REVIEW

Journal of Cachexia, Sarcopenia and Muscle 2016; **7**: 110–125
Published online 15 October 2015 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12058

Diet composition as a source of variation in experimental animal models of cancer cachexia

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Abstract

Background A variety of experimental animal models are used extensively to study mechanisms underlying cancer cachexia, and to identify potential treatments. The important potential confounding effect of dietary composition and intake used in many preclinical studies of cancer cachexia is frequently overlooked. Dietary designs applied in experimental studies should maximize the applicability to human cancer cachexia, meeting the essential requirements of the species used in the study, matched between treatment and control groups as well as also being generally similar to human consumption.

Methods A literature review of scientific studies using animal models of cancer and cancer cachexia with dietary interventions was performed. Studies that investigated interventions using lipid sources were selected as the focus of discussion.

Results The search revealed a number of nutrient intervention studies (n = 44), with the majority including n-3 fatty acids (n = 16), mainly eicosapentaenoic acid and/or docosahexaenoic acid. A review of the literature revealed that the majority of studies do not provide information about dietary design; food intake or pair-feeding is rarely reported. Further, there is a lack of standardization in dietary design, content, source, and overall composition in animal models of cancer cachexia. A model is proposed with the intent of guiding dietary design in preclinical studies to enable comparisons of dietary treatments within the same study, translation across different study designs, as well as application to human nutrient intakes.

Conclusion The potential for experimental endpoints to be affected by variations in food intake, macronutrient content, and diet composition is likely. Diet content and composition should be reported, and food intake assessed. Minimum standards for diet definition in cachexia studies would improve reproducibility of pre-clinical studies and aid the interpretation and translation of results to humans with cancer.

Keywords Cancer; Cachexia; Animal model; Dietary design

Received: 26 January 2015; Revised: 24 May 2015; Accepted: 16 June 2015
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Introduction

Cancer cachexia is a wasting syndrome characterized by ongoing loss of skeletal muscle mass (with or without loss of fat mass) that leads to progressive functional impairment.[1] A global feature of cachexia is negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism. Experimental models are used extensively to study mechanisms underlying cachexia and to identify potential treatments. Preclinical studies enable control of many factors such as genetics, age, tumour type, and load that would be expected to influence the results of an intervention in a human population. Paradoxically, the important potential confounding effect of dietary intake and composition used in many pre-clinical studies of cancer cachexia is frequently overlooked. Where details of dietary intake in animal experiments of cancer cachexia have been included, the majority of animal diets are imbalanced or lack certain essential nutrients, and frequently bear no resemblance to those normally consumed by humans with respect to macronutrient and micronutrient levels.[2]

Where nutrient interventions have been used for cancer cachexia studies, there is wide variation in timing and duration of supplementation, as well as route and frequency

of administration of any given nutrient, making it difficult to compare results across different animal models, study designs, and laboratory groups. Key metabolic differences between rodents and humans are not widely appreciated. Moreover the importance of background diet is often ignored. Thus, animals fed nutrient-deficient diets may be particularly sensitive to nutritional stress induced by the cancer-bearing state, and demonstrate corresponding exaggerated beneficial responses to supplements containing the missing nutrients. Finally it can be argued that, to maximize the applicability to human cachexia, attention should be paid to making the composition of diets used for experimental animals both sufficient for essential baseline requirements for the species under study but also generally similar to human diets.

To illustrate these points we have reviewed studies performed in rodents that used a dietary intervention for cancer or cancer cachexia. Evidence to support the consideration of diet as an important experimental factor is presented. Moreover, we suggest that minimum standards for diet definition in cachexia studies should be adopted to improve reproducibility of preclinical studies and aid interpretation and translation of results to humans with cancer.

Challenges in using animal models of human cancer cachexia

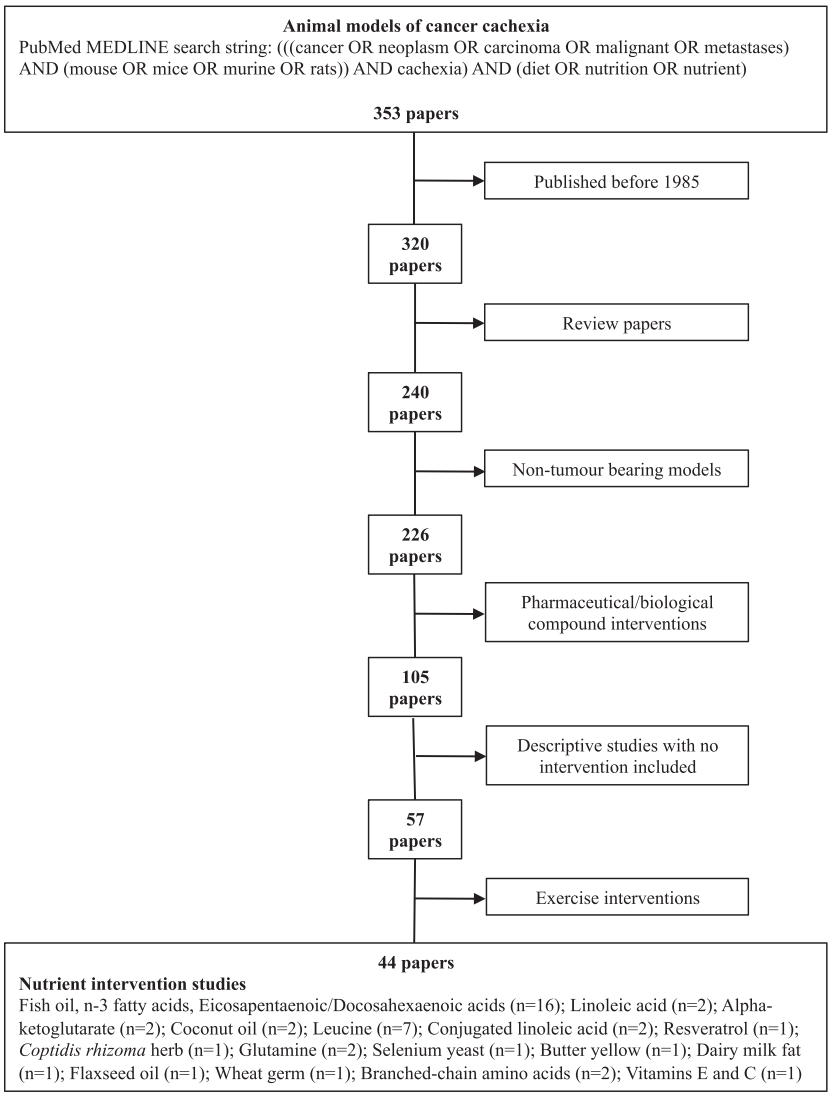
In humans, the mechanisms underlying cancer cachexia are complex, heterogeneous, and largely poorly defined. This complexity cannot be fully reproduced in animal models of cachexia, despite attempts to try and bridge the gap between human disease and animal models of cancer. A limited number of tumour cell types are used in rodent experiments [Lewis lung carcinoma (LLC); Colon 26 adenocarcinoma (C26)] but these are often chosen to be representative of tumour-types (i.e. lung and colorectal cancers) which are more frequently associated with cachexia in humans. Tumour cell lines are typically implanted subcutaneously into experimental animal models, and are left to grow until the tumour-burden induces cachexia symptoms.[3] Although tumour cell implantation induces cachexia, these tumours rarely metastasize, a key difference from cachexia-causing neoplasms in humans.[3] A variety of cachexia models are available, and methodological variations within models also make study comparisons difficult.[4] The majority of animal models for cancer and cancer cachexia use mice and each strain may have important differences relevant to their metabolic responses to cancer. Thus, some strains of in-bred animals are more susceptible to developing obesity and diabetes such as the C57BL/6J strain,[5] which can significantly affect study outcomes in cachexia. Furthermore, sex differences in growth responses are recognized in rodents, which may also limit generalizability of results to humans. Female rodents are preferred over male rodents in studies with endpoints involving body weight changes since females have a slower growth curve and are less susceptible to rapid changes in body weight. In contrast, studies attempting to limit hormonal fluctuations within an animal prefer male rodents.[6]

