Effect of a Fish Oil-Enriched Nutritional Supplement on Metabolic Mediators in Patients With Pancreatic Cancer Cachexia

Matthew D. Barber, Kenneth C. H. Fearon, Michael J. Tisdale, Donald C. McMillan, and James A. Ross

Abstract: Weight loss in advanced cancer patients is refractory to conventional nutritional support. This may be due to metabolic changes mediated by proinflammatory cytokines, hormones, and tumor-derived products. We previously showed that a nutritional supplement enriched with fish oil will reverse weight loss in patients with pancreatic cancer cachexia. The present study examines the effect of this supplement on a number of mediators thought to play a role in cancer cachexia. Twenty weight-losing patients with pancreatic cancer were asked to consume a nutritional supplement providing 600 kcal and 2 g of eicosapentaenoic acid per day. At baseline and after 3 wk, patients were weighed and samples were collected to measure serum concentrations of interleukin (IL)-6 and its soluble receptor tumor necrosis factor receptors I and II, cortisol, insulin, and leptin, peripheral blood mononuclear cell production of IL-1β, IL-6, and tumor necrosis factor, and urinary excretion of proteolysis inducing factor. After 3 wk of consumption of the fish oil-enriched nutritional supplement, there was a significant fall in production of IL-6 (from median 16.5 to 13.7 ng/ml, \( P = 0.015 \)), a rise in serum insulin concentration (from 3.3 to 5.0 mU/l, \( P = 0.0064 \)), a fall in the cortisol-to-insulin ratio (\( P = 0.0084 \)), and a fall in the proportion of patients excreting proteolysis inducing factor (from 88% to 40%, \( P = 0.008 \)). These changes occurred in association with weight gain (median 1 kg, \( P = 0.024 \)). Various mediators of catabolism in cachexia are modulated by administration of a fish oil-enriched nutritional supplement in pancreatic cancer patients. This may account for the reversal of weight loss in patients consuming this supplement.

Introduction

Previous studies of nutritional intervention in patients with advanced cancer have failed to show any nutritional benefit, despite an increase in caloric and protein intake (1,2). It has been suggested that the failure of conventional nutritional intervention to affect weight loss in advanced cancer is due to a combination of metabolic changes that prevent the efficient use of the nutrients supplied (3). Mediators of these changes are thought to include proinflammatory cytokines, hormones, and tumor-specific products. In weight-losing patients with cancer, upregulation of interleukin (IL)-6 and tumor necrosis factor (TNF) production (4), elevation of cortisol-to-insulin ratio (5), and urinary excretion of proteolysis inducing factor (PIF), a newly described glycoprotein that causes muscle protein breakdown when administered to animals (6), have been observed. Taken together, these changes create a catabolic state, which may contribute to the aforementioned apparent block to the accretion of lean tissue, despite the supply of apparently adequate nutrition.

Fish oil is rich in the n-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA). EPA and fish oil have been shown to reduce the production of proinflammatory cytokines by isolated peripheral blood mononuclear cells (PBMCs) in healthy volunteers (7,8) and pancreatic cancer patients (9). We also observed previously that the acute-phase protein response (mediated at least in part by cytokines) may be stabilized or reduced in patients with advanced pancreatic cancer by fish oil or EPA (9–11). It has also been suggested that EPA may modulate the activity of PIF on muscle in vitro (12). We have reported that a nutritional supplement enriched with fish oil will reverse weight loss in a group of patients with advanced pancreatic cancer (13).

In the present study, we report the effects of a fish oil-enriched nutritional supplement on 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 the restoration of body composition toward normality (11,13).

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Methods

Patients

Men or nonpregnant, nonlactating women between 18 and 80 yr of age with histological confirmation or unequivocal operative or radiological diagnosis of unresectable adenocarcinoma of the pancreas with evidence of ongoing weight-loss were recruited. Patients had a life expectancy of >2 mo and a World Health Organization performance status ≤2 at enrollment. Written informed consent was obtained from all patients. Patients were excluded if they had undergone surgery or endoscopic stenting during the previous 4 wk, had other active medical conditions or another malignancy, or were receiving medication that could modulate metabolism or weight. The study protocol was approved by the Lothian Ethical Committee.

A total of 20 patients were recruited to the study. On enrollment, none of the patients were jaundiced, pyrexial, ascitic, edematous, or severely anemic or had clinical or radiological evidence of infection. No patient underwent cytotoxic chemotherapy or radiotherapy at any stage in their disease. All patients had adequate pain control at the time of study. Pancreatic enzyme supplements were administered if patients had or developed clinical evidence of steatorrhea.

