ORIGINAL ARTICLE

Omega-3 and omega-3/curcumin-enriched fruit juices decrease tumour growth and reduce muscle wasting in tumour-bearing mice

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Abstract

Background
The aim of the present investigation was to evaluate the effects of the administration of a juice containing essential nutrients (marine omega-3 fatty acids: EPA and DHA, a polyphenol-rich juice, vitamin D3, essential amino acids and dietary fibre) (CAX) and a juice also enriched with curcumin (CUR), alone or in combination with a chemotherapeutic agent (*sorafenib*) in a mouse cancer cachexia model.

Methods
Administration of CAX and CUR in the form of jellified pellets to mice bearing the Lewis lung carcinoma.

Results
Administration of CAX and CUR in the form of jellified pellets to mice bearing the Lewis lung carcinoma resulted in a 12 and 18% reduction in tumour weight, respectively, but no additive effect in combination with sorafenib was seen. No effects on metastases measurements were observed. In spite of the reduction of the primary tumour, the chemotherapy treatment alone did not result in any changes in body weight. Conversely, in combination with sorafenib, both juices had an important effect on body weight loss. CUR also had an effect without chemotherapy. Concerning muscle weight, tibialis muscle mass was increased as a result of CAX and CUR treatment.

Conclusions
It is concluded that administration of omega-3/protein and omega-3/protein/curcumin-enriched fruit juices may have beneficial effects on muscle wasting and could be part of a multi-modal therapy for cancer cachexia.

Introduction

Cancer cachexia is perhaps the most common manifestation of advanced malignant disease. Indeed, cachexia occurs in the majority of cancer patients before death, and accordingly to Warren[1] it is responsible for the death of 22% of cancer patients. The abnormalities associated with cancer cachexia include anorexia, weight loss, muscle loss and atrophy, anaemia and alterations in carbohydrate, lipid and protein metabolism[2]. The degree of cachexia is inversely correlated with the overall survival of the patient and it always implies a poor prognosis[3–5].

The mouse Lewis lung carcinoma is a suitable model system to study the mechanisms involved in the establishment of cachexia. The tumour has been described as anaplastic and epidermoid with a marked haemorrhagic tendency, which produces multiple lung metastases regularly, spontaneously and consistently[6]. Moreover these tumours are extremely refractory to most chemotherapeutic agents[7]. Basically the Lewis lung cancer model is based on a well-known neoplasia with a short infective cycle linked to rapid tumour growth and development of lung metastases, which soon leads to death[6]. The rapid growth causes progressive weight loss and tissue waste, particularly of skeletal muscle. In fact, accelerated tissue protein breakdown accounts for most of the wasting in Lewis lung carcinoma bearers [8].

Both tumour growth and chemotherapy contribute to muscle loss by activating common signaling

pathways[9]. For example, loss of skeletal muscle during neoadjuvant chemotherapy is related to decreased survival in ovarian cancer patients[10]. There is, therefore, a need for improved combination strategies that both aim to counteract tumour growth and to reduce chemotherapy side effects on skeletal muscle.

The chemotherapeutic drug *Sorafenib* is a kinase inhibitor used in humans for the treatment of primary kidney cancer (advanced renal cell carcinoma), advanced primary liver cancer (hepatocellular carcinoma) and radioactive iodine-resistant advanced thyroid carcinoma. Sorafenib is a multikinase inhibitor that has shown efficacy against a wide variety of tumours in pre-clinical models[11]. It inhibits cell proliferation by targeting the Raf/MEK/ERK signalling pathways and exerts an anti-angiogenic effect by inhibition of tumour angiogenesis through vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR)[12,13]. Sorafenib is already approved for treatment of patients with advanced hepatocellular carcinoma[14] and advanced renal cell carcinoma[15]. Previous studies in our laboratory have shown that sorafenib indeed decreases tumour growth in different rat and mouse tumour models[16,17].

