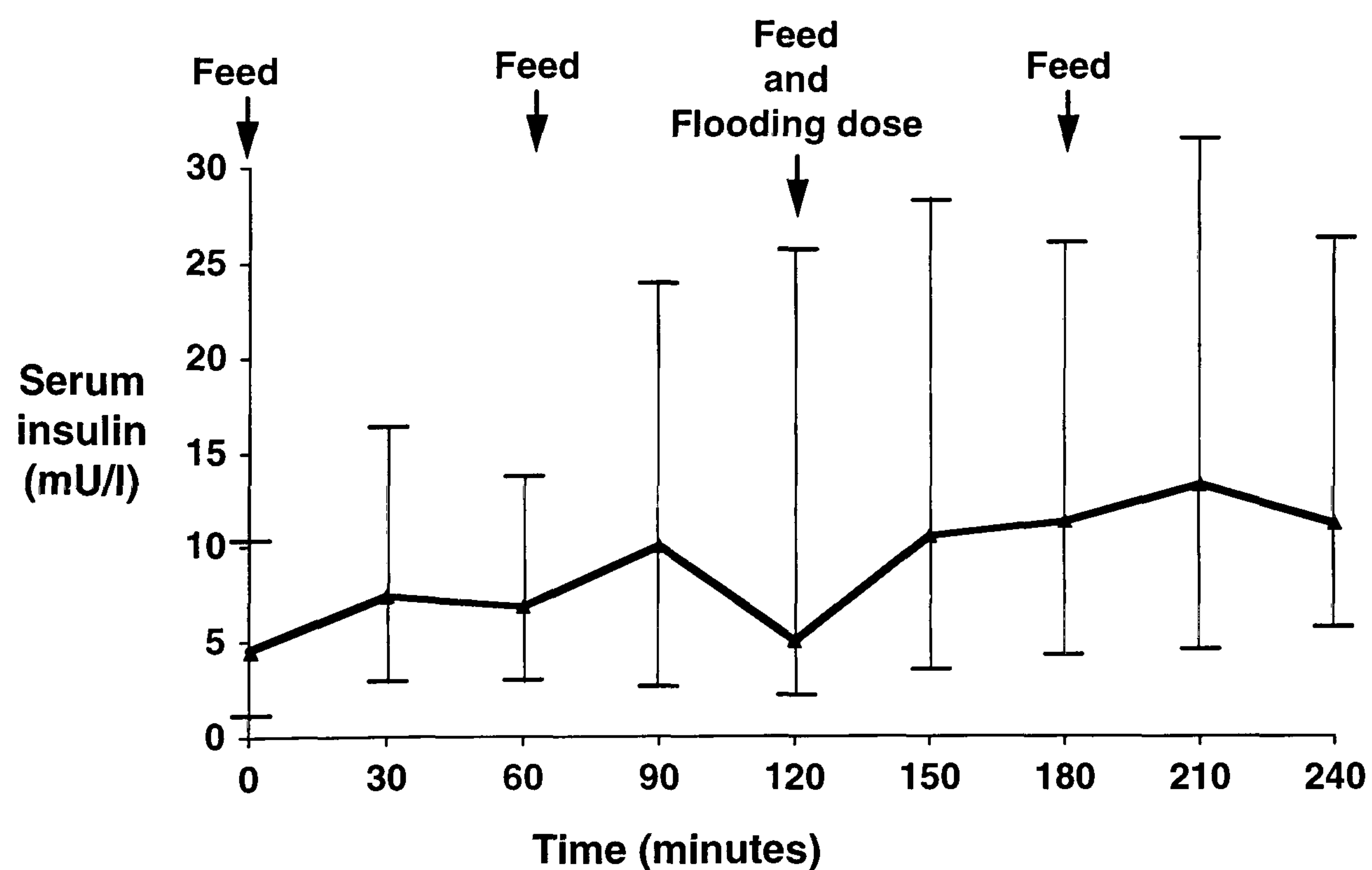


Figure 10.3. Insulin concentration in 8 patients with advanced pancreatic cancer with feeding. Median and range presented.

Albumin synthesis rates are shown in table 10.2. Changes in albumin synthesis between the fasted and fed state are presented in figure 10.4. There was no difference between albumin synthesis rates between weight-losing cancer patients and healthy controls in the fasted (median 14.2g/day versus 15.7g/day ($p=0.30$)) or fed (total synthetic rate median 11.3g/day versus 13.9g/day ($p=0.70$)) state. Both cancer patients and controls showed an identical pattern of significant stimulation of albumin synthesis in the fed state of median 29 and 24% respectively.

Fibrinogen synthesis rates are shown in table 10.3. Changes in fibrinogen synthesis between the fed and fasted state are presented in figure 10.5. Cancer patients had substantially and significantly elevated rates of fibrinogen synthesis in both the fasting (median 3.3g/day versus 1.1g/day ($p=0.0019$)) and fed (median 4.5g/day versus 1.3g/day ($p=0.0019$)) state compared with controls. There was no significant change in fibrinogen synthesis with feeding in control patients (median 14%, $p=0.12$). By contrast there was a statistically significant stimulation of fibrinogen synthesis with feeding in the cancer patients (median 38%, $p=0.012$).

Table 10.2. Baseline albumin kinetics of in weight losing cancer patients and healthy controls and of patients after 3 weeks consumption of a fish oil-enriched nutritional supplement.

| | Cancer patients at baseline (n=8) | Healthy controls (n=6) | p (patients vs controls) | Cancer patients after 3 week supplementation n period | p (vs baseline) | p (vs controls) |
|--|--------------------------------------|---------------------------|-----------------------------|---|--------------------|--------------------|
| FSR Fasting (%/day) | 9.6 (6.1-15.6) | 10.1 (5.5-14.8) | 0.80 | 8.8 (6.9-12.5) | 0.40 | 0.52 |
| FSR Fed (%/day) | 12.2 (8.67-21.0) | 12.4 (11.0-15.7) | 0.85 | 10.7 (7.6-17.7) | 0.16 | 0.16 |
| ASR Fasting (mg/kg/day) | 200.1 (102.7-311.6) | 144.0 (103.8-286.1) | 0.44 | 182.9 (103.6-221.5) | 0.16 | 0.25 |
| ASR Fed (mg/kg/day) | 251.8 (188.3-419.2) | 190.5 (156.2-304.0) | 0.16 | 197.3 (119.7-381.9) | 0.093 | 0.093 |
| TSR Fasting (g/day) | 11.3 (6.9-22.0) | 13.9 (6.0-20.1) | 0.70 | 10.5 (6.5-13.7) | 0.21 | 0.70 |
| TSR Fed (g/day) | 14.2 (10.6-29.6) | 15.7 (11.9-21.4) | 0.30 | 11.2 (7.5-22.2) | 0.12 | 0.80 |
| Percentage difference fasted to fed | 29.1 (6.4-121.4) | 24.0 (-2.7-106.8) | 0.70 | 3.0 (-6.0-+91.4) | 0.21 | 0.24 |
| p value fasting versus fed | 0.012 | 0.046 | | 0.29 | | |

Median (range).

Comparison between patients and controls by Mann Whitney U test and between patients over time by Wilcoxon signed rank test.

FSR - Fractional synthesis rate

ASR - Absolute synthesis rate

TSR - Total synthesis rate

Table 10.3. Baseline fibrinogen kinetics of in weight losing cancer patients and healthy controls and of patients after 3 weeks consumption of a fish oil-enriched nutritional supplement.

| | Cancer patients at baseline (n=8) | Healthy controls (n=6) | p (patients vs controls) | Cancer patients after 3 week supplementation n period | p (vs baseline) | p (vs controls) |
|-----------------------|--------------------------------------|---------------------------|-----------------------------|---|--------------------|--------------------|
| FSR | 22.6 | 15.0 | 0.028 | 24.9 | 0.89 | 0.093 |
| Fasting (%/day) | (14.0-32.5) | (11.6-18.0) | | (10.2-34.5) | | |
| FSR | 28.5 | 15.8 | 0.0019 | 29.3 | 0.26 | 0.0098 |
| Fed | (25.3-84.7) | (13.6-24.6) | | (17.2-39.4) | | |
| (%/day) | | | | | | |
| ASR | 58.0 | 14.2 | 0.0019 | 55.6 | 0.67 | 0.0098 |
| Fasting | (28.8-75.6) | (12.2-15.1) | | (17.0-96.7) | | |
| (mg/kg/day) | | | | | | |
| ASR | 68.8 | 16.6 | 0.0019 | 62.7 | 0.12 | 0.0067 |
| Fed | (64.3-196.8) | (12.3-22.0) | | (24.7-110.4) | | |
| (mg/kg/day) | | | | | | |
| TSR | 3.3 | 1.0 | 0.0019 | 2.9 | 0.48 | 0.0019 |
| Fasting | (1.8-4.3) | (0.8-1.5) | | (1.1-5.1) | | |
| (g/day) | | | | | | |
| TSR | 4.5 | 1.3 | 0.0019 | 3.7 | 0.12 | 0.0019 |
| Fed | (3.6-11.2) | (1.0-1.7) | | (1.6-5.8) | | |
| (g/day) | | | | | | |
| Percentage difference | 37.8 | 14.1 | 0.12 | 17.3 | 0.26 | 0.37 |
| fasted to fed | (3.2-160.3) | (-15.7-57.0) | | (-6.4-84.4) | | |
| p value | 0.012 | 0.12 | | 0.017 | | |
| fasting versus fed | | | | | | |

Median (range).

Comparison between patients and controls by Mann Whitney U test and between patients over time by Wilcoxon signed rank test.

FSR - Fractional synthesis rate

ASR - Absolute synthesis rate

TSR - Total synthesis rate

Study after 3 weeks fish oil-enriched nutritional supplement

After 3 weeks administration of the fish oil-enriched nutritional supplement to the eight pancreatic cancer patients weight stabilised (median +1.0kg (range -1.5-+6.5), $p=0.23$ versus baseline, $p=0.012$ versus prestudy rate of weight loss. Albumin and fibrinogen concentrations did not change (0 (-3-+3), $p=0.67$ and -0.4 (-1.78-+0.81), $p=0.16$ respectively).

Albumin synthesis rates are shown in table 10.2. Changes in albumin synthesis between the fasted and fed state are presented in figure 10.2. At baseline there was a significant rise in albumin synthesis in the fed state of median 29% ($p=0.012$). However, after administration of the fish oil-enriched nutritional supplement for three weeks cancer patients no longer had significant stimulation of albumin synthesis on feeding (median rise 3%, $p=0.21$).

Fibrinogen synthesis rates are shown in table 10.3. Changes in fibrinogen synthesis between the fed and fasted state are presented in figure 10.3. At baseline there was a substantial rise in fibrinogen synthesis in the fed state of median 38% ($p=0.012$). After administration of the fish oil-enriched nutritional supplement for three weeks the scale of stimulation of fibrinogen synthesis in the fed state was substantially reduced (median 17%) although this difference did not reach statistical significance ($p=0.26$).

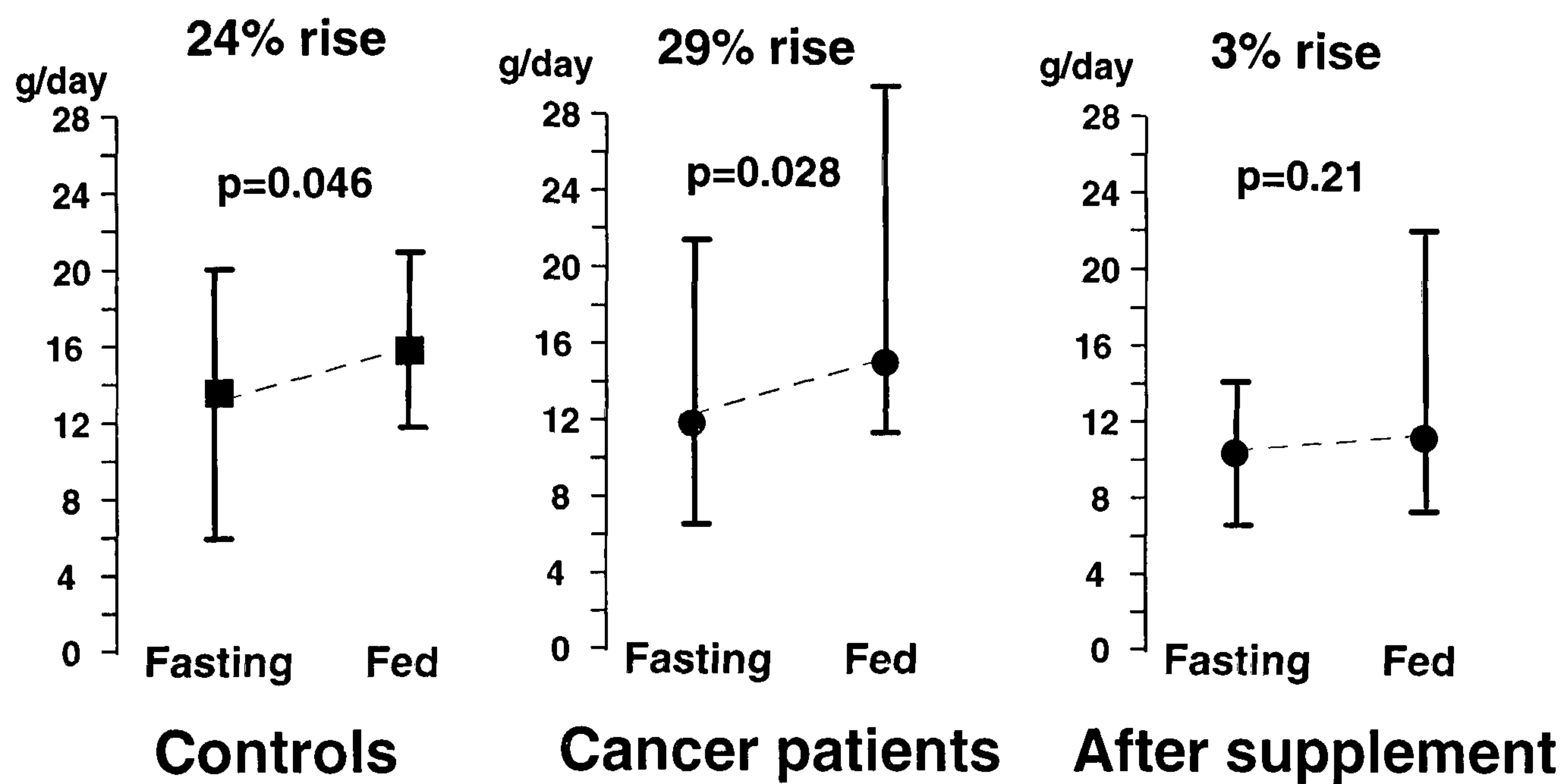


Figure 10.2. Albumin total synthesis rate in the fasted and fed state in 6 healthy controls and 8 weight losing cancer patients at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Graph presents median and range. Comparison between fasting and fed values by Wilcoxon signed rank test.