Patients were studied at baseline and after 3 wk. 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, OH). The composition of this product is shown in Table 1. Patients were requested to store the product in the refrigerator.

Patients were asked to consume two cans per day (providing 610 kcal, 32.2 g protein, 2.2 g EPA, and 0.96 g docosahexaenoic acid). Compliance was assessed by a diary of consumption and return of labels from empty cans.

Cytokine Measurement

Serum concentrations of IL-6 and soluble TNF receptors (sTNF-R) I and II were measured by enzyme-linked immunosorbent assay (ELISA; Quantikine, R & D Systems, Abingdon, UK). Limits of detection were 0.5 pg/ml, 0.156 ng/ml, and 0.78 ng/ml, respectively. Soluble IL-6 receptor (sIL-6R) concentration was measured by ELISA (CLB, Amsterdam, The Netherlands). The lower limit of detection was 4 ng/ml. Coefficient of variation for all assays was <8.8% across the concentration range studied.

Cytokine Production

PBMCs were separated from 20 ml of heparin-anticoagulated blood on a Hypaque gradient (Histopaque 1077, Sigma, Poole, UK) by centrifugation at 1,500 rpm for 30 min. Cells from the interface were removed and washed three times in culture medium (Roswell Park Memorial Institute medium 1640, Life Technologies, Paisley, UK) to which penicillin, streptomycin, and glutamine (2 mmol/l) (Sigma) were added. Cells were resuspended, counted, and cultured in 96-well, flat-bottomed tissue culture plates (Costar, Cambridge, MA) at 2 × 10^5/well in 200 µl of cell culture medium with 10% fetal calf serum or autologous plasma in the presence or absence of 10 µg/ml Escherichia coli lipopolysaccharide (Sigma). Supernatants from PBMC cultures were removed at 24 h 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). Limits of detection were 195, 240, and 13.9 pg/ml, respectively, for stimulated cells and 19.5, 30, and 13.9 pg/ml, respectively, for unstimulated cells. Coefficient of variation for all assays was <8.8% across the concentration range studied.

Hormone Measurement

All samples for the measurement of hormone concentration were taken at 8 AM after an overnight fast. Insulin and

<table>
<thead>
<tr>
<th>Nutrient</th>
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<tr>
<td>Energy, kcal</td>
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<tr>
<td>Protein, g</td>
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<tr>
<td>Carbohydrate, g</td>
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<td>Fat, g</td>
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<tr>
<td>Molybdenum, µg</td>
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Table 1. Composition of the Trial Fish Oil-Enriched Nutritional Supplement
cortisol concentrations were analyzed by radioimmunoassay as previously described (14). Leptin concentrations were measured by radioimmunoassay (Linco Research, St. Charles, MO). Limit of detection was <1 ng/ml. Coefficient of variation was <8.3% across the concentration range.

PIF Measurement

The isolation and measurement of PIF has been described previously (6). Briefly, urine was treated with 80% (NH₄)₂SO₄ overnight, and the precipitated protein was recovered by centrifugation, dialyzed against water, and concentrated. An immunoblot was performed using a monoclonal antibody to PIF prepared from MAC16 tumor-bearing mouse splenocytes. This procedure was used for determining the presence or absence of PIF in urine.

Weight

Subjects, without shoes and wearing light clothing, were weighed using a beam scale (Avery, Birmingham, UK).

Statistical Analysis

Data are presented as the median and interquartile range. Because of clinical deterioration, 2 of the 20 patients enrolled initially were unavailable for assessment at Week 3. Paired comparisons with baseline values were performed using Wilcoxon signed rank test or the χ² test for categorical data. P < 0.05 was taken to denote significance.

Results

Of the 20 patients enrolled, 10 were male and 10 were female. Median age was 62 yr (range 51–75). Tumor stages were as follows: UICC Stage 2, eight patients; Stage 3, three patients; and Stage 4, nine patients. Eight patients had undergone nonresectional palliative surgical procedures, and eight patients had endobiliary stenting before the study. Seventeen patients had cancers of the head of the pancreas and 3 had cancers of the body of the pancreas. Confirmation of unresectable pancreatic adenocarcinoma was by histology (16 of 20) or unequivocal operative or radiological findings (4 of 20). Of the 20 patients enrolled, 18 were available for analysis at Week 3. Deterioration of the clinical condition of the other two patients prevented further assessment and consumption of the supplement.

The surviving 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 an estimated rate of weight loss of 2.9 kg/mo (interquartile range −3.9 to −2.1). After 3 wk of consumption of the fish oil-enriched supplement, patients had a median weight gain of 1.0 kg (interquartile range −0.1 to +2.0, P = 0.024 vs. baseline). Further nutritional details of these patients have been reported previously (11,13).