In addition to metabolic and growth differences between sexes and strains of rodents, it is important to remember that there are also several important differences between human and rodent metabolism. Young and healthy rodents are commonly utilized in experimental studies; however young, healthy animals may not accurately reflect the manifestation of disease in adult or aging humans. For example, cancer patients may present with a number of comorbidities such as obesity, diabetes, heart disease, and are typically over the age of 50 years old.[7] Furthermore, the progression and timeline of aging in humans are not equivalent to that in rodents. Quinn (2005) determined that the ratio of rat to human life span is 1 to 30, making the age of the host species an important factor to consider in the study design.[8] Moreover, rats reach mature skeletal size much earlier than humans.[9] Rodents also differ from humans in several aspects of metabolism. For example, rats have a higher activity of liver enzymes involved in altering the chemical structure of fats [10] and liver CYP enzymes involved in drug metabolism.[11] Compared to human hepatocytes, rat hepatocytes are much less susceptible to carcinogenic factors and mutagenicity.[12] Absence of a gall bladder in rats enables certain fats to be secreted directly into the intestine;[13] thus, rats have an increased ability to eliminate cholesterol from the body. Unlike humans, rats are capable of liver vitamin C synthesis *de novo*,[14] resulting in 2.5-fold higher levels of vitamin C in their bodies compared to humans.[15] Rats also have a higher metabolic rate than other mammals and indeterminant growth, thus have a higher demand for energy relative to their small body size compared to human requirements.[16] In spite of all this, the similarities between rodents and humans are still sufficient to justify use of rodents as models of human disease,[8] but attention in design and interpretation of each experiment is required.

Methods

Scientific articles were obtained through PubMed MEDLINE searches containing the following search strings: (((cancer OR neoplasm OR carcinoma OR malignant OR metastases) AND (mouse OR mice OR murine OR rats)) AND cachexia) AND (diet OR nutrition OR nutrient). Papers that did not include a nutrient intervention were excluded as well as interventions that involved pharmaceutical agents (*Figure* 1). In the 1970s the American Institute of Nutrition (AIN) began to develop semi-purified diets, with these diets becoming more readily used and commercially available after 1985.

Figure 1 Search results and paper exclusion.



Note: Disparities in *n* number due to combination studies using multiple interventions.

For this reason, papers before 1985 were not included in the present review.

Results

The search captured 353 papers in the initial search (*Figure* 1). After removal of studies not meeting the inclusion criteria, 44 papers investigating a number of nutrient interventions remained (see *Figure* 1). The majority of studies applying a nutrient intervention involved n-3 fatty acids, mainly eicosapentaenoic acid (EPA; 20:5n-3) and/or docosahexaenoic acid (DHA; 22:6n-3) (n = 16 studies). This review will focus in detail on the influences of dietary content on the results and interpretation of studies, with an emphasis on study designs applying interventions with various lipid sources.

Important variations in the delivery of experimental diets

The majority of pre-clinical models reviewed in the literature provide diets *ad libitum* (free access), while fewer studies control daily food intakes with fixed amounts.[17,18] Certain animal strains may require food to be delivered in controlled daily amounts to prevent over-feeding.[17] In contrast, providing diets *ad libitum* when food intake is also recorded

may enable researchers to associate the amount of nutrient intake with outcome measures. Form of diet delivery may explain differences in study outcomes if animals ate different amounts of food during the study. Typical outcomes in cachexia studies include muscle mass and weight loss, both of which are intimately related to food intake. Regardless of the diet control, food intake is an essential measure particularly when nutrient intakes are associated with study outcomes. The use of rodent models also enables pair feeding which would not be ethically conceivable in human subjects and allows precise manipulation of dietary intakes while accounting for the effects of food intake per se on experimental outcomes.

Composition of experimental animal diets

Animal diets used in the laboratory are categorized into three main groups: cereal-based (non-purified) chow, purified or semi-purified, and chemically defined diets.[19] Diets either have an open formula, indicating that the dietary composition has been published and is available to the scientific community, or diets can have a closed formula, indicating the dietary composition and ingredients are known only to the manufacturer.[20] Chemically defined, or elemental diets, are made of purified triglycerides, free fatty acids, free amino acids, sugars, vitamins, and minerals.[21] Semi-purified diets are composed of purified proteins, starch, sugars, and defined lipid components from specified fats and oils.

Standard laboratory chow diets

From the body of literature reviewed, it is clear that the majority of diets used are standard laboratory chow from commercial sources provided *ab libitum* (free access). Two of the most common laboratory rodent chows are the *Standard Rat and Mouse Breeding Diet* from Pilsbury (Birmingham, UK) and *Panlab Laboratory Chow* (Barcelona, Spain). Laboratory chow does not have a standard macronutrient composition, and is formulated from plant materials, meat meal, fishmeal, and fats that are rarely defined.[21] Standard laboratory chow diets vary from batch to batch, and individual ingredients may also vary over time.[22,23] Chows with closed formulas are incompletely characterized, making it nearly impossible to determine exactly what is in a particular batch.[21] These factors make it difficult to report the composition of lab chow, aside from the manufacturers name, since a batch fed five years ago will be different from a batch fed one year ago making results from individual studies difficult to replicate in future studies. Laboratory chow aims to meet the essential requirements for a rodent, but is delivered pre-mixed, making it difficult to modify.[21] Most laboratory chows provide the minimum requirement for essential fatty acids for rodents, usually 1.5% total diet,[24,25] and the primary source of fat in standard laboratory chow diet is most often corn oil.[26]

Laboratory chow diets also contain micronutrients or compounds that are unknown to researchers and may impact study endpoints and results. For example, many commonly used natural-ingredient laboratory chows use soy as a protein source, inadvertently adding phytoestrogens to the diet in the form of isoflavones.[27] A number of studies have investigated the effects of using laboratory chow in animal models of breast cancer and endocrine/hormonal organ systems [28,29] have determined that the variability in commercial rodent chow diets interferes with the value of these animal models and experimental results.[27] The variation in isoflavone content between formulations of rodent chow and between batches of the same formulation has been shown to be several fold different.[27] A number of studies have also found chemicals present in rodent chow such as arsenic,[30] mercury,[31] and polychlorinated dioxins,[32] with these chemicals having the potential to exert effects on gene expression and phenotypic outcomes. Chow diets may significantly alter effects of experimental treatments, and in particular, affect gene and protein expression.

