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Can Coenzyme Q10 Supplementation effectively reduce human tumor necrosis factor-α and interluekine-6 levels in chronic diseases? Protocol for a systematic review and meta-analysis of randomized controlled trial

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Contributions: AA, MV, AK and LJ were responsible for the systematic review protocol design process and formulation the research question for this work. FF and JH searched the electronic databases and reviewed the collected data. NM and PI participated in the assessment of full text papers and data collection.

Amendments: To date, we do not find a previously completed or published protocol.

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Keywords: Interleukin-6; Tumor necrosis factor-α; Coenzyme Q10; Inflammatory response; Cytokines

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Abstract

Introduction: Inflammation, as a critical factor, can cause a numerous chronic disease by creating various pro-inflammatory cytokines. Coenzyme Q10 (CoQ10) can potentially exert an anti-inflammatory agent, in turn, that can inhibit and reduce the systemic inflammatory response. Our aims of this study is to conduct a comprehensive systematic review and a meta-analysis for determining the CoQ10 efficacy on changes in serum interluekine-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels in unhealthy subjects.

Method and analysis: We will conduct an electronic search for articles published between January 1990 and January 2017 using a pre-specified search strategy in MEDLINE, Scopus, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, WHO International Clinical Trials Registry Platform, ClinicalTrials.gov and ISRCTN registry.

Our search will focus on only randomized controlled clinical trials in unhealthy subjects (≥18) that employ either a parallel or cross-over design and with concurrent control groups. The primary outcomes of the literature are to determine the CoQ10 efficacy on changes in serum IL-6 and TNF-α levels in unhealthy subjects. Secondary outcomes such as body mass index, serum adiponectin and hs-CRP levels, lipid profile and the heterogeneity assessment of primary studies will also be evaluated. The stages of screen articles, extract relevant data and assess study quality using the Cochrane risk of bias tool will be conducted independently by two reviewers. Any disagreement will resolve by discussion and/or consultation with a third person. If the number of eligible studies is an adequate, we will carry out a meta-analysis according to both outcomes.

Ethics and dissemination: This research is a protocol for a systematic review and no ethics approval is needed. The findings from the full systematic review will be published in a peer-reviewed journal and will also be exhibited at national/international academic and clinical conferences.

Review registration number: CRD42016052200

Article summary

- The benefits of this systematic review with meta-analysis are because of a comprehensive search strategy, designed to retrieve as many articles relevant to our primary and secondary objectives as possible.
- The evidence highest levels for informed decisions about the role of CoQ10 in chronic disease will be provided from results of this study.
- The protocol of this study has been prepared in accordance with the PRISMA-P guidelines, including description of key methodological steps.
- The main limitation of this study is that the conclusions will be limited by the number and quality of primary studies.
- One limitation of this study is related to published articles in other languages that needs to a translator.

Introduction

Much evidence indicates that inflammation has a major role in many human chronic diseases pathogenesis, such as cardiovascular, pulmonary, autoimmune, degenerative diseases, cancer, diabetes, Alzheimer disease, insulin resistance (IR), endothelial dysfunction and obesity (1-3). For this reason, inflammation is considered as one of the main causes of mortality in the western countries (2). Under conditions of inflammation process, numerous cytokines are produced by various of cell types, particularly macrophages and monocytes at inflammatory sites (4). These inflammatory cytokines include interluekine-6 (IL-6), tumor necrosis factor-α (TNF-α), interferon-γ and others, are major inducers of acute phase proteins production (4). Among cytokines, TNF-α, as known one of the first inflammatory mediators, has been shown to be a chief stimulator in systemic inflammatory response via activation of nuclear factor κB (NF-κ B) (3). Hence, the intensive production of TNF-α is closely linked to the obesity-associated metabolic syndrome, development of IR, obesity, and diabetes (3).

IL-6, as a multi-functional cytokine, is another key inflammatory mediator that elicits several effects, ranging from specific cellular and humoral immune defenses development to systematic inflammation and tissue injury (4, 5). It therefore can have a dual effect in human (4). Elevated levels of IL-6 are closely linked with numerous human chronic diseases including multiple myeloma, rheumatoid arthritis, AIDS, psoriasis, sepsis, osteoporosis, Crohn's disease, IR and others (3-5).

Chemically coenzyme Q10 (CoQ10), referred to as ubiquinone (7), is composed of a lipophilic benzoquinone structure with a side-chain of 10 isoprenoid units (8). It is endogenously synthesized in tissue from tyrosine and mevalonic acid (9) and can also be obtained in much of the human diet (8). CoQ10 is a critical intermediate of the mitochondrial electron transport chain for the synthesis of ATP (8, 10). The biological importance of CoQ10 related to antioxidant activity which can scavenge free radicals as well as restores antioxidant defense system (7). Furthermore, several studies from both in vitro and animal models have suggested that CoQ10 acts as an anti-inflammatory agent, in turn, that can inhibit the inflammatory response (7) by blocking the action and expression of NF-κB (12) as well as through activating the PPAR mediated anti-inflammatory responses (7).

As referred above, CoQ10 supplementation with a natural dietary antioxidant having anti-inflammatory activity would be beneficial against numerous human chronic diseases (8-14). Although a recent study by Liu et al had observed that CoQ10 (300 mg/day) could not significantly decrease inflammatory cytokines (TNF-α, P = 0.29, IL-6, P = 0.13) in the progression of hepatocellular carcinoma after surgery patients at 12 weeks (10). The another recent clinical trial showed that the serum level of TNF-α significantly reduced in rheumatoid arthritis patients who received 100 mg/day capsules of CoQ_{10} for 8 weeks, but any significant effect on serum IL-6 concentration was not seen (14). Consistent with results of this study, Farsi et al observed that CoQ10 supplement at 100 mg/day could be significantly reduce the serum levels of TNF-α (P= 0.049) after 3 months of treatment in nonalcoholic fatty liver disease

patients. However, IL-6 blood levels no significant changed after intervention duration (11). In this regard, Sanoobar et al examined the efficacy of CoQ10 administration in the patients with multiple sclerosis. They observed that daily intake CoQ10 supplement at a dosage of 500 mg has favorable effects in reduction plasma inflammatory markers levels (TNF-α, P = 0.003, IL-6, P = 0.037) after 12 weeks of intervention (9). Overall, on the basis of the available evidence, researchers have reported inconclusive results of the CoQ10 supplement effectiveness on TNF-α and IL-6 serum levels (9-15). These discrepancies might be related to the various dosing regimens of CoQ10 and the differences of supplementation duration, studied subjects with variations clinical inclusion criteria by using small sample sizes and plasma TNF-α and IL-6 levels at baseline have been used. Despite several clinical trials, the efficacy of CoQ10 on circulating TNF-α and IL-6 levels remain questionable and ambiguous. Based on our knowledge and understanding, until now, we found two recent systematic review and meta-analysis of the evidence in relation to CoQ10 efficacy for changing in pro-inflammatory factors levels (CRP, IL-6, or TNF-α) (16, 17). Fan et al in this issue observed that significant lowering effects of CoQ10 on CRP, IL-6, or TNF-α, which included mixed patient and healthy subjects (16). Besides, another recent review reported that CoQ10 was helpful in significant decreasing TNF-α levels, but they no found significantly changes in IL-6 and CRP serum levels (17). However, none of these studies did not have specifically focused the evidence for only the effectiveness of CoQ10 on both TNF- α and IL-6 serum levels (17). Furthermore, they were based on a limited search database search and restricted to studies published in English language (16, 17). Therefore, based on available literature, we believe that a systematic review and meta-analysis is required about only the CoQ10 supplementation efficacy on concurrent changes in serum TNF- α and IL-6 levels in unhealthy subjects. In order to extend our work, we will review more database and gray literature without language limitation of publication. Thus, it appears that the results of this study can potentially help to determine the net effect of oral CoQ10 supplementation on TNF-α and IL-6 serum levels.