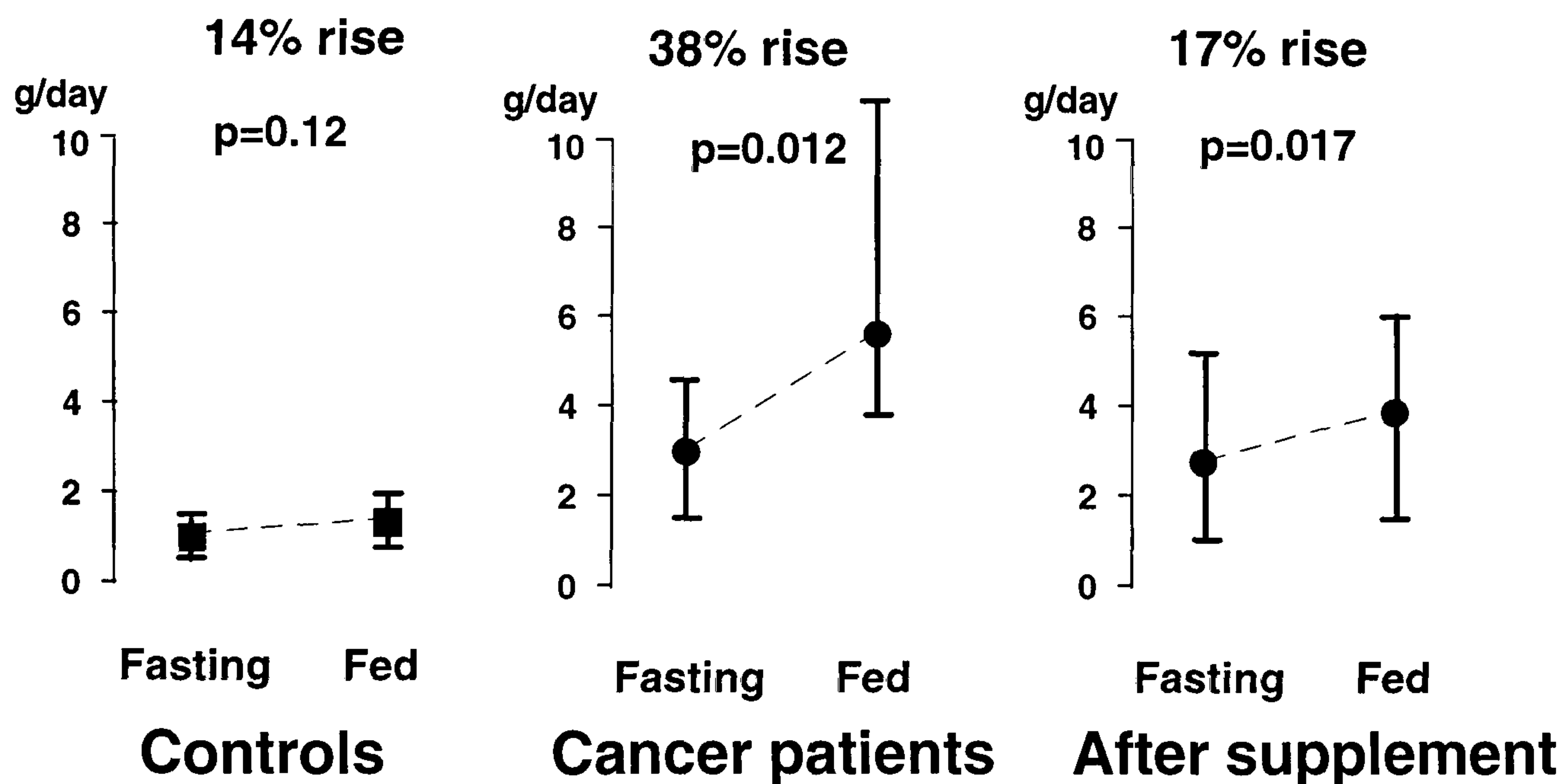


Figure 10.3. Fibrinogen total synthesis rate in the fasted and fed state in healthy controls and weight losing cancer patients at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Graph presents median and range. Comparison between fasting and fed values by Wilcoxon signed rank test.

When the synthetic rates of albumin and fibrinogen were combined (table 10.4 and figure 10.4) there was no difference in synthetic rates of patients in the fasting state before and after 3 weeks consumption of the fish oil-enriched nutritional supplement. Whereas, at baseline, there had been a substantial stimulation of hepatic export protein synthesis of median 33%, after administration of the fish oil-enriched nutritional supplement there was now no significant change, with a significant reduction in synthetic rate ($p=0.012$).

Table 10.4. Combined albumin plus fibrinogen total synthetic rates in cancer patients before and after administration of fish oil-enriched nutritional supplement for 3 weeks.

| | Baseline (n=8) | After intervention period | p value |
|---|---------------------|---------------------------------|---------|
| TSR | 14.7 | 12.3 | 0.093 |
| Fasting (g/day) | (9.5-25.3) | (9.1-18.4) | |
| TSR | 18.7 | 14.6 | 0.012 |
| Fed (g/day) | (15.2-34.2) | (11.0-25.2) | |
| Percentage difference fasted to fed | 33.5 (9.6-112.4) | 10.2 (-0.6-+77.6) | 0.12 |
| p value fasting versus fed | 0.012 | 0.093 | |

Values are median (range).

Comparison by Wilcoxon signed rank test.

FSR - Fractional synthesis rate

ASR - Absolute synthesis rate

TSR - Total synthesis rate

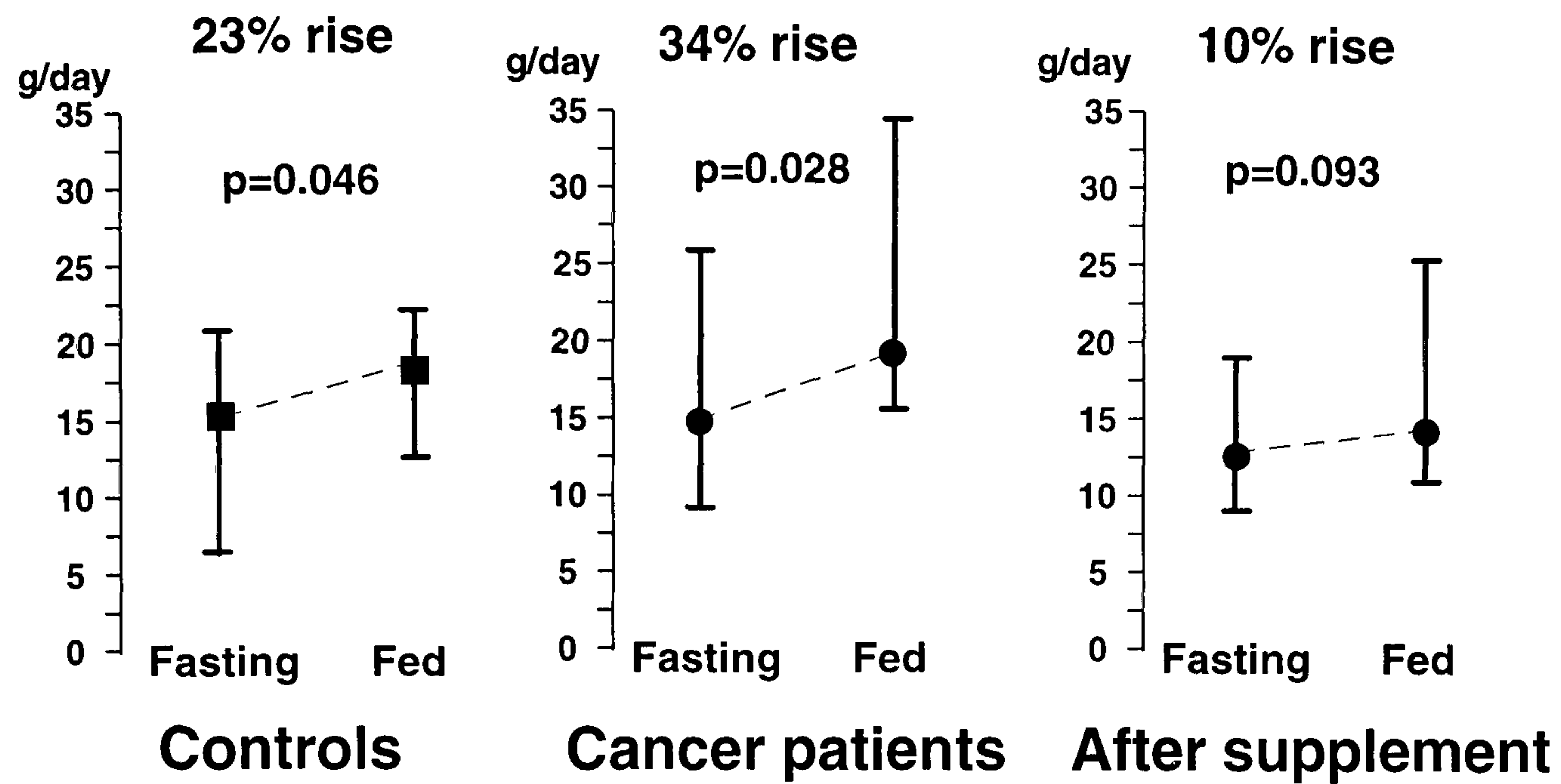


Figure 10.4. Combined albumin and fibrinogen total synthesis rate in the fasted and fed state in 6 healthy controls and 8 pancreatic cancer patients at baseline and after 3 weeks consumption of a fish oil-enriched nutritional supplement. Graph presents median and range. Comparison between fasting and fed values by Wilcoxon signed rank test. Comparison of fasting synthesis rate at baseline and after supplement $p=0.093$. Comparison of fed synthesis rate at baseline and after supplement $p=0.012$.

Discussion

This chapter has shown that weight-losing patients with pancreatic cancer undergo stimulation of albumin synthesis with feeding and that this is similar to healthy controls despite significantly lower circulating albumin concentrations and the presence of an APPR in the cancer patients. Circulating fibrinogen concentrations were significantly elevated in the cancer patients and their fibrinogen synthesis was elevated significantly in both the fasting and fed state. While controls show no change in fibrinogen synthesis in the fed state there was a significant increase with feeding in cancer patients.

In addition the present chapter has shown that the administration of this supplement results a significant reduction in the combined synthetic rate of the hepatic export proteins albumin and fibrinogen in the fed state in cachectic pancreatic cancer patients in association with beneficial nutritional effects, modulation of metabolic mediators and stabilisation of acute phase protein concentrations as described in Chapters 6-9.

The acute phase response is observed following a variety of insults including trauma, infection and malignancy. The APPR is thought to be stimulated by pro-inflammatory cytokines such as interleukin-6 (Castell et al, 1990). The synthesis of acute phase proteins by hepatocytes is also known to be influenced by endocrine hormones such as insulin and cortisol (O'Riordain et al, 1995). Increased production of interleukin-6 is observed in patients with advanced pancreatic cancer (Falconer et al, 1994a) and Chapter 4 demonstrated elevated circulating IL-6 concentrations in such patients. Alterations in the balance of neuroendocrine hormones have been shown previously (Fearon et al, 1998 and Chapter 9).