Successful treatment of cancer cachexia remains a challenge in clinical practice. The syndrome involves not just anorexia but also important metabolic abnormalities[18,19]. For this reason, currently used nutritional strategies are simply insufficient to reverse the cachexia syndrome. Among the pharmacological strategies tested, the use of megestrol acetate has shown some positive effects on cancer cachexia. However, the drug seems primarily to act by increasing the food intake, although some additional effects on the skeletal muscle have been described recently. Thus, altogether megestrol acetate does not address several of the key aspects of the cachectic syndrome [20,21]. Another very important factor in the treatment of cachexia is timing. In cancer patients, any therapy (nutritional/metabolic/pharmacological) has to be started at the earliest possible stage of the disease, i.e. before the weight loss becomes irreversible.

Omega-3-Polyunsaturated (omega-3) fatty acids (PUFAs), present in large amounts in fish oil, have been proposed to reduce both tumour growth[22,23] and tissue wasting, in particular adipose tissue[24]. In fact, the interest in omega-3-PUFA originated from the observation that populations consuming a diet rich in PUFAs showed a far lower incidence of certain types of cancer. One plausible reason is that in addition to suppressing tumour growth[22,23] omega-3 fatty acids also reduce systemic inflammation[25] by interfering with cytokine production[26]. For that reason, omega-3 fatty acids have also been used to fight muscle wasting during cancer. Indeed, different studies using nutritional supplements containing fish oil in cancer cachexia have reported positive, although partly controversial, results. Back in 1990, Tisdale described positive effects of omega-3 fatty acids in an experimental model of cancer

cachexia by demonstrating that EPA could block the enhanced protein degradation present in skeletal muscle during cancer[27]. However, Costelli et al. did on the other hand report that daily intragastric administration of EPA(1.5 g/kg body wt) to AH-130 bearing rats was completely ineffective in preventing tissue waste as well as in reducing tumour growth[28]. It may be speculated that the low degree of tumour differentiation and the rapid tumour growth rate of the AH-130 hepatomas could have accounted for the lack of efficacy in this latter case[28]. Moreover, several clinical trials have shown beneficial effects of nutritional supplements containing fish oil[29,30] while others have failed to demonstrate positive effects[31]. Among the positive effects observed has been an increase in lean body mass and an improved quality of life, as demonstrated in a randomised doubled blind trial using a protein and energy dense omega-3 fatty acid-enriched oral supplement, provided its consumption was equal or superior to 2.2 g EPA[30]. However, data arising from a large multicentre double-blind placebo-controlled trial indicate that EPA administration alone is not successful in the treatment of weight-losing patients with advanced gastrointestinal or lung cancer[32]. Moreover, a metaanalysis based on five trials concluded that there were insufficient data to establish whether oral EPA was better than placebo. Comparisons of EPA combined with a protein energy supplementation versus a protein energy supplementation (without EPA) in the presence of an appetite stimulant (megestrol acetate) provided no evidence that EPA improves symptoms associated with the cachexia syndrome often seen in patients with advanced cancer[33].

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenil)1,6-heptadiene-3,5-dione], present in turmeric (a traditional spice used in Indian cookery), has been shown to have important anti-inflammatory, antioxidant, and free radical-scavenging properties[34–36]. In addition to being present in turmeric, curcumin is also the major yellow pigment in curry and mustard. Indeed, its use as a colouring agent in foods and cosmetics has been very wide. In addition to its antioxidant and anti-inflammatory properties, curcumin has been shown to have anti-mutagenic and anti-carcinogenic effects[37]. Thus, curcumin seems to be able to induce apoptosis in some tumour cells[38,39], and to inhibit tumour proliferation[40].

Bearing all this in mind, the aim of the present investigation was to evaluate the effects of Smartfish Targeted Medical Nutrition, an omega-3 polyunsaturated fatty acid enriched fruit juice containing whey protein and vitamin D, alone or in combination with a chemotherapeutic agent (*Sorafenib*) in a mouse cancer cachexia model.

Experimental

Animals and tumour inoculation

Male C57BL6 mice (Interfauna, Barcelona, Spain) weighing about 20 g were used. The animals were maintained in individual cages on a regular light-dark cycle (light on from 08:00 a.m. to 08:00 p.m.) and had free access to food and water. The diet (Panlab, Barcelona, Spain) consisted of 54% carbohydrate, 17% protein and 5% fat (the residue was non digestible material); the food intake was measured daily.