Objectives

The primary objective of this study of the literature is therefore to evaluate CoQ10 supplementation efficacy on changes in serum TNF-α and IL-6 levels in unhealthy subjects.

Secondary aims of our study are to:

1- To evaluate CoQ10 supplementation efficacy on BMI in comparison control group in unhealthy subjects.
2- To evaluate CoQ10 supplementation efficacy on changes in serum adiponectin concentrations in comparison with control group in unhealthy subjects.
3- To assess CoQ10 supplementation efficacy on changes in serum hs-CRP levels in comparison with control group in unhealthy subjects.
4- To determine and summarize the evidence about the efficacy of CoQ10 supplementation to reduce lipid profile (LDL, cholesterol and TG) in comparison with control group in unhealthy subjects.
5- To investigate the heterogeneity assessment of primary studies and other sources.

Method and analysis

The design of this systematic review has been developed according to the recommended detail on the 2015 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines checklist (18). Moreover, the flowchart of PRISMA will be applied to explain the number of included and excluded primary studies in the different stages of this systematic review (supplementary 1). This systematic review protocol has been prepared in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015) guidelines (19). This protocol is registered in the international prospective register of systematic reviews (PROSPERO 2016:CRD42016052200; http://www.crd.york.ac.uk/PROSPERO).

Eligibility criteria

Study design/characteristics: Primary studies will be included in the systematic review if they had to be a randomized clinical trial (RCTs) at least single-blind with either parallel or cross-over design and with concurrent control groups as well as published in any language between January 1990 and January 2017. Reviewers will exclude other than RCT conducted in human including review articles, case studies, case series, observational studies (cross-sectional, case-control and cohort), experimental studies with animals or in vitro and studies on healthy subjects, proceedings, editorials/commentaries, letters as well as reporting insufficient data on baseline or follow-up TNF-α and IL-6 in each group. We will exclude studies reporting CoQ10 in combination with other substances.

Subject types: In order to be eligible for inclusion, primary studies will be considered if the tested intervention targets unhealthy adult (≥18 years) or at least one of the population subgroups and at least one of the two males and females.

Intervention(s): Our target will be RCT studies that used oral CoQ10 supplementation (capsule form or in any form) daily in divided dosage or single doses (amount/day) for any intervention duration.

Comparator(s)/ control: Studies will be eligible to compare the oral CoQ10 supplementation versus oral supplementation without CoQ10 (control) (if relevant):

- Other natural or pharmacological agents
- No intervention or no control
- Standard therapy alone
- Placebo

Outcome(s): Studies will be included in the review if they report the effect of the intervention on primary outcomes in terms of serum/plasma TNF-α and IL-6 levels at baseline and at the end of treatment duration in the study groups.

Secondary outcomes of project: We will also record the results of intervention effectiveness on one or several of the following outcomes if available:

- BMI
- Serum adiponectin and levels
- Serum hs-CRP levels
- Lipid profile measurements (TG, cholesterol, LDL-c)
- The heterogeneity assessment of primary studies and other sources

Data sources

The searching of electronic bibliographic databases for this systematic review will be conducted by two reviewers between January1990 and January 2017. Bibliographic and electronic databases will be searched using the following assess words in topics and abstracts, as follows:

1. Cochrane Central Register of Controlled Trials (CENTRAL)
2. Web of Science
3. Medline (http://www.ncbi.nlm.nih.gov/pubmed)
4. SCOPUS
5. EMBASE

Database of ongoing clinical trials

Registers of clinical trials will be searched in the following databases.

- www.ClinicalTrials.gov
- ISRCTN registry
- www.who.int/trialsearch/

Other resources

To ensure research saturation, the other resources will be manual reviewing to find additional eligible studies that are key journals and the reference list of included relevant research articles, meta-analyses, and review publications on CoQ10. In regard to the recommendations of the Institute of Medicine Standards and the Cochrane Handbook for Systematic Reviews of Interventions, we will evaluate some of informally published in academic sources (the grey literature) include thesis data and abstracts of papers presented at different conferences for further comprehensiveness we will contact to authors of papers with available abstracts by email and will request to the full text if necessary.

Search strategy

The aims of literature search strategies will be to find all relevant RCTs conducted in human using an appropriate set of key search terms to delimit the concepts ‘coenzyme Q10’, ‘IL-6’ and ‘TNF-α’. The search terms of any component of this study were found in the medical subject headings (MeSH) tags of PubMed database, EMTREE and free text word, a combination of these was used to create proper electronic search strategy, in regard to each database, search strategy

will be adopted. The search strategy and syntax of PubMed database in details are presented in online supplementary appendix 2.

Study records

Data management

Two primary researchers (FF and JH) will perform the initial search of electronic databases using the strategy search and guided by the PRISMA-P statement. FF and JH will also manual review the reference lists of all included studies. In order to data management, Endnote X7 software will be used. The main reviewer will import the results of literature searches into an EndNote library and will delete duplicate records.

Selection process

The selection phase of this systematic review is compliant the PRISMA guidelines. The selection of relevant studies for inclusion in the review will be performed in a three-step process. The two independent reviewers (FF and PI) will first screen titles and abstracts of all records identified by the database searches in line with the inclusion criteria/exclusion criteria in order to identify a subset of potentially eligible articles. Any discrepancies about inclusion in each step of the screen process (title/ abstract and then full text review) will resolve by discussion and/or consultation with a third researcher specific expertise in chronic diseases and CoQ10.

We will then obtain the full texts of potentially eligible article that appear to meet our inclusion criteria on the basis of their title/ abstract, or where there is uncertainty. The full-text screening process of included abstracts will be also carried out by two independent reviewers (FF and PI). At each step of the selection process, a record will be kept of the reasons for excluding studies.

Data extraction

A standard data extraction form designed by the primary reviewer will be used for data extraction from all selected studies (see online supplementary 2). The data extraction form will be pilot test by our team on three selected studies and will be refined as necessary in order to ensure the reliability of the data extraction process. The two reviewers (FF and NM) will independently extract the information from included studies. Data extraction will be completed using the full text of published reports or via the study authors, if the data provided in the published articles was inadequate to complete the extraction process. Principal investigator involved in the process of data extraction will have practice using the form and will receive appropriate training if deemed necessary. Similarly, any disagreement in the extracted data process which cannot be resolved through consultation will be referred to a third reviewer specific expertise in order to reach consensus.

Data items

The Participants, Interventions, Comparisons, Outcomes, Study characteristics (PICOS) criteria will be applied to systematize our information extraction.

- Study characteristics: name of the first author, study design, place and time of the study, country of origin, year of publication and size of the sample to separate groups.
- Participant sociodemographic characteristics: age, ethnicity, sex, number of participants, disease type, initial healthy status.
- Intervention and their specific: dosage, length of follow-up, type of administration, treatment group sample size, blinding procedure, withdrawals and dropouts.
- Outcomes: definition and measures of primary (TNF-α and IL-6 levels) and secondary outcomes (BMI, serum adiponectin, hs-CRP levels, lipid profile, heterogeneity assessment in primary studies and other miscellaneous points)

Outcomes and prioritization

We will consider pooling studies which include TNF-α and IL-6 levels in unhealthy subjects as our primary clinical outcomes and other outcomes as secondary outcomes. Our prioritization will also be given to studies with a RCT design which examined the effect of CoQ10 supplementation alone in inflammation conditions. The way of articles presentation in the review will be ultimately improved by prioritization of the search strategy items.