In the present chapter, despite evidence of an acute phase protein response and reduced circulating albumin concentrations in the cancer patients, there was no evidence of a reduction in albumin synthesis in the fasting state. These findings are in keeping with other studies in cancer (Fearon et al, 1998), during surgery (van Acker et al, 1998, Barle et al, 1998), after vaccination (Cayol et al, 1995), acutely after stress hormone infusion (McNurlan et al, 1996) or in sepsis (Dahn et al, 1995). Thus the mechanisms controlling hepatic albumin synthesis are complex and the importance of the role of pro-inflammatory cytokines in suppressing synthesis *ex vivo* must be called into question.

Albumin synthesis has been shown to be stimulated significantly by feeding in healthy individuals (De Feo et al, 1992, Hunter et al, 1995). The administration of protein appears to provide a significantly greater stimulus to albumin synthesis than a protein-free feed (Cayol et al, 1996, Cayol et al, 1997), suggesting that dietary amino acids are readily used to synthesise

hepatic export proteins. Different methods of measurement of protein synthesis and different routes of feeding have been used in the above studies. Using a primed constant infusion method and intraduodenal feeding an increase of around 90% in albumin synthesis in the fed state was found (De Feo et al, 1992), while a flooding dose protocol and oral feeding produced an increase of around 20% (Hunter et al, 1995). The present study utilised the flooding dose protocol for measurement of protein synthesis (Garlick et al, 1989, Ballmer et al, 1990, Garlick et al, 1994). This technique is convenient for both staff and experimental subjects, allowing the present study to be performed on an outpatient basis. It also allows more certainty of enrichment of the appropriate precursor pool for protein synthesis (Preston et al, 1998). The flooding dose technique for measuring protein synthesis has been criticised as it has been suggested that the rapid infusion of amino acids, particularly essential amino acids, stimulates insulin secretion and therefore protein synthesis (Rennie et al, 1994). The present study suggests that any stimulus of protein synthesis due to tracer infusion does not disguise the stimulus provided by feeding. In addition, measurement of circulating insulin concentrations revealed no obvious increase after the infusion of labelled phenylalanine. This is particularly clear in the fasted state (figure 2). Similarly there is no difference in insulin concentrations between subjects receiving amino acid infusion and those not in the results presented in Chapter 9. Insulin samples were taken every 20 minutes and so a transient rise in insulin cannot be ruled out.

Albumin synthesis has not previously been studied in the fed state in cancer patients but the present study using a flooding dose method and oral feeding suggests that synthesis is stimulated to a similar degree to control subjects by around 25%. Thus, an abnormally low albumin synthetic response to feeding is unlikely to explain the low circulating concentrations in these patients. Head-injured patients with an acute phase response also have higher albumin synthesis rates than controls in the fed state (Mansoor et al, 1997). In the present study the size of the feeding stimulus was determined by measured resting energy expenditure. As in previous studies (Hunter et al, 1995) the size of the feeding stimulus in the present study was determined by measured resting energy expenditure. As the cancer patients had a significantly lower body weight than controls with a similar resting energy expenditure, the feeding stimulus was larger per unit body weight in the cancer patients (cancer patients median 3.15kcal/kg (range 2.73-3.37) versus controls 2.36kg/kcal (1.92-2.71), $p=0.0019$). It is possible that this may have led to a relative increase in the stimulation of protein synthesis by feeding in the cancer patients.

Circulating fibrinogen concentrations are increased during an acute phase response. Synthesis of fibrinogen has been shown to be elevated approximately 2-fold in the fasting state in cancer patients compared with controls (Preston et al, 1998). Fibrinogen fractional synthesis rates of around 26% (Stein et al, 1978) and around 39% (Preston et al, 1998), with similar ranges to that in the present study, have been shown in fasting cancer patients. A similar degree of elevation of fibrinogen synthesis has also been demonstrated in trauma and burns patients (Thompson et al, 1989). In healthy subjects, feeding has been shown to have either no effect (De Feo et al, 1992, Cayol et al, 1996) or a small (around 20%) stimulatory effect (Boirie et al, 1998) on fibrinogen synthesis similar to the findings in the healthy controls in the present study. In contrast, feeding caused a marked further increase in fibrinogen synthesis in the cancer patients in the present study. A similar observation has been made in head-injured patients although the synthesis rate in controls was rather lower than that seen in other studies and the effect of feeding was not discriminated from that of disease (Mansoor et al, 1997). The present study, therefore, suggests that in cancer patients the synthesis of both positive and negative acute phase proteins is stimulated by feeding.

There was no obvious relationship between circulating protein concentrations and rates of synthesis of the relevant protein with the numbers in the present study. In addition there was no significant change in concentration of albumin or fibrinogen over the 4 hour study period. With a fasting fibrinogen fractional synthesis rate of around 1% per hour, a fed rate of around 1.25% per hour and a fractional breakdown rate of at least 1% one would not expect an increase in fibrinogen concentration within the precision of the assay. With its larger pool and slower turnover any change in albumin concentration would be even smaller.

Patients with advanced cancer exhibit marked loss of weight, a large component of which is skeletal muscle (Fearon & Preston, 1990). This weight loss is particularly marked in the presence of an acute phase protein response (Falconer et al, 1994a, Staal-van den Brekel et al, 1995, Wigmore et al, 1997b) and appears to contribute to worsened survival (Blay et al, 1992, Falconer et al, 1995). If the synthesis of negative acute phase proteins such as albumin is not changed during the acute phase response and the synthesis of positive acute phase proteins is markedly elevated (when patients are in the fasted or fed state) then the demand for amino acids in order to manufacture these proteins may accelerate the breakdown of skeletal muscle especially if nutritional intake is inadequate. It has been suggested that the differences in the amino acid composition of acute phase proteins and skeletal muscle may further increase the need for muscle breakdown to supply particularly aromatic amino acids (Reeds et

al, 1994). To represent a continuing drain on amino acid resources recycling of amino acids from acute phase proteins would have to be inefficient. There has been no measure of amino acid return from acute phase protein breakdown. In any event the synthesis and recycling of this protein requires energy and may contribute to the hypermetabolism previously observed in association with the acute phase response (Falconer et al, 1994, Staal-van den Brekel et al, 1995, Wigmore et al, 1997b).

Albumin may account for 5-7% of whole body protein synthesis in the fasted state in healthy subjects (De Feo et al, 1992). This may increase to over 10% in the fed state (De Feo et al, 1992, Cayol et al, 1997). It has been estimated that synthesis of all acute phase proteins may account for 30% of total protein synthesis during infection in the fasted state (Waterlow, 1991). While this total may be less in the chronic inflammatory state such as cancer, with the stimulation of both positive and negative acute phase protein synthesis by feeding, acute phase protein synthesis may represent a substantial proportion of total body protein synthesis. The present study suggests that a proportion of dietary amino acids may be used for accelerated acute phase protein synthesis and thus not be available for peripheral tissue anabolism. In addition, the supply of additional dietary amino acids delivered first to the liver via the portal system and used for acute phase protein production will result in consumption of particular amino acids. The remaining amino acid mixture may thus be unbalanced further inhibiting efficient skeletal muscle anabolism.

Protein synthesis rates are only one factor in determining plasma protein concentrations but at present there is limited information on acute phase protein breakdown and transcapillary escape. There is indirect evidence of increased fibrinogen breakdown in cancer with elevated concentrations of fibrin degradation products (Dvorak et al, 1987, McMillan et al, 1994), however, the role of fibrinogen in coagulation complicates the relevance of this finding. Transcapillary escape has been shown to occur at an elevated rate in weight-losing cancer patients and been suggested to contribute to the hypoalbuminemia of the acute phase response (Fleck et al, 1985). However, lymphatic return must be similarly increased as there is no relationship between transcapillary escape and albumin concentration (Ballmer et al, 1994) and no change in the intravascular albumin pool with elevated transcapillary escape (Ballmer-Weber et al, 1995). Limited work has suggested a moderate increase in albumin breakdown in cancer patients although it is not clear whether the patients studied were losing weight (Rossing, 1968). It has also been suggested that the tumour itself may consume albumin (Stehle et al, 1997) although the significance of this phenomenon in

human disease remains unclear. Further study of acute phase protein breakdown in weight-losing cancer patients is required.

The present study also demonstrated a difference in the pattern of stimulation of protein synthesis by feeding in pancreatic cancer patients after the consumption of a fish oil-enriched nutritional supplement for three weeks. There was a loss of stimulation of albumin synthesis with feeding and a halving of the magnitude of the stimulation of fibrinogen synthesis. This meant that there was now no significant stimulation by feeding of synthesis of the two proteins combined and that there was a significant reduction in the combined protein synthesis in the fed state.

The magnitude of the changes in protein synthetic rate are small in terms of whole body protein kinetics, but may be larger if all positive and negative acute phase proteins behave in a similar manner.

Thus the stimulation of the synthesis of hepatic export proteins by feeding in cancer patients provides one new mechanism which may contribute to the suboptimal response to conventional nutritional supplementation observed in some patients with cancer. In addition, the demonstration that the provision of a fish oil-enriched nutritional supplement will abolish this provides a potential mechanism to explain in part the beneficial effects seen on weight and lean body mass seen in Chapter 6. Other potential actions of eicosapentaenoic acid may, of course, have contributed to the conservation of lean tissue observed such as a reduction in the activity of proteolysis inducing factor (PIF) at the skeletal muscle level (Tisdale, 1996) and a reduction in resting energy expenditure (Chapter 6).

Discussion

In contrast to those otherwise well individuals who have lost weight due to inadequate dietary intake, patients with cancer cachexia frequently do not regain weight and functional ability when supplied with additional nutrients (Roubenoff et al, 1997). This thesis has attempted to explore some of the possible mechanisms for this phenomenon together with studying some of the mediators responsible and the importance of cachexia to patients' survival and performance. In addition an attempt has been made to modulate the mechanisms and mediators responsible for cachexia to allow patients to regain weight with the provision of a fish oil-enriched nutritional supplement.

The importance of cancer cachexia to patient well-being

It was demonstrated in Chapter 3 that patients with pancreatic cancer progressively lose weight and associated with this is a deterioration in performance status. Others have previously reported that cancer patients with weight loss have an inferior performance status and quality of life compared with weight stable cancer patients (DeWys et al, 1980, Ovesen et al, 1993a, O'Gorman et al, 1998).

It has been shown that advanced cancer patients have a marked deficit in lean tissue compared with healthy controls (Heymsfield & McManus, 1985, Fearon & Preston, 1990). Longitudinal studies using total body potassium measurement have demonstrated that a major proportion of the weight lost by cancer patients is accounted for by loss of lean tissue (McMillan et al, 1998). The data presented in Chapter 3 is consistent with this finding, although the bioelectrical impedance technique of body composition analysis used in the studies presented in this thesis is not as precise.

Loss of protein in cancer patients has been associated with reduced muscle strength and an increased frequency of infective episodes and shortened survival (Nixon et al, 1980, Windsor & Hill, 1988a, Windsor & Hill, 1988b). The ongoing loss of lean tissue is, therefore, a crucial step in the progression of cachexia and a vital target for intervention in cachexia. While some agents used in the treatment of cachexia such as steroids will transiently improve a few measures of quality of life (Robustelli della Cuna et al, 1989, Popiella et al, 1989), they probably act by a direct central effect and no intervention studied previously has been shown to influence loss of lean tissue in cachexia.

Pro-inflammatory cytokines and their role in cachexia

When considering intervention in cachexia it is important to try to understand the mediator pathways involved which are absent in benign weight loss and may therefore be responsible for the relative lack of response to refeeding in cachexia. Elevated ex vivo peripheral blood mononuclear cell production of the pro-inflammatory cytokines interleukin-6 and tumour necrosis factor α has previously been demonstrated in advanced pancreatic cancer (Falconer et al, 1994a) although the present study has failed to confirm this (Chapter 7). Elevating circulating concentrations of interleukin-6 have previously been shown in solid adult tumours (Fearon et al, 1991, Scott et al, 1996) and this has now been demonstrated in pancreatic cancer in Chapter 7. Concentrations of interleukin-6 have also been found to be associated with the acute phase response (Fearon et al, 1991, Scott et al, 1996 and Chapters 4 and 7) and with weight loss in cancer patients. Chapter 4 has also demonstrated an association between tumour necrosis factor receptor concentrations (suggested to reflect tumour necrosis factor activity) and the acute phase protein response.

It is not clear why no obvious difference was demonstrated between peripheral blood mononuclear cell cytokine production in cancer patients and healthy controls when increased circulating concentrations of interleukin-6 and tumour necrosis factor receptors were found in cancer patients (Chapter 7). Previous studies in pancreatic cancer patients have shown increased pro-inflammatory cytokine production but not circulating concentrations (Falconer et al, 1994a, Wigmore et al, 1997d). Similar techniques were used for measuring cytokine production and concentrations in the previous and present studies, the only difference being the ELISA kit used. The different kits may have had differing sensitivity for the cytokines from serum and cell culture medium. The patients examined for the current thesis were recruited at a relatively early stage in their disease compared with previous studies and it is possible that this may have affected the results obtained. Further study of cytokine production and circulating concentrations in advanced cancer patients is required to clarify the significance of these conflicting observations.

