Single and combined supplementation of glutamine and *n*-3 polyunsaturated fatty acids on host tolerance and tumour response to 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil chemotherapy in rats bearing Ward colon tumour

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Prior reports suggest that during irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin; CPT-11) chemotherapy in laboratory rats, the anti-tumour efficacy and diarrhoea toxicity could be modulated by *n*-3 PUFA and glutamine, respectively. We further examined how these two dietary elements, when provided individually and in combination, would affect the efficacy of a cyclical regimen of CPT-11/5-fluorouracil (5-FU), an accepted combination regimen for colorectal cancer. Prior to initiating chemotherapy, diets enriched either with glutamine (2 %, w/w total diet) or *n*-3 PUFA (0·88 %, w/w total diet) alone, inhibited Ward colon tumour growth ($P < 0.05$). These diets also completely or partially normalized the changes in peripheral leucocyte counts associated with the tumour-bearing state (e.g. neutrophil proportion/concentration and lymphocyte proportion). During chemotherapy, either glutamine- or *n*-3 PUFA-enriched diet enhanced tumour chemo-sensitivity, and reduced body weight loss, anorexia and muscle wasting (*v.* animals fed control diet, $P < 0.05$). Surprisingly, providing both glutamine and *n*-3 PUFA together did not confer a greater benefit on tumour inhibition either in the presence or absence of chemotherapy; individual benefits associated with single treatments, particularly in respect to host nutritional status (i.e. body weight, food intake and muscle weight) and immune (peripheral leucocyte counts) features were instead partially or completely lost when these two nutrients were combined. These results draw into question the common assumption that there are additive or synergistic benefits of combinations of nutrients, which are beneficial on an individual basis, and suggest that co-supplementation with glutamine and *n*-3 PUFA is not indicated during chemotherapy with CPT-11 and 5-FU.

Glutamine: *n*-3 PUFA: CPT-11: 5-Fluorouracil

Increasing evidence has emerged suggesting a promising role for certain nutritional factors in modulating efficacy and/or toxicity of cancer chemotherapy. Using nutritional adjuncts to chemotherapy is based on the premise that tumour and host responses to chemotherapy could potentially be favourably influenced (i.e. sensitization of the tumour to chemotherapy and protection of the host against chemotherapy-related toxicity). Glutamine and long-chain *n*-3 PUFA are two nutrients reported to modify efficacy or toxicity of cancer chemotherapy[1–3]. Glutamine mitigates chemotherapy toxicity by improving gastrointestinal-related symptoms, gut barrier function and whole-body nitrogen balance[2–4]. *n*-3 PUFA,

such as EPA (20:5*n*-3) and DHA (22:6*n*-3), are reported to enhance the cytotoxicity of several widely used anti-neoplastic agents including anthracyclines, cisplatin and bleomycin via several different mechanisms[5,6]. There is also some evidence that *n*-3 PUFA may alleviate chemotherapy-related toxicities[1,5]. Both glutamine and *n*-3 PUFA are recognized for their immunomodulatory roles by providing substrates for immune cells, regulating redox status and affecting the inflammatory cytokine/eicosanoid networks[7]. Evidence for their therapeutic utility in modifying immunity in the tumour-bearing state, with and without chemotherapy, is yet to be substantiated.

Interactions between some of the different nutrients that have been suggested to favourably affect host response to tumour and chemotherapy have not been explored, to the best of our knowledge. While there already exist some enteral 'immunonutrition' formulae featuring glutamine and *n*-3 PUFA combinations for applications in clinical nutrition[8–10], most research on *n*-3 PUFA or glutamine nutrition has focused on the individual effects of these two nutrients[11–16]. It is commonly assumed that a greater benefit could be achieved by combining individually beneficial nutrients; however, evidence regarding the efficacy of nutrient combinations is lacking[17].

We established a system to study interactions amongst tumour, chemotherapy and diet (rats bearing the Ward colon tumour), treated with irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin; CPT-11; Camptosar®)[3]. Within our controlled dietary design, glutamine treatment mitigated late diarrhoea, the dose-limiting toxicity for CPT-11, whereas *n*-3 PUFA enhanced tumour response to CPT-11[3,18]. The current study aimed at comparing the effects of *n*-3 PUFA and glutamine, alone or in combination, on the response of the tumour and host to a combination chemotherapy similar to that used in the current treatment of colorectal cancer. The measured outcomes were mortality, food intake, weight loss, host leucocyte concentration, tumour growth and response to therapy. As oxidative stress is involved in anti-tumour activity as well as the pathophysiology of chemotherapy toxicity[19–23], we further tested effects of the dietary interventions on redox status in host and tumour tissues.

Experimental methods

Animal treatments

Animal use was reviewed and approved by the Institutional Animal Care Committee and conducted in accordance with the Guidelines of the Canadian Council on Animal Care. Fisher 344 rats (150–180 g body weight and 11–12 weeks old) obtained from Charles River (St. Constant, QC, Canada) were used. Female animals were used for all studies, to avoid potential confounding effects of sex and to allow direct comparison with the body of prior work in the same tumour model[3]. Rats were housed two per cage in a temperature (22°C) and light (12 h light) controlled room; water and food were available for *ad libitum* consumption. Rats were separated into individual cages 1 week before chemotherapy. The Ward colorectal carcinoma was provided by Dr Y. Rustum, Roswell Park Institute[24]. Tumour pieces (0·05 g) were transplanted subcutaneously on the flank of the rats via trocar under slight isoflurane anaesthesia. A major consideration in selecting this tumour site was to facilitate continuous evaluation of tumour growth and response to CPT-11 treatment. CPT-11 was provided by Pfizer as a ready-to-use clinical formulation. Atropine (0·6 mg/ml) was a clinical injectable formulation.

Diet

Tested nutrients were incorporated into a nutritionally complete diet (Table 1). The diets were based on an American Institute of Nutrition-76 (AIN-76) modified basal diet with

Table 1. Composition of the experimental diets*

Ingredient		Control diet (g/100 g diet)	*n*-3 PUFA (FO) diet (g/100 g diet)†	Glutamine (GLN) diet (g/100 g diet)	Glutamine + *n*-3 PUFA (GLN + FO) diet (g/100 g diet)†
Constant portion					
Modified AIN-76 basal mix (46 g/100 g)	Casein	25·2	25·2	25·2	25·2
	Methionine	0·25	0·25	0·25	0·25
	Glucose	13·95	13·95	13·95	13·95
	Vitamins AIN-76	1	1	1	1
	Minerals AIN-76	5	5	5	5
	Inositol	0·6	0·6	0·6	0·6
	Choline	0·3	0·3	0·3	0·3
Fibre (10 g/100 g)	Cellulose	10	10	10	10
Variable portion					
Lipids (20 g/100 g)	Soyabean stearine	15·22	13·84	15·22	13·84
	Linseed oil	0·4	0·4	0·4	0·4
	Sunflower oil	0	3·46	0	3·46
	Safflower oil	4·38	0	4·38	0
	Fish oil	0	2·3	0	2·3
Amino acids (24 g/100 g)	Control amino acid mixture‡	1·5	1·5	0	0
	Glutamine	0	0	2	2
	Maize starch	22·2	22·2	21·7	21·7
Total		100	100	100	100

AIN, American Institute of Nutrition.