Missing data

In order to data management in certain conditions, according to the Cochrane Institute our investigators will contact the corresponding authors of studies by email to obtain clarification if data are incomplete in the study report. We again will have to send reminder email up to three times if the authors do not reply to email. Reviewers will consider as the missing data if not receive a response after three emails.

Risk of bias assessment (in individual studies)

Risk of bias assessment of the individual studies will be carried out by two independent review authors (FF, JH) using the guidelines of the tool developed by the Cochrane Collaboration to assess and report risk of bias in the following 7 criteria:

1. Sequence generation 2. Allocation concealment 3. Blinding of participants and personnel 4. Blinding of outcome assessment 5. Incomplete outcome data 6. Selective outcome reporting 7. other potential sources of biases.

Two principal investigators (FF, JH) will first test pilot Cochrane tool items on three primary articles. In case of disagreement in risk of bias assessment will be resolved through consultation with the third person. The options of yes, no or unclear will be used for each component of our chosen domains and then risk of bias will be documented as a risk of bias category from the following: low, unclear or high. We then will describe reasons for each assessment.

Data synthesis

If the number of eligible studies is an adequate, we will carry out a meta-analysis according to both outcomes. Based on conditions the primary studies in terms of methodology, one of the two models (fixed or random effects model) will be used.

A narrative data synthesis of all the include studies after a systemic review will be performed and it will be assigned in a text and separate tables. For the purposes of data synthesis, all data from continuous outcomes analyses will be presented as mean difference or standardized mean difference (SMD), both with their 95% CI and also risk ratio (RR) index will be calculated for qualitative and categorical data.

To investigate clinical and methodological heterogeneity, we will undertake in order to determine meta-analysis feasibility. For this purpose, we will consider the main sources of heterogeneity including different study design (cross over or parallel and year of publication), population characteristics (gender, ethnicity, age, disease types and stage distribution), duration of follow-up, sampling interval and test characteristics. The Q Cochrane test will be applied to check be statistically the extent of heterogeneity among primary studies as recommended by the Cochrane Handbook for Systematic Reviews of RCT. The I^2 statistic will also be used to determine the extent of heterogeneity between studies and to guide our choice of model (fixed or random effects model). I^2 >50% will be considered to be severe heterogeneity. If, however we find that substantial heterogeneity and the sufficient number of eligible studies, meta-regression and subgroup analyses according to gender, BMI, the sample size and SMD will be conducted in order to identify heterogeneity sources. If some studies are at high risk of bias, we will conduct a sensitivity analysis in order to assess the impact of methodological quality and studies with a lower sample size on the power of review conclusions. All data from sensitivity analyses will be presented and summaries with table. The forest plots will be used for the graphical representation and final synthesis of primary studies. Stata V.12 software (Stata Corp LP, College Station, Texas, USA) will be used for all the mentioned analyses.

Assessment of possible reporting bias

We will investigate the likelihood of outcome reporting bias (publication and other reporting biases) using funnel plots (ie, plots of study results against precision), if the number of studies included in a meta-analysis is sufficient (~10 studies). Begg's and Egger's tests will also be used in order to assess for asymmetry. When this is non-negligible, we will use Trim and fill method to correct results. The statistically significant correlation will be indicated using a p-value of ≤ 0.05.

Ethics and dissemination

This research is a protocol for a systematic review and no ethics approval is needed. The findings from the full systematic review will be published in a peer-reviewed journal and will also be exhibited at national/international academic and clinical conferences.

As mentioned, inflammation is recognized as common causes of numerous human chronic diseases pathogenesis. Since, among of the pharmacotherapy, anti-inflammatory medicines or inflammatory blockers are currently being applied in extended range these blockers are highly expensive and make more side effects. Hence, natural component such as CoQ10 is needed that are not only safe, but also is cost effective and readily available (6). CoQ10 can also consider important agent for the conservative management of this condition.

The purpose of this systematic review will be comprehensively identified and summarized studies reporting which demonstrate whether CoQ10 supplementation effectively can reduce serum TNF-α and IL-6 levels in unhealthy subjects. The results of this study will assistance to notification future research on the anti-inflammatory effect of CoQ10 as treatment approach in many human chronic diseases. Furthermore, our results of this systematic review with meta-analysis will be important to aware clinicians, therapists and patients to attain a better understanding of the CoQ10 effectiveness.

Confidence in cumulative evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) (20) guidelines will be apply in order to assess the quality of the evidence in relation to the effect of CoQ10 on the primary and secondary outcomes. The quality assessment of evidence will be performed based on the following domains: design and risk of bias, consistency, directness, precision and publication bias.

Contributors: AA, MV, AK and LJ were responsible for the systematic review protocol design process and formulation the research question for this work. FF and JH searched the electronic databases and reviewed the collected data. NM and PI participated in the assessment of full text papers and data collection.

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Competing interests: None declared.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: Results from the completed will be disseminated through peer-reviewed publications and social networks.

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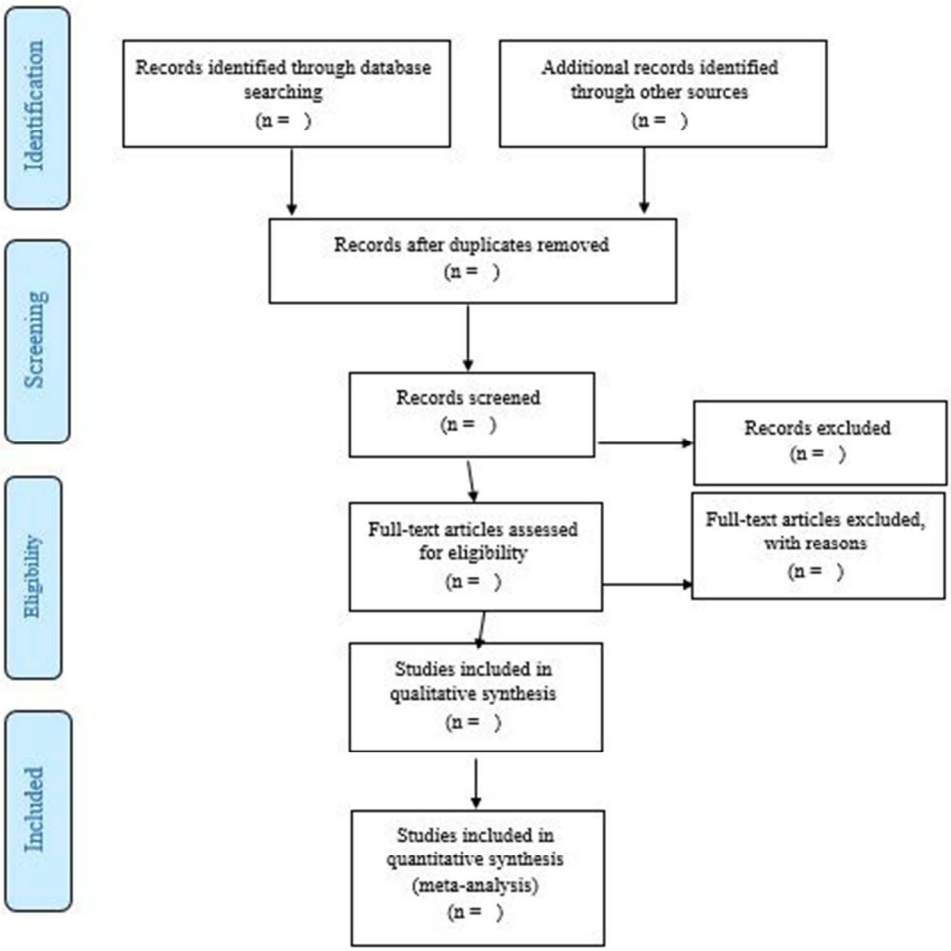
Supplementary 1. PRISMA Flow Diagram



131x136mm (96 x 96 DPI)

Supplementary appendix 2.