The data presented in Chapter 5 suggests that production of interleukin-1 β is associated with the acute phase protein response, that interleukin-1 β production is genetically determined in pancreatic cancer and that these factors both influence survival. This raises the question of whether we can ascribe effects observed to a single cytokine acting alone and whether the loss of weight observed in cancer is a direct action of such cytokines or whether they represent a small corner of a much larger network of mediators culminating in the cachectic syndrome. The observations in Chapters 4, 5 and 7 that concentrations of interleukin-6, markers of tumour

necrosis factor activity (tumour necrosis factor receptor concentrations) and production of interleukin- 1β all seem to be related to each other, to the inflammatory response, and, by extension, to the progression of cachexia makes it unlikely that any one cytokine acts alone in this condition. Experimental work also seems to support this with antibody blockade of single cytokines having only a very limited effect on cachexia in animal models (Gershenwald et al, 1990, Gelin et al, 1991, Strassmann et al, 1992, Smith & Kluger, 1993). Administration of antibodies against single cytokines will also affect concentrations of others (Gershenwald et al, 1990) and injection of single cytokines into human and animal subjects will induce the production of others (Yasumoto et al, 1995, Mori et al, 1996). Thus it seems that pro-inflammatory cytokines form a complicated web of overlapping effects, mutual stimulation and redundancy in their action. An important result of this is that pharmacological agents aimed at downregulating only one or a few cytokines are unlikely to be of benefit in cachexia.

While cytokines may have direct effects in the hypothalamus to decrease appetite and increase resting energy expenditure (Mahony & Tisdale, 1988, Moldawer et al, 1988, Hellerstein et al, 1989, Langstein et al, 1991, Matthys et al, 1991, Smith & Kluger, 1993) and they may also act at this site via effects on neurotransmitters such as serotonin, neuropeptide Y and corticotrophin releasing hormone (Cangiano et al, 1994, Schwarz et al, 1995) there is no evidence that cytokines alone in culture with muscle will cause protein breakdown (Goodman, 1990, Tisdale, 1997). Thus, while pro-inflammatory cytokines may be a significant part of the inflammatory cascade ultimately resulting in cachexia and protein breakdown, it is important to examine the other mediators and mechanisms influenced by cytokines.

Other mediators and their interaction with pro-inflammatory cytokines

The data presented in Chapter 9 demonstrated that weight-losing patients with advanced pancreatic cancer have a reduced fasting concentration of the anabolic hormone insulin compared with healthy controls. The concentration of the catabolic hormone cortisol was not different in cancer patients and controls. Such findings have been noted previously in a similar group of patients (Fearon et al, 1998). It has been suggested that the ratio of these hormones may give an impression of the anabolic / catabolic balance and in the present study the cortisol / insulin ratio was substantially increased in cancer patients compared with controls suggesting a catabolic picture. In response to feeding at baseline, the concentration of insulin rose by a similar proportion and absolute amount to that seen in healthy subjects suggesting that it is not an absolute lack of insulin in the fed state which prevents anabolism with feeding. Administration of supraphysiological doses of pro-inflammatory cytokines will influence the

concentrations of conventional endocrine hormones, with increases in cortisol and glucagon and a decrease in insulin concentrations (Selby et al, 1987, Warren et al, 1987, Michie et al, 1988, Starnes et al, 1988, Stouthard et al, 1996). It is not clear from the data available at present whether the changes observed in cancer patients are a response to cytokine stimulation or whether both are reflections of the wider inflammatory and catabolic state.

Another suggested mediator of weight loss in advanced cancer is proteolysis inducing factor (PIF), a glycoprotein identified in a cachectic mouse model which causes protein breakdown (Todorov et al, 1996a). Clinical measurement of PIF is in its infancy. Circulating concentrations are not readily measurable but assessments are made of urinary excretion (Todorov et al, 1996a). These measurements are not quantitative but excretion has so far only been found in weight-losing cancer patients but not in weight-stable cancer patients, those losing weight with benign disease or in healthy subjects (Todorov et al, 1996a, Todorov et al, 1996b, Cariuk et al, 1997). It was shown in Chapter 7 that proteolysis inducing factor was found in the urine of 89% of the weight losing pancreatic cancer patients examined and not in healthy controls. It has been suggested that proteolysis inducing factor may work in concert with pro-inflammatory cytokines to produce weight loss in cancer (Fujiki et al, 1997).

The role of energy balance in cancer cachexia

It has previously been suggested that increased resting energy expenditure may contribute to weight loss in advanced cancer (Fredrix et al, 1991, Staal-van den Breckel et al, 1994, Falconer et al, 1994a). This is perhaps due to small daily energy costs of increased protein and carbohydrate turnover (Edén et al, 1984, Fearon et al, 1988). The data presented in Chapter 9 suggests that resting energy expenditure per unit weight was elevated in the group of pancreatic cancer patients studied. However, Chapter 9 also demonstrated that there was no increased energy cost of feeding in these patients. The physical activity component of energy expenditure was not examined in the present study but it has been suggested that this may be reduced in advanced cancer patients (Gibney et al, 1997). Those examined in the present study did have limitations to their functional activity brought about by their disease reflected in reduced performance status (Chapter 3) and thus the present study provides no evidence that overall energy expenditure was increased. While the small increase in relative resting energy expenditure may reflect futile metabolic cycles known to be occurring it would appear that the reduction in the energy cost of feeding observed in Chapter 9 is appropriate to the weight-losing state of these patients.

Before intervention only a few patients had an apparently inadequate dietary intake that would result in a marked negative energy balance based on estimated total energy expenditure (Chapter 9). While previous studies have shown an inadequate dietary intake in pancreatic cancer patients, differences tend to be small or limited to a few patients (Wigmore et al, 1997b). This means that for many patients, particularly the group examined in the present study who were relatively heterogeneous in terms of energy balance, a large increase in energy expenditure (in the fasted and fed state) or a major decrease in energy intake was not observed and large energy imbalances do not explain the weight loss observed. Indeed the small reduction in the energy cost of feeding would appear to be an appropriate adaptation to the weight-losing state. Thus it is possible that specific metabolic pathways may drive the progressive loss of lean tissue in cancer patients and prevent anabolism with feeding rather than a particularly marked energy imbalance exacerbated by feeding.

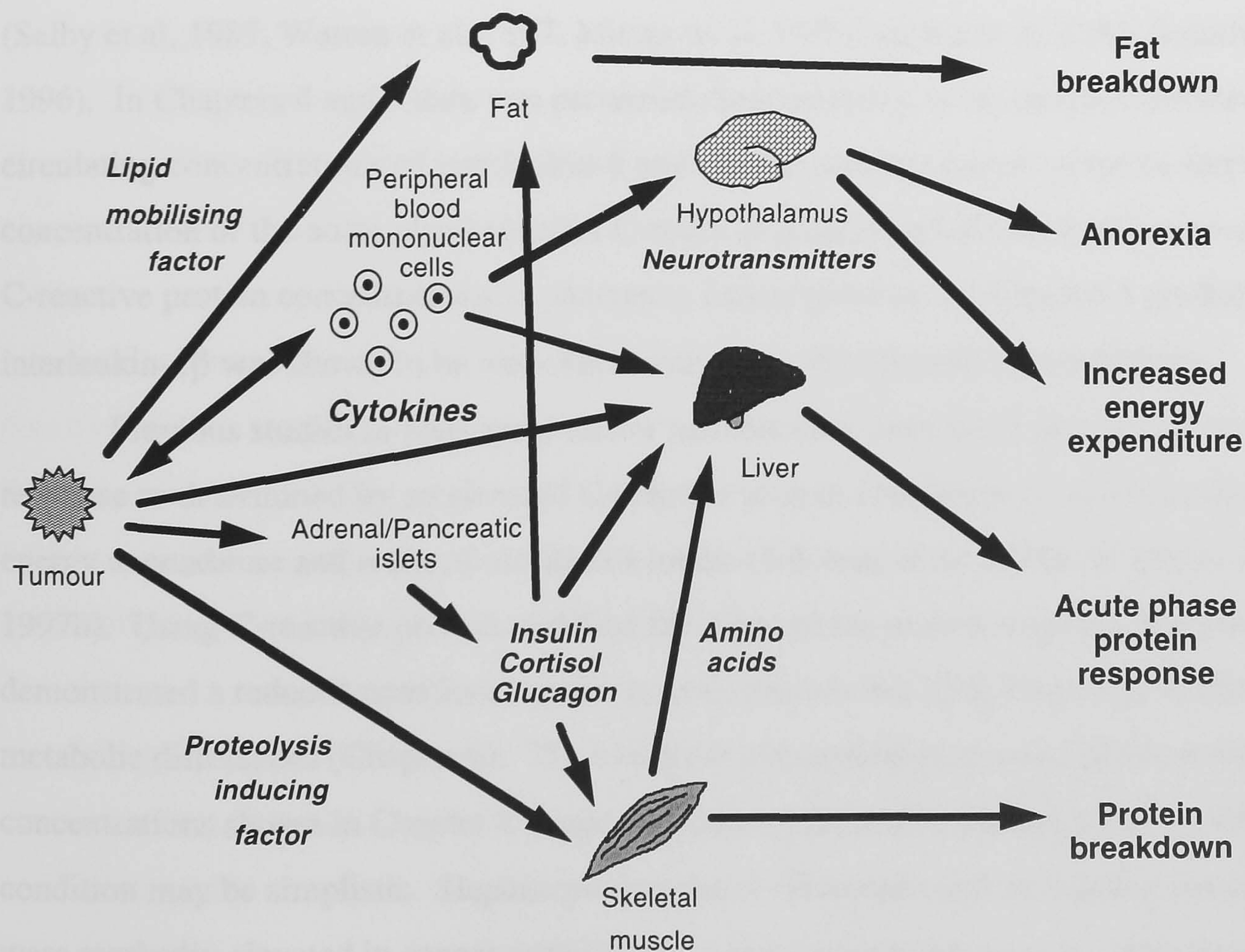


Figure 11.1. Potential network of mediators and mechanisms in cancer cachexia.

The acute phase protein response as a contributor to cachexia

It is increasingly being recognised that associated with loss of weight and progress of disease in many advanced malignancies is an inflammatory response characterised by changes in plasma protein concentrations (Falconer et al, 1994a, Staal-van den Breckel et al, 1995, Wigmore et al, 1997b, O’Gorman et al, 1998, McMillan et al, 1998) while no such changes are observed in simple starvation. This acute phase protein response, with increases in the concentrations of positive acute phase proteins such as C-reactive protein and fibrinogen and decreases in negative acute phase proteins such as albumin, has been associated with poor survival in advanced pancreatic cancer (Falconer et al, 1995) and this association has also been demonstrated in Chapter 5. The data presented in Chapter 8 confirms marked changes in the concentrations of a broad spectrum of acute phase proteins in advanced pancreatic cancer patients compared with healthy controls while both Chapter 3 and Chapter 8 suggest that the relevant positive and negative changes become more marked with progress of disease.

The acute phase protein response has been shown to be stimulated in cultured hepatocytes by pro-inflammatory cytokines (Castell et al, 1990). The administration of pro-inflammatory cytokines also stimulates an acute phase protein response in human subjects (Selby et al, 1987, Warren et al, 1987, Michie et al, 1988, Starnes et al, 1988, Stouthard et al, 1996). In Chapters 4 and 7 data was presented demonstrating an association between circulating concentrations of interleukin-6 and tumour necrosis factor receptors and the concentration of the acute phase reactant C-reactive protein and of interleukin-6 production and C-reactive protein concentrations in pancreatic cancer patients. In Chapter 5 production of interleukin-1 β was shown to be associated with C-reactive protein concentrations.

Previous studies in pancreatic cancer patients have associated the acute phase protein response as determined by an elevated C-reactive protein concentration with increased resting energy expenditure and reduced nutritional intake (Falconer et al, 1994a, Wigmore et al, 1997b). Using C-reactive protein to define the acute phase protein response, the present study demonstrated a reduced nutritional intake in such patients but little in the way of other metabolic differences (Chapter 9). The variety of abnormalities in acute phase protein concentrations shown in Chapter 8 suggest that using C-reactive protein alone to define this condition may be simplistic. Hepatic production of fibrinogen and circulating concentrations were markedly elevated in cancer patients with normal C-reactive protein concentrations (Chapter 10), thus an acute phase protein response may apparently be present without changes in the concentrations of all acute phase proteins. Further longitudinal studies would be required to determine how the various acute phase protein concentrations change quantitatively and

temporally with the progress of disease. However, Chapter 8 also suggests a progressive increase in the concentration of positive acute phase proteins and a progressive decrease in the concentration of negative acute phase proteins with time.