The mice were randomized and divided into two groups, namely controls (C) and tumour bearers (TB). The TB mice received an intramuscular (hind leg) inoculum of 5×10^5 Lewis lung carcinoma cells obtained from exponential tumours. The Lewis lung carcinoma is a highly cachectic rapidly growing mouse tumour containing poorly differentiated cells, with a relatively short doubling time[6]. At day 18 after tumour transplantation, the animals were weighed and anesthetized with a ketamine/xylacine mixture. The tumour was harvested from the hind leg, its volume and mass evaluated, and the number of lung metastasis evaluated under the microscope. Blood was collected from the abdominal aorta into heparinized tubes and centrifuged ($3,500\ g$, 10 min, 4ºC) to obtain plasma. Tissues were rapidly excised, weighed, and frozen in liquid nitrogen. The Ethical Committee for Animal Experimentation of the University of Barcelona approved the experimental protocol. All animal manipulations were made in accordance with the European Community Guidelines for the Use of Laboratory Animals and the *Policy on Humane Care and Use of Laboratory Animals* (ILAR 2011) and in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Treatment

Tumour-bearing animals were divided into two major groups, untreated (no chemotherapy) and treated (chemotherapy) daily with sorafenib (90mg/kg body weight, intraperiteoneally (i.p.) from day 4 after tumour injection). The animal groups (treated and non-treated with sorafenib) were divided into subgroups of 8 animals: control group: sham (vehicle)-treated animals (PLAC), and treated groups: Remune (CAX) and Nutrifriend 3000/Curcumin (CUR). CAX contains essential nutrients: marine omega-3 fatty acids (approximately 2g/200 mL eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), polyphenol rich juice, vitamin D3, essential amino acids and dietary fibre). CUR contains the same compounds that CAX but with an enrichment of curcumin (760mg/200mL). The PLAC treatment is milk-based, isocaloric and contains fat (2g/200 mL sunflower oil), carbohydrates and proteins.

Administration of the different solutions started the day of tumour inoculation and continued until day 18. The solutions were given as jellified blocks: CAX: 826 mg EPA/kg in 2 g of jellified juice and CUR: 616 mg/kg in 1 g of jellified juice. Jellified juices were prepared daily, using ice-cube moulds of gelatine-juice drink (1g / 25mL).

Total physical activity

Total physical activity was measured during the last 24h before the sacrifice of the animals using the *IR ACTIMETER System and ACTITRAK software* from Panlab-Harvard Apparatus (Barcelona, Spain). This system uses activity sensors that translate individual changes in the infrared pattern caused by movements of the animals into arbitrary activity counts (automated system)[41]. In order to carry out the measurements, animals remained in their home cage with free access to food and water, and a frame containing an infrared beam system was placed on the outside of the cage. Data were collected for a total period of 24h.

Muscle force assessment

Skeletal muscle strength in mice was quantified by the grip-strength test[41] once a week. The grip strength device (Panlab-Harvard Apparatus, Barcelona, Spain) comprised a pull bar connected to an isometric force transducer (dynamometer). Basically, the grip strength meter was positioned horizontally and the mice are held by the tail and lowered towards the device. The animals are allowed to grasp the pull bar and were then pulled backwards in the horizontal plane. The force applied to the bar just before the animals lost grip was recorded as the peak tension. At least three measurements were taken per mouse on both baseline and test days, and the results were averaged for analysis. This force was measured in grams/grams of initial body weight.

Tumour mass and metastasis

The tumour was harvested from the hind leg, its volume and mass evaluated, and number of lung metastasis evaluated under the microscope. For visualization of metastasis, lungs were removed after killing of the mice. Metastatic nodules were counted using an anatomic microscope equipped with a 40x objective. The weights of the metastases were evaluated according to the method used by Donati et al.[42]. Briefly, metastases were classified according to area: (1) 1 mm^2; (2) 4 mm^2; (3) 9 mm^2; (4) 16 mm^2; and (5) 25 mm^2. Their weights were determined using the formula $4/3\pi r^3$, where r is the radius if considering the metastases as a sphere. The volume is converted to weight considering that the tumour density is around 1 g/mL and the relation between metastasis classification and weight is: (1) 0.5 mg; (2) 4.2 mg; (3) 14.1 mg; (4) 33.5 mg; and (5) 65.4 mg.