After 3 wk of the trial supplement, there was a significant fall in IL-6 production by PBMCs stimulated with lipopolysaccharide and cultured in fetal calf serum (P = 0.015). There was a trend toward a fall in production of IL-1β in cells cultured under these conditions (P = 0.068) but no change in TNF production (P = 0.55; Fig. 1). There was no significant change in the production of IL-1β, IL-6, or TNF cultured without lipopolysaccharide or in autologous plasma (data not shown). There was no significant change in the serum concentration of IL-6, sTNF-RI, sTNF-RII, or sIL-6R over the 3-wk supplementation period (Fig. 2).

Fasting serum insulin concentrations rose significantly by a median of 1.3 mU/l (P = 0.0064) over the 3-wk supplementation period. Correspondingly, fasting serum concent-

![Figure 1.](image-url) Figure 1. Peripheral blood mononuclear cell production of interleukin-6 (IL-6), tumor necrosis factor (TNF), and interleukin-1β (IL-1β) in 18 patients with advanced pancreatic cancer at baseline and after 3 wk of consumption of a fish oil-enriched nutritional supplement. Medians and interquartile ranges are shown. Values were compared by Wilcoxon signed rank test.
Concentrations of cortisol fell over the study period by a median of 40 nmol/l, but this change did not reach statistical significance \( (P = 0.063) \). When the two results were combined, there was a significant fall in the cortisol-to-insulin ratio \( (P = 0.0084) \). There was no significant change in the serum concentration of leptin over the supplementation period \( (P = 0.38) \); Fig. 3).

Urine samples were examined for excretion of PIF in 17 of the 18 patients at baseline and 15 of the 18 patients at Week 3 because of sample transport problems. There was a significant fall in the proportion of patients with detectable excretion of PIF in urine from 88\% (15 of 17) at baseline to 40\% (6 of 15) after 3 wk of the fish oil-enriched nutritional supplement \( (P = 0.008) \).}

### Discussion

As previously reported, the administration of a fish oil-enriched nutritional supplement to severely cachectic pancreatic cancer patients can result in reversal of weight loss \( (13) \). The present study demonstrates that this anabolism is associated with a significant fall in PBMC IL-6 production, a rise in serum insulin concentrations, a fall in the cortisol-to-insulin ratio, and a fall in the proportion of patients excreting PIF. Clearly, these findings are preliminary in nature, inasmuch as the study did not include a placebo arm, and the results could, conceivably, indicate a caloric effect. However, previous studies of nutritional intervention in pa-

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**Figure 2.** Serum concentrations of IL-6, soluble TNF receptors I (sTNF-R1) and II (sTNF-R2), and soluble IL-6 receptor (sIL-6R) in 18 patients with advanced pancreatic cancer at baseline and after 3 wk of consumption of a fish oil-enriched nutritional supplement. Medians and interquartile ranges are shown. Values were compared by Wilcoxon signed rank test.

**Figure 3.** Serum concentration of insulin, cortisol, and leptin and cortisol-to-insulin ratio in 18 patients with advanced pancreatic cancer at baseline and after 3 wk of consumption of a fish oil-enriched nutritional supplement. Medians and interquartile ranges are shown. Values were compared by Wilcoxon signed rank test.
tients with advanced cancer have failed to show any nutritional benefit, despite an increase in caloric and protein intake (1,2). Ascribing the observed results to the n–3 component of the supplement, therefore, remains speculative until double-blind placebo-controlled trials are undertaken.

A variety of proinflammatory cytokines, including TNF, IL-6, and IL-1, have been implicated in the weight loss associated with cancer. The precise importance of their involvement remains unclear, but individually they will all induce anorexia, weight loss, and an acute-phase protein response (15–22). Circulating concentrations of proinflammatory cytokines are difficult to measure and interpret in human health and disease. In cancer cachexia, TNF and IL-1 are infrequently detected, whereas IL-6 is more frequently found to be elevated (4,23). Thus, in the present study, only serum IL-6 concentrations were measured and were found not to be affected by consumption of the fish oil-enriched supplement.