From in vitro studies which demonstrated increased production of positive acute phase proteins in parallel with decreased production of negative acute phase proteins it has been assumed that the process did not greatly affect overall nitrogen balance (Castell et al, 1990). However, it has recently been shown that although the albumin concentration may be reduced in advanced cancer its production remains unchanged compared with controls in the fasting state (Fearon et al, 1998). Fibrinogen concentrations are elevated in cancer patients and in the fasting state its synthesis is substantially increased compared with controls (Preston et al, 1998). It has been shown in Chapter 10 that in the fed state there is a very similar increase in albumin synthesis in both cancer patients and controls and that while controls have no change in fibrinogen synthesis with feeding there is a substantial stimulation of the already elevated fibrinogen synthesis in cancer patients. Thus if these results apply to the other positive and negative acute phase proteins there is no apparent nitrogen saving from decreased synthesis of negative acute phase proteins. In addition, feeding serves to stimulate positive (and negative) acute phase protein synthesis. In cachectic cancer patients this may thus represent a potential mechanism for continued amino acid demand which must be supplied from lean tissue stores in the fasting state. In addition it may contribute to the lack of anabolic response to feeding in cancer patients if a proportion of supplied nutrients are diverted to acute phase protein synthesis.

Despite the imbalance in skeletal muscle and acute phase protein amino acid composition (Reeds et al, 1994) the overall quantity of protein involved is small and factors such as amino acid recycling have not been examined, however, there appears to be strong evidence to link the acute phase protein response to the ongoing process of cachexia and potentially to the lack of anabolic response to feeding.

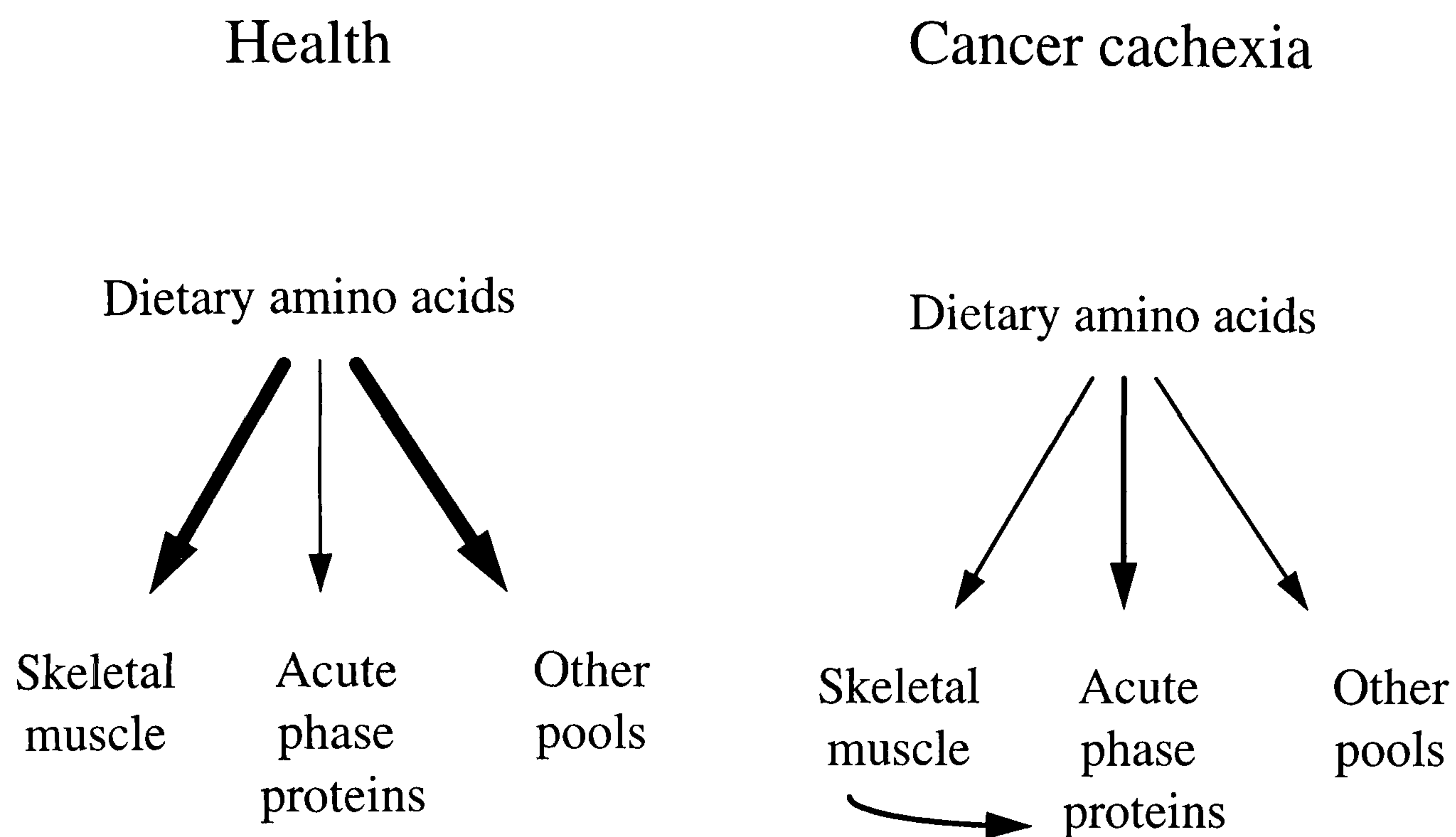


Figure 11.2. Potential role of the acute phase protein response as a drain on amino acid resources contributing to ongoing lean tissue breakdown not reversed by feeding.

Intervention in cancer cachexia

Curative treatment of patients with advanced cancer remains rare thus it is left to try to treat the symptoms suffered by patients in an attempt to improve their quality and length of life. The treatment of cachexia would seem a legitimate target for treatment as it has a substantial influence on patients well-being and survival. The supply of additional nutrients does not appear to influence the progress of cachexia for the reasons outlined above.

Options for intervention in cancer cachexia and other pro-inflammatory cytokine mediated weight losing conditions such as sepsis have included direct anticytokine therapy with specific antibodies (Wherry et al, 1993, Smith, 1998), general anti-cytokine agents such as pentoxifylline and thalidomide (Goldberg et al, 1995, Haslett, 1998), agents acting on specific enzymes in carbohydrate metabolism such as hydrazine sulphate (Loprinzi et al, 1994a, Loprinzi et al, 1994b) and agents affecting hypothalamic neurotransmitters such as cyproheptadine (Kardinal et al, 1990). Steroids and progestogenic agents may also act centrally to affect appetite and well being (Robustelli della Cuna et al, 1989, Popiela et al, 1989, Bruera et al, 1998). Unfortunately none of these approaches appears to be wholly successful in reversing the metabolic changes responsible for weight loss and some, such as steroids, may exacerbate them. It is likely that these agents are either too specific, acting only

on a small corner of the huge network of mediators of cachexia, or act too far down the chain of events to effect an overall change.

The interventions which have shown some promise in cachexia have been suggested to normalise the metabolic state of cachectic patients to some degree. The non-steroidal anti-inflammatory ibuprofen has been shown to reduce concentrations of pro-inflammatory cytokines and acute phase proteins and to normalise whole body protein kinetics (McMillan et al, 1995, Preston et al, 1995, Wigmore et al, 1995). Indomethacin may stabilise performance status and improve survival. The n-3 fatty acid eicosapentaenoic acid and fish oil rich in eicosapentaenoic acid have been shown to reduce pro-inflammatory cytokine production and acute phase protein concentrations (Wigmore et al, 1996, Wigmore et al, 1997d) and may stabilise weight and prolong survival (Wigmore et al, 1996, Barber et al, 1997, Gogos et al, 1998). While these agents have shown potential in manipulating some of the presumed mediators of cachexia none will reverse the process of cachexia itself on their own.

A new approach has been the combination of an anti-inflammatory agent to affect the inflammatory process with an agent to increase nutritional intake. The only trial previously published taking this approach in cancer cachexia examined the combination of ibuprofen with the appetite stimulant megestrol acetate compared with megestrol acetate alone (McMillan et al, 1999). Seventy-three patients with gastrointestinal cancer were studied over 12 weeks. Although nutritional intake was not measured, appetite improved in both groups. However, in the group taking megestrol acetate alone weight loss continued at a rate of around 1kg/month while the group also receiving ibuprofen had a significant weight gain of around 1kg after 6 weeks and 2.3kg after 12 weeks. Quality of life studies also suggested some benefits to the group receiving the combination treatment. The studies presented in Chapters 6-10 took a similar approach using a nutritional supplement to increase nutritional intake in combination with fish oil, rich in eicosapentaenoic acid, to down-regulate the inflammatory process.

The effect of a fish oil-enriched nutritional supplement in cancer cachexia

Previous studies have suggested that fish oil or eicosapentaenoic acid will affect various mediators believed to have a role in cachexia. Production of or circulating concentrations of the pro-inflammatory cytokines interleukin-1, interleukin-6 and tumour necrosis factor have been suggested to be reduced by these agents in humans in health and disease (Endres et al, 1989, Meydani et al, 1991, Meydani et al, 1993, Cooper et al, 1993, Purasiri et al, 1994, Caughey et al, 1996, Wigmore et al, 1997d). However, another group using apparently similar techniques and n-3 fatty acid doses have found prompt rises in pro-inflammatory cytokine

production (Demols et al, 1998). The present study demonstrated that there was a significant reduction in the production of interleukin-6 by weight-losing pancreatic cancer patients after the administration of a fish oil-enriched nutritional supplement for three weeks under certain culture conditions while there was no change in the concentration of circulating cytokines or their receptors (Chapter 7). As discussed above the significance of this finding is difficult to gauge in view of the elevated circulating concentration of interleukin-6 observed in cancer patients compared with controls and the similar ex vivo interleukin-6 production in the two groups. Further study in this area is required.

It has previously been suggested that eicosapentaenoic acid may inhibit the end organ effects of PIF in vitro (Tisdale et al, 1996). The effects of an eicosapentaenoic acid-based intervention on PIF have not previously been studied in human subjects. The present study demonstrated a reduction in the proportion of patients with detectable PIF in urine from 89% to 40% after provision of a fish oil enriched nutritional supplement for 3 weeks. Measurement of PIF activity in vivo is not yet possible and it is not yet clear in any detail how urinary excretion of PIF relates to its concentration or activity in circulation but the observation of reduced urinary excretion may suggest that the concentration of this mediator in circulation may be reduced with or without any effect on end-organ activity.

Fasting insulin concentration increased significantly in the weight-losing cancer patients after intervention to a level similar to that seen in healthy controls (Chapter 9). The cortisol / insulin ratio also fell significantly suggesting a change to a more anabolic hormonal balance. The effect of a fish oil-derived intervention on hormone concentrations has not previously been studied. However, Chapter 9 also demonstrated that the insulin response to feeding changed little after supply of the fish oil-enriched nutritional supplement suggesting that it is not an increased amount of insulin in the fed state that is responsible for allowing anabolism. This observation also supports the supposition made above that it is not a lack of insulin in the fed state which prevents anabolism in cachexia. It therefore seems likely that insulin concentrations appropriately reflect the general metabolic state rather than being a major causative factor of the cachexia.

After intervention there was a reduction in resting energy expenditure but an increase in the energy cost of feeding (Chapter 9). These changes were seen in conjunction with a relative normalisation of substrate utilisation and suggest patients were in a more stable metabolic state similar to the healthy controls examined. This again suggests that these factors are a reflection of the metabolic changes of these patients rather than a cause.

The weight-losing pancreatic cancer patients achieved a significant increase in their nutritional intake with the provision of the fish oil-enriched nutritional supplement (Chapter 6). This occurred despite an initially poor appetite and was accompanied by an improvement in appetite. Patients had a substantial ongoing weight-loss prior to intervention with all patients losing more than 1.6kg/month. Fourteen of the eighteen surviving patients began regaining weight after receiving the fish oil-enriched nutritional supplement and the other four patients had a substantial slowing of their rate of weight-loss. Previous studies in cancer patients have succeeded in increasing the nutritional intake of patients to a similar degree to that which was achieved in the present thesis (Evans et al, 1987, Ovesen et al, 1993b). However, the provision of additional nutrients in these studies failed to affect weight. Similarly, while the administration of anti-inflammatory agents such as ibuprofen and fish oil-based preparations have reduced concentrations of inflammatory mediators and stabilised weight none have been shown to reverse weight loss in cachectic cancer patients (McMillan et al, 1995, Preston et al, 1995, Wigmore et al, 1995, Wigmore et al, 1996, Barber et al, 1997). Thus it appears that the combination of the fish oil as an anti-inflammatory agent with nutritional supplementation was responsible for the effects observed.