Analytical procedures

Haematocrit

Total blood was withdrawn from anaesthetized mice by cardiac puncture and collected in heparinized tubes. A drop was used to fill haematocrit capillary tubes that were centrifuged in a haematocrit centrifuge for 5 min at 800 x g. Haematocrit was calculated as percentage of packed cell volume of the total blood.

IL-6

Post-prandial serum levels of IL6 were quantified by ELISA kit (IL-6: Diaclone 670010192, Spain).

Statistical analysis

Average (arithmetic mean) and standard error of the mean (SEM) were calculated for each studied variable. Intergroup differences were evaluated statistically using analysis of variance (ANOVA). Fixed factors: juice treatment and sorafenib treatment). *Post hoc* pairwise comparisons (Duncan test) were performed when appropriated. All statistical tests were performed using SPSS version 21.

Results

As can be seen in Figure 1A, juice treatment both, without curcumin (CAX) or with curcumin (CUR), significantly reduced primary tumour growth. Sorafenib treatment also reduced primary tumour growth although no additive effect was observed on this parameter when juice treatment was applied to sorafenib-treated mice. No effects on the number of metastases (either by chemotherapy or juice administration) were observed (Figure 1B). In spite of the tumour reduction, the chemotherapy treatment did not result in any changes in body weight (measured as growth decrease) (Figure 2A). However, the haematocrit was improved in sorafenib-treated animals (Table 1).

In combination with sorafenib, CAX had and important effect on reducing body weight loss. CUR treatment also had an effect without chemotherapy (Figure 2A). CUR treatment (with or without chemotherapy) significantly increased food and water intake (Figure 2B). A clear statistically significant increase was observed in tibialis muscle when the animals were treated with either CAX or CUR. In combination with sorafenib, CAX treatment also resulted in an increased tibialis muscle mass (Table 2). No significant effects were observed on any of the other studied skeletal muscles (Table 2). In sorafenib-treated mice, treatment with neither CAX nor CUR juices resulted in a significant change in grip force (Figure 3). Similarly, no changes were observed when the total physical activity or parameter related to mobility were examined (Table 3).

Concerning adipose tissue, the juice treatment resulted in a significant increase in both epididymal (eWAT) and dorsal (dWAT) white adipose tissue (CAX) and an increase in interscapular brown adipose tissue (BAT) mass (CUR), respectively (Table 4). This effect of CAX on dWAT was observed also in combination with chemotherapy. Liver, heart, kidney, spleen and carcass weights were not significantly affected as a result of the chemotherapy (Table 5).

We also examined the levels of IL-6 following the different treatments. Neither juice administration nor sorafenib had any effects on the plasma levels of the inflammatory biomarker IL-6 (Table 1).

Table 1 Haematocrit and IL-6 plasma levels in treated tumour-bearing mice.

	Haematocrit	IL-6
PLAC	25.4 ± 1.9 (8)	100.2 ± 5.0 (8)
CAX	24.3 ± 1.8 (8)	98.9 ± 4.0 (8)
CUR	23.3 ± 2.1 (7)	97.9 ± 5.1 (8)
PLAC+S	28.9 ± 2.7 (8)	95.8 ± 4.7 (8)
CAX+S	29.9 ± 1.7 (7)	97.6 ± 4.1 (8)
CUR+S	30.3 ± 2.2 (9)	100.9 ± 4.1 (9)
Chemo	**0.004**	ns
Juice	ns	ns
Chemo+juice	ns	ns

Results are mean ± S.E.M. for the number of animals indicated in parentheses. Haematocrit was calculated as percentage of packed cell volume of the total blood. IL-6 is expressed in pg/mL. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib. Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice) and sorafenib treatment (Chemo)).