Purified, semi-purified, and nutritionally made diets

The most common commercial source for semi-purified diets is the American Institute of Nutrition (AIN) formulation. The AIN first developed these diets to reduce the variance when researchers developed their own formulations. Results from studies prior to AIN-developed diets were difficult to compare across studies and nutrition disciplines. The original AIN diet was the AIN-76A formulation, which was later revised and replaced by AIN-93.[33] AIN-93 diets are offered in Growth (G) or Maintenance (M) forms, depending on the age of the animals being fed. AIN semi-purified diets are available without macronutrients and micronutrients, which enables researchers to formulate and alter the diet according to the particular study objectives. As opposed to corn oil, which was used in AIN diets prior to 1993, soybean oil has been used since 1993 which, compared to corn oil, has a more balanced fatty acid composition [15% saturated fat, 54% linoleic acid (n-6 fatty acid), 8% alpha linoleic acid (n-3 fatty acid), 23% oleic acid (n-9 fatty acid)]. Unlike protein meal used in chow diets, the protein source is purified and contains high quality defined protein sources.

In comparison to laboratory chow, the ingredients included in purified or semi-purified diets are considered open formulas and are well characterized. There are several benefits to using semi-/purified diets over laboratory chow in murine study designs. When diet modifications occur in nutrient interventions, semi-purified diets provide a benefit over laboratory chow; if the nutrient intervention involves a modification of the fat source, semi-purified diets enable fats to be added and modified by the researcher. For example, corn oil can be substituted for olive oil, safflower oil, fish

oil, or individual fatty acids of interest to determine effects of fatty acids or to enable matching of fatty acid composition between experimental groups. Furthermore, semi-purified diets allow researchers to modify fat quantity, and the proportion each type of fat contributes to the diet as a whole.

The influence of background diet on results of nutritional interventions

In nutrient intervention studies, little attention is paid to background diets for their particular nutrient of interest. The phrase, 'a standard rodent chow was used' is written frequently, indicating that the content and composition of the animal diet are either unknown to researchers, or considered unimportant.[27] Knowing the composition and quantity of the diet fed is required to interpret findings relative to human disease states. After meeting nutritional requirements of the rat, the first criteria in dietary design are formulating the comparison diets to be isocaloric and isonitrogenous between treatment and control diets within the same study (*Figure* 2). Although studies may define dietary compositions in detail, fundamental matching of calories and nitrogen content between diets is often overlooked [34–36] (*Table* 1). Second, proportions of macro- and micronutrients could be made similar to human intakes (*Figure* 2).

A review of the literature reveals that many dietary designs do not subscribe to the criteria outlined in *Figure* 2. In three similar studies, male NMRI mice were implanted with MAC16 tumour cells, and supplemented with medium-chain

Figure 2 Proposed dietary design flow chart.

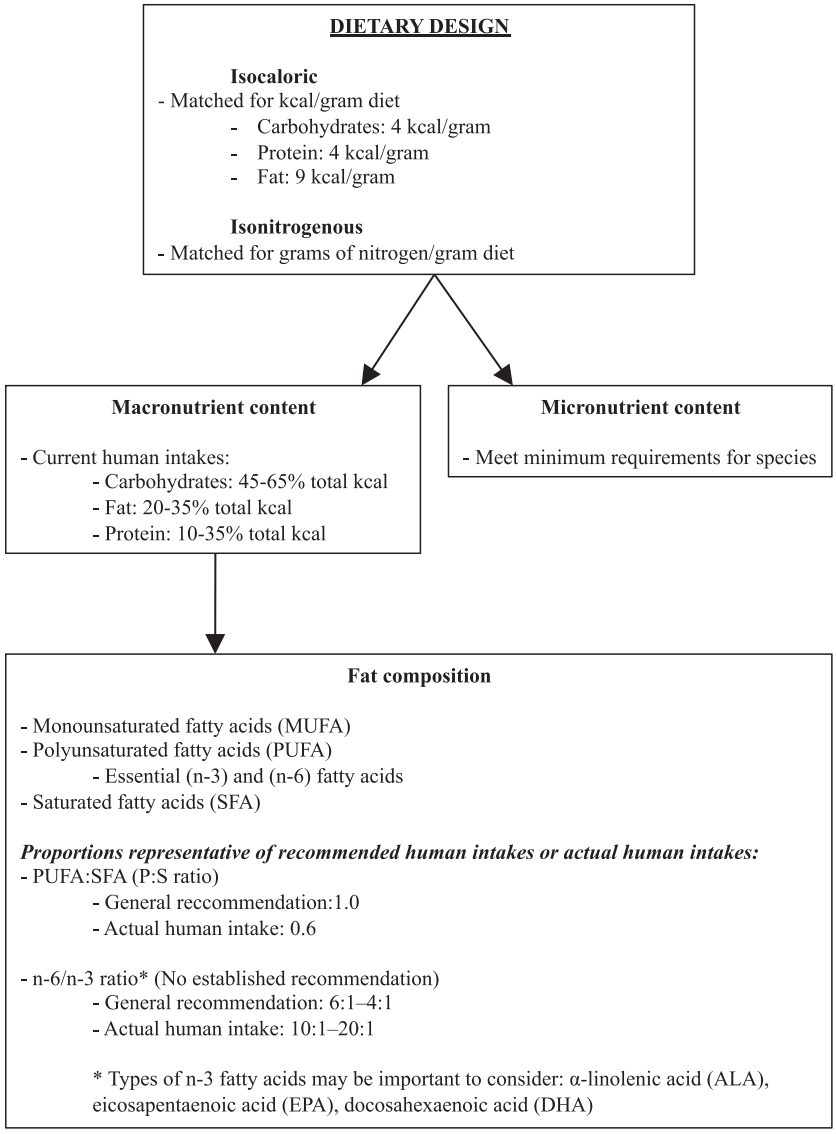


Table 1 Preclinical studies with a nutrient intervention

Reference [#]	Animal + tumour model	Nutrient intervention	Isonitrogenous/ isocaloric	Diet control	Diet composition
			Fish Oil, EPA ± DHA interventions		
Costelli *et al.*, 1995 [37] Lack of effect of EPA in preventing cancer cachexia and inhibiting tumour growth	Male Wistar rats bearing Yoshida AH-130 ascites hepatoma	EPA as fatty acid methyl esters	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: standard laboratory chow Source/brand: Panlab (Barcelona, Spain) (1) Control diet: standard laboratory chow (2) Treatment diet: daily intragastric dose of EPA (1.5 g/kgBW); - Complete diet compositions not defined
Dagnelie *et al.*, 1994 [24] Effects of fish oil on cancer cachexia and host liver metabolism in rats with prostate tumours	Copenhagen–Fisher F1 hybrid rats inoculated with MATLy-Lu prostate tumour cells	EPA	Isocaloric and isonitrogenous	*Ad libitum*	Background diet: standard laboratory chow (control diet); semi-purified fish oil (FO) diet Source/brand: Rat and mouse breeding diet, Pilsbury, Birmingham, United Kingdom (control diet); source undefined (FO diet) (1) Control diet: standard laboratory chow containing 50% total kcal carbohydrates, 20% total kcal protein, 11.5% total kcal fat (2) FO diet: semi-purified diet, undefined, containing 50% total kcal coming from fish oil, which substituted for carbohydrates - Complete diet compositions not defined
Dumas *et al.*, 2010 [38] N-3 PUFA-enriched diet delays the occurrence of cancer cachexia in rat with peritoneal carcinosis	Male Berlin Druckery IX (BDIX) rats bearing DHD/K12 cells from a dimethyl-hydrazine induced colon tumour	Fish oil (FO) EPA/DHA	Isocaloric and isonitrogenous Representative of human intakes	*Ad libitum*	Background diet: semi-purified diet Source/brand: undefined (1) Control diet (g/kg): casein (220), sucrose (187), cellulose (20), DL-methionine (1.6), mineral mixture (40), vitamin mixture (no vitamin E; 10), cornstarch (371.4), lipids (150): 12% peanut oil, 3% rapeseed oil (2) FO diet (g/kg): casein (220), sucrose (187), cellulose (20), DL-methionine (1.6), mineral mixture (40), vitamin mixture (no vitamin E; 10), cornstarch (371.4), lipids (150): 8% peanut oil, 2% rapeseed oil, 5% fish oil (3) Pair-fed group: control diet - Complete diet composition defined - Both diets (1) and (2) contain 4460 kcal/kg diet - Nature of lipids changed in both diets - Fat accounts for ~28% total kcal diets (1) and (2)
Fini *et al.*, 2010 [17] Highly purified EPA as free fatty acids strongly suppresses Polyps in $Apc^{Min/+}$ mice	Male $Apc^{Min/+}$ mice on a C57BL/6J background and corresponding wild-type (wt) mice	99% pure preparation of EPA as n-3 free fatty acids	Isocaloric and isonitrogenous	Controlled	Background diet: semi-purified Source/brand: AIN-93G diet: 18.8% total kcal protein, 16.4% total kcal fat, 65.1% total kcal carbohydrates (Research Diets) (1) Control diet: modified AIN-93G diet, corn oil substituted for soybean oil (2) EPA diet: modified AIN-93G diet, soybean oil substituted for EPA free fatty acids at 2.5% weight/weight (*w/w*) (3) EPA diet: modified AIN-93G diet, soybean oil substituted for EPA free fatty acids at 5% *w/w* - Complete diet compositions defined - 99% EPA-FFA in fish oil diets - Soybean oil substituted for EPA in varying amounts (*w/w*) in diets (2) and (3)
Griffini *et al.*, 1998 [65] Dietary omega-3 PUFAs promote colon carcinoma metastasis in rat liver	Male and female Wag-rij rats bearing CC531 colon carcinoma cells	Fish oil (FO) EPA/DHA	Not isocaloric or isonitrogenous	*Ab libitum*	Background diet: semi-purified Source/brand: Hope Farms (Woerden, The Netherlands); standard diets contain 41% total kcal carbohydrates, 38% total kcal fat, 20% total kcal protein