* All diets contained 262 g protein and $15·48 \times 10^3$ kJ energy/kg. The constant portion consisted of the pre-mixed modified AIN-76 basal ingredients (Harlan Teklad, Madison, WI, USA); the variable portion was formulated to allow the addition of selected fat/fibre/amino acid elements. Other ingredients were supplied: soyabean stearine (ICN Biomedicals Inc., Cleveland, OH, USA), safflower oil (Canadian Superstore, President's Choice, AB, Canada), linseed oil (Planet Organic, Gold Top, AB, Canada), fish oil (Ocean Nutrition Canada, Dartmouth, NS, Canada) and oligofructose-enriched inulin (Beneo Synergy I®), kindly supplied by Orafti, Tienen, Belgium.

† *n*-3 PUFA-enriched diets (FO and GLN + FO diets) contained 18 : 3(3), 20 : 5(3), 22 : 5(3) and 22 : 6(3), respectively, at 0·04, 0·64, 0·04, 0·16 % of total diet (w/w, or 0·2, 3·2, 0·2, 0·8 % of total fat, respectively), and had an *n*-6:*n*-3 ratio of 3·8. All other diets contained 18 : 3(3) as the only *n*-3 PUFA (0·2 % of total diet, w/w) and had an *n*-6:*n*-3 ratio of 21·0.

‡ The control amino acid mixture contained an equimolar mixture of alanine, serine, glycine and histidine, and was isonitrogenous with glutamine-enriched diet.

40 % of energy from fat. The modified fat component is for-
mulated to be similar to typical North American dietary pat-
terns in man (40 % of energy; polyunsaturated to saturated
fat ratio of 0·35) and have been described elsewhere[3]. Ani-
mals were initially fed Rodent Laboratory Chow (Harlan
Teklad, Madison, WI, USA). During adaptation, this chow
was mixed with our control diet (50:50, w/w) for 1 week,
followed by full transition to experimental diets.

Experimental design

Two weeks prior to tumour implantation, rats were randomly
assigned to one of four diets: (1) control diet (*n* 12); (2) glu-
tamine diet (*n* 10); (3) *n*-3 PUFA (fish oil) diet (*n* 10); (4)
glutamine + *n*-3 PUFA diet (GLN + FO; *n* 10).

When tumours reached approximately 2·3 cm^3 (1·2 % of
body weight) a modified regimen of CPT-11/5-fluororuracil
(5-FU) combination chemotherapy[24] was initiated. Drugs
were administrated intravenously once a week for 2 weeks.
The day when chemotherapy was initiated was designated as
day 0. CPT-11 (50 mg/kg) was administered on days 0 and 7,
whereas 5-FU (50 mg/kg) was administered on days 1 and 8.
Atropine (1 mg/kg, subcutaneously) was administered
immediately before each CPT-11 injection to alleviate the
early onset cholinergic symptoms[3].

Another set of rats of the same age as the tumour-bearing
rats were assigned to a reference group (*n* 7) for comparison.
The REF group did not receive tumour implantation or
chemotherapy and was on control diet throughout the study.

Outcome measures

Body weight and food intake were monitored every second
day. Food intake and body weight at day 0 for each animal
was considered the baseline value, and subsequent changes
were expressed relative to that initial value. Tumour volume
was measured at time-points indicated in the figures, in
three dimensions with a caliper, the length (*L*), width (*W*)
and height (*H*). Tumour volume was calculated:
tumour volume (cm^3) = 0·5 × *L* (cm) × *W* (cm) × *H* (cm)[3].
Tumour response was expressed as relative tumour volume,
calculated relative to the volume at the start of chemotherapy,
for each rat. Calculation of tumour growth inhibition was as
described previously[3].

Sample collection and assays

Animals were killed by CO_2 asphyxia followed immediately
by exsanguination by cardiac puncture 13 d after completion
of chemotherapy. The colonic mucosal tissue was scraped
off from the first 6 cm section of the proximal colon and
immediately frozen in liquid nitrogen for glutathione (GSH)
assay. Tumour and tibialis anterior muscles were collected,
weighed and then immediately frozen in liquid nitrogen.

Blood was collected by jugular vein puncture into hepari-
nized tubes 5 d prior to chemotherapy (as baseline) and at
the end of the study (13 d after completion of chemotherapy)
and was used to measure leucocyte concentrations. The com-
plete blood count and automated differential were performed
on whole blood using a Hemavet instrument (CDC Technol-
ogies, Oxford, CT, USA).

Reduced glutathione (rGSH) and glutathione disulphide
(GSSG) were determined with an Oxford rGSH/GSSG kit
(product no. GT-30), which features 1-methyl-2-vinyl-pyridi-
nium trifluoromethane sulphonate as a scavenger of rGSH[18].

Statistical analysis

Data are expressed as means and their standard errors. Statisti-
cal analysis was performed using univariate or multivariate
linear models of SPSS version 12.0 (SPSS Inc., Chicago, IL,
USA). The models included glutamine, *n*-3 PUFA and time,
and their interactions. Unless specified in the text, treatment
differences in leucocyte counts, GSH, GSSG and muscle
weight were analysed using one-way ANOVA followed by
post hoc Tukey's test; whereas treatment differences in
tumour growth, body weight and food intake changes were
tested using two-way repeated-measures ANOVA (dietary
treatment × time) followed by *post hoc* Tukey's test. Survival
curves were obtained using the Kaplan–Meier method, and
differences in survival between groups were analysed using
the log-rank test. A probability of $P < 0.05$ was accepted as
being statistically significant.

Results

*Effects of dietary treatments during the pre-chemotherapy
period*

After tumour implantation but prior to chemotherapy, there
was no significant effect of diet treatment on body weight
and the food intake of tumour-bearing rats (data not shown).
Although the total concentration of leucocytes did not differ
between treatments, an effect of diet on the proportions of
different leucocyte populations was observed. Tumour-bearing
rats fed the control diet had a higher concentration and pro-
portion of neutrophils and a lower proportion of lymphocytes
in peripheral blood compared with the reference rats ($P < 0.05$;
Table 2). Feeding *n*-3 PUFA diet alone partially corrected
these alterations; feeding glutamine also lowered blood neu-
trophil concentration while increasing the proportion of lym-
phocytes in the tumour-bearing rats towards the values seen
in the reference rats. Co-supplementation of glutamine and
n-3 PUFA (GLN + FO) resulted in a lower proportion and
concentration of monocytes as compared with reference rats
($P < 0.05$) and a lower lymphocyte concentration as compared
with *n*-3 PUFA diet alone ($P < 0.05$).

