SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Effect of Ghrelin on food intake and body composition in experimental rat and mice models of cancer cachexia.	
2.	Authors (names, affiliations, contributions)	1. Mahalaqua Nazli Khatib, India (MNK), DMIMS (DU), India, Drafting the protocol, Interpreting analysis, writing manuscript 2. Anuraj Shankar (AS), HSPH, The Harvard University, USA, Obtain copies of studies, Resolving discrepancies in inculsion of studies and Risk of Bias , Interpreting analysis , Supervising SR process, revising manuscript. 3. Richard K (RK), South Asian Cochrane Centre, CMC, India, Carry out the analysis 4. Padam Simkhada (PS), Liverpool John Moores University, UK, Interpret the analysis , Develop and run the search strategy, 5. Shilpa Gaidhane (SG), DMIMS (DU), India, Extract data from studies. 6. Abhay Gaidhane (AG) DMIMS (DU), India, Assessing risk of bias, Develop and run the search strategy, Select which studies to include 7. Quazi Syed Zahiruddin (SZQ), DMIMS (DU), India, Select which studies to include, Assessing risk of bias, Enter data into RevMan, Writing manuscript. 8. Judith van Luijk (JvL), SYRCLE – Radboudumc the Netherlands, Supervising SR process, revising manuscript	
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5.	Funding sources/sponsors	None	
6.	Conflicts of interest	NIL	
7.	Date and location of protocol registration	SYRCLE website	
8.	Registration number (if applicable)	Awaited	
9.	Stage of review at time of registration	Not yet started	
	B. Objectives		

	Background		
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	**Description about condition:** According to the online database of The International Agency for Research on Cancer (IARC); GLOBOCAN 2012, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012 (1). Abnormalities in energy metabolism are universal in this population and frequently lead to cachexia (2). Though underestimated and under-recognised medical corollary of cancer; it remains an significant cause of morbidity and mortality among these patients (3). The prevalence of cachexia is 60% to 80% in the later stages of cancer (4)(2). It has been estimated amongst cancer patients, more than 30% of patients die due to cachexia and more than 50% of patients with cancer die with cachexia being present (5). "Cancer cachexia was defined as a multifactorial syndrome characterised by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment"(6). The pathophysiology of cancer cachexia is typified by a negative protein and energy balance determined by a variable combination of decreased food intake and abnormal metabolism (of energy, fat and muscle)(6)(5)(7). Cachexia is characterised by wasting of muscle adipose tissue with weight loss greater than 5% or sarcopenia (skeletal muscle mass) and inflammation associated with anorexia (6)(8). **Description about intervention:** Though associated with increased mortality and poor quality of life; there are currently no effective therapies for cancer cachexia. Keeping in view the complex pathophysiology of this condition, therapies which have a potential anabolic as well as anti-inflammatory effect should be targeted to counter this condition. One such possible therapeutic agent is a gut hormone ghrelin which affects numerous key pathways in the regulation of body weight and body composition through increased appetite and growth hormone (GH) secretion(2)(9)(10)(11)(12). Several studies in rodent models and some human clinical trials have demonstrated that ghrelin promotes appetite and is therefore useful in the treatment of cancer cachexia (13). It has also been demonstrated that, ghrelin levels are elevated in patients with cancer cachexia and that it plays a significant role in controlling the mediators involved in the cachectic process (8). **Importance of the review:** Although ghrelin is sometimes used for the treatment of cancer cachexia, few studies	