("CoQ 10" OR CoQ10 OR Ubidecarenone OR "co-enzyme Q10" OR "ubiquinone Q10" OR "Bio-Quinone Q10" OR 2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone OR "ubisemiquinone radical" OR Q-ter OR ubisemiquinone OR ("coenzyme Q10" AND (Z,Z,Z,Z,Z,Z,E,E,E)-isomer) OR ("coenzyme Q10" AND "ion (1-)" AND (all-E)-isomer) OR "2, 3 dimethoxy 5 methyl 6 decaprenylbenzoquinone" OR caomet OR "coenzyme 910" OR "coenzyme q 10" OR "coenzyme Q10" OR decorenone OR mitocor OR neuquinone OR "quinone q 10" OR ubimaior OR "ubiquinone (10)" OR "ubiquinone 10" OR "ubiquinone 50" OR ubiten) AND (("26 k protein" OR "b cell stimulating factor 2" OR "B cell stimulatory factor 2" OR "b lymphocyte stimulating factor 2" OR "beta 2 interferon" OR "beta2 interferon" OR "bsf 2" OR bsf2 OR "hepatocyte stimulating factor" OR "il 6" OR "interferon beta 2" OR "interferon beta2" OR "interleukin b" OR "interleukin hp1" OR interleukin-6 OR "liver cell stimulating factor" OR "plasmacytoma growth factor" OR "protein 26k" OR "Interleukin 6" OR "B-Cell Differentiation Factor" OR "B Cell Differentiation Factor" OR "B-Cell Differentiation Factor-2" OR "B-Cell Stimulatory Factor 2" OR "B-Cell Stimulatory Factor-2" OR BSF-2 OR ("Differentiation Factor" AND B-Cell) OR ("Differentiation Factor" AND "B Cell") OR ("Differentiation Factor-2" AND B-Cell) OR ("Differentiation Factor 2" AND "B Cell") OR "Hepatocyte-Stimulating Factor" OR "Hybridoma Growth Factor" OR ("Growth Factor" AND Hybridoma) OR "IFN-beta 2" OR IL-6 OR IL6 OR MGI-2 OR "Myeloid Differentiation-Inducing Protein" OR ("Differentiation-Inducing Protein" AND Myeloid) OR "Myeloid Differentiation Inducing Protein" OR ("Growth Factor" AND Plasmacytoma) OR "B Cell Stimulatory Factor-2") OR ("mhr 24" OR "TNF alfa" OR "TNF alpha" OR "tumor necrosis factor alfa" OR "tumor necrosis factor-alpha" OR "tumour necrosis factor alfa" OR "tumour necrosis factor alpha" OR "tumour necrosis factor-alpha" OR "Tumor Necrosis Factor alpha" OR "Cachectin-Tumor Necrosis Factor" OR "Cachectin Tumor Necrosis Factor" OR TNFalpha OR TNF-alpha OR "Tumor Necrosis Factor" OR "Tumor Necrosis Factor Ligand Superfamily Member 2" OR ("TNF Superfamily" AND "Member 2") OR Cachectin)) AND 1990/01/01:2017/01/15 [dp]

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	1
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	1
Support:			
Sources	5a	Indicate sources of financial or other support for the review	1
Sponsor	5b	Provide name for the review funder and/or sponsor	1
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	5, 6
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	6
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	6
Study records:			

Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	7
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7,8
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	10

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

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Can Coenzyme Q10 Supplementation effectively reduce human tumor necrosis factor-α and interluekine-6 levels in chronic diseases? Protocol for a systematic review and meta-analysis of randomized controlled trials

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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	Tumor necrosis factor-α, Interleukin-6, Coenzyme Q10, Inflammatory response, Cytokines

Can Coenzyme Q10 Supplementation effectively reduce human tumor necrosis factor-α and interluekine-6 levels in chronic diseases? Protocol for a systematic review and meta-analysis of randomized controlled trials

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Review registration number: CRD42016052200

Contributions: AA, MV, AK and LJ were responsible for the systematic review protocol design process and formulation the research question for this work. FF and JH searched the electronic databases and reviewed the collected data. NM and PI participated in the assessment of full text papers and data collection.

Amendments: To date, we do not find a previously completed or published protocol.

Support:
Sources: This work does not have access to funding from any funding agency in developing the protocol.

Sponsor: This work does not have any review funder and/or sponsor.

Keywords: Interleukin-6; Tumor necrosis factor-α; Coenzyme Q10; Inflammatory response; Cytokines

Word count: 3688

Abstract

Introduction: Inflammation, as a critical factor, can cause numerous chronic diseases by creating various pro-inflammatory cytokines. Coenzyme Q10 (CoQ10) can potentially exert an anti-inflammatory agent; in turn, this agent can reduce the systemic inflammatory response. The aims of this study are to conduct a comprehensive systematic review, and a meta-analysis for the determination of the CoQ10 efficacy on the changes in serum interluekine-6 (IL-6) and the tumor necrosis factor-α (TNF-α) levels in unhealthy subjects.

Method and analysis: We will conduct an electronic search for articles published between January 1990 and January 2017 using a pre-specified search strategy in MEDLINE, Scopus, EMBASE, the Cochrane Central Register of Controlled Trials, Web of Science.

Our search will focus only on randomized controlled clinical trials in unhealthy subjects that employ either a parallel or a crossover design; this search will involve concurrent control groups. The primary outcomes of the literature are to determine the CoQ10 efficacy on the changes in the serum IL-6 and the TNF-α levels in unhealthy subjects. Secondary outcomes such as body mass index, serum adiponectin and hs-CRP levels, lipid profile, and the heterogeneity assessment of the primary studies will be evaluated. The stages of screen articles, the extracts of relevant data, and the assessment of study quality using the Cochrane risk of bias tool will be conducted independently by the two reviewers. Any disagreement will be resolved by discussion with a third person. If the number of eligible studies is sufficient, we will carry out a meta-analysis according to both the outcomes.

Ethics and dissemination: This study is the protocol for a systematic review and no ethics approval is needed. The findings from the full systematic review will be published in a peer-reviewed journal, and they will also be exhibited at national/international academic and clinical conferences.

Review registration number: CRD42016052200

Article summary

- The benefits of this systematic review with meta-analysis are because of a comprehensive search strategy, designed to retrieve as many articles relevant to our primary and secondary objectives as possible.
- The evidence highest levels for informed decisions about the role of CoQ10 in chronic disease will be provided from results of this study.
- The protocol of this study has been prepared in accordance with the PRISMA-P guidelines, including description of key methodological steps.
- The main limitation of this study is that the conclusions will be limited by the number and quality of primary studies.
- One limitation of this study is related to published articles in other languages that needs to a translator.

Introduction

Much evidence indicates that systematic inflammation has a major role in many human chronic disease pathogeneses, such as cardiovascular, pulmonary, autoimmune, and degenerative diseases as well as cancer and metabolic diseases (1–3). Elevated levels of Interluekine-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α), as inflammation mediators, are closely linked to numerous chronic diseases (3–5). Therefore, it is essential to reduce inflammation in order to reduce the risk of chronic diseases.

Coenzyme Q10 (CoQ10), referred to as 'ubiquinone' (6), is composed of a lipophilic benzoquinone structure with a sidechain of 10 isoprenoid units (7). It is endogenously synthesized by the mevalonate pathway in the human body (8) and it is obtained in much of human diet (9). CoQ10 is a critical intermediate of the mitochondrial electron transport chain for the synthesis of adenosine triphosphate (7,10). The biological importance of CoQ10 is related to antioxidant activity, which can scavenge free radicals as well as restore the antioxidant defence system (9). Furthermore, several studies from both in vitro and animal models have suggested that CoQ10 acts as an anti-inflammatory agent, inhibiting the inflammatory response (9) by blocking the expression of NF-κB (10) as well as activating the PPAR mediated anti-inflammatory responses (9).