Weight gain has been achieved in previous studies of interventions in cachectic cancer patients, notably megestrol acetate, however, body composition analysis has revealed this to be due to the accumulation of water or fat (Loprinzi et al, 1993b, Simons et al, 1998). As discussed above, it would appear to be the loss of lean tissue that contributes to the loss of functional ability and shortened survival of cachectic cancer patients and thus weight gain alone is not an appropriate endpoint in studies of intervention in cachexia. In the present thesis body composition was assessed by bioelectrical impedance analysis and a significant increase in lean body mass was observed after intervention with no change in percentage body water or fat mass. The observed body composition changes may be questioned due to the relatively imprecise nature of the bioimpedance method used (Kotler et al, 1996, Jensen et al, 1997). The method relies on equations (largely based on weight and height) to derive body composition. Body composition changes with disease (Fearon & Preston, 1990) so it is important to use equations derived from study of a similarly diseased group such as was used in the present study (Hannan et al, 1995). There are a number of potentially more precise methods of measuring body composition such as hydrodensitometry, measurement of total body potassium, isotope dilution and dual energy X-ray absorptiometry, however, these are much more costly and less convenient than bioimpedance analysis and may be subject to their own inaccuracies (McMillan et al, 1994, Paton et al, 1995). However, the body composition findings in the

present study were reinforced by the lack of change in serum leptin concentration suggesting a no change in fat mass and a reversal of nitrogen balance from -0.8g/day to +1.9g/day consistent with a gain in lean body mass. While no change was seen in anthropometric measures but this may represent a change from the expected pattern of a progressive fall in arm muscle circumference and triceps skinfold thickness previously described in weight-losing pancreatic cancer patients (Wigmore et al, 1997a). The improvement in lean body mass with an intervention in cancer cachexia has not previously been demonstrated and future confirmation using a more rigorous technique of body composition analysis would be valuable.

While detailed studies of quality of life were not performed in all patients in the present study, Karnofsky performance status, a measure of functional ability was recorded and demonstrated a significant improvement (Chapter 6) in contrast to a progressive decline in untreated patients (Chapter 3). The limited quality of life and other functional studies performed did, however, also suggest that particularly functional aspects of quality of life may have been improved. Thus it seems that the provision of a nutritional supplement enriched with fish oil to weight-losing advanced pancreatic cancer patients resulted in a reversal of weight-loss with a gain in lean tissue resulting in functional benefit for the patients.

Any survival benefit produced by the fish oil-enriched nutritional supplement could not be formally addressed in the present study although survival was at the top end of that expected for patients with advanced pancreatic cancer (Ahlgren, 1996). However, it is conceivable that if deterioration in lean body mass contributes to shortened survival then a reversal of this process may have a survival benefit. Lengthened survival in patients given Maxepa, providing 3g eicosapentaenoic acid daily, has been shown in a randomised study of advanced cancer patients (Gogos et al, 1998). These patients were reported as not showing weight change over the course of the study but it is not clear whether this represents a change from the expected course in this group. A randomised controlled trial in circumstances similar to those of the present study is required to address survival.

In association with the beneficial nutritional effects of the nutritional supplement enriched with fish oil, a stabilisation of acute phase protein concentrations was shown while progressive increases and decreases in positive and negative acute phase protein concentrations respectively were seen without intervention (Chapter 8). The stabilisation in the concentrations of negative acute phase proteins such as albumin (Chapter 8) despite the relative reduction in their synthesis (Chapter 10) suggests that factors such as plasma protein breakdown and redistribution are probably also changing. This suggests that measurements of circulating

protein concentrations alone are of limited value for studying changes in amino acid balance without knowledge of their synthesis.

Conventional nutritional supplementation does not result in anabolism in cachectic cancer patients and in Chapter 10 it has been observed that overall synthesis of acute phase proteins may be stimulated by feeding in cancer patients but not in controls. A hypothesis examined in this thesis was that this stimulation of acute phase protein synthesis by feeding may contribute to the lack of anabolic effect of feeding in advanced cancer patients. After consumption of the fish oil-enriched nutritional supplement for 3 weeks, stimulation of albumin synthesis was abolished and the degree of stimulation of fibrinogen synthesis was halved resulting in no significant overall stimulation of acute phase protein synthesis with feeding and a significant reduction in acute phase protein synthesis in the fed state. This observation in association with the reversal of weight loss, positive nitrogen balance, and gain in lean body mass (Chapter 6) suggests that the potential amino acid sparing effect of the reduction in acute phase protein synthesis may allow dietary amino acids to be used for lean tissue synthesis. This represents a substantial change in the metabolic milieu compared with the condition before intervention. Fish oil-derived interventions will influence pro-inflammatory cytokine production (Endres et al, 1989, Meydani et al, 1991, Meydani et al, 1993, Cooper et al, 1993, Purasiri et al, 1994, Caughey et al, 1996, Wigmore et al, 1997d and Chapter 7) which may effect acute phase protein synthesis. However, pro-inflammatory cytokine synthesis and acute phase protein synthesis are stimulated by the same nuclear transcription factor (Beauparlant & Hiscot, 1996) and it is not clear whether fish oil is acting via suppression of the pro-inflammatory state or by a direct effect on acute phase protein synthesis. Either way the present study provides evidence that the normally irreversible weight-loss of advanced cancer may be at least partly due to the diversion of amino acids for acute phase protein synthesis and that the reversal of weight-loss by the fish oil-enriched nutritional supplement may be at least partly due to suppression of the stimulation of acute phase protein synthesis by feeding.

Limitations of the present studies

There were some disappointing aspects to the work described in this thesis. The controls recruited were, in general, younger than the cancer patients studied. It was decided to try to recruit controls over the age of 50 years in an attempt to minimise potential physiological differences between cancer patients and controls on the basis of age but it remains possible that age may have influenced results to some extent.

The inability to demonstrate elevated cytokine production in cancer patients compared with controls, despite this having been shown previously (Falconer et al, 1995, Wigmore et al, 1997d) would appear to threaten the hypothesis that such increased cytokine production is of crucial importance to driving the metabolic changes in cancer. As discussed above this may have been due to limitations of the cell culture technique or an earlier disease stage in the patients examined in the present study. However, increased circulating concentrations, particularly of interleukin-6, were shown and the relative clinical importance of ex vivo cytokine production and in vivo circulating concentrations of cytokines remains to be determined.

The design of the present study did not allow for an assessment of the effects of the various components of the fish oil-enriched supplement. It is assumed from previous studies that nutritional intervention alone does not affect weight change in cachectic cancer patients (Evans et al, 1987, Ovesen et al, 1993b). The relative role of eicosapentaenoic acid and docosahexaenoic acid could not be determined and no dose response relationship could be ascertained.

Other problems result from the relative success of the study. Because changes in mediators, mechanisms and the progress of cachexia were demonstrated relatively unequivocally, analysis of other factors in more detail became more relevant. Effective measures of quality of life and functional ability were not identified until well into the study and if examined in all patients may have been able to demonstrate benefit in these, arguably more important, factors. The technique of bioimpedence analysis, while cheap, quick, easy and well tolerated, has many critics and additional or alternative measures would have been value. However, these factors leave several opportunities for future study particularly in the context of a randomised controlled trial.

Potential value of fish oil-based intervention in other weight-losing conditions

The non-toxic nature of n-3 fatty acids and their widespread availability means they have enormous potential not only as an anti-cachectic agent in cancer but also in other circumstances where an inflammatory metabolic milieu has an apparently detrimental effect, such as trauma and sepsis and after surgery. A nutritional supplement enriched with n-3 fatty acids in addition to arginine, glutamine and ribonucleotides has been shown to reduce infectious complications and hospital stay in a randomised trial of severely injured patients compared to a standard supplement (Kudsk et al, 1996). A study of a similar preparation postoperatively in patients undergoing surgery for upper abdominal malignancy suggested that

patients had fewer complications and shorter hospital stay compared with those given standard supplements (Daly et al, 1995). A recent meta-analysis of studies comparing nutritional supplements enriched with n-3 fatty acids, arginine, glutamine and ribonucleotides with standard supplements has also suggested a shorter hospital stay in cancer and critically ill patients and a reduction in infective complications in cancer patients (Heys et al, 1999). However, a study in postoperative upper gastrointestinal cancer patients demonstrated no difference between those on such an enriched supplement and those receiving intravenous crystalloid alone (Heslin et al, 1997). The relative contributions of the substances used to enrich such supplements is unclear, indeed some may have opposing immunological actions. Arginine, glutamine and ribonucleotides have been suggested to cause relative immune enhancement while n-3 fatty acids may cause immunosuppression (Meydani et al, 1991, Brittenden et al, 1994, O'Riordain et al, 1994, Khan et al, 1995). However, it is likely that the effects of these agents is to produce a more subtle immunomodulation damping down the catabolic inflammatory response rather than a straight forward immune enhancement or suppression (Furukawa et al, 1999).

Two trials of eicosapentaenoic acid based preparations have been undertaken in patients with AIDS, a condition often associated with weight loss. In an uncontrolled study of 20 HIV infected weight-losing men administered 18g/day of Maxepa, providing around 3 g eicosapentaenoic acid per day, for 10 weeks, weight was unchanged over the trial period. It is not clear whether this represented a change from the state before intervention (Hellerstein et al, 1996). A randomised trial of 64 HIV infected individuals of oral nutritional supplementation or supplementation enriched with n-3 fatty acids (1.7g/day) and arginine for 6 months produced weight gain in both groups. An increase in body weight of around 2kg and of fat mass of around 1kg were seen over 6 months with a trend towards greater weight gain with the n-3 fatty acid enriched supplement. However, the patients examined in this study were not weight losing at baseline (Pichard et al, 1998). These studies in AIDS unfortunately add little to our understanding of the role of eicosapentaenoic acid in cachexia due to limitations of the studies. It has also been suggested that the pattern of weight loss in AIDS differs from that in cancer. While weight loss appears to proceed in a fairly constant manner in cancer in association with a chronic inflammatory state, in AIDS weight loss occurs in a more stepwise fashion associated with acute infections (Grunfeld et al, 1992, Mulligan & Bloch, 1998).

Further study of the potential effects of eicosapentaenoic acid-based preparations in cachexia in non-malignant conditions is clearly required.

Potential for future studies of fish oil-based preparations in cancer cachexia

There is much further work to be carried out in this field to further define the nature of cachexia and the role of n-3 polyunsaturated fatty acids in affecting this process.

The present thesis formed a pilot study for planned larger, randomised, controlled studies. A randomised trial of the effects of eicosapentaenoic acid in cachexia is required to consolidate and confirm the findings of the present work. Ideally, to assess the effect of nutritional supplementation and eicosapentaenoic acid, a randomised trial would involve patients receiving placebo alone, eicosapentaenoic acid alone, a conventional nutritional supplement alone and a nutritional supplement enriched with eicosapentaenoic acid. Such a study would require the collaboration of several centres with the involvement of a few hundred patients. Necessarily such a study would not be able to examine metabolic factors in such detail as the present study but measures of functional ability and quality of life would be of great interest. For this, reliable and reproducible measures which are able to reflect changes in the well being of patients are essential.

Such clinical studies should also address the body composition of patients, perhaps using more sophisticated measures than bioimpedence analysis. The effect of cachexia on total energy expenditure is of interest together with any effect of intervention. If total energy expenditure increased with the reversal of cachexia it may represent an objective measure of improvement in quality of life due to increased physical activity.

Further work may also consider fine tuning the dosage of the fish oil and nutritional components used in the treatment of cachexia. Previous studies have revealed no obvious difference in the metabolic modulation produced by 2g and 6g of eicosapentaenoic acid daily (Wigmore et al, 1997d, Barber et al, 1997). The role of other components of the fish oil such as docosahexaenoic acid also require further elucidation.

The role of the nuclear transcription factor NF- κ B, in driving cachexia and the agents responsible for its activation and inhibition deserve further investigation. The effects of n-3 fatty acids on activation of NF- κ B are also of interest.

The present thesis examined too few individuals to allow assessment of which patients may gain particular benefit from this form of therapy. It is not clear whether intervening early in the course of the disease, before substantial weight loss and a measurable inflammatory response may be more beneficial than trying to intervene when patients are already severely catabolic. It is also not clear whether measuring factors such as C-reactive protein and interleukin-1 β polymorphism genotype may allow determination of patients who would gain

particular benefit or who would continue to decline even with intervention. Hopefully future studies may help clarify this.

Conclusion

Cancer cachexia is of profound importance to the deterioration in condition of many patients with advanced cancer. The syndrome produces a metabolic state in which patients will not regain weight even with the supply of apparently adequate nutrition. This thesis has described several components of the inflammatory reaction culminating in cachexia and drawn particular attention to the acute phase protein response. The demand for amino acids for the synthesis of acute phase proteins may contribute to the lean tissue breakdown seen in cachectic cancer patients and the supply of additional nutrients may be diverted due to stimulation of acute phase protein synthesis. This thesis has also described the effects of a nutritional supplement enriched with fish oil and shown that it will normalise many aspects of the metabolic state. Patients receiving the supplement regained lean tissue with an apparent improvement in functional ability in association with an abolition of the stimulation of acute phase protein synthesis by food. This approach, combining an anti-inflammatory agent and additional nutrients, shows considerable potential as a safe, well tolerated treatment for patients suffering from cancer cachexia.

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Appendix 1

Fatty acid nomenclature

Fatty acids are unbranched, acyclic monocarboxylic acids usually with an even number of carbon atoms. They are designated according to the number of carbon atoms they contain by a prefix, eg eicosa (20), docosa (22). They are also classified according to the number of double bonds as saturated (no double bonds), monounsaturated (one double bond), and polyunsaturated (two or more double bonds). Depending on the number of double bonds they are called dienoic (2), trienoic (3), tetraenic (4), pentaenoic (5) or hexaenoic (6) (figure A.1).

The carbon atoms are numbered from the terminal methyl group (the omega (n, w or •) system) and the abbreviated term uses this numbering to denote the position of the first double bond from the methyl end.