Inhibition of tumour growth was observed with *n*-3 PUFA
(−23·6 (SEM 5·9) %) or glutamine (−18·4 (SEM 5·8) %) sup-
plemented individually, at 16 d after tumour implantation,
compared with rats fed the control diet ($P < 0.05$; Fig. 1(A)).
Co-supplementation of these two nutrients in the diet
(GLN + FO) led to an identical tumour growth inhibition as
seen with the single *n*-3 PUFA supplementation (Fig. 1(A));
tumour inhibition in this case was −23·9 (SEM 6·2) %
($P < 0.05$ *v.* control diet).

*Effects of dietary treatments during the post-chemotherapy
period*

Tumour response to chemotherapy. CPT-11/5-FU therapy
was initiated for each treatment group when the average

tumour burden in each diet group was identical (approximately 1·2 % of body weight). Both glutamine and n-3 PUFA diets significantly enhanced anti-tumour activity of CPT-11/5-FU chemotherapy as compared with control diet ($P < 0.05$; Fig. 1(B)). However, these effects were not additive in the glutamine and n-3 PUFA co-enriched diet, which resulted in a similar potentiation of anti-tumour efficacy as n-3 PUFA diet without the addition of glutamine (52·9 (SEM 16·7) % tumour inhibition by n-3 PUFA alone and 53·7 (SEM 13·0) % inhibition by GLN + FO as compared to the control diet on day 11; $P < 0.05$).

Host responses to chemotherapy. *Survival*: some mortality occurred during days 9–17 of the study (Fig. 2). As tumour burden was markedly reduced with CPT-11/5-FU treatment, the observed short-term mortality was considered to be attributable to the chemotherapy rather than cancer progression. The mortality was more prevalent in rats fed the control diet (five out of twelve, 41·7 %). Mortality for glutamine, n-3 PUFA and GLN + FO groups were 20 % (two out of ten), 30 % (three out of ten) and 10 % (one out of ten), respectively (Fig. 2); difference as compared to the control diet did not reach significance for any of these groups.

Blood cell counts: at 13 d following the last chemotherapy, there was a marked increase in the total leucocyte count in rats fed the control diet, compared to the baseline level before chemotherapy. This was primarily attributable to expansion of the neutrophil population and to a lesser extent of the monocytes ($P < 0.05$, paired t test); lymphocyte concentration did not change significantly from the pre-chemotherapy baseline values (Table 2). These changes in the post-chemotherapy leucocyte concentrations were completely or partially mitigated in rats fed either n-3 PUFA or glutamine alone. However, the rats fed the combination diet had a similar post-chemotherapy increase in neutrophils, monocytes and total leucocytes as the rats fed the control diet.

Body weight: rat body weights during CPT-11/5-FU treatment displayed a loss–regain pattern concordant with the cyclic chemotherapy administration (Fig. 3(A)). Body weight loss was much greater upon administration of the second cycle of chemotherapy than after the first cycle for rats in all diet groups. For instance, rats fed control diet initially lost 5·5 (SEM 0·5) % of their weight following the
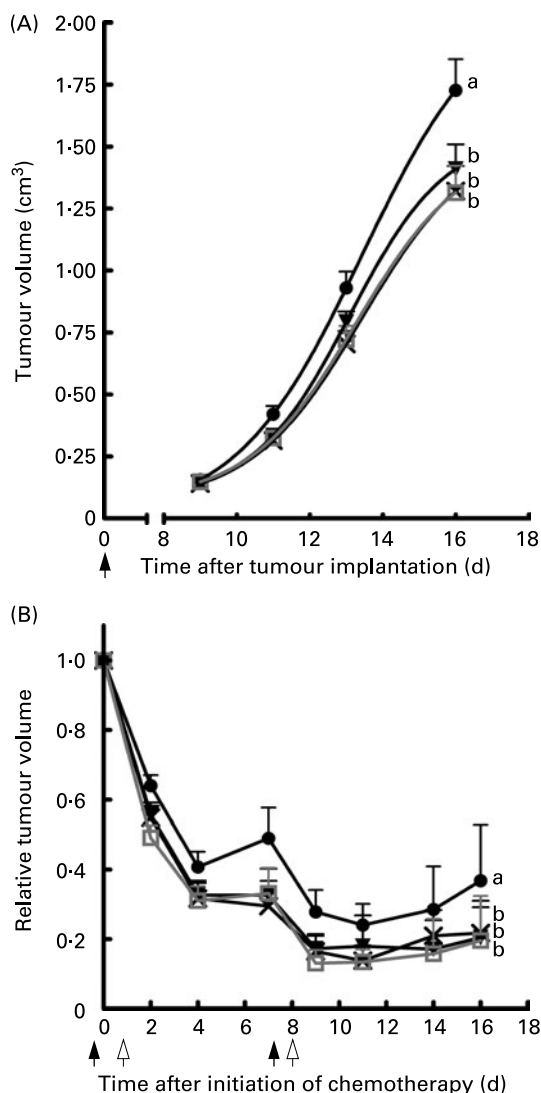

Fig. 1. Dietary effects on Ward colon tumour growth and anti-tumour efficacy of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil (5-FU) chemotherapy in vivo. Values are means with their standard errors depicted by vertical bars. (A), Effect of dietary treatment per se on Ward colon tumour growth in vivo (●, control diet (CON); ×, n-3 PUFA (fish oil) diet (FO); ▼, glutamine diet (GLN); □, glutamine + n-3 PUFA diet (GLN + FO)). Fisher rats were implanted with Ward colon tumour and changes in tumour volume were followed (day 0, tumour implantation). Co-supplementation of glutamine and n-3 PUFA in the diet (GLN + FO) led to an identical tumour growth inhibition as the single n-3 PUFA (FO) supplementation, and the tumour growth curves of these two groups overlap. (B), Dietary modification of anti-tumour efficacy of CPT-11/5-FU therapy. CPT-11/5-FU treatment was initiated when rats of all the dietary treatment groups had tumours of approximately 2·3 cm³ in volume. Relative tumour volume is compared to the baseline volume when chemotherapy was initiated (day 0). ▲, Single CPT-11 injection at 50 mg/kg; △, single 5-FU injection at 50 mg/kg. ^{a,b} Dietary treatments with unlike letters were significantly different ($P < 0.05$, post hoc Tukey's).
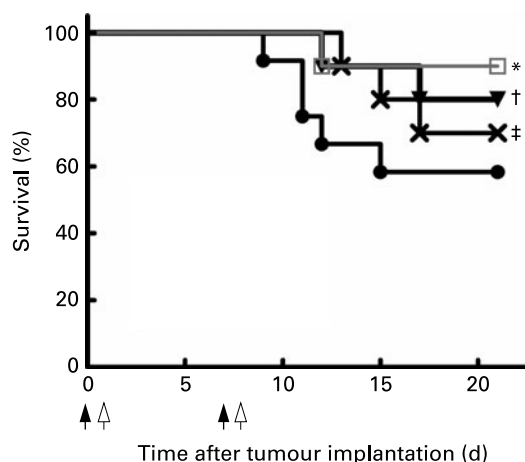

Fig. 2. Dietary effect on 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil (5-FU)-induced mortality. Post-chemotherapy survival associated with different dietary treatments (●, control diet (CON); ×, n-3 PUFA (fish oil) diet (FO); ▼, glutamine diet (GLN); □, glutamine + n-3 PUFA diet (GLN + FO)) was analysed using the Kaplan–Meier method and overall survival between groups was analysed using the log-rank test. ▲, Single CPT-11 injection at 50 mg/kg; △, single 5-FU injection at 50 mg/kg. Differences as compared with the control diet did not reach significance for any of these groups: $*P = 0.43$, $†P = 0.23$, $‡P = 0.10$.