It has been suggested that these proinflammatory cytokines act locally, and so measurement of ex vivo production may be a better gauge of activity than circulating concentrations (4). Fish oil and EPA have been shown to reduce production of IL-1, IL-6, and TNF from stimulated PBMCs isolated from healthy volunteers and patients with advanced cancer (7–9). Production of IL-6 and TNF tends to be elevated in weight-losing patients with advanced pancreatic cancer (4,9). The present study has shown a significant reduction in IL-6 production and a trend to a reduction in IL-1β production by lipopolysaccharide-stimulated PBMCs in fetal calf serum after 3 wk of fish oil supplementation in pancreatic cancer patients receiving the equivalent of 2 g EPA/day. There was, however, no change in TNF production. The absence of significant changes in PBMC production of proinflammatory cytokines when cultured in the presence of autologous plasma may be explained by the presence of high levels of confounding factors, such as additional fatty acids or alterations in human soluble receptors/cytokines during the study. The use of fetal calf serum from a single homogeneous batch provides an identical milieu in which to compare PBMC cytokine production before and after dietary supplementation. The composition of the autologous plasma will have changed with diet and the intake of nutritional supplement over the course of the study.

TNF is frequently not detected in the serum of cachectic cancer patients (25), and it has been suggested that circulating concentrations of the TNF receptors may give a better estimate of TNF production in vivo (26). The role of soluble IL-6 receptors in the inflammatory state remains unclear, although it has been suggested that it may help mediate the IL-6 signal in cells lacking membrane IL-6 receptors (27). However, the present study did not reveal any changes in the circulating concentrations of sIL-6R or sTNF receptors in pancreatic cancer patients given a fish oil-enriched nutritional supplement. The observed lack of a modulatory effect on sTNF receptors is consistent with the findings with regard to TNF production. Taken together, these changes suggest a complex modulation by EPA of the cytokine milieu in cancer cachexia, the overall pattern being a trend toward downregulation of certain proinflammatory elements.

Circulating concentrations of the catabolic hormone cortisol are often elevated in weight-losing cancer patients (28–30), whereas concentrations of the anabolic hormone insulin tend to be reduced (5). The resulting large rise in the cortisol-to-insulin ratio would seem to reflect the catabolic state of these patients (5). In the present study, with the administration of the fish oil-enriched nutritional supplement for 3 wk, there was a significant rise in serum insulin concentrations. The observed fall in cortisol concentrations did not reach statistical significance, but there was a significant fall in the cortisol-to-insulin ratio. Thus a change to a less catabolic hormonal state was seen in parallel with a gain in weight. Interestingly, the change in serum cortisol concentration and the cortisol-to-insulin ratio were the only two factors to correlate significantly with a change in lean body mass in the present study (r = −0.54, P = 0.038 and r = −0.52, P = 0.044, respectively, Spearman rank correlation coefficient). It is not clear whether this change in the hormonal balance reflects a response to the improved nutritional state of patients or whether the fish oil-enriched supplement was responsible for a more direct modulation of hormone production, resulting in the more anabolic balance observed.

The present study found no change in the circulating concentration of leptin after the patients had received the trial supplement for 3 wk. Leptin, a hormone produced by fat, decreases food intake and increases energy expenditure (31). It has been suggested that leptin concentrations are increased during inflammation and, thus, contribute to weight loss in animal models (32,33). However, leptin concentrations would appear to be appropriately low in human cancer cachexia (34). 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 (13). Thus no substantial change in leptin concentrations would have been expected.

PIF was found initially to be produced by an experimental murine cancer cell line and was shown to be capable of inducing skeletal muscle protein breakdown in vitro (6). An identical glycoprotein has been isolated from the urine of weight-losing cancer patients but not those losing weight for other reasons (35,36). EPA has been shown to inhibit muscle protein breakdown by PIF in vitro (12). 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.

Previous studies of conventional oral supplements in cancer patients have failed to demonstrate any improvement in nutritional measures (1,2). It has been suggested that this is due to metabolic changes driven by mediators such as proinflammatory cytokines, hormones, and PIF, which prevent the effective use of the nutrients supplied. EPA and fish oil
have been shown to affect many of these mediators (7–9,12). Thus the ability of the supplement used in the present study to reduce cytokine production and PIF excretion suggests that the fish oil component may downregulate some of these metabolic factors, allowing more efficient use of the calories supplied and, therefore, allow a gain in weight.

In summary, the present study has demonstrated that, in a group of cachectic pancreatic cancer patients, the administration of a fish oil-enriched nutritional supplement resulted in a significant fall in PBMC IL-6 production, an increase in insulin concentration, and a fall in the proportion of patients excreting PIF. It is suggested that the fish oil component of this supplement normalizes some of the metabolic changes associated with cancer that normally prevent weight gain with nutritional supplementation in cancer patients. Clearly, the results of the present study need to be confirmed in future prospective, randomized, controlled studies. Further research is required to address possible mechanisms whereby EPA may modulate anabolic and catabolic mediators in vivo.

Acknowledgments and Notes

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