Many fatty acids retain older nonsystematic names.

Examples

Palmitic acid (from palm oil) with 16 carbon and no double bonds - 16:0

Oleic acid (from olive oil) with 18 carbon atoms and one double bond nine carbons from the methyl end - 18:1n-9

Linoleic acid with 18 carbon atoms and two double bonds, the first 6 carbons from the methyl end - 18:2n-6

Eicosapentaenoic acid with 20 carbon atoms and 5 double bonds, the first 3 carbons from the methyl end - 20:5n-3

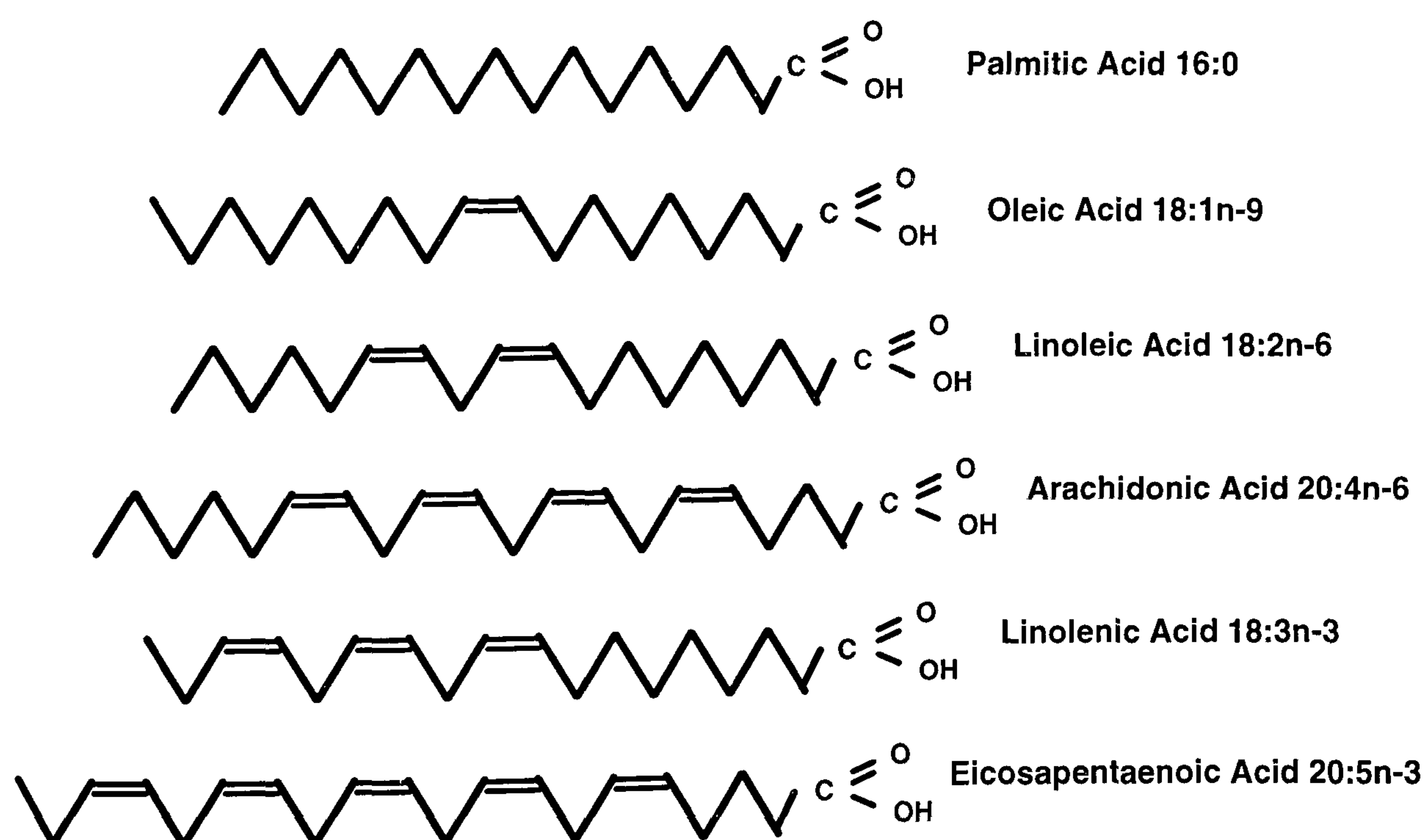


Figure A.1. Structure and nomenclature of fatty acids.

Appendix 2

Patient details

The patients studied were all diagnosed with unresectable pancreatic cancer having been referred to the Royal Infirmary of Edinburgh. Data was collected on all patients referred but advanced stage of disease, travel distance and patient choice affected the studies patients underwent. The core group of 20 patients with appropriate diagnosis, ongoing weight-loss, performance status, life expectancy and willingness to participate were studied in depth for Chapters 6-9. Two patients were unfit for further study and were excluded from Chapters 7-9. Two further diabetic patients were excluded from Chapter 9. Eight of this core group underwent study of protein synthesis mainly due to lack of availability of the labelled phenylalanine.

This appendix describes basic characteristics of all patients studied.

% weight loss - percentage weight loss at diagnosis

Survival - from diagnosis

Surgery - stent = endoscopic or percutaneous biliary stenting

bypass = Surgical bypass of stomach and biliary tree

Histology - histological confirmation of diagnosis - y = yes, n = no

| Age | Sex | %weight loss | IUCC stage | Survival (days) | Surgery | Histolog y | Chapter |
|-----|-----|-----------------|---------------|--------------------|---------|---------------|------------------|
| 69 | m | 33 | IV | 168 | none | y | 3,4,5,6,7,8,9,10 |
| 70 | m | 19 | II | 256 | none | n | 5 |
| 77 | f | 6 | IV | 310 | bypass | y | 3,4,5 |
| 58 | f | 6 | IV | 242 | stent | y | 3,5,8 |
| 58 | m | 18 | II | 82 | bypass | y | 3,5,6,7,8,9,10 |
| 77 | f | 6 | II | 136 | none | y | 5 |
| 58 | m | 19 | III | 115 | stent | n | 5,6,7,8,9,10 |
| 69 | f | 11 | IV | 83 | none | y | 3,5 |
| 58 | f | 18 | II | 277 | stent | y | 3,4,5,6,7,8,9 |
| 59 | f | 9 | IV | 77 | bypass | y | 3,5 |
| 64 | f | 29 | II | 15 | none | y | 5 |
| 64 | f | 18 | II | 265 | stent | n | 5,6,7,8,9,10 |
| 78 | m | 14 | IV | 256 | stent | n | 3,5 |
| 61 | m | 16 | II | 223 | none | y | 3,5 |
| 68 | f | 22 | II | 334 | none | y | 5 |
| 60 | f | 13 | II | 281 | bypass | y | 3,4,5,6,7,8,9 |
| 75 | m | 6 | II | 112 | stent | n | 5 |
| 74 | m | 6 | IV | 280 | none | y | 5 |
| 62 | m | nk | II | 768 | bypass | y | 5 |
| 77 | f | 11 | IV | 333 | bypass | y | 4,5,6,7,8,9 |
| 55 | f | 26 | III | 113 | none | y | 3,4,5,6,7,8,9 |
| 77 | f | 13 | II | 246 | stent | y | 3,5,8 |
| 69 | f | 6 | II | >360 | bypass | y | 5 |
| 42 | f | 5 | IV | 1696 | bypass | y | 5 |
| 74 | m | 6 | II | 112 | stent | y | 5,8 |
| 73 | m | 10 | IV | 539 | bypass | n | 4,5 |
| 61 | f | 19 | II | 165 | bypass | y | 3,5,8 |
| 66 | m | 24 | III | 111 | stent | y | 3,5,6,8 |
| 64 | f | 8 | II | 295 | bypass | y | 3,4,5,6,7,8,9 |
| 37 | m | 17 | IV | 122 | stent | y | 5 |
| 72 | m | 16 | IV | 465 | stent | n | 3,5,6,7,8,10 |
| 52 | m | 14 | IV | 445 | bypass | y | 5 |
| 59 | m | 12 | IV | 97 | none | y | 3,4,5,6 |
| 61 | m | 12 | III | 441 | bypass | y | 5 |
| 66 | f | 3 | III | 273 | stent | y | 5 |
| 66 | m | 9 | IV | 221 | bypass | n | 5 |
| 68 | f | nk | IV | 76 | none | y | 5 |
| 29 | m | 33 | III | 106 | none | y | 5 |
| 54 | m | 6 | IV | 42 | none | y | 3,5,8 |
| 67 | f | 4 | IV | 221 | stent | y | 3,5,8 |
| 69 | f | 31 | III | 144 | bypass | y | 5 |
| 56 | f | 8 | II | 63 | none | n | 5 |
| 67 | f | 12 | IV | 307 | bypass | y | 5,6,7,8,9,10 |
| 81 | m | 8 | II | 496 | bypass | n | 5 |
| 85 | m | nk | II | 57 | stent | y | 5 |
| 66 | m | 14 | IV | 202 | bypass | y | 5,6,7,8,9,10 |
| 76 | f | 6 | I | 214 | stent | y | 5 |
| 60 | m | nk | IV | 65 | stent | n | 5 |
| 62 | m | 18 | II | 185 | stent | y | 3,4,5,6,7,8,9 |
| 59 | m | 17 | II | >900 | bypass | y | 5 |
| 56 | f | 15 | II | 243 | bypass | y | 3,4,5,6,7,8 |
| 48 | m | 7 | III | 927 | bypass | y | 5 |
| 67 | m | 12 | IV | 153 | none | y | 3,5,6,7,8,9,10 |
| 47 | f | 20 | IV | 126 | none | y | 5 |
| 74 | m | 25 | II | 227 | stent | y | 5 |
| 68 | f | 2 | IV | 38 | none | y | 5 |
| 76 | m | nk | II | 181 | stent | n | 5 |
| 78 | m | nk | IV | 50 | none | n | 5 |
| 72 | m | 24 | II | 266 | stent | n | 3,4,5,6,7,8,9 |
| 53 | f | 22 | II | 316 | stent | y | 3,4,5,6,7,8,9 |
| 66 | m | 11 | IV | 94 | bypass | y | 3,5,8 |
| 67 | f | 16 | IV | >300 | bypass | y | 5,6,7,8,9 |
| 56 | m | 19 | II | 559 | stent | n | 5 |
| 65 | f | 30 | II | 67 | none | y | 5 |

Appendix 3**Composition of the trial fish oil-enriched nutritional supplement**

Between batch coefficients of variation in the proportion of EPA and DHA in the supplement were <1% and <1.5% respectively.

| Nutrient | Amount per can (237ml) |
|-----------------------|------------------------|
| Energy (kcal) | 310 |
| Protein (g) | 16.1 (21% of calories) |
| Carbohydrate (g) | 49.7 (61% of calories) |
| Fat (g) | 6.5 (18% of calories) |
| EPA | 1.09 |
| DHA | 0.46 |
| Vitamin A (IU) | 1320 |
| Vitamin D (IU) | 192 |
| Vitamin E (IU) | 72 |
| Vitamin K (IU) | 32 |
| Vitamin C (mg) | 156 |
| Folic acid (mcg) | 456 |
| Thiamin (mg) | 1.6 |
| Riboflavin (mg) | 1.2 |
| Vitamin B6 (mg) | 1.2 |
| Vitamin B12 (mcg) | 4.32 |
| Niacin (mg) | 9.6 |
| Choline (mg) | 126 |
| Biotin (mcg) | 187 |
| Pantothenic acid (mg) | 6 |
| Sodium (mg) | 360 |
| Potassium (mg) | 480 |
| Chloride (mg) | 365 |
| Calcium (mg) | 432 |
| Phosphorus (mg) | 300 |
| Magnesium (mg) | 108 |
| Iodine (mcg) | 42 |
| Copper (mg) | 1.5 |
| Manganese (mg) | 0.6 |
| Zinc (mg) | 7 |
| Iron (mg) | 5.3 |
| Selenium (mg) | 22 |
| Chromium (mcg) | 30 |
| Molybdenum (mcg) | 49.4 |

Appendix 4

Quality of life questionnaires

Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983)

Rotterdam Symptom Checklist (de Haes et al, 1990, Watson et al, 1992)

European Organisation for Research and Treatment of Cancer QLQ-C30 (Aaronson et al, 1993)

EuroQOL EQ-5D

Hospital Anxiety and Depression Scale

FOLD HERE

NAME:

DATE:

Doctors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he will be able to help you more.

This questionnaire is designed to help your doctor to know how you feel. Ignore the numbers printed on the left of the questionnaire. Read each item and underline the reply which comes closest to how you have been feeling in the past week.

Don't take too long over your replies; your immediate reaction to each item will probably be more accurate than a long thought out response.