		have investigated the efficacy of this intervention, and to our knowledge, no systematic review has been published specifically addressing the effectiveness of ghrelin for promoting food intake and improving body composition in cancer cachexia. This review aims to collect and combine all the pragmatic evidences and investigate the efficacy and safety of ghrelin in animal models of cancer cachexia.	
	Research question		
11.	Specify the disease/health problem of interest	Experimentally induced cancer cachexia (irrespective of the type of cancer) in animal models where cancer is induced by implantation/ inoculation of cancer cells in the body and cachexia is induced by any chemotherapeutic agent.	
12.	Specify the population/species studied	All animal models of cancer cachexia will be included irrespective of their species, gender, type, age and body weight.	
13.	Specify the intervention/exposure	Administration of Ghrelin in any forms; at any dose; at any frequency; for any duration will be considered for inclusion in the review. Following comparisons will be included and established according to intervention: • Ghrelin versus placebo • Ghrelin versus no treatment	
14.	Specify the control population	Animal studies on cancer cachexia which include a group with that is deprived of Ghrelin and are administered saline/ placebo.	
15.	Specify the outcome measures	Primary outcomes: 1. Food consumption 2. Total body weight 3. Lean body mass 4. Fat mass Secondary outcomes: 1. Plasma Ghrelin levels 2. Plasma Growth hormone levels 3. Serum IGF-1 levels	
16.	State your research question (based on items 11-15)	What is the efficacy of Ghrelin on food intake and body composition in experimental animal models of cancer cachexia ?	
	C. Methods		
	Search and study identification		
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	**√ MEDLINE via PubMed** **√EMBASE**	
18.	Define electronic search strategies (*e.g.* use the step by step search guide [1] and animal search filters [2, 3])	1. Electronic search strategies : PubMed We will search PubMed for original articles concerning the effects of ghrelin on experimental animal models of cancer cachexia. The search strategy will be composed of	

		following four search components (SC) as suggested by Leenaars et.al (14): • SC1: intervention/exposure; Ghrelin • SC2: disease of interest/health problem; Cancer Cachexia • SC3: animal/animal species/population studied; and animals 2. Electronic search strategies: EMBASE	
19.	Identify other sources for study identification	**√ Reference lists of included studies** **√Reference lists of relevant reviews**	
20.	Define search strategy for these other sources	References of primary studies and reviews will be screened for additional studies. No language restriction will be imposed. Furthermore, we will also conduct hand-searching for books, journals and conference proceedings to find additional primary studies. Manufacturers of Ghrelin preparations, experts and authors working in this field will be contacted through e-mails and will be requested to contribute additional information.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	First phase screening by title and abstract, second phase screening by full text of the eligible articles	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	First Phase: Two reviewers (ABG, SZQ) will independently screen the studies initially on the basis of title and abstract. Differences of opinion will be resolved by consulting the third reviewer (AS). Second phase: full texts of all the studies that appear to meet our selection criteria will be obtained Two reviewers (ABG, SZQ) will independently screen all full text papers for inclusion criteria. Differences amongst primary reviewers will be resolved by a third reviewer (AS).	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	**Inclusion criteria:** Studies will be included if they evaluated the effects of Ghrelin or any of its form on food intake and body composition in experimental animal model of cancer cachexia. **Exclusion criteria:** Papers will be excluded if they fulfilled one of the following criteria: 1. Duplicate publication: If a paper is published more than once. Only the original manuscript will be included. 2. Not an original research article. (e.g. letter/ editorial etc.) 3. studies with no appropriate control group 4. Ghrelin supplementation combined with other	