Table 2. Effects of dietary treatments on peripheral leucocyte counts before and after 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil (5-FU) chemotherapy†

(Mean values with their standard errors)

Dietary treatment	Leucocyte ($\times 10^9$/l)		Neutrophils (% of leucocyte)		Neutrophils ($\times 10^9$/l)		Lymphocytes (% of leucocyte)		Lymphocytes ($\times 10^9$/l)		Monocytes (% of leucocyte)		Monocytes ($\times 10^9$/l)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pre-chemotherapy														
REF (n 11)	8.81	1.06	19.84[a]	1.91	1.56[a]	0.18	73.02[b]	1.46	6.36[ab]	0.82	5.51[b]	0.46	0.52[b]	0.08
CON (n 12)	8.53	0.31	26.28[b]	1.48	2.29[b]	0.19	64.18[a]	2.18	5.45[ab]	0.21	4.18[ab]	0.25	0.36[ab]	0.03
GLN (n 10)	9.12	0.29	24.86[b]	1.68	2.15[ab]	0.16	69.44[ab]	1.31	6.18[ab]	0.16	3.98[a]	0.20	0.36[ab]	0.02
FO (n 10)	9.83	0.19	22.52[ab]	0.91	2.22[ab]	0.11	70.01[ab]	1.13	7.01[b]	0.12	4.69[ab]	0.33	0.46[ab]	0.04
GLN + FO (n 10)	7.56	0.66	23.96[b]	1.54	1.64[ab]	0.16	68.94[ab]	1.13	5.18[a]	0.41	3.93[a]	0.48	0.31[a]	0.06
Post-chemotherapy														
REF (n 11)	8.81[a]	1.06	19.84[a]	1.91	1.56[a]	0.18	73.02[c]	1.46	6.36	0.82	5.51	0.46	0.52[a]	0.08
CON (n 7)	14.53*[ab]	2.76	41.43*[c]	5.17	6.07*[b]	1.53	47.00*[a]	6.24	6.83	1.54	7.68	0.76	1.13*[b]	0.25
GLN (n 8)	7.95[a]	1.52	24.08[ab]	1.60	1.95[a]	1.53	67.25[bc]	1.08	5.32	0.98	6.80	0.82	0.51[a]	0.07
FO (n 7)	9.38[ab]	2.46	30.53[b]	2.16	2.99*[a]	0.90	59.30*[ab]	1.20	5.50	1.40	7.75	1.05	0.66[a]	0.10
GLN + FO (n 9)	16.68*[b]	1.84	40.43*[c]	2.76	6.59*[b]	0.29	48.80*[a]	3.22	8.30	1.53	8.30	0.84	1.34*[b]	0.04

CON, control diet; FO, n-3 PUFA (fish oil) diet; GLN, glutamine diet; GLN + FO, n-3 PUFA + glutamine diet; REF, reference group.

[a,b,c] For both pre-chemotherapy and post-chemotherapy data, mean values within a column with unlike superscript letters were significantly different ($P<0.05$).

Mean values were significantly different from those prior to chemotherapy (paired t test): *$P<0.05$.

† For details of procedures and diets, see the Experimental methods section and Table 1. Whole blood was collected prior to initiation of CPT-11/5-FU chemotherapy when all groups had tumours of approximately $2.0\,cm^3$ in volume (1.0 % of body weight) and $13\,d$ after completion of CPT-11/5-FU chemotherapy (at the end of the study).

first cycle at day 2 (nadir of the first cycle), and further suffered an additional 9·6 (SEM 1·8) % weight loss following the second cycle at day 11 (nadir of the second cycle) ($P<0.05$, paired t test). Rats receiving either glutamine, n-3 PUFA treatment or both had significantly less body weight loss following two cycles of chemotherapy as compared to the rats fed the control diet ($P<0.05$). Glutamine diet appeared to have a greater benefit in limiting post-chemotherapy body weight loss compared to n-3 PUFA and combination (GLN + FO) diets ($P<0.05$). By the end of the study, rats fed the glutamine diet had entirely caught up and exceeded their baseline body weight by + 2·1 (SEM 0·7) % ($P<0.01$ v. control diet, Bonferroni post-test) and rats fed the n-3 PUFA diet also ended the study above baseline weight by + 2·6 (SEM 1·4) % ($P<0.05$ v. control diet, Bonferroni post-test) above their baseline body weight. However, rats fed GLN + FO had a slower post-chemotherapy body weight catch-up, ended the study with a − 1·7 (SEM 1·1) % weight loss overall, which was significantly different from the glutamine and n-3 PUFA groups ($P<0.05$, Bonferroni post-test) but not different from the control group.

Food intake: the time course of the food intake change during the chemotherapy is illustrated in Fig. 3(B). Both single glutamine and n-3 PUFA treatments significantly alleviated the anorexia that occured following CPT-11/5-FU chemotherapy, as compared with the control diet ($P<0.05$). However, food intake of rats fed GLN + FO were not different from rats on the control diet. At day 10, where rats from all groups had the lowest daily food intake, rats fed either glutamine or n-3 PUFA diet had a higher food intake level as compared with rats fed control diet ($P<0.05$, Bonferroni post-test). However, feeding diet co-enriched with glutamine and n-3 PUFA was not associated with a higher food intake on day 10.

Muscle weight: cancer chemotherapy could be a potent catabolic stimulus for muscle wasting[25]. At the end of the study, rats fed the control diet had a significantly lower relative weight of tibialis anterior muscle than reference rats (Fig. 3; $P<0.05$). Feeding either glutamine or n-3 PUFA diet completely prevented this muscle loss. However, feeding the GLN + FO diet did not prevent the muscle loss and the relative tibialis weight for this group was comparable to that of the rats fed control diet and considerably lower than the reference group or rats with dietary glutamine or n-3 PUFA monotherapy ($P<0.05$).

Effects of dietary treatments on glutathione stores in host and tumour tissues following 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin/5-fluorouracil therapy

At 13 d after completing CPT-11/5-FU chemotherapy, rGSH/GSSG ratio was lowered by 50 % in colonic mucosa of control diet-fed rats (v. reference rats, $P<0.05$; Table 3). This was mainly attributed to a higher GSSG level ($P<0.05$). By contrast, this ratio for the other dietary treatment groups was maintained at a comparable level as the reference rats and was significantly higher for glutamine and n-3 PUFA dietary treatments (but not for the combination treatment) as compared with rats on control diet ($P<0.05$). In tumour tissue (Table 3), glutamine supplementation, either alone or in combination with n-3 PUFA, resulted in a lower rGSH/GSSG ratio, compared to the rats on control diet ($P<0.05$).