Figure 1 Tumour weight and metastases number in treated tumour-bearing animals.
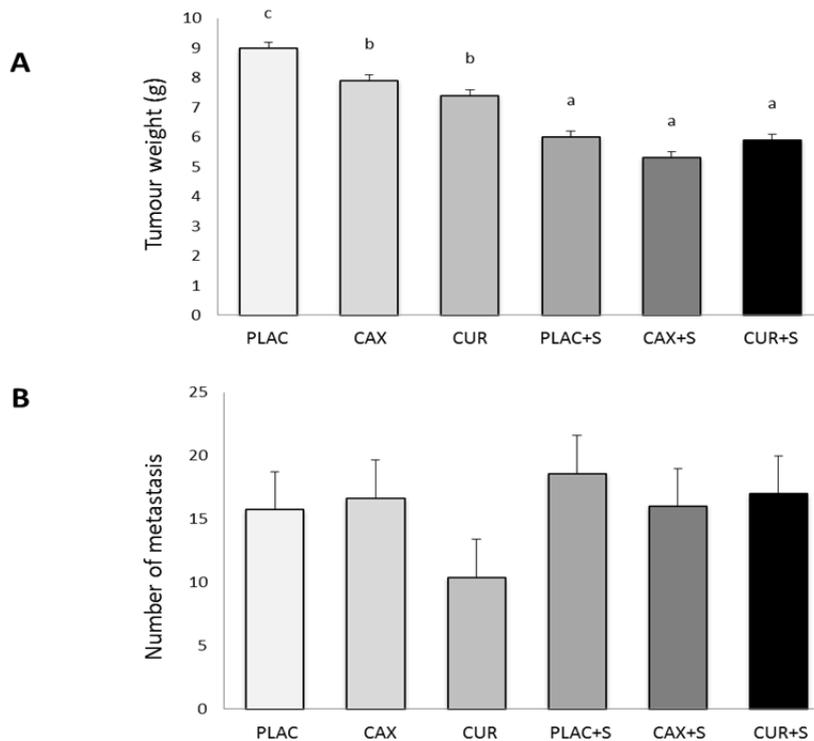
Results are mean ± S.E.M. for the number of animals indicated in parentheses. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib.

> **A.** Tumour weight (g). Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice p≤0,001), sorafenib treatment (Chemo p≤0,001) and interaction Chemo+Juice p=0,013). Post hoc Duncan tests were performed; different superscripts indicate significant differences between groups.
>
> **B.** Number of metastases. Non significant differences.

Table 2 Muscle weights in treated tumour-bearing mice.

	EDL	Soleus	Tibialis	GSN
PLAC	33.8 ± 1.2 (8)	32.7 ± 1.6 (8)	127.1 ± 3.0 (8) **a**	455.1 ± 15.5 (8)
CAX	34.5 ± 1.6 (8)	31.2 ± 0.2 (8)	141.4 ± 3.3 (8) **bc**	466.5 ± 12.6 (8)
CUR	37.8 ± 2.9 (8)	36.0 ± 1.1 (8)	144.9 ± 3.8 (8) **bc**	459.9 ± 8.6 (8)
PLAC+S	31.0 ± 2.5 (8)	34.2 ± 2.9 (8)	135.0 ± 3.3 (8) **ab**	464.6 ± 12.9 (8)
CAX+S	34.7 ± 1.8 (8)	37.5 ± 1.6 (8)	149.3 ± 5.0 (8) **c**	473.6 ± 25.2 (8)
CUR+S	32.6 ± 1.6 (9)	36.8 ± 2.1 (9)	138.0 ± 3.5 (9) **abc**	485.4 ± 17.7 (9)
Chemo	ns	ns	ns	ns
Juice	ns	ns	**0.001**	ns
Chemo+juice	ns	ns	**< 0.1**	ns

Results are mean ± S.E.M. for the number of animals indicated in parentheses. GSN: gastrocnemius muscle. Tissue weights are expressed as mg/100 g of initial body weight. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib. Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice) and sorafenib treatment (Chemo)). Post hoc Duncan tests were performed; different superscripts indicate significant differences between groups.