As previously mentioned, CoQ10 supplementation, a natural dietary antioxidant, possessing anti-inflammatory activity, would be beneficial for numerous human chronic diseases (8–14). However, a recent study by Liu et al. indicated that CoQ10 (300 mg/day) could not significantly decrease the inflammatory cytokines in the progression of the hepatocellular carcinoma after 12 weeks of performing surgery (10). Another recent clinical trial showed that the serum level of TNF-α was significantly reduced in rheumatoid arthritis patients who received capsules of CoQ_{10} (100 mg/day) for 8 weeks, but no significant effect was seen on serum IL-6 concentration (14). Consistent with the results of this study, Farsi et al. observed that CoQ10 supplement at a dose of 100 mg/day could significantly reduce the serum levels of TNF-α ($P = 0.04$) after three months of treatment in patients suffering from non-alcoholic fatty liver disease. IL-6 blood levels, however, had significantly changed after the intervention duration (11). In this regard, Sanoobar et al. examined the efficacy of CoQ10 administration in patients with multiple sclerosis. The researchers observed that the daily intake of CoQ10 supplement at a dosage of 500 mg has favourable effects on the reduction of the plasma inflammatory marker levels (TNF-α, $P = 0.003$, IL-6, $P = 0.03$) after 12 weeks of intervention (8). Overall, on the basis of the available evidence, researchers have reported inconclusive results of CoQ10 supplement effectiveness on TNF-α and IL-6 serum levels (8–15). These discrepancies might be related to the various dosing regimens of CoQ10 and the differences in supplementation duration, a variation of the clinical inclusion criteria in the studied samples as a result of using small sample sizes, and plasma TNF-α and IL-6 levels at the baseline. Despite several clinical trials, the efficacy of CoQ10 on circulating TNF-α and IL-6 levels remains questionable and ambiguous. Based on our knowledge and understanding, until now, we have found two systematic reviews and meta-analyses of the

evidence in relation to the CoQ10 efficacy for changing the pro-inflammatory factors levels (CRP, IL-6, or TNF-α) (16, 17). Fan et al., in this issue, observed the significant lowering effects of CoQ10 on CRP, IL-6, or TNF-α, in a sample that comprised a combination of patients and healthy subjects (16). Another recent review reported that CoQ10 was helpful in significantly decreasing TNF-α levels, but found no substantial change in IL-6 and CRP serum levels (17).

However, none of these studies had specifically focused on the evidence solely in order to study the effectiveness of CoQ10 on both the TNF-α and the IL-6 serum levels (16, 17). Furthermore, they were based on a limited database search and were restricted to studies published in English (16, 17). Therefore, based on the available literature, we believe that a systematic review and meta-analysis is required only for the CoQ10 supplementation efficacy on the concurrent changes in serum TNF-α and IL-6 levels in unhealthy subjects. In order to extend our work, we will review more database and grey literature without setting a language limitation on publications. It, therefore, appears that the results of this study can potentially help determine the net effect of the oral CoQ10 supplementation on the TNF-α and the IL-6 serum levels.

Objectives

The primary objective of this study of the literature is, therefore, to evaluate the CoQ10 supplementation efficacy on the changes in serum TNF-α and IL-6 levels in unhealthy subjects.
The secondary aims of our study are as follows:

1- To evaluate CoQ10 supplementation efficacy on BMI in comparison to the control group in unhealthy subjects.
2- To evaluate CoQ10 supplementation efficacy on the changes in serum adiponectin concentrations in comparison to the control group in unhealthy subjects.
3- To assess CoQ10 supplementation efficacy on the changes in serum hs-CRP levels in comparison to the control group in unhealthy subjects.
4- To determine and summarize the evidence about the efficacy of CoQ10 supplementation to reduce the lipid profile (LDL, cholesterol, and TG) in comparison to the control group in unhealthy subjects.
5- To investigate the heterogeneity assessment of primary studies and other sources.

Method and analysis

The design of this systematic review has been developed according to the recommended details presented on the 2015 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines checklist (18). Moreover, the flowchart of PRISMA will be applied to explain the number of included and excluded primary studies in the different stages of this systematic review (supplementary Appendix 1). This systematic review protocol has been prepared in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015) guidelines (18). This protocol is registered in the international prospective register of systematic reviews (PROSPERO 2016: CRD42016052200; website: http://www.crd.york. ac.uk/PROSPERO).

Eligibility criteria

Study design/characteristics: Primary studies will be included in the systematic review if they contain Randomized Clinical Trials (RCTs) at least single-blind with either parallel or crossover designs, comprising concurrent control groups, which were published in any language between January 1990 and January 2017. Reviewers will exclude methods other than RCTs conducted on humans, such as review articles, case studies, case series, observational studies (cross-sectional, case-control, and cohort), experimental studies with animals or in vitro, and studies on healthy subjects, proceedings, editorials/commentaries, letters as well as reports comprising insufficient data on baseline or follow-up TNF-α and IL-6 in each group. We will exclude studies reporting CoQ10 in combination with other substances.

Subject types: In order to be eligible for inclusion, primary studies will be considered if the tested intervention targets unhealthy adults (≥ 18 years) or at least one of the population subgroups, and at least at least one of the two sexes.

Intervention(s): Our target will be RCT studies that used oral CoQ10 supplementation (in capsule form or in any other form) daily in divided dosage or single doses (amount/day) for any intervention duration.

Comparator(s)/control: Studies will be eligible that compared oral CoQ10 supplementation versus control (if relevant):

- Other natural or pharmacological agents
- No intervention or no control
- Standard therapy alone
- Placebo

Outcome(s): Studies will be included in the review if they report the effect of the intervention on the primary outcomes in terms of serum/plasma TNF-α and IL-6 levels at the baseline and at the end of treatment duration in the study groups.

Secondary outcomes of project: We will also record the results of intervention effectiveness on one or several of the following outcomes if available:

- BMI
- Serum adiponectin and levels
- Serum hs-CRP levels
- Lipid profile measurements (TG, cholesterol, and LDL-c)
- The heterogeneity assessment of primary studies and other sources

Data sources

Searching electronic bibliographic databases for this systematic review will be conducted by two reviewers between January1990 and January 2017. Bibliographic and electronic databases will be searched using the following assess words within topics and abstracts:

1. Cochrane Central Register of Controlled Trials (CENTRAL)
2. Web of Science
3. Medline (http://www.ncbi.nlm.nih.gov/pubmed)
4. SCOPUS
5. EMBASE

Database of ongoing clinical trials

Registers of clinical trials will be searched for in the following databases:

- www.clinicaltrials.gov
- ISRCTN registry
- www.who.int/trialsearch/

Other resources

To ensure research saturation, the other resources will be manually reviewed to find additional eligible studies such as key journals and the reference list of all the included relevant research articles, meta-analyses, and review publications on CoQ10. In regard to the recommendations of the Institute of Medicine Standards and the Cochrane Handbook for Systematic Reviews of Interventions, we will evaluate some of informally published content in academic sources (the grey literature), including thesis data and abstracts of papers presented at different conferences. For further comprehensiveness, we will contact the authors of these papers by email on the basis of the available abstracts; we will request for the full text if necessary.

Search strategy

The aims of literature search strategies will be to find all the relevant RCTs conducted on humans using an appropriate set of key search terms to delimit the concepts ‘Coenzyme Q10’, ‘IL-6’, and ‘TNF-α’. The search terms of any component of this study were found in the medical subject headings (MeSH) tags of the PubMed database, EMTREE, and a free text word; a combination of these were used to create a proper electronic search strategy. In regard to each database, a search strategy will be adopted. The search strategy and the syntax of the PubMed database are presented in detail in the online supplementary Appendix 2.