I feel tense or 'wound up':

- Most of the time
- A lot of the time
- From time to time, occasionally
- Not at all

I still enjoy the things I used to enjoy:

- Definitely as much
- Not quite so much
- Only a little
- Hardly at all

I get a sort of frightened feeling as if something awful is about to happen:

- Very definitely and quite badly
- Yes, but not too badly
- A little, but it doesn't worry me
- Not at all

I can laugh and see the funny side of things:

- As much as I always could
- Not quite so much now
- Definitely not so much now
- Not at all

| | |
|---|---|
| | A |
| | 3 |
| | 2 |
| | 1 |
| | 0 |
| D | |
| 0 | |
| 1 | |
| 2 | |
| 3 | |
| | A |
| | 3 |
| | 2 |
| | 1 |
| | 0 |
| D | |
| 0 | |
| 1 | |
| 2 | |
| 3 | |

| | | FOLD HERE |
|---|---|-----------|
| | A | |
| | 3 | |
| | 2 | |
| | 1 | |
| | 0 | |
| D | | |
| 3 | | |
| 2 | | |
| 1 | | |
| 0 | | |
| | A | |
| | 0 | |
| | 1 | |
| | 2 | |
| | 3 | |
| D | | |
| 3 | | |
| 2 | | |
| 1 | | |
| 0 | | |
| | A | |
| | 0 | |
| | 1 | |
| | 2 | |
| | 3 | |
| D | | |
| 3 | | |
| 2 | | |
| 1 | | |
| 0 | | |

Worrying thoughts go through my mind:
 A great deal of the time
 A lot of the time
 From time to time but not too often
 Only occasionally

I feel cheerful:
 Not at all
 Not often
 Sometimes
 Most of the time

I can sit at ease and feel relaxed:
 Definitely
 Usually
 Not often
 Not at all

I feel as if I am slowed down:
 Nearly all the time
 Very often
 Sometimes
 Not at all

I get a sort of frightened feeling like
 'butterflies' in the stomach:
 Not at all
 Occasionally
 Quite often
 Very often

I have lost interest in my appearance:
 Definitely
 I don't take so much care as I should
 I may not take quite as much care
 I take just as much care as ever

(Continued overleaf)

| | | - FOLD HERE - | |
|---|---|---------------|---|
| | A | | I feel restless as if I have to be on the move: |
| | 3 | | Very much indeed |
| | 2 | | Quite a lot |
| | 1 | | Not very much |
| | 0 | | Not at all |
| D | | | I look forward with enjoyment to things: |
| 0 | | | As much as ever I did |
| 1 | | | Rather less than I used to |
| 2 | | | Definitely less than I used to |
| 3 | | | Hardly at all |
| | A | | I get sudden feelings of panic: |
| | 3 | | Very often indeed |
| | 2 | | Quite often |
| | 1 | | Not very often |
| | 0 | | Not at all |
| D | | | I can enjoy a good book or radio or TV programme: |
| 0 | | | Often |
| 1 | | | Sometimes |
| 2 | | | Not often |
| 3 | | | Very seldom |

Now check you have answered all questions

FOR HOSPITAL USE ONLY

D (8 - 10) -----

A (8 - 10) -----

Rotterdam Symptom Checklist

Confidential

date of completion _____ 19 _____

In this questionnaire you will be asked about your symptoms. Would you please, for all symptoms mentioned, indicate to what extent you have been bothered by it, by circling the answer most applicable to you. The questions are related to the past week.

Example: Have you been bothered, during the past week, by

| | | | | |
|---|------------|---------------------|-------------|-----------|
| headaches | not at all | <div>a little</div> | quite a bit | very much |
| <i>Have you, during the past week, been bothered by</i> | | | | |
| lack of appetite | not at all | a little | quite a bit | very much |
| | | | | |
| irritability | not at all | a little | quite a bit | very much |
| | | | | |
| tiredness | not at all | a little | quite a bit | very much |
| | | | | |
| worrying | not at all | a little | quite a bit | very much |
| | | | | |
| sore muscles | not at all | a little | quite a bit | very much |
| | | | | |
| depressed mood | not at all | a little | quite a bit | very much |
| | | | | |
| lack of energy | not at all | a little | quite a bit | very much |
| | | | | |
| low back pain | not at all | a little | quite a bit | very much |
| | | | | |
| nervousness | not at all | a little | quite a bit | very much |
| | | | | |
| nausea | not at all | a little | quite a bit | very much |
| | | | | |
| despairing about the future | not at all | a little | quite a bit | very much |
| | | | | |
| difficulty sleeping | not at all | a little | quite a bit | very much |
| | | | | |
| headaches | not at all | a little | quite a bit | very much |
| | | | | |
| vomiting | not at all | a little | quite a bit | very much |
| | | | | |
| dizziness | not at all | a little | quite a bit | very much |
| | | | | |
| decreased sexual interest | not at all | a little | quite a bit | very much |
| | | | | |
| tension | not at all | a little | quite a bit | very much |
| | | | | |
| abdominal (stomach) aches | not at all | a little | quite a bit | very much |
| | | | | |
| anxiety | not at all | a little | quite a bit | very much |
| | | | | |
| constipation | not at all | a little | quite a bit | very much |
| | | | | |

| | | | | |
|---------------------------------|------------|----------|-------------|-----------|
| diarrhoea | not at all | a little | quite a bit | very much |
| acid indigestion | not at all | a little | quite a bit | very much |
| shivering | not at all | a little | quite a bit | very much |
| tingling hands or feet | not at all | a little | quite a bit | very much |
| difficulty concentrating | not at all | a little | quite a bit | very much |
| sore mouth/pain when swallowing | not at all | a little | quite a bit | very much |
| loss of hair | not at all | a little | quite a bit | very much |
| burning/sore eyes | not at all | a little | quite a bit | very much |
| shortness of breath | not at all | a little | quite a bit | very much |
| dry mouth | not at all | a little | quite a bit | very much |

A number of activities is listed below. We do not want to know whether you actually do these, but only whether you are able to perform them presently. Would you please mark the answer that applies most to your condition of the past week.

| | | | | |
|--------------------------------|--------|----------------|-------------------------------|--------------|
| | unable | only with help | without help, with difficulty | without help |
| care for myself (wash etc.) | 0 | 0 | 0 | 0 |
| walk about the house | 0 | 0 | 0 | 0 |
| light housework/household jobs | 0 | 0 | 0 | 0 |
| climb stairs | 0 | 0 | 0 | 0 |
| heavy housework/household jobs | 0 | 0 | 0 | 0 |
| walk out of doors | 0 | 0 | 0 | 0 |
| go shopping | 0 | 0 | 0 | 0 |
| go to work | 0 | 0 | 0 | 0 |

- All things considered, how would you describe your quality of life during the past week?
- 0

excellent
- 0

good
- 0

moderately good
- 0

neither good nor bad
- 0

rather poor
- 0

poor
- 0

extremely poor

Would you please check whether you answered all questions?

Thank you for your help.

patient number _____



EORTC QLQ-C30 (version 2.0.)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

Your birthdate (Day, Month, Year):

| | | | | | | |
|--|--|--|--|--|--|--|
| | | | | | | |
|--|--|--|--|--|--|--|

Today's date (Day, Month, Year):

| | | | | | | |
|--|--|--|--|--|--|--|
| | | | | | | |
|--|--|--|--|--|--|--|

| | No | Yes |
|--|----|-----|
| 1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase? | 1 | 2 |
| 2. Do you have any trouble taking a <u>long</u> walk? | 1 | 2 |
| 3. Do you have any trouble taking a <u>short</u> walk outside of the house? | 1 | 2 |
| 4. Do you have to stay in a bed or a chair for most of the day? | 1 | 2 |
| 5. Do you need help with eating, dressing, washing yourself or using the toilet? | 1 | 2 |

| During the past week: | Not at All | A Little | Quite a Bit | Very Much |
|--|------------|----------|-------------|-----------|
| 6. Were you limited in doing either your work or other daily activities? | 1 | 2 | 3 | 4 |
| 7. Were you limited in pursuing your hobbies or other leisure time activities? | 1 | 2 | 3 | 4 |
| 8. Were you short of breath? | 1 | 2 | 3 | 4 |
| 9. Have you had pain? | 1 | 2 | 3 | 4 |
| 10. Did you need to rest? | 1 | 2 | 3 | 4 |
| 11. Have you had trouble sleeping? | 1 | 2 | 3 | 4 |
| 12. Have you felt weak? | 1 | 2 | 3 | 4 |
| 13. Have you lacked appetite? | 1 | 2 | 3 | 4 |
| 14. Have you felt nauseated? | 1 | 2 | 3 | 4 |
| 15. Have you vomited? | 1 | 2 | 3 | 4 |



| During the past week: | | Not at All | A Little | Quite a Bit | Very Much |
|-----------------------|---|---------------|-------------|----------------|--------------|
| 16. | Have you been constipated? | 1 | 2 | 3 | 4 |
| 17. | Have you had diarrhea? | 1 | 2 | 3 | 4 |
| 18. | Were you tired? | 1 | 2 | 3 | 4 |
| 19. | Did pain interfere with your daily activities? | 1 | 2 | 3 | 4 |
| 20. | Have you had difficulty in concentrating on things, like reading a newspaper or watching television? | 1 | 2 | 3 | 4 |
| 21. | Did you feel tense? | 1 | 2 | 3 | 4 |
| 22. | Did you worry? | 1 | 2 | 3 | 4 |
| 23. | Did you feel irritable? | 1 | 2 | 3 | 4 |
| 24. | Did you feel depressed? | 1 | 2 | 3 | 4 |
| 25. | Have you had difficulty remembering things? | 1 | 2 | 3 | 4 |
| 26. | Has your physical condition or medical treatment interfered with your <u>family</u> life? | 1 | 2 | 3 | 4 |
| 27. | Has your physical condition or medical treatment interfered with your <u>social</u> activities? | 1 | 2 | 3 | 4 |
| 28. | Has your physical condition or medical treatment caused you financial difficulties? | 1 | 2 | 3 | 4 |

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1234567

Very poor

Excellent

30. How would you rate your overall quality of life during the past week?

1234567

Very poor

Excellent

EuroQOL EQ-5D

By placing a tick in one box in each group below, please indicate which statement best describes your own health state today.

Do not tick more than one box in each group.

Mobility

- I have no problems in walking about
- I have some problems in walking about
- I am confined to bed

| |
|--------------------------|
| <input type="checkbox"/> |
| <input type="checkbox"/> |
| <input type="checkbox"/> |

Self-Care

- I have no problems with self-care
- I have some problems washing or dressing myself
- I am unable to wash or dress myself

| |
|--------------------------|
| <input type="checkbox"/> |
| <input type="checkbox"/> |
| <input type="checkbox"/> |

Usual Activities (eg. work, study, housework, family or leisure activities)

- I have no problems with performing my usual activities
- I have some problems with performing my usual activities
- I am unable to perform my usual activities

| |
|--------------------------|
| <input type="checkbox"/> |
| <input type="checkbox"/> |
| <input type="checkbox"/> |

Pain/Discomfort

- I have no pain or discomfort
- I have moderate pain or discomfort
- I have extreme pain or discomfort

| |
|--------------------------|
| <input type="checkbox"/> |
| <input type="checkbox"/> |
| <input type="checkbox"/> |

Anxiety/Depression

- I am not anxious or depressed
- I am moderately anxious or depressed
- I am extremely anxious or depressed

| |
|--------------------------|
| <input type="checkbox"/> |
| <input type="checkbox"/> |
| <input type="checkbox"/> |

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is.

**Your own
health state
today**

**Best
imaginable
health state**

100

90

80

70

60

50

40

30

20

10

0

**Worst
imaginable
health state**

Appendix 5**Publications arising from work contained in this thesis**

Barber MD, Ross JA, Voss AC, Tisdale MJ, Fearon KCH. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *Br J Cancer* 81:80-86, 1999.

Barber MD, Powell JJ, Lynch SF, Gough NJ, Fearon KCH, Ross JA. Two polymorphisms of the tumor necrosis factor gene do not influence survival in pancreatic cancer. *Clin Exp Immunol* 117:425-429, 1999.

Barber MD, Fearon KCH, Ross JA. Relationship of serum levels of interleukin-6, soluble interleukin-6 receptor and tumour necrosis factor receptors to the acute phase protein response in advanced pancreatic cancer. *Clin Sci* 96:83-87, 1999.

Barber MD, Ross JA, Preston T, Shenkin A, Fearon KCH. Fish oil-enriched nutritional supplement attenuates progression of the acute phase response in weight-losing patients with advanced pancreatic cancer. *J Nutr* 129:1120-1125, 1999.

Barber MD, Ross JA, Fearon KCH. Changes in nutritional, functional and inflammatory markers in advanced pancreatic cancer. *Nutr Cancer* 35:106-110, 1999.

Barber MD, Fearon KCH, McMillan DC, Slater C, Ross JA, Preston T. Liver export protein synthetic rates are increased by oral meal feeding in weight-losing cancer patients. *Am J Physiol* 279:E707-714, 2000.

Barber MD, McMillan DC, Preston T, Ross JA, Fearon KCH. The metabolic response to feeding in weight-losing pancreatic cancer patients and its modulation by a fish oil-enriched nutritional supplement. *Clin Sci* 98:389-399, 2000.

Barber MD, Powell JJ, Lynch SF, Fearon KCH, Ross JA. A polymorphism of the interleukin-1 β gene influences survival in pancreatic cancer. *Br J Cancer* 83:1443-1447, 2000.