Figure 2 Body weight loss and food and water intake in treated tumour-bearing mice.
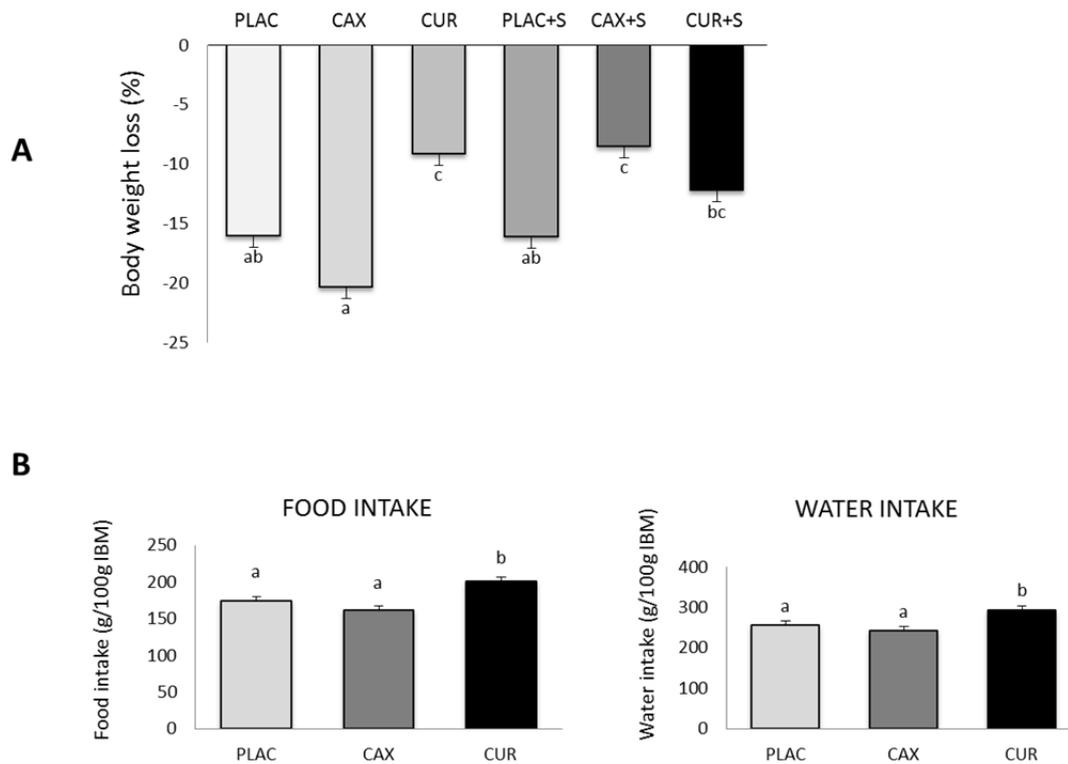
Results are mean ± S.E.M. for the number of animals indicated in parentheses. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin,

PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib.

A. Body weight loss (%). Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice: ns), sorafenib treatment (Chemo: ns) and interaction Chemo+Juice p=0,01). Post hoc Duncan tests were performed; different superscripts indicate significant differences between groups.

B. Food and water intake (data are expressed in g /100 g IBW and refers to the water or food ingestion during the period of the experiment prior to sacrifice (corrected by the IBW)). Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice p≤0,001 (food) p≤0,01 (water)), sorafenib treatment (Chemo: ns) and interaction Chemo+Juice: ns). Post hoc Duncan tests were performed; different superscripts indicate significant differences between groups.

Table 3. Physical activity in treated tumour-bearing mice.

	Total activity	Stereotyped movements	Locomotor movements	Max velocity	Mean velocity	Travelled Distance
PLAC	22727 ± 999 (4)	1986 ± 259 (4)	20741 ± 1161 (4)	11.7 ± 1.3 (4)	0.2 ± 0 (4)	15352 ± 660 (4)
CAX	17237 ± 827 (3)	2702 ± 902 (3)	14536 ± 1565 (3)	26.4 ± 6 (3)	0.2 ± 0 (3)	15570 ± 2642 (3)
CUR	22789 ± 2252 (4)	3551 ± 1275 (4)	19239 ± 2959 (4)	21.3 ± 8.2 (4)	0.3 ± 0 (4)	16249 ± 2062 (3)
PLAC+S	23475 ± 3006 (4)	2322 ± 461 (4)	21154 ± 2609 (4)	18.8 ± 1.7 (4)	0.2 ± 0 (4)	15963 ± 2131 (4)
CAX+S	20130 ± 1840 (4)	2252 ± 284 (4)	17878 ± 1879 (4)	20.3 ± 4.9 (4)	0.2 ± 0 (4)	14484 ± 1218 (4)
CUR+S	24574 ± 1563 (4)	2273 ± 103 (4)	22301 ± 1581 (4)	13.6 ± 1.1 (3)	0.2 ± 0 (4)	17007 ± 1090 (4)
Chemo	ns	ns	ns	ns	ns	ns
Juice	**0.05**	ns	ns	ns	ns	ns
Chemo+juice	ns	ns	ns	ns	ns	ns