(Continues)

Table 1 (Continued)

Reference [#]	Animal + tumour model	Nutrient intervention	Isonitrogenous/ isocaloric	Diet control	Diet composition
					(1) Low fat diet: 69% total kcal carbohydrates, 20% total kcal protein; 11% total kcal fat (2) n-6 PUFA diet (safflower oil): 41% total kcal carbohydrates, 38% total kcal fat, 20% total kcal protein (3) n-3 PUFA diet (fish oil): 41% total kcal carbohydrates, 38% total kcal fat, 20% total kcal protein - Complete dietary composition defined - Fat source in n-6 PUFA diet is safflower oil; fat source in n-3 PUFA diet is fish oil
Jho *et al.*, 2002 [18] Eicosapentaenoic acid supplementation reduces tumour volume and attenuates cachexia in a rat model of progressive non-metastasizing malignancy	Fischer 344 rats bearing the methylchol-anthrene-induced fibrosarcoma (MCA) tumour	EPA	Not isocaloric or isonitrogenous	Controlled	Background diet: undefined Source/brand: undefined (1) Control diet: undefined, oral gavage isovolemic 5.0 g/kg per day of corn oil (isocaloric) combined with 10 IU vitamin E/g fat, or isovolemic but nonisocaloric normal saline (NS) combined with 10 IU vitamin E/g NS (2) EPA diet: undefined, oral gavage twice daily, providing 5.0 g/kg per day of EPA with 10 IU vitamin E/g fat - Complete dietary compositions undefined
Mannini *et al.*, 2009 [26] Dietary n-3 polyunsaturated fatty acids enhance metastatic dissemination of murine T lymphoma cells	C57B1/6 mice bearing (i) highly metastatic S11 cell line or (ii) low-metastatic 164T2 lymphoma cell line	Fish oil (FO) EPA/DHA	Isonitrogenous and isocaloric	*Ab libitum*	Background diet: semi-purified Source/brand: undefined (1) Maize oil (MO) diet: 70% sucrose, 16% casein, 3% alpha-cellulose, 1% choline chloride, 1% vitamins, 4% salt mixture, 5% maize oil (2) FO diet: 70% sucrose, 16% casein, 3% alpha-cellulose, 1% choline chloride, 1% vitamins, 4% salt mixture, 5% fish oil - Complete dietary composition defined - Both diets contain: 72% total kcal carbohydrates, 16% total kcal protein, 12% total kcal fat
Pizato *et al.*, 2005 [39] Ratio of n-6 to n-3 fatty acids in the diet affects tumour growth and cachexia in Walker 256 tumour-bearing rats	Male Wistar rats bearing the Walker 256 tumour	Fish oil (FO)	Isonitrogenous, not isocaloric	*Ab libitum*	Background diet: standard laboratory chow (control group), semi-purified diet (treatment diets) Source/brand: Nuvital CR-1, Curitiba, Brazil (control diet); undefined semi-purified source (1) Control group: standard chow diet, 62% total kcal carbohydrates, 29% total kcal protein, 9% total kcal fat
Pizato *et al.*, 2005 [39] Ratio of n-6 to n-3 fatty acids in the diet affects tumour growth and cachexia in Walker 256 tumour-bearing rats	Male Wistar rats bearing the Walker 256 tumour	Fish oil (FO)	Isonitrogenous, not isocaloric	*Ab libitum*	Background diet: standard laboratory chow (control group), semi-purified diet (treatment diets) Source/brand: Nuvital CR-1, Curitiba, Brazil (control diet); undefined semi-purified source (1) Control group: standard chow diet, 62% total kcal carbohydrates, 29% total kcal protein, 9% total kcal fat (2) High-fat diet: modified standard laboratory chow, 49% total kcal fat, 29% total kcal carbohydrates, 22% total kcal protein; 33% of fat substituted as coconut oil (3) N-3 diet high-fat FO diet: same background as (2) High-fat diet, but 66% of fat as substituted as FO (4) N-6 diet high fat sunflower oil diet: same background as (2) High-fat diet, but 66% fat substituted as sunflower oil

(Continues)

Table 1 (Continued)