		(nutritional) components 5. Clinical (human) studies	
24.	Type of animals/population (*e.g.* age, gender, disease model)	**Inclusion criteria:** All animal -studies on models of cancer cachexia will be included irrespective of species, strain, gender, age and body weight. Any type of cancer model (any induction method) will be acceptable for inclusion in review. **Exclusion criteria:** Human studies cancer cachexia will be excluded from the review.	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Any form, dose, duration, frequency and route of administration of Ghrelin will be acceptable for inclusion in the review.	
26.	Outcome measures	**Inclusion criteria:** Food intake; body weight; lean mass; fat mass;; GH levels; Ghrelin levels and IGF-1.	
27.	Language restrictions	**Inclusion criteria:** No restriction of language will be imposed. Studies published in other languages will be translated to English. **Exclusion criteria:** Nil	
28.	Publication date restrictions	**Inclusion criteria:** Studies published after 1999 will be included as Ghrelin was first isolated by Kojima in 1999.	
29.	Other	Inclusion criteria: Exclusion criteria:	
30.	Sort and prioritize your exclusion criteria per selection phase	1. Duplicate publication 2. Not an original research article 3. Uncontrolled studies 4. Ghrelin supplementation combined with other (nutritional) components 5. Studies not done on animal models of cancer cachexia 6. Outcomes measured not of interest	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Authors, year	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Type of study, Duration of study, experimental groups, number of animals in each group.	
33.	Animal model characteristics (*e.g.* species, gender, disease induction)	Species/ strain, gender, age and body weight of animals at the beginning of the study. Method of induction of cancer cachexia.	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Form, dose, duration, frequency and route of administration of Ghrelin. Timing of supplementation of Ghrelin with respect to induction of cancer cachexia, timing of data collection	
35.	Outcome measures	Outcome measures included in the review: Food consumption, total body weight, lean body mass, fat mass, signs of drug-related toxicity, plasma Ghrelin levels, plasma Growth hormone levels and serum IGF-1 levels Outcome measures not included in the review: Will be enumerated.	
36.	Other (*e.g.* drop-outs)	Drop- outs with reasons. Was missing data (if any) handled appropriately? , Country and funding source (if any).	

	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	The risk of bias will be independently assessed by two reviewers (MK; SG)using SYRCLE's Risk of Bias tool (15). The judging criteria will be as: • Score ‘‘yes’’ will indicate low risk of bias, • Score ‘‘no’’ indicates high risk of bias and • ‘‘?’’ indicates unclear risk of bias. Discrepancies between the two reviewers will be resolved through mediation of a third reviewer (PS)	
38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	√ **By use of** SYRCLE's Risk of Bias tool (15) □By use of SYRCLE’s Risk of Bias tool, adapted as follows: □ By use of CAMARADES' study quality checklist, e.g. [5] □By use of CAMARADES' study quality checklist, adapted as follows:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	Food consumption: Continuous data; Total body weight: Continuous data. Lean body mass: Continuous data. Fat mass: Continuous data. Plasma Ghrelin levels: Continuous data Plasma Growth hormone levels: Continuous data Serum IGF-1 levels: Continuous data Descriptive – drug related toxicity	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	From the studies included, number of events or mean, standard deviation (SD) or standard error of mean(SE) as well as total number of animals in each group will be noted. If data is only presented in graphs, it will be measured using digital ruler software wherever possible. If not possible the authors will be contacted and requested to provide data.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	Two reviewers (SG, MK) will extract data and discrepancies (if any) will be resolved by consulting the third reviewer (DS).	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis will be performed (using Review Manager (version 5.3)) with subgroup analysis and sensitivity analysis for all outcome measures if possible. Otherwise descriptive summary (eg. toxicity measures).	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	A meta analysis will be performed if there are a minimum of 3 independent comparisons per outcome measure.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	All the outcome measures are continuous variables. They will express as mean difference (MD) or as standardized mean difference (SMD). Where outcomes are measured repeatedly on different points of time in the same	

		animals, we will use the time point at which the measured effect is greatest.	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Anticipating diversity in experimental design of animal studies; we will apply random effects model for all the Outcomes.	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	I2 (the proportion of total variance explained by heterogeneity)	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	We will try to explore the possible causes for heterogeneity (if any) by subgroup analyses. It will be planned for form, dose, duration, timing of Ghrelin supplementation. Animal model, species, strain, sex	
48.	Any sensitivity analyses you propose to perform	We will try to explore the effect of study quality for each comparison by excluding studies per quality item rated by 'High Risk of [type of] bias' and restricting to those trials rated as 'low risk of [type of] bias'.	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)		
50.	The method for assessment of publication bias	We will visually inspect the Funnel plot to determine the publication bias if outcome contained at least ten or more studies.	
Final approval by (names, affiliations): Dr Quazi Syed Zahiruddin		Date:	

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