Study records

Data management

Two primary researchers (FF and JH) will perform the initial search of the electronic databases using the strategy search and guided by the PRISMA-P statement. FF and JH will also manually review the reference lists of all the included studies. In order to conduct data management, the

Endnote X7 software will be used. The main reviewer will import the results of literature searches into an EndNote library and then delete the duplicate records.

Selection process

The selection phase of this systematic review is compliant with the PRISMA guidelines. The selection of relevant studies for inclusion in the review will be performed in a three-step process. The two independent reviewers (FF and PI) will first screen the titles and the abstracts of all the records identified by the database searches in line with the inclusion/exclusion criteria in order to identify a subset of potentially eligible articles. Any discrepancies relating to inclusion in each step of the screen process (title/abstract, and then, full text review) will be resolved by discussion and/or consultation with a third researcher with specific expertise in chronic diseases and CoQ10.

We will then obtain the full texts of potentially eligible articles that appear to meet our inclusion criteria on the basis of their title/abstract. The full-text screening process of the included abstracts will be carried out by two independent reviewers (FF and PI). At each step of the selection process, a record of the reasons behind excluding certain studies will be maintained.

Data extraction

A standard data extraction form designed by the primary reviewer will be used for data extraction from all the selected studies (see online supplementary Appendix 2). The data extraction form will be pilot tested by our team on three selected studies and will be refined as necessary in order to ensure the reliability of the data extraction process. The two reviewers (FF and NM) will independently extract the information from the included studies. Data extraction will be completed using the full text of the published reports or via correspondence with the study authors if the data provided in the published articles was inadequate to complete the extraction process. The principal investigator involved in the process of data extraction will have practice using the form and will receive appropriate training if deemed necessary. Similarly, any disagreement in the extracted data process which cannot be resolved through consultation will be referred to a third reviewer with specific expertise in order to reach a consensus.

Data items

The Participants, Interventions, Comparisons, Outcomes, Study characteristics (PICOS) criteria will be applied to systematize our information extraction.

- Study characteristics: name of the first author, study design, place and time of the study, country of origin, year of publication, and size of the sample divided into separate groups.
- Participant sociodemographic characteristics: age, ethnicity, sex, number of participants, disease type, and initial healthy status.
- Intervention and their specific: dosage, length of follow-up, type of administration, treatment group sample size, blinding procedure, withdrawals, and dropouts.

- Outcomes: definition and measures of primary (TNF-α and IL-6 levels) and secondary outcomes (BMI, serum adiponectin, hs-CRP levels, lipid profile, heterogeneity assessment in primary studies, and other miscellaneous points).

Outcomes and prioritization

We will consider pooling studies that include TNF-α and IL-6 levels in unhealthy subjects as our primary clinical outcomes and other outcomes as secondary outcomes. Our prioritization will also be given to studies with a RCT design, which examined the effect of CoQ10 supplementation alone under inflammation conditions. Article presentation in the review will be ultimately improved by prioritization of the search strategy items.

Missing data

In order to perform data management in certain conditions set by the Cochrane Institute, our investigators will contact the corresponding authors of the studies by email to obtain clarification if the data provided are incomplete in the study reports. We will have to send reminder emails (up to three times) if the authors do not reply to the initial email. Reviewers will consider the incomplete information as missing data if a response is not received after three emails.

Risk of bias assessment (in individual studies)

Risk of bias assessment of the individual studies will be carried out by two independent review authors (FF, JH) using the guidelines of a tool developed by Cochrane Collaboration to assess and report the risk of bias in the following seven criteria:

1. Sequence generation
2. Allocation concealment
3. Blinding of participants and personnel
4. Blinding of outcome assessment
5. Incomplete outcome data
6. Selective outcome reporting
7. Other potential sources of biases.

Two principal investigators (FF, JH) will first test pilot the Cochrane tool items on three primary articles. In case of disagreement on the risk of bias assessment, consultation with the third person will be employed to reach a resolution. The options of yes, no, or unclear will be used for each component of our chosen domains, and then, the risk of bias will be documented as a risk of bias category from the following: low, unclear, or high. We will then describe reasons for each assessment.

Data synthesis

If the number of eligible studies is an adequate, we will carry out a meta-analysis according to both outcomes. Based on the conditions of the primary studies in terms of methodology, one of the two models (fixed or random effects model) will be used.

A narrative data synthesis of all the included studies after a systemic review will be performed and it will be assigned in a text as well as separate tables. For the purposes of data synthesis, all the data from continuous outcomes analyses will be presented as mean difference or Standardized Mean Difference (SMD), both with 95% CI; the Risk Ratio (RR) index will be calculated for the qualitative and the categorical data.

To investigate clinical and methodological heterogeneity, we will undertake the determination of the feasibility of meta-analysis. For this purpose, we will consider the main sources of heterogeneity including different study design (crossover or parallel and year of publication), population characteristics (gender, ethnicity, age, disease types and stage distribution), duration of follow-up, sampling interval, and test characteristics. The Q Cochrane test will be applied to statistically check the extent of heterogeneity among primary studies as recommended by the Cochrane Handbook for Systematic Reviews of RCT. The I^2 statistic will also be used to determine the extent of heterogeneity between studies and to guide our choice of the model (fixed or random effects model). $I^2 > 50\%$ will be considered as severe heterogeneity. However, if we find substantial heterogeneity and a sufficient number of eligible studies, meta-regression, and subgroup analyses according to gender, BMI, and the sample size, SMD will be conducted in order to identify the heterogeneity of the sources. If some studies are at high risk of bias, we will conduct a sensitivity analysis in order to assess the impact of the methodological quality and the effect of studies with a lower sample size on the power of review conclusions. All data from sensitivity analyses will be presented and summarized in tables. The forest plots will be used for the graphical representation and the final synthesis of primary studies. The Stata (Version 12) software (Stata Corp LP, College Station, Texas, USA) will be used for all the mentioned analyses.

Assessment of possible reporting bias

We will investigate the likelihood of outcome reporting bias (publication and other reporting biases) using funnel plots (i.e., plots of study results against precision) if the number of studies included in a meta-analysis is sufficient (~10 studies). Begg's test and Egger's test will also be used in order to assess for asymmetry. When this is non-negligible, we will use the trim and fill method to correct the results. The statistically significant correlation will be indicated using a p-value of ≤ 0.05.

Ethics and dissemination

This research is a protocol for a systematic review and no ethics approval is needed. The findings from the full systematic review will be published in a peer-reviewed journal and will also be exhibited at national/international academic and clinical conferences.

As previously mentioned, inflammation is recognized as a common cause of numerous chronic disease pathogeneses in humans (16, 17). Among the constituents of pharmacotherapy, as anti-inflammatory medicines or inflammatory blockers are currently being applied in extended ranges,

these blockers are highly expensive and have more side effects (1, 17). Hence, a natural component such as CoQ10 is needed, which is not only safe but also is cost-effective and readily available (16). CoQ10 can also be considered as an important agent for the conservative management of this condition.

The purpose of this systematic review will be comprehensively identified and summarized in studies reporting whether CoQ10 supplementation effectively can reduce serum TNF-α and IL-6 levels in unhealthy subjects. The results of this study will assist future research that studies the anti-inflammatory effect of CoQ10 as a treatment approach in many human chronic diseases. Furthermore, the results of this systematic review with meta-analysis will be important to create awareness among clinicians, therapists, and patients on the topic of CoQ10 effectiveness.