Results are mean ± S.E.M. for the number of animals indicated in parentheses. Physical activity is expressed in number of movements. Stereotyped movements include movements without displacement (eating and cleaning movements); conversely, locomotor movements include movements with displacement. Mean velocity and maximum velocity are expressed in cm/s. Travelled distance is expressed in cm. The thresholds of time are the following: time involving resting (sleeping, cleaning and eating time): [0–2] cm/s, time involving slow movements: cm/s and time involving fast movements: [>5] cm/s. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib. Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice) and sorafenib treatment (Chemo)).

Figure 3 Grip force in cachectic tumour-bearing mice.
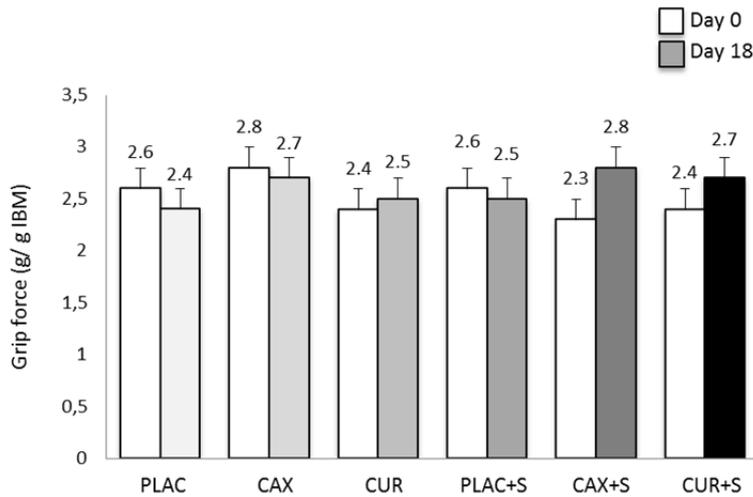
Results are mean ± S.E.M. for the number of animals indicated in parentheses. GSN: gastrocnemius muscle. Results are expressed as g/g initial body weight. White bars: day 0, gray bars: day 18. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib. Non significant differences of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice=0,195) and sorafenib treatment (Chemo=0,163)).

Table 4 Adipose tissue weights in treated tumour-bearing mice.

	dWAT	eWAT	dWAT + eWAT	BAT
PLAC	10.0 ± 5 (8) **a**	111.6 ± 18 (8)	122 ± 23 (8) **a**	158.2 ± 12 (8)
CAX	16.4 ± 5 (8) **a**	143.4 ± 33 (8)	160 ± 37 (8) **ab**	194.6 ± 8 (8)
CUR	26.1 ± 6 (8) **a**	170.8 ± 43 (8)	197 ± 47 (8) **ab**	206.9 ± 19 (8)
PLAC+S	22.8 ± 9 (8) **a**	177.7 ± 33 (8)	200 ± 39 (8) **ab**	225.8 ± 22 (8)
CAX+S	71.7 ± 18 (8) **b**	371.2 ± 50 (8)	443 ± 60 (8) **c**	231.1 ± 15 (8)
CUR+S	27.5 ± 7 (9) **a**	256.6 ± 41 (9)	284 ± 47 (9) **b**	247.3 ± 13 (9)
Chemo	**0.007**	**0.001**	**0.001**	**0.001**
Juice	**0.045**	**0.018**	**0.011**	**0.005**
Chemo+juice	**0.044**	ns	**0.042**	ns

Results are mean ± S.E.M. for the number of animals indicated in parentheses. Tissue weights are expressed as mg/100 g of initial body weight. dWAT: dorsal white adipose; eWAT: epididimal white adipose, dWAT+eWAT: sum of the two adipose tissue pads, BAT: interscapular brown adipose tissue. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib. Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice) and sorafenib treatment (Chemo)). Post hoc Duncan tests were performed; different superscripts indicate significant differences between groups.

Table 5 Tissue weights in treated tumour-bearing mice.