Discussion

Individual effects of glutamine and n-3 PUFA monotherapy on the tumour and host

The Ward colon tumour caused an array of changes in peripheral leucocytes, i.e. elevation of neutrophil count and skewed relative abundance of neutrophils and lymphocytes, consistent with findings in patients with various malignant solid tumours[26–28]. These cancer-related abnormalities in peripheral blood leucocyte concentrations and proportions are suggested to negatively correlate with clinical prognosis in terms of response rate to chemotherapy, incidence of
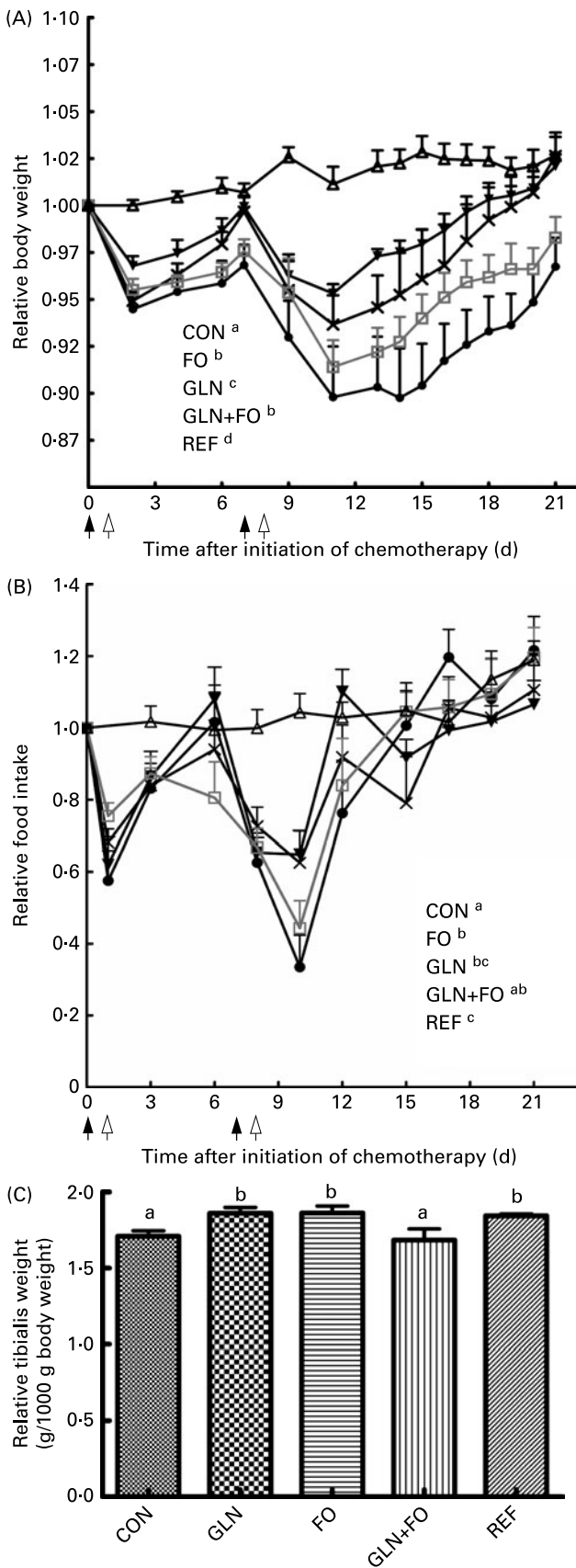
post-treatment infectious complications and survival[29–31]. The present results show that these changes in the leucocyte concentration and populations that appear prior to chemotherapy could be at least partially normalized by diets with *n*-3 PUFA or glutamine. This may convert into advantage favouring *n*-3 PUFA or glutamine-fed rats during subsequent CPT-11/5-FU treatment. At 13 d after completion of the chemotherapy, we observed a marked neutrophilic and monocytic leucocytosis observed in rats on control and GLN + FO diets, but not on glutamine or *n*-3 PUFA diets. More detailed evaluations of peripheral leucocyte dynamics will be required to discern whether the neutrophilia and monocytosis associated with the control and GLN + FO diets were merely a reflection of 'rebound-overshoot' immunological recovery after immunosuppressive chemotherapy[32], or myelopoiesis/neutrophil mobilization related to pathological conditions such as infection and inflammation[33].

Supplementing the diet with either *n*-3 PUFA or glutamine inhibited Ward tumour growth *in vivo*. A direct growth-inhibitory effect of dietary *n*-3 PUFA is abundantly documented in various tumour types[34,35] including Ward colon tumour[3]. Incorporation of *n*-3 fatty acids in tumour membrane phospholipids affects a wide range of biological functions, such as biosynthesis of eicosanoids, membrane lipid peroxidation, signal transduction, membrane fluidity and cell interaction[5]. Compared to *n*-3 PUFA, which has been increasingly accepted as an anti-tumour nutrient, utility of glutamine supplementation is often challenged due to a prevalent notion that tumours are 'glutamine traps'[36] and exogenous glutamine may promote tumour growth. This notion is based on findings in tissue culture with abundant oxygen supply[37,38] and indirect evidence (i.e. tumour glutaminase activity)[39] and lowered plasma glutamine concentration differences in tumour-bearing hosts[40]. There is currently no direct evidence indicating glutamine can stimulate tumour growth *in vivo*, either in animals[41,42] or clinical trials[43]. Glutamine supplementation may conversely exert inhibitory effects on tumour growth[44–46], possibly involving reduced production of PGE$_2$, enhanced natural kill cell activity, altered tumour GSH stores and reduced expression of apoptosis effectors[45–47].

The Ward colon tumour model recapitulates the response of human colon cancer to the prevailing chemotherapy regimens based on CPT-11 alone or in combination with 5-FU[3,24]. Dietary supplementation with *n*-3 PUFA or glutamine has been shown to modulate the anti-tumour efficacy of various drugs including doxorubicin, cytosine arabinoside, mitomycin C, CPT-11 and methotrexate[48,49]. We additionally demonstrate

Fig. 3. Dietary effect on host nutritional features following 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil (5-FU) therapy. Values are means with their standard errors depicted by vertical bars. (A), Body weight change. Body weight is relative to baseline value when the chemotherapy was initiated (day 0). (B), Food intake change. Daily food intake level is relative to that prior to chemotherapy initiation (day 0). [a–d] Dietary treatments with unlike superscript letters were significantly different ($P < 0.05$, *post hoc* Tukey's). (C), Muscle weight. At the end of the study, left tibialis anterior muscle was isolated from the killed rats and weighed. Muscle weight is relative to the whole body weight at kill. [a,b] Mean values with unlike letters were significantly different ($P < 0.05$, *post hoc* Tukey's). △, Reference group (REF); ●, control diet (CON); ×, *n*-3 PUFA (fish oil) diet (FO); ▼, glutamine diet (GLN); □, glutamine + *n*-3 PUFA diet (GLN + FO); ▲, Single CPT-11 injection at 50 mg/kg; ⬆, single 5-FU injection at 50 mg/kg.