Reference [#]	Animal + tumour model	Nutrient intervention	Isonitrogenous/ isocaloric	Diet control	Diet composition
					(5) 6:1 diet: 49% total kcal fat, 29% total kcal carbohydrates, 22% total kcal protein; 33% of fat substituted as coconut oil and 66% as blend of FO and sunflower oil to yield n-6 to n-3 PUFA ratios of approximately 6:1 (6) 30:1 diet: same background as [6:1 diet]; 66% of fat substituted as a blend of FO and sunflower oil to yield n-6 to n-3 PUFA ratios of approximately 30:1 (7) 60:1 diet: same background as [6:1 diet]; 66% of fat substituted as a blend of FO and sunflower oil to yield n-6 to n-3 PUFA ratios of approximately 60:1 - Complete dietary composition defined - Oils blended to maintain linoleic acid content consistency; n-6 to n-3 PUFA ratio was changed by altering n-3 PUFA content - All diets contained the same amounts of protein (230 g/kg), fibre (60 g/kg), and vitamins and minerals (10 g/kg)
Pizato *et al.*, 2006 [40]Fish oil alter T-lymphocyte proliferation and macrophage responses in Walker 256 tumour-bearing rats	Male Wistar rats bearing the Walker 256 tumour	Fish oil (FO)	Isonitrogenous, not isocaloric	*Ab libitum*	Same diet as Pizato *et al.*, 2005; See reference #9.
		Fish oil, EPA/DHA in combination with other nutrients			
Hudson *et al.*, 1994 [41] Comparison of the effectiveness of eicosapentaenoic acid administered as either the free acid or ethyl ester as an anticachectic and antitumour agent	NMRI mice bearing the MAC16 colon adenocarcinoma	EPA as free fatty acids or ethyl ester Medium chain triglycerides (MCT)	Not isocaloric or isonitrogenous	*Ab libitum*	Background diet: standard laboratory chow Source/brand: rat and mouse breeding diet, Pilsbury, Birmingham, United Kingdom (1) Control diet: standard laboratory chow, 11.5% total kcal as fat (2) EPA diet: modified standard laboratory chow with 80% as EPA ethyl esters (3) MCT diet: modified standard laboratory chow with 80% as MCT - Complete dietary compositions undefined - Unidentified route of oral administration of EPA as free fatty acid form or ethyl ester form - Complete dietary compositions undefined - Unidentified route of oral administration of EPA as free fatty acid form or ethyl ester form
Tisdale *et al.*, 1990 [25] Inhibition of weight loss by n-3 fatty acids in an experimental cachexia model	NMRI mice bearing the MAC16 tumour	Fish oil (FO) Medium chain triglycerides (MCT)	Isocaloric and isonitrogenous	*Ab libitum*	Background diet: standard laboratory chow (control diet); semi-purified fish oil (FO) diet Source/brand: rat and mouse breeding diet, Pilsbury, Birmingham, United Kingdom (control diet); source undefined (FO and MCT diets) (1) Standard breeding diet for all groups; 50% total kcal carbohydrates, 20% total kcal protein, 11.5% total kcal fat Total kcal from fat: (2) 5% FO (3) 10% FO (4) 25% FO (5) 25% FO + 55% MCT (6) 50% FO

(Continues)

Table 1 (Continued)

Reference [#]	Animal + tumour model	Nutrient intervention	Isonitrogenous/ isocaloric	Diet control	Diet composition
					(7) 50% FO + 30% MCT - Complete dietary composition defined - Isocaloric and isonitrogenous diets were prepared by decreasing the carbohydrate content and supplying the remaining energy from either FO or MCT
Bonatto *et al.*, 2006 [42] Lifelong exposure to dietary fish oil alters macrophage responses in Walker 256 tumor-bearing rats	Male wister rats, bearing Walker-256 tumour	Fish oil (FO) Coconut oil (CO)	Not isocaloric or isonitrogenous	*Ab libitum*	Background diet: standard laboratory chow Source/brand: undefined (1) Control diet: Standard laboratory chow (2) CO Diet: Standard laboratory chow + oral micropipette bolus dose of coconut oil (1 g/kgBW) (3) FO Diet: Standard laboratory chow + oral micropipette bolus dose of fish oil (1 g/kgBW) - Complete dietary composition not defined - Fish oil is mixed marine triacylglycerol preparation containing 180 g EPA, 120 g DHA per kg
Busquets *et al.*, 2007 [43] Resveratrol does not ameliorate muscle wasting in different types of cancer cachexia models	Male Wistar rats bearing Yoshida AH-130 ascites hepatoma or Lewis Lung C57Bl/6 mice bearing Yoshida AH-130 ascites hepatoma or Lewis Lung	Resveratrol Fish oil (FO)	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: undefined Source/brand: undefined *Yoshida AH-130 ascites hepatoma diets*: (1) Resveratrol-treated: Daily i.p. dose of resveratrol (1 mg/kgBW) (2) Untreated: saline i.p. dose (1 mg/kgBW) (3) Combination treatment, FO + resveratrol: intragastric injection of 1 mL FO + 3 mg/kgBW resveratrol dissolved in FO (4) Control treatment: intragastric injection of 1 mL olive oil *Lewis lung carcinoma diets*: (1) Resveratrol-treated: i.p. dose of resveratrol (5 or 25 mg/kg BW) (2) Untreated: i.p. dose of saline (5 or 25 mg/kg BW)
Cremades *et al.*, 2007 [44] Nutritional treatment of cancer cachexia in rats	Male Wistar rats bearing Yoshida AH-130 ascites hepatoma	Crayfish enzymatic extract (CFEE) containing n-3 fatty acid	Isocaloric and isonitrogenous	*Ad libitum*	Background diet: semi-purified Source/brand: AIN-76A diet (Research Diets) (1) Standard diet: consisting of 63.5% carbohydrate (corn starch, sucrose), 12% protein (casein) and 5% fat (olive oil); the difference to 100% comprised of minerals, vitamins and non-digestible material (2) CFEE diet: consisting of 63.5% carbohydrate (corn starch, sucrose), 12% protein (crayfish enzymatic extract) and 5% fat (olive oil); the difference to 100% comprised of minerals, vitamins and non-digestible material - Complete dietary composition defined - Both diets contain: 79% total kcal carbohydrates, 15% total kcal protein, 6% total kcal fat - CFEE contains high-quality proteins, essential amino acids, n-3 fatty acids, and carotenoids
Faber *et al.*, 2008 [34] Beneficial immune modulatory effects of a specific nutritional combination in a murine model for cancer cachexia	Male CD2F1 (BALB/c × DBA/2) mice bearing C26 adenocarcinoma	Fish oil (FO) Specific oligo- saccharide mixture (SOM) High protein leucine formulation (HPLeu)	*Experiment A*: Not isonitrogenous or isocaloric *Experiment B*: not isonitrogenous or isocaloric Representative of human intakes	*Ad libitum*	*Experiments A*: Background diet: semi-purified diet Source/brand: AIN93-M diet (Research Diets) (1) Control diet (g/kg): 126 g protein (100% casein), 727 g carbohydrates and 40 g fat (100% soy oil) (2) FO diet: control diet + 22.1 g fish oil (6.9 g EPA and 3.1 g DHA) per kg food

(Continues)

Table 1 (Continued)