Confidence in cumulative evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) (19) guidelines will be applied in order to assess the quality of the evidence in relation to the effect of CoQ10 on the primary and secondary outcomes. The quality assessment of evidence will be performed based on the following domains: design and risk of bias, consistency, directness, precision, and publication bias.

Contributors: AA, MV, AK, and LJ were responsible for the systematic review protocol design process and the formulation the research question for this work. FF and JH searched the electronic databases and reviewed the collected data. NM and PI participated in the assessment of the full text papers and data collection.

Funding: This research is not supported by a specific grant from any funding agency.

Competing interests: None declared.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: Results from the completed work will be disseminated through peer-reviewed publications and social networks.

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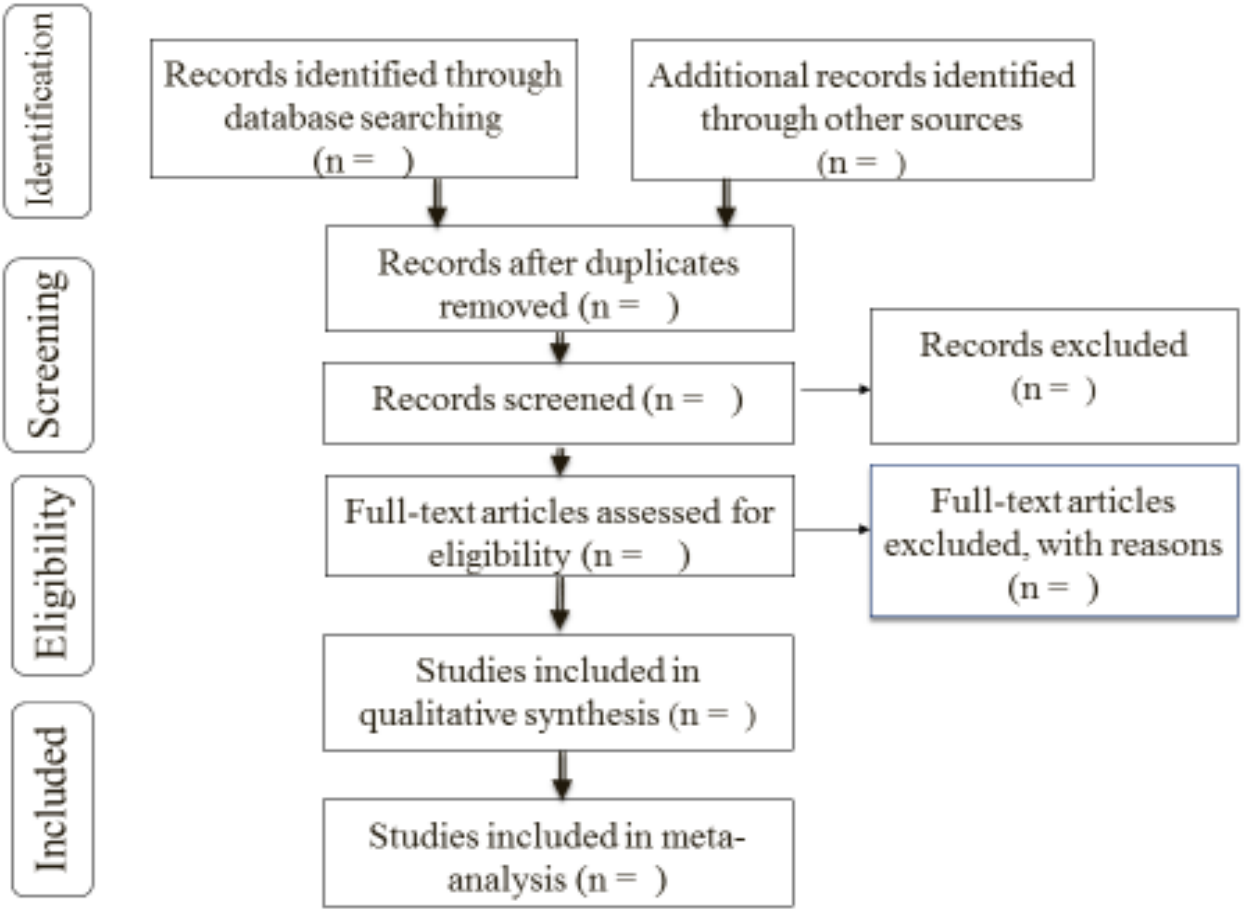
Supplementary 1. PRISMA Flow Diagram



Supplementary appendix 2.

("CoQ 10" OR CoQ10 OR Ubidecarenone OR "co-enzyme Q10" OR "ubiquinone Q10" OR "Bio-Quinone Q10" OR 2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone OR "ubisemiquinone radical" OR Q-ter OR ubisemiquinone OR ("coenzyme Q10" AND (Z,Z,Z,Z,Z,Z,E,E,E)-isomer) OR ("coenzyme Q10" AND "ion (1-)" AND (all-E)-isomer) OR "2, 3 dimethoxy 5 methyl 6 decaprenylbenzoquinone" OR caomet OR "coenzyme 910" OR "coenzyme q 10" OR "coenzyme Q10" OR decorenone OR mitocor OR neuquinone OR "quinone q 10" OR ubimaior OR "ubiquinone (10)" OR "ubiquinone 10" OR "ubiquinone 50" OR ubiten) AND (("26 k protein" OR "b cell stimulating factor 2" OR "B cell stimulatory factor 2" OR "b lymphocyte stimulating factor 2" OR "beta 2 interferon" OR "beta2 interferon" OR "bsf 2" OR bsf2 OR "hepatocyte stimulating factor" OR "il 6" OR "interferon beta 2" OR "interferon beta2" OR "interleukin b" OR "interleukin hp1" OR interleukin-6 OR "liver cell stimulating factor" OR "plasmacytoma growth factor" OR "protein 26k" OR "Interleukin 6" OR "B-Cell Differentiation Factor" OR "B Cell Differentiation Factor" OR "B-Cell Differentiation Factor-2" OR "B-Cell Stimulatory Factor 2" OR "B-Cell Stimulatory Factor-2" OR BSF-2 OR ("Differentiation Factor" AND B-Cell) OR ("Differentiation Factor" AND "B Cell") OR ("Differentiation Factor-2" AND B-Cell) OR ("Differentiation Factor 2" AND "B Cell") OR "Hepatocyte-Stimulating Factor" OR "Hybridoma Growth Factor" OR ("Growth Factor" AND Hybridoma) OR "IFN-beta 2" OR IL-6 OR IL6 OR MGI-2 OR "Myeloid Differentiation-Inducing Protein" OR ("Differentiation-Inducing Protein" AND Myeloid) OR "Myeloid Differentiation Inducing Protein" OR ("Growth Factor" AND Plasmacytoma) OR "B Cell Stimulatory Factor-2") OR ("mhr 24" OR "TNF alfa" OR "TNF alpha" OR "tumor necrosis factor alfa" OR "tumor necrosis factor-alpha" OR "tumour necrosis factor alfa" OR "tumour necrosis factor alpha" OR "tumour necrosis factor-alpha" OR "Tumor Necrosis Factor alpha" OR "Cachectin-Tumor Necrosis Factor" OR "Cachectin Tumor Necrosis Factor" OR TNFalpha OR TNF-alpha OR "Tumor Necrosis Factor" OR "Tumor Necrosis Factor Ligand Superfamily Member 2" OR ("TNF Superfamily" AND "Member 2") OR Cachectin)) AND 1990/01/01:2017/01/15 [dp]

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	1
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	1
Support:			
Sources	5a	Indicate sources of financial or other support for the review	1
Sponsor	5b	Provide name for the review funder and/or sponsor	1
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	5
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5, 6
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	6
Study records:			

Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	10

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

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Can Coenzyme Q10 Supplementation effectively reduce human tumor necrosis factor-α and interluekine-6 levels in chronic diseases? Protocol for a systematic review and meta-analysis of randomized controlled trials

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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	Tumor necrosis factor-α, Interleukin-6, Coenzyme Q10, Inflammatory response, Cytokines

Can Coenzyme Q10 Supplementation effectively reduce human tumor necrosis factor-α and interluekine-6 levels in chronic diseases? Protocol for a systematic review and meta-analysis of randomized controlled trials

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Review registration number: CRD42016052200

Contributions: AA, MV, AK and LJ were responsible for the systematic review protocol design process and formulation the research question for this work. FF and JH searched the electronic databases and reviewed the collected data. NM and PI participated in the assessment of full text papers and data collection.