Table 3. Dietary effects on glutathione (GSH) content in host colonic mucosa and tumour tissues following 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11)/5-fluorouracil (5-FU) chemotherapy*

(Mean values with their standard errors)

Treatment group	tGSH (μmol/g tissue)		GSSG (μmol/g tissue)		rGSH (μmol/g tissue)		rGSH/GSSG ratio	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Colonic mucosa (*n* 6)								
REF	1·86	0·12	0·014[a]	0·002	1·83	0·12	136[b]	13
CON	1·65	0·06	0·033[b]	0·010	1·58	0·05	68[a]	18
GLN	1·57	0·10	0·012[a]	0·001	1·55	0·10	136[b]	13
FO	1·72	0·12	0·015[ab]	0·003	1·69	0·11	124[b]	13
GLN + FO	1·74	0·06	0·015[ab]	0·002	1·71	0·11	118[ab]	13
Tumour (*n* 5)								
CON	1·56	0·10	0·017	0·003	1·52	0·09	99[b]	15
GLN	1·20	0·17	0·024	0·004	1·15	0·16	55[a]	10
FO	1·26	0·14	0·023	0·004	1·22	0·14	59[ab]	11
GLN + FO	1·10	0·12	0·028	0·006	1·04	0·12	47[a]	13

CON, control diet; FO, *n*-3 PUFA (fish oil) diet; GLN, glutamine diet; GLN+FO, *n*-3 PUFA+glutamine diet; REF, reference group; rGSH, reduced GSH; tGSH, total GSH.
[a,b] For both colonic mucosa and tumour tissues, means within a column with unlike superscript letters were significantly different ($P < 0.05$).
* For details of procedures and diets, see the Experimental methods section and Table 1. GSH concentration was analysed in the host colonic mucosa and tumour tissue at the end of the study (13 d after completion of CPT-11/5-FU treatment).

that supplementation with either nutrient enhanced the responsiveness of Ward colon tumour to CPT-11/5-FU treatment. GSH is the major intracellular anti-oxidant that protects cells from injury caused by excessive oxidative stress, and is one possible effector of this altered tumour response. Reduced GSH/GSSG ratio is the most important regulator of intracellular redox status (the lower the ratio, the higher the oxidative burden)[50]. Tumour GSH levels correlate with tumour cell proliferation and resistance to chemotherapy[50]. Consistent with their chemo-sensitizing effects, feeding glutamine either alone or in combination with *n*-3 PUFA lowered GSH/GSSG ratio in tumour tissue. *n*-3 PUFA have been suggested to predispose tumour cells to oxidative injury caused by chemotherapy[48,51]. Although serving as a precursor for GSH synthesis, glutamine treatment paradoxically lowered the rGSH/GSSG ratio, consistent with some earlier reports[18,52]. Feeding *n*-3 PUFA alone also tended to lower rGSH/GSSG ratio in tumours, which could be ascribed to enhanced oxidative stress associated with *n*-3 PUFA incorporation in membranes[1,53].

Adding either glutamine or *n*-3 PUFA to the diet improved clinical symptoms associated with CPT-11/5-FU therapy such as body weight loss, anorexia and muscle wasting. This work adds to previous findings on benefits associated with individual glutamine or *n*-3 PUFA supplementation in the setting of CPT-11 chemotherapy[18,54]. Improvement of these manifestations may contribute to the trend to increased survival after intensive CPT-11/5-FU treatment by animals in these diet treatment groups. Anorexia and accompanying weight loss could be a direct reflection of gastrointestinal toxicity of chemotherapy. In particular, a compromised gut barrier integrity has been suggested to serve as a pivotal mechanism by which chemotherapy triggers endotoxaemia, bacterial translocation and systemic inflammatory responses[55]. Effects of dietary glutamine and *n*-3 PUFA on the endogenous intestinal GSH store have been suggested to play a role in preserving the structural and functional integrity of the gut barrier against exogenous insults[52,56]. Consistent with our previous finding with bolus

glutamine treatment, continuous glutamine feeding normalized colonic GSH stores and redox status, which was deteriorated by chemotherapy[18]. Feeding *n*-3 PUFA also normalized the GSH-related redox state following CPT-11/5-FU treatment. Both these nutrients regulated the host and tumour GSH-related redox status in a differential manner. In the tumour, glutamine and *n*-3 PUFA were pro-oxidative, whereas in the colon these two factors alleviated chemotherapy-related oxidative stress. This differential effect has been previously reported during glutamine feeding of tumour-bearing rats[18,52]. *n*-3 PUFA supplementation has been shown to reduce colonic oxidative burden in patients with inflammatory bowel disease or animal models of colitis[57,58]. This could be secondary to its anti-inflammatory effect by inhibiting the production of pro-inflammatory *n*-6 series eicosanoids, TNF-α, IL-6 and IL-1β[59,60]. Decreased generation of reactive oxygen species could result from reduction of the inflammation process, e.g. respiratory burst of immune cells[61].

Interaction of glutamine and n-3 PUFA when combined

Despite the individual benefits of supplementing the diet with either glutamine or *n*-3 PUFA, additive benefits were notably lacking. When these two nutrients were combined there was no additive benefit on tumour inhibition either in the presence or absence of chemotherapy. Surprisingly, individual benefits associated with single supplementation of glutamine or *n*-3 PUFA, particularly in respect to host nutritional (i.e. body weight, food intake and muscle weight) and immune (peripheral leucocyte counts) features were instead partially or completely lost when these two nutrients were combined. The present results do not support supplementation of both glutamine and *n*-3 PUFA during CPT-11/5-FU chemotherapy. Taken together, the present study suggests the need to test any assumption that there are additive or synergistic benefits of nutrients that are beneficial on an individual basis. This has important ramifications for clinical nutrition. Enteral

food formulations containing both glutamine and *n*-3 PUFA have been marketed for various conditions such as sepsis, surgery, trauma and burns[8-10]. However, clear scientific evidence for combining these is lacking.

Mixtures of different nutrients may have antagonistic effects[62-64] and the specific mechanisms underlying these are not well understood. In a rat model of head injury, feeding arginine alone reduced bacterial translocation more efficiently than the combination of arginine and *n*-3 PUFA[65]. The same group also showed that combination of arginine and *n*-3 PUFA may exert some conflicting effects on gut barrier function and macrophage reactivity in a rat model of inflammation induced by turpentine. Co-supplementation resulted in increased bacterial translocation and impaired pro-inflammatory Th1 cytokine production compared with arginine alone (C Moinard, personal communication). Since enteral glutamine administration elevates arginine levels in tissue and plasma[18,66] it is possible that arginine contributed to the negative interaction between glutamine and *n*-3 PUFA in the present study.