Reference [#]	Animal + tumour model	Nutrient intervention	Isonitrogenous/ isocaloric	Diet control	Diet composition
					(3) SOM diet: control diet + 18 g short-chain galacto-oligosaccharides and 2 g short-chain fructo-oligosaccharides per kg food (4) HPLeu: control diet + 151 g casein and 16 g leucine per kg food *Experiments B*: Background diet: semi-purified diet (1) Control diet (g/kg): 126 g protein (100% casein), 699 g carbohydrates and 52.6 g fat (100% corn oil) (2) Nutritional mixture diet: 210 g protein (189 g intact protein of which 68% casein, 32% whey, 21 g free leucine), 561 g carbohydrates, 52.5 g fat (20.2 g corn oil, 10.2 g canola oil and 22.1 g fish oil (providing 6.9 g EPA and 3.1 g DHA)), 18 g short-chain galacto-oligosaccharides and 2 g short-chain fructo-oligosaccharides - Complete dietary compositions defined - Category B experiment control diet was a more humanized diet - Both control diets in Experiments A and B were isonitrogenous and isocaloric (Exp. A: 3773 kcal/kg diet; Exp. B: 3772 kcal/kg diet) with varying proportions of macronutrients: •Experiment A: 74% total kcal carbohydrates, 13% total kcal protein, 13% total kcal fat •Experiment B: 77% total kcal carbohydrates, 13% total kcal protein, 10% total kcal fat
Van Norren *et al.*, 2009 [36] Dietary supplementation with a specific combination of high protein, leucine, and fish oil improves muscle function and daily activity in tumour-bearing cachectic mice	Male CD2F1 (BALB/c × DBA/2) mice bearing C26 adenocarcinoma	Fish oil (FO) Specific oligo-saccharide mixture (SOM) High protein leucine formulation (HPLeu)	*Experiment A*: Not isonitrogenous or isocaloric *Experiment B*: not isonitrogenous or isocaloric Representative of human intakes	*Ad libitum*	Same diet as Faber *et al.*, 2008; See reference #15.
Togni *et al.*, 2003 [45] Cancer cachexia and tumour growth reduction in Walker 256 tumour-bearing rats supplemented with n-3 polyunsaturated fatty acids for one generation	Female Wistar rats bearing the Walker 256 tumour	Fish oil (FO) Coconut oil (CO)	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: standard laboratory chow Source/brand: undefined (1) Control diet: standard laboratory chow (2) FO treatment: standard laboratory chow, supplemented orally with fish oil (3) CO treatment: standard laboratory chow, supplemented orally with coconut oil - Complete dietary compositions not defined - Oils were supplemented at a level of 1 g/kg body weight per day and were provided as a single bolus using a pipette
			Other nutrient interventions		
Beck *et al.*, 1989 [46] Nitrogen excretion in cancer cachexia and its modification by a high fat diet in mice	NMRI mice bearing MAC16 tumour	Medium-chain triglycerides (MCT)	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: standard laboratory chow Source/brand: rat and mouse breeding diet, Pilsbury, Birmingham, United Kingdom (1) Control diet: standard laboratory chow; 11.5% total kcal as fat (2) MCT diet: modified standard laboratory chow with 80% total kcal from MCT (3) Standard diet, water supplemented with sodium D-(−)-3-hydroxybutyrate at 30 μmol/mL - Complete dietary compositions not defined

(Continues)

Table 1 (Continued)

Reference [#]	Animal + tumour model	Nutrient intervention	Isonitrogenous/ isocaloric	Diet control	Diet composition
Tisdale *et al.*, 1987 [47] Reduction of weight loss and tumour size in a cachexia model by a high fat diet	Male NMRI mice bearing MAC16 tumour	Medium-chain triglycerides (MCT)	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: standard laboratory chow (control diet); semi-purified fish oil (FO) diet Source/brand: rat and mouse breeding diet, Pilsbury, Birmingham, United Kingdom (control diet); source undefined (MCT diet) (1) Control diet: standard laboratory chow; 50% total kcal carbohydrates, 20% total kcal protein, 11.5% total kcal fat (2) High-fat diet: authors indicated there was a reduced carbohydrate proportion, and 80% total kcal from medium-chain triglyceride - Complete dietary compositions not defined
Graves *et al.*, 2005 [6] Conjugated linoleic acid preserves gastrocnemius muscle mass in mice bearing the colon-26 adenocarcinoma	Female CD2F1 mice bearing C26 adenocarcinoma	Conjugated linoleic acid (CLA)	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: standard laboratory chow Source/brand: undefined (1) Control diet: pulverized rodent chow (2) CLA diet: pulverized rodent chow supplemented with 0.5% CLA - Complete dietary compositions not defined
Gomes-Marcondes *et al.*, 2002 [35] A leucine-supplemented diet improved protein content of skeletal muscle in young tumour-bearing rats	Male Wistar rats bearing Walker 256 carcinoma	Leucine	Isocaloric	*Ad libitum*	Background diet: semi-purified Source/brand: AIN-93G (Research Diets) (1) Control diet: modified AIN-93G diet, containing 15% protein (2) Leucine diet: modified AIN-93G diet, containing 15% protein supplemented with 3% leucine - Complete dietary compositions not defined since AIN-93G standard diet was modified for protein
Iizuka *et al.*, 2002 [48] Anticachectic effects of the natural herb *Coptidis rhizoma* and berberine on mice bearing colon 26/clone-20 adenocarcinoma	Male BALB/c mice bearing C26 adenocarcinoma	*Coptidis rhizoma* (CR) herb + berberine	Isonitrogenous and isocaloric	*Ad libitum*	Background diet: standard laboratory chow Source/brand: F1 breeding diet (Funabashi Farm, Funabashi, Japan) (1) Control diet: standard laboratory chow, Funabashi Farms containing 21.3% protein, 57.1% carbohydrates, 5.6% fat, 3.3% fibre, 5.7% ash, and 7.0% moisture (2) Treatment: standard laboratory chow (same as control diet); *C. rhizoma* was incorporated into breeding diet at a final concentration of 1% (10 mg/g) or 2% (20 mg/g); berberine was added at a final concentration of 0.1% (1 mg/g) to 0.4% (4 mg/g) at Funabashi Farms (3) Pair-fed group: standard laboratory chow - Complete dietary composition defined - Both diets (1) and (2) contained 63% total kcal carbohydrates, 23% total kcal protein, 14% total kcal fat
Carbo *et al.*, 1999 [49] Resveratrol, a natural product present in wine, increases tumour growth in a rat tumour model	Male Wistar bearing Yoshida AH-130 ascites hepatoma	Resveratrol	Not isocaloric or isonitrogenous	*Ad libitum*	Background diet: standard laboratory show Source/brand: Panlab (Barcelona, Spain) (1) Control diet: standard laboratory chow, 71% total kcal carbohydrates, 22% total kcal protein, 7% total kcal fat (2) Resveratrol treatment: standard laboratory chow + i.p. injection of 1 mg/kgBW resveratrol per day - Complete dietary composition not defined

triglycerides [46,47] and eicosapentaenoic acid (EPA; *Table* 1).[41] Mice were fed a rat/mouse breeding diet *ad libitum* for 2 weeks before a dietary intervention began. Mice were randomized into diet groups containing 11.5% total energy from fat (source and composition unspecified by Beck and Tisdale, 1989 [46] and Hudson and Tisdale, 1994; [41] palm oil used in control diet in by Tisdale, Brennan and Fearon, 1987 [47]). All three studies modified the control diet to consist of 80% of total energy from medium-chain triglycerides, and Hudson *et al*. [41] included a second treatment diet group, which consisted of 80% total energy from EPA ethyl esters. The proportions of fat in the diets within the same study are not similar to each other, nor are they congruent with human intakes. In another study, male Wistar rats bearing the Yoshida AH-130 ascites hepatoma, animals were randomized to receive resveratrol injections, or saline injections while consuming standard laboratory chow.[49] The diet consisted of 71% total kcal carbohydrates, 22% total kcal protein, and 7% total kcal fat. In contrast to the very high contribution of fat to the diets in Beck *et al*.,[46] Hudson *et al*.,[41] and Tisdale *et al*.,[47] the macronutrient proportion of this diet compared to a typical human diet would be considered low in fat. Thus, results are difficult to extrapolate to humans, since the macronutrient imbalance results in either a high fat/low carbohydrate diets or low fat/high carbohydrate diets. These examples represent the majority of typical animal dietary designs in the preclinical cachexia literature, where the first criteria of matching kilocalories between diets are not met.

Important considerations related to fat quantity and composition in dietary models

The following discussion will aim to provide a framework focused on fat content and composition of experimental diets. The principles and underlying considerations applied to fat as a macronutrient can also be applied to protein and carbohydrate quantity and composition.