	LIVER	HEART	KIDNEY	SPLEEN	CARCASS
PLAC	6275 ± 266 (8)	564 ± 13 (8)	1342 ± 26 (8)	1430 ± 51 (8)	48 ± 1.8 (8)
CAX	6177 ± 125 (8)	571 ± 20 (8)	1434 ± 31 (8)	1538 ± 103 (8)	45 ± 2.6 (8)
CUR	6785 ± 218 (8)	602 ± 27 (8)	1441 ± 42 (8)	1646 ± 68 (8)	50 ± 2.8 (8)
PLAC+S	5138 ± 165 (8)	511 ± 10 (8)	1326 ± 22 (8)	1394 ± 84 (8)	54 ± 1.7 (8)
CAX+S	5423 ± 171 (8)	579 ± 83 (8)	1356 ± 27 (8)	1416 ± 100 (8)	54 ± 2.4 (8)
CUR+S	5361 ± 164 (9)	525 ± 50 (9)	1388 ± 71 (9)	1405 ± 31 (9)	54 ± 0.7 (9)
Chemo	**0.001**	**0.001**	**0.004**	**0.038**	**0.001**
Juice	ns	ns	ns	ns	ns
Chemo+juice	ns	ns	ns	ns	ns

Results are mean ± S.E.M. for the number of animals indicated in parentheses. Tissue weights are expressed as mg/100 g of initial body weight. Carcass weight is expressed as g/100 g of initial body weight. PLAC: placebo, CAX: Remune, CUR: Nutrifriend 3000+curcumin, PLAC+S: placebo+sorafenib, CAX+S: Remune+sorafenib, CUR+S: Nutrifriend 3000+curcumin+sorafenib. Statistical significance of the results by full factorial two-way ANOVA (fixed factors: juice treatment (Juice) and sorafenib treatment (Chemo)

Discussion

Recent clinical studies showed improved body weight and performance status in cancer patients using specific medical foods that included fish oil supplementation[29]. The results presented here clearly show the beneficial effects of CAX supplements, specifically the combination of low-oxidized fish oil, protein and vitamin D, i.e. not the fish oil alone, in relation to the body weight loss in chemotherapy-treated tumour-bearing mice versus an isocaloric placebo. In addition, the administration of the fish oil-rich targeted medical nutrition also induced an increase in muscle mass (tibialis) and adipose tissue mass. The only muscle that benefited from the treatment was tibialis and this is an interesting observation since the tibialis muscle has a high content of type II white, or muscle fast-twitch glycolytic, fibers.

Halder et al. have shown that curcuminoids and omega-3 fatty acids potentiate the cytotoxic effects of NK cells on pancreatic tumour cells [43]. In addition, Menon et al. [44] have demonstrated that curcumin also has anti-metastatic effects due to its inhibition of tumour cell metalloproteinases, i.e. proteolytic enzymes that facilitate invasion. We have previously described the anti-tumoural effect of curcumin in a rat model of tumour growth[45]. In our study we clearly show that curcumin supplementation, in addition to contributing to a reduced tumour growth, increased body weight, and enhanced appetite in tumour-bearing mice. It also contributed to an increase in brown adipose tissue mass. Interestingly, a recent report suggests that curcumin promotes browning of white adipose tissue[46]. This process transforms white adipose cells into ones where uncoupling protein-1 is expressed. This may have taken place in the present study but further investigations will be needed to confirm this hypothesis.

The data obtained with both CAX and CUR treatment show an effect on both tumour growth and weight loss. It is therefore difficult to draw clear conclusions as regards the anti-cancer effects of the investigated compounds as we cannot discriminate between their anti-cachexia activity of the compounds and their indirect effects, resulting in reduced tumour growth. Thus, the present study has some limitations when it comes to understanding the specific mode of action underlying these anti-cancer effects.

In conclusion, future treatment of the cachectic syndrome will no doubt be based on a combined, multimodal approach, including different therapeutic approaches to efficiently reverse the metabolic changes and, at the same time, ameliorate the anorexia of the patients. Defining this therapeutic combination of nutrition and drugs is an exciting project. Future studies will, no doubt, benefit from well-defined end-points and improved measures of cachexia, providing new insight into the disease. This, in combination with the elucidation of cachexia underlying mechanisms, will provide new treatment strategies in the near future.

Conflict of Interest & Statement of Authorship

Acknowledgements

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