The current study reflects a single attempt to evaluate potential higher-order interactions between host physiology and dietary elements. Taking into account the large variety of dietary factors that are suggested to interact with tumour growth and response to therapy, as well as the potential for various nutrients to interact with one another, the number of relevant combinations of all of these elements is somewhat daunting. Experimental models, possibly involving expression of key proteins or genes, that could be useful in predicting the types of interactions shown here, would be a valuable asset for future work.

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References

1. Hardman WE (2002) Omega-3 fatty acids to augment cancer therapy. *J Nutr* **132**, 3508S–3512S.
2. Savarese DM, Savy G, Vahdat L, *et al.* (2003) Prevention of chemotherapy and radiation toxicity with glutamine. *Cancer Treat Rev* **29**, 501–513.
3. Xue H, Sawyer MB, Field CJ, *et al.* (2007) Nutritional modulation of antitumor efficacy and diarrhea toxicity related to irinotecan chemotherapy in rats bearing the ward colon tumor. *Clin Cancer Res* **13**, 7146–7154.
4. Ziegler TR (2002) Glutamine supplementation in bone marrow transplantation. *Br J Nutr* **87**, Suppl. 1, S9–S15.
5. Baracos V, Mazurak V & Ma D (2004) n-3 Polyunsaturated fatty acids throughout the cancer trajectory: influence on disease incidence, progression, response to therapy and cancer-associated cachexia. *Nutr Res Rev* **17**, 1–17.
6. Conklin KA (2002) Dietary polyunsaturated fatty acids: impact on cancer chemotherapy and radiation. *Altern Med Rev* **7**, 4–21.
7. Grimble RF (2001) Nutritional modulation of immune function. *Proc Nutr Soc* **60**, 389–397.
8. Choudhry MA, Haque F, Khan M, *et al.* (2003) Enteral nutritional supplementation prevents mesenteric lymph node T-cell suppression in burn injury. *Crit Care Med* **31**, 1764–1770.
9. Chuntrasakul C, Siltham S, Sarasombath S, *et al.* (2003) Comparison of an immunonutrition formula enriched arginine, glutamine and omega-3 fatty acid, with a currently high-enriched enteral nutrition for trauma patients. *J Med Assoc Thai* **86**, 552–561.
10. Pearce CB, Sadek SA, Walters AM, *et al.* (2006) A double-blind, randomised, controlled trial to study the effects of an enteral feed supplemented with glutamine, arginine, and omega-3 fatty acid in predicted acute severe pancreatitis. *Jop* **7**, 361–371.
11. Yoshida S, Matsui M, Shirouzu Y, *et al.* (1998) Effects of glutamine supplements and radiochemotherapy on systemic immune and gut barrier function in patients with advanced esophageal cancer. *Ann Surg* **227**, 485–491.
12. Garrel D (2004) The effect of supplemental enteral glutamine on plasma levels, gut function, and outcome in severe burns. *JPEN J Parenter Enteral Nutr* **28**, 123, author reply 123.
13. Lai YN, Yeh SL, Lin MT, *et al.* (2004) Glutamine supplementation enhances mucosal immunity in rats with gut-derived sepsis. *Nutrition* **20**, 286–291.
14. Roy N, Barnett M, Knoch B, *et al.* (2007) Nutrigenomics applied to an animal model of Inflammatory Bowel Diseases: transcriptomic analysis of the effects of eicosapentaenoic acid- and arachidonic acid-enriched diets. *Mutat Res* **622**, 103–116.
15. Lin MT, Hsu CS, Yeh SL, *et al.* (2007) Effects of omega-3 fatty acids on leukocyte Th1/Th2 cytokine and integrin expression in rats with gut-derived sepsis. *Nutrition* **23**, 179–186.
16. Hsu CS, Chiu WC, Yeh CL, *et al.* (2006) Dietary fish oil enhances adhesion molecule and interleukin-6 expression in mice with polymicrobial sepsis. *Br J Nutr* **96**, 854–860.
17. Roth E & Kudsk KA (2004) Immunonutrition: back to science. *JPEN J Parenter Enteral Nutr* **28**, 278, author reply 279–280.
18. Xue H, Sawyer MB, Field CJ, *et al.* (2008) Bolus oral glutamine protects rats against CPT-11-induced diarrhea and differentially activates cytoprotective mechanisms in host intestine but not tumor. *J Nutr* **138**, 740–746.
19. Hwang PM, Bunz F, Yu J, *et al.* (2001) Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells. *Nat Med* **7**, 1111–1117.
20. Yanez JA, Teng XW, Roupe KA, *et al.* (2003) Chemotherapy induced gastrointestinal toxicity in rats: involvement of mitochondrial DNA, gastrointestinal permeability and cyclooxygenase-2. *J Pharm Pharm Sci* **6**, 308–314.
21. Sadzuka Y & Hirota S (1997) Effect of CPT-11 on lipid peroxide level in mouse tissues. *Jpn J Cancer Res* **88**, 512–516.
22. Timur M, Akbas SH & Ozben T (2005) The effect of Topotecan on oxidative stress in MCF-7 human breast cancer cell line. *Acta Biochim Pol* **52**, 897–902.
23. Wessner B, Strasser EM, Koitz N, *et al.* (2007) Green tea polyphenol administration partly ameliorates chemotherapy-induced side effects in the small intestine of mice. *J Nutr* **137**, 634–640.
24. Cao S & Rustum YM (2000) Synergistic antitumor activity of irinotecan in combination with 5-fluorouracil in rats bearing advanced colorectal cancer: role of drug sequence and dose. *Cancer Res* **60**, 3717–3721.
25. Le Bricon T, Gugins S, Cynober L, *et al.* (1995) Negative impact of cancer chemotherapy on protein metabolism in healthy and tumor-bearing rats. *Metabolism* **44**, 1340–1348.

26. Melichar B, Touskova M, Solichova D, *et al.* (2001) CD4 + T-lymphocytopenia and systemic immune activation in patients with primary and secondary liver tumours. *Scand J Clin Lab Invest* **61**, 363–370.

27. Johnson RA & Roodman GD (1989) Hematologic manifestations of malignancy. *Dis Mon* **35**, 721–768.

28. Melichar B, Touskova M & Vesely P (2002) Effect of irinotecan on the phenotype of peripheral blood leukocyte populations in patients with metastatic colorectal cancer. *Hepatogastroenterology* **49**, 967–970.

29. Tabuchi T, Ubukata H, Saniabadi AR, *et al.* (1999) Granulocyte apheresis as a possible new approach in cancer therapy: a pilot study involving two cases. *Cancer Detect Prev* **23**, 417–421.

30. Zaloudik J, Lauerova L, Janakova L, *et al.* (1999) Significance of pre-treatment immunological parameters in colorectal cancer patients with unresectable metastases to the liver. *Hepatogastroenterology* **46**, 220–227.

31. Hannisdal E & Engan T (1991) Blood analyses and survival in symptom- and survey-detected lung cancer patients. *J Intern Med* **229**, 337–341.