Fat content and composition are important to consider, especially in studies focused on 'tumour × host × nutrient' interactions. As a recommendation, it is important for fats included in the diet to first meet the nutrient requirements for the species being used, and second, for the composition and types of fat in the diet to be matched between diet treatments, and finally to represent what is commonly consumed by humans (*Figure* 2). Most laboratory chow diets use corn oil as the only source of fat,[50] while fat blends that include more than one type of oil or fat source are used only in semi-purified or nutritionally made diets.[21] A corn oil-based diet will result in a higher proportion of n-6 fatty acids and n-6/n-3 fatty acid ratio than other oil types. In Wistar rats bearing the Walker 256 tumour, rats were fed high-fat diets (49% total kcals), low-fat diets (9% total kcals), or diets with fish oil and sunflower oil with varied ratios of n-6/n-3, 6:1; 30:1; and 60:1 (*Table* 1).[39,40] Pizato *et al*. [39,40] aimed to demonstrate that a lower n-6/n-3 ratio (6:1) was more effective in preventing cachexia compared to higher n-6/n-3 ratio diets; however the diets were not matched for calories. Higher ratios of n-6/n-3 fatty acids have been associated with greater inflammation and enhanced tumour growth,[51,52] emphasizing the importance of considering the n-6/n-3 ratio in dietary design. In addition to metabolic differences between n-6 and n-3 fatty acids, the length of fatty acid chains also has an impact on metabolism and energy expenditure. Compared to long chain fatty acids, medium chain fatty acids are absorbed more efficiently and have been associated with weight loss or reduced weight gain in long-term studies. Thus, fatty acid chain length is an additional factor to consider in dietary design given the differences in metabolism between long and medium chain fatty acids.[53] A number of studies to date have reported that promotion of mammary tumours in rodents fed a high-fat diet, compared with those fed a low-fat diet, may be due to specific metabolic activities of the type of fatty acids in the diet, independent of calories.[54] These findings reiterate the importance of designing macronutrient intakes to be representative of the research question at hand, and to further take into account the composition of the macronutrient class in addition to matching diets for kilocalories.

When adding or replacing fat in diets it is also important to note that this frequently alters other aspects of diet composition. The majority of studies that provide high-fat diets used sources of fat, with or without another nutrient, added to a background diet of standard laboratory chow.[37,43] These results should be interpreted cautiously since the capacity for chow diets to be modified is limited, and adding nutrients into the diet may reduce concentrations of the other nutrients.[23] This can result in a diet that is deficient for example, in protein per gram of chow. When animals are fed *ad libitum*, they will consume to a specific number of calories per day, therefore a high-fat diet, which is more calorie dense per gram of diet, also affects protein intake. The addition of nutrients to standard chow also makes it difficult to compare to the original lab chow. The control group may be receiving adequate proportions of macro- and micronutrients, whereas the experimental group may be deficient in certain nutrients.

EPA and DHA nutrient interventions

Although there is an abundance of evidence that suggests the fatty acid profile is of importance for the efficacy of EPA and DHA *in vivo*, most preclinical models have not considered the fatty acid profile of the background diet when designing nutrient intervention diets. The effectiveness of long-chain fatty acids EPA and DHA on enhancing drug antitumour activity has been demonstrated in several rodent models of cancer.[55] Interestingly, a comparison of studies has shown that the effectiveness of EPA and DHA for antineoplastic activities depends on the background diet, with effects

greatest in studies where control diets contain fat sources of palm or corn oil, and subsequently low levels of total polyunsaturated fatty acids.[56,57] Control diets with fat sources from safflower or sunflower oil, and therefore higher levels of total PUFA, do not give the same results.[21,55,58] N-3 fatty acids may exert different biological effects depending on the background diet fat composition, n-6/n-3 ratio, and polyunsaturated/saturated fat ratio.[39,59,60] Collectively, studies suggest that amount of total PUFA in the diet may be an important factor in determining efficacy of EPA and DHA on cancer-related outcomes. These findings emphasize the importance of a well-developed background diet in studies of nutrient interventions, particularly those with EPA and DHA. It is difficult to compare studies and interpret findings when the majority of studies fail to provide appropriate background diets.

Dietary designs to represent macronutrient content of human diets

Many dietary designs do not reflect macronutrient proportions typical of human consumption. While it is appreciated the high degree of variability in dietary consumption patterns of cancer patients over the disease trajectory,[61] it is still possible and probably advisable to use diets which are at least approximately in line with typical human diet macronutrient composition to facilitate interpretation between studies as well as translation to human populations. Cremades *et al*. [44] aimed to investigate whether a diet formulated with a crayfish enzymatic extract enriched in essential amino acids and n-3 fatty acids would be effective for the treatment of cancer-associated cachexia by decreasing mortality, morbidity and lengthened survival. A semi-purified diet using the AIN-76A Vitamin and Mineral mixture was developed with a macronutrient distribution of: 79% total kcal carbohydrates, 15% total kcal protein, and 6% total kcal fat. The source of fat in this diet was solely from olive oil, being high in n-9 fatty acids, and relatively low in n-6 and saturated fat, with minimal n-3 fatty acids. Although the fatty acid profile was not given much consideration, Cremades *et al*. [44] carefully considered the nitrogen balance of the diet. Since crayfish enzymatic extract is mainly protein and contains all essential amino acids, the casein from the standard diet was removed and replaced by the enzyme for the crayfish diet to achieve isonitrogenous diets. While the dietary design from this study includes a source of high quality protein, it would be considered low in fat compared to typical human intakes that fall in the range of 20–35% total kcal from fat.[62] Moreover, a low fat diet is not ideal in an experiment with weight gain or maintenance as a study endpoint.

Nutrients provided via gavage

The majority of nutrient intervention studies administer the intervention via the diet, either by incorporation into semi-purified diets, or the addition of a nutrient to a standard chow diet. However, some studies administer a nutrient gavage, where oral pipettes are used to deliver an exact amount of the nutrient to the animal ensuring that the nutrient is ingested regardless of food intake. The dietary design upon which the gavage is administered is still a key consideration.

Wistar rats inoculated with Walker 256 tumour cells were randomized to receive a standard laboratory chow diet, or treatment with coconut oil or fish oil (*Table* 1).[45] An undefined source and amount of standard laboratory chow was fed to all groups; rats were given bolus doses of either coconut oil or fish oil at 1 g/kgBW/day. Since the route of administration was gavage, the addition of fat kilocalories on top of the background diet of standard laboratory would result in diets being dissimilar between control and treatment groups. Feeding coconut oil (>90% saturated fat) on top of laboratory chow (rich in n-6 fatty acids) does not achieve a dietary intake resembling a human diet, which consists of approximately 10% saturated fat. Moreover, the authors do not provide any information about the background diet, proportion of macronutrients, kcals consumed, or food intake, so it is not possible to determine the amount of fat consumed in total or the intake of other macronutrients in respect to the fat, such as the n-6/n-3 ratio. The fatty acid composition of the background diet is particularly important when the nutrient of interest is fat.

Costelli *et al*. [37] fed male Wistar rats bearing the Yoshida ascites hepatoma (YAH-130) standard laboratory chow (Panlab, Barcelona, Spain) and similar to Togni *et al*. [45] treatment animals received intragastric injections of EPA (1.5 g/kgBW/day). The composition of the background diet of standard laboratory chow was not defined, and food intake was not reported. While EPA was administered in a consistent dose, food intake may have been reduced during the study period, which would change other source of nutrients during the study period that are known to interfere with EPA effectiveness such as the ratio of n-3/n-6 fatty acids. Several other studies have administered EPA/DHA interventions intragastrically [43] (*Table* 1), or by bolus micropipette dose,[45,42] with only one of these studies administering the background diet in a controlled daily amount and reporting food intake [18] (*Table* 1). In addition to the amount of n-6 fatty acids, research also suggests that the absorption of long chain n-3 fatty acids may be improved through the meat, fish, and poultry factor or when consumed with monounsaturated fatty acids.[63–65] Chow diets are high in n-6 fatty acids, and unbalanced for n-6/n-3 ratio which may alter the absorption of EPA and/or DHA. It is difficult to know, however, to what extent food intake is a factor when it is not reported.

Overall fat content and n-6/n-3 ratio in dietary design

Evidence suggests that the consumption of n-3 fatty acids may exert antineoplastic effects through the incorporation of n-3 fatty acids into membranes, replacing the n-6 fatty acid, arachidonic acid. A higher intake of n-3 fatty acids, therefore, may reduce pro-inflammatory mediators.[66] On

the basis of this hypothesis, Griffini *et al*. [67] fed Wag-rij rats bearing CC531 colon carcinoma cells (i) low fat diet (11% total kcal fat), (ii) n-6 PUFA diet (38% total kcal from fat as safflower oil), or (iii) n-3 PUFA diet (38% total kcal from fat as fish oil). The sole source of fat in the n-6 and n-3 fatty acid treatment diets was safflower oil and fish oil respectively. When evaluating this diet, one must first consider that n-6 and n-3 fatty acids are dietary essential nutrients. When providing fat sources with minimal amounts of either n-6 or n-3 fatty acids, there is a risk of essential fatty acid deficiency. Results could be attributed to the deficiency, since the effects of n-3 fatty acids are being interpreted against a diet containing only n-6 fatty acids as the PUFA source, and additionally, results may be exaggerated when comparing treatment and control groups.[50,56] Further, diets are not matched for calories, and food intake was not reported.

In three similar studies, Tisdale *et al*. [25,47] investigated the effects of feeding a fish oil or high fat diet on cachexia and tumour growth in female NMRI mice bearing the MAC16 tumour, and Dagnelie *et al*. [24] investigated the effects of feeding fish oil on cancer cachexia and host liver metabolism in Fisher F1 hybrid rats injected with MAT-Ly-Lu prostate tumour cells. All three studies used the same dietary design. Animals were fed a standard diet of laboratory chow, containing 50% total kcal from carbohydrates, 20% total kcal from protein, and 11.5% total kcal from fat (Pilsbury's, Birmingham, United Kingdom), or semi-purified treatment diets with varying amounts of medium-chain triglycerides or fish oil supplemented up to 80% total kcal supplied as medium-chain triglycerides or 50% total kcal supplied as fish oil (*Table* 1). The authors indicate that the treatment diets were developed to be isonitrogenous and isocaloric by reducing the carbohydrate content and supplementing the remaining energy from the fish oil or medium-chain triglycerides. However, this is not possible given the macronutrient composition as fat provides 9 kcal/g, whereas carbohydrates provide 4 kcal/g. Similar to Cremades *et al*.,[44] the standard diet in both studies conducted by Tisdale *et al*. [25,47] and Dagnelie *et al*. [24] was low in fat, which is not ideal when the study endpoints involve weight loss or gain. Moreover, the proportion of fat in the high fat and fish-oil diets aforementioned, 68–80% medium chain triglycerides and 50% fish oil respectively, are not proportions that are applicable to human dietary intakes where very few medium-chain triglycerides or fish oil lipids are consumed in the daily diet. Similar to Griffini *et al*. [67] feeding fish oil as the sole source of fat eliminates n-6 fatty acids from the diet and places animals at a risk for essential fatty acid deficiency. Both studies fed a high dose of fish oil, accounting for 50% of total energy in the absence of n-6 fatty acids. Rats had free access to food throughout the study period. Dagnelie *et al*. [24] did report that the fish oil group had significantly lower food intake compared to control animals, however, this could be related to caloric density of the diets.

One additional consideration for dietary intervention studies where fish oils or other lipids are used is the susceptibility of the prepared diets with the oils to peroxidation. Peroxidation can change the flavour of the food, making it less palatable, and can also result in a loss of the bioactive lipid being studied. Therefore, it is recommended that diets be prepared fresh, especially when using nutrients that are susceptible to oxidation and a quality control measure is implemented at various points throughout the study (i.e. diet composition analysis at the beginning, middle and end of the study) to ensure the diet components do not change over the course of the study, and/or contribute to changes in food intake.

Dietary design: case study

To illustrate a dietary design that meets the criteria outlined in *Figure* 2, the study conducted by Dumas *et al*. [38] will be outlined. Dumas *et al*. [38] developed isocaloric and isonitrogenous semi-purified diets and included a pair-fed group to discriminate the effects of food intake reduction per se from those of the tumour or cachexia. The review of the literature revealed that there was only one other fat supplementation study that included a pair-fed group.[48] Dumas *et al*. [38] aimed to prevent severe cachexia by limiting a decline in food intake, weight loss, and by reducing the inflammatory marker, TNF-alpha in Berlin–Druckrey IX rats inoculated with DHD/K12 colon tumour cells. Animals were randomized to the semi-purified control diet or fish oil supplemented diet with free access (*Table* 1). Diets were matched for calories and nitrogen content, differing only in fatty acid composition. Dietary composition was defined in g/kg diet: casein (220 g), sucrose (187 g), cellulose (20 g), DL-methionine (1.6 g), mineral mixture (40 g), vitamin mixture (without vitamin E; 10 g), corn starch (371.4 g), and lipids (150 g). A variety of lipid sources were used to match diets for fat amount and composition. The lipid mixture in the control diet was composed of 12% peanut oil and 3% canola oil; lipid mixture in the fish oil diet was 8% peanut oil, 2% canola oil, and 5% fish oil. Peanut oil and canola oil are high in oleic acid (n-9 fatty acids) and linoleic acid (n-6 fatty acids), while peanut oil is also a major source of saturated fat. The main difference in composition of these diets is the EPA and DHA in the treatment diet, allowing researchers to attribute differences in the outcomes to the treatments. The lipid profile and major macronutrient proportions of the control diet are within the range of human dietary intakes, within a range of fish oil intake that would be achievable in humans, rendering these results applicable to human populations. Macronutrient proportions that are representative of typical human intakes, though rare in preclinical studies, remain advantageous in enabling translation of findings to human populations.

Conclusion

There is a lack of standardization in dietary design, content, source, and overall composition in animal studies of cancer cachexia. In many cases, information about the diet is not included. Standard lab chow may be easy and convenient to use, but the uncertainty about diet content when using a closed label diet is poorly appreciated. Knowledge that different groups of animals within the same experimental study are consuming diets divergent in nutrient content may be critical to fully interpret the effectiveness of a given intervention. Many studies using preclinical models frequently fail to report food intake, or total energy and protein content of the diet. The role of a variety of dietary and other interventions cannot be effectively evaluated without more detailed dietary disclosure and information about potential confounding effect of dietary factors. Correct interpretation of outcomes with a particular intervention is enabled when full dietary disclosure accompanies rigorous dietary designs. At minimum, diet content and composition should be reported and food intake assessed during the study. Implementation of a standard dietary design as set out in *Figure* 2 that meets minimum basic criteria of dietary design would facilitate comparison of preclinical studies and replication of studies between research groups. Standardizing dietary designs in studies that are using preclinical models to explore important questions regarding mechanisms and interventions for cancer cachexia would be an important step to moving preclinical findings to human application.

Conflict of interest

The authors declare no conflicts of interest, and that this work has not been previously published elsewhere. The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle (von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle. 2010;1:7-8).

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