32. Harris J, Sengar D, Stewart T, *et al.* (1976) The effect of immunosuppressive chemotherapy on immune function in patients with malignant disease. *Cancer* **37**, 1058–1069.

33. Stock W & Hoffman R (2000) White blood cells 1: non-malignant disorders. *Lancet* **355**, 1351–1357.

34. Ramos EJ, Middleton FA, Laviano A, *et al.* (2004) Effects of omega-3 fatty acid supplementation on tumor-bearing rats. *J Am Coll Surg* **199**, 716–723.

35. Roynette CE, Calder PC, Dupertuis YM, *et al.* (2004) n-3 polyunsaturated fatty acids and colon cancer prevention. *Clin Nutr* **23**, 139–151.

36. Knox WE, Horowitz ML & Friedell GH (1969) The proportionality of glutaminase content to growth rate and morphology of rat neoplasms. *Cancer Res* **29**, 669–680.

37. Kovacevic Z & McGivan JD (1983) Mitochondrial metabolism of glutamine and glutamate and its physiological significance. *Physiol Rev* **63**, 547–605.

38. Ollenschlager G, Simmel A & Roth E (1989) Availability of glutamine from peptides and acetylglutamine for human tumor-cell cultures. *Metabolism* **38**, 40–42.

39. Knox WE, Linder M & Friedell GH (1970) A series of transplantable rat mammary tumors with graded differentiation, growth rate, and glutaminase content. *Cancer Res* **30**, 283–287.

40. Moyer GH & Pitot HC (1974) Static and dynamic aspects of amino acid pools in rat liver and Morris hepatomas 9618A and 7800. *Cancer Res* **34**, 2647–2653.

41. Austgen TR, Dudrick PS, Sitren H, *et al.* (1992) The effects of glutamine-enriched total parenteral nutrition on tumor growth and host tissues. *Ann Surg* **215**, 107–113.

42. Yoshida S, Kaibara A, Yamasaki K, *et al.* (1995) Effect of glutamine supplementation on protein metabolism and glutathione in tumor-bearing rats. *JPEN J Parenter Enteral Nutr* **19**, 492–497.

43. Wang WS, Lin JK, Lin TC, *et al.* (2007) Oral glutamine is effective for preventing oxaliplatin-induced neuropathy in colorectal cancer patients. *Oncologist* **12**, 312–319.

44. Kaufmann Y, Kornbluth J, Feng Z, *et al.* (2003) Effect of glutamine on the initiation and promotion phases of DMBA-induced mammary tumor development. *JPEN J Parenter Enteral Nutr* **27**, 411–418.

45. Fahr MJ, Kornbluth J, Blossom S, *et al.* (1994) Harry M. Vars Research Award. Glutamine enhances immunoregulation of tumor growth. *JPEN J Parenter Enteral Nutr* **18**, 471–476.

46. Klimberg VS, Kornbluth J, Cao Y, *et al.* (1996) Glutamine suppresses PGE2 synthesis and breast cancer growth. *J Surg Res* **63**, 293–297.

47. Todorova VK, Harms SA, Kaufmann Y, *et al.* (2004) Effect of dietary glutamine on tumor glutathione levels and apoptosis-related proteins in DMBA-induced breast cancer of rats. *Breast Cancer Res Treat* **88**, 247–256.

48. Pardini RS (2006) Nutritional intervention with omega-3 fatty acids enhances tumor response to anti-neoplastic agents. *Chem Biol Interact* **162**, 89–105.

49. Rubio IT, Cao Y, Hutchins LF, *et al.* (1998) Effect of glutamine on methotrexate efficacy and toxicity. *Ann Surg* **227**, 772–778, discussion 778–780.

50. Balendiran GK, Dabur R & Fraser D (2004) The role of glutathione in cancer. *Cell Biochem Funct* **22**, 343–352.

51. Germain E, Chajes V, Cognault S, *et al.* (1998) Enhancement of doxorubicin cytotoxicity by polyunsaturated fatty acids in the human breast tumor cell line MDA-MB-231: relationship to lipid peroxidation. *Int J Cancer* **75**, 578–583.

52. Rouse K, Nwokedi E, Woodliff JE, *et al.* (1995) Glutamine enhances selectivity of chemotherapy through changes in glutathione metabolism. *Ann Surg* **221**, 420–426.

53. Hardman WE, Avula CP, Fernandes G, *et al.* (2001) Three percent dietary fish oil concentrate increased efficacy of doxorubicin against MDA-MB 231 breast cancer xenografts. *Clin Cancer Res* **7**, 2041–2049.

54. Hardman WE, Moyer MP & Cameron IL (2002) Consumption of an omega-3 fatty acids product, INCELL AAFA, reduced side-effects of CPT-11 (irinotecan) in mice. *Br J Cancer* **86**, 983–988.

55. Khan SA & Wingard JR (2001) Infection and mucosal injury in cancer treatment. *J Natl Cancer Inst Monogr* **29**, 31–36.

56. Hoensch H, Morgenstern I, Petereit G, *et al.* (2002) Influence of clinical factors, diet, and drugs on the human upper gastrointestinal glutathione system. *Gut* **50**, 235–240.

57. Barbosa DS, Cecchini R, El Kadri MZ, *et al.* (2003) Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. *Nutrition* **19**, 837–842.

58. Camuesco D, Galvez J, Nieto A, *et al.* (2005) Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. *J Nutr* **135**, 687–694.

59. Simopoulos AP (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* **21**, 495–505.

60. Calder PC (2003) n-3 Polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* **38**, 343–352.

61. Pavlick KP, Laroux FS, Fuseler J, *et al.* (2002) Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radic Biol Med* **33**, 311–322.

62. Exner R, Tamandl D, Goetzinger P, *et al.* (2003) Perioperative GLY-GLN infusion diminishes the surgery-induced period of immunosuppression: accelerated restoration of the lipopolysaccharide-stimulated tumor necrosis factor-alpha response. *Ann Surg* **237**, 110–115.

63. Gennari R, Alexander JW & Eaves-Pyles T (1995) Effect of different combinations of dietary additives on bacterial translocation and survival in gut-derived sepsis. *JPEN J Parenter Enteral Nutr* **19**, 319–325.

64. Wessner B, Strasser EM, Spittler A, *et al.* (2003) Effect of single and combined supply of glutamine, glycine, N-acetylcysteine, and R,S-alpha-lipoic acid on glutathione content of myelomonocytic cells. *Clin Nutr* **22**, 515–522.

65. Hamani D, Charrueau C, Butel MJ, *et al.* (2007) Effect of an immune-enhancing diet on lymphocyte in head-injured rats: what is the role of arginine? *Intensive Care Med* **33**, 1076–1084.

66. Boelens PG, Melis GC, van Leeuwen PA, *et al.* (2006) Route of administration (enteral or parenteral) affects the contribution of L-glutamine to de novo L-arginine synthesis in mice: a stable-isotope study. *Am J Physiol Endocrinol Metab* **291**, E683–E690.