Amendments: To date, we do not find a previously completed or published protocol.

Support:
Sources: This work does not have access to funding from any funding agency in developing the protocol.

Sponsor: This work does not have any review funder and/or sponsor.

Keywords: Interleukin-6; Tumor necrosis factor-α; Coenzyme Q10; Inflammatory response; Cytokines

Word count: 3666

Abstract

Introduction: Inflammation, as a critical factor, can cause numerous chronic diseases by creating various pro-inflammatory cytokines. Coenzyme Q10 (CoQ10) can potentially exert an anti-inflammatory agent; in turn, this agent can reduce the systemic inflammatory response. The aims of this study are to conduct a comprehensive systematic review, and a meta-analysis for the determination of the CoQ10 efficacy on the changes in serum interluekine-6 (IL-6) and the tumor necrosis factor-α (TNF-α) levels in unhealthy subjects.

Method and analysis: We will conduct an electronic search for articles published between January 1990 and January 2017 using a pre-specified search strategy in MEDLINE, SCOPUS, EMBASE, CENTRAL and Web of Science.

Our search will focus only on randomized controlled clinical trials in unhealthy subjects that employ either a parallel or a crossover design; this search will involve concurrent control groups. The primary outcomes of the literature are to determine the CoQ10 efficacy on the changes in the serum IL-6 and the TNF-α levels in unhealthy subjects. Secondary outcomes such as body mass index, serum adiponectin and high-sensitivity c-reactive protein levels, lipid profile, and the heterogeneity assessment of the primary studies will be evaluated. The stages of screen articles, the extracts of relevant data, and the assessment of study quality using the Cochrane risk of bias tool will be conducted independently by the two reviewers. Any disagreement will be resolved by discussion with a third person. If the number of eligible studies is sufficient, we will carry out a meta-analysis according to both the outcomes.

Ethics and dissemination: This study is the protocol for a systematic review and no ethics approval is needed. The findings from the full systematic review will be published in a peer-reviewed journal, and they will also be exhibited at national/international academic and clinical conferences.

Review registration number: CRD42016052200

Article summary

- The benefits of this systematic review with meta-analysis are because of a comprehensive search strategy, designed to retrieve as many articles relevant to our primary and secondary objectives as possible.
- The evidence highest levels for informed decisions about the role of CoQ10 in chronic disease will be provided from results of this study.
- The protocol of this study has been prepared in accordance with the PRISMA-P guidelines, including description of key methodological steps.
- The main limitation of this study is that the conclusions will be limited by the number and quality of primary studies.
- One limitation of this study is related to published articles in other languages that needs to a translator.

Introduction

Much evidence indicates that systematic inflammation has a major role in many human chronic disease pathogeneses, such as cardiovascular, pulmonary, autoimmune, and degenerative diseases as well as cancer and metabolic diseases (1–3). Elevated levels of Interluekine-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α), as inflammation mediators, are closely linked to numerous chronic diseases (3–5). Due to adverse effects and health problems of existing anti-inflammatory therapies, the usage of natural compounds as antioxidant and anti-inflammatory agents has considered more attention in scientific researches (6).

Coenzyme Q10 (CoQ10), referred to as 'ubiquinone' (7), is composed of a lipophilic benzoquinone structure with a sidechain of 10 isoprenoid units (8). It is endogenously synthesized by the mevalonate pathway in the human body (9) and it is obtained in much of human diet (10). CoQ10 is a critical intermediate of the mitochondrial electron transport chain for the synthesis of adenosine triphosphate (8,11). The biological importance of CoQ10 is related to antioxidant activity, which can scavenge free radicals as well as restore the antioxidant defence system (10). Furthermore, several studies from both in vitro and animal models have suggested that CoQ10 acts as an anti-inflammatory agent, inhibiting the inflammatory response (10) by blocking the expression of NF-κB (11) as well as activating the PPAR mediated anti-inflammatory responses (10).

As previously mentioned, CoQ10 supplementation, a natural dietary antioxidant, possessing anti-inflammatory activity, would be beneficial for numerous human chronic diseases (8–15). However, a recent study by Liu et al. indicated that CoQ10 (300 mg/day) could not significantly decrease the inflammatory cytokines in the progression of the hepatocellular carcinoma after 12 weeks of performing surgery (8). Another recent clinical trial showed that the serum level of TNF-α was significantly reduced in rheumatoid arthritis patients who received capsules of CoQ_{10} (100 mg/day) for 8 weeks, but no significant effect was seen on serum IL-6 concentration (15). Consistent with the results of this study, Farsi et al. observed that CoQ10 supplement at a dose of 100 mg/day could significantly reduce the serum levels of TNF-α ($P = 0.04$) after three months of treatment in patients suffering from non-alcoholic fatty liver disease. IL-6 blood levels, however, had significantly changed after the intervention duration (12). In this regard, Sanoobar et al. examined the efficacy of CoQ10 administration in patients with multiple sclerosis. The researchers observed that the daily intake of CoQ10 supplement at a dosage of 500 mg has favourable effects on the reduction of the plasma inflammatory marker levels (TNF-α, $P = 0.003$, IL-6, $P = 0.03$) after 12 weeks of intervention (9). Overall, on the basis of the available evidence, researchers have reported inconclusive results of CoQ10 supplement effectiveness on TNF-α and IL-6 serum levels (9–16). These discrepancies might be related to the various dosing regimens of CoQ10 and the differences in supplementation duration, a variation of the clinical inclusion criteria in the studied samples as a result of using small sample sizes, and plasma TNF-α and IL-6 levels at the baseline. Despite several clinical trials, the efficacy of CoQ10 on circulating TNF-α and IL-6 levels remains questionable and ambiguous. Based on our knowledge and

understanding, until now, we have found two systematic reviews and meta-analyses of the evidence in relation to the CoQ10 efficacy for changing the pro-inflammatory factors levels (C-Reactive Protein (CRP), IL-6, or TNF-α) (17, 18). Fan et al., in this issue, observed the significant lowering effects of CoQ10 on CRP, IL-6, or TNF-α (17). Another recent review reported that CoQ10 was helpful in significantly decreasing TNF-α levels, but found no substantial change in IL-6 and CRP serum levels (18).

However, they were based on a limited database search and were restricted to studies published in English (17, 18). Therefore, based on the available literature, we believe that a systematic review and meta-analysis is required only for the CoQ10 supplementation efficacy on the concurrent changes in serum TNF-α and IL-6 levels in unhealthy subjects. In order to extend our work, we will review more database and grey literature without setting a language limitation on publications. It, therefore, appears that the results of this study can potentially help determine the net effect of the oral CoQ10 supplementation on the TNF-α and the IL-6 serum levels.

Objectives

The primary objective of this study of the literature is, therefore, to evaluate the CoQ10 supplementation efficacy on the changes in serum TNF-α and IL-6 levels in unhealthy subjects.
The secondary aims of our study are as follows:

1- To evaluate CoQ10 supplementation efficacy on Body Mass Index (BMI) in comparison to the control group in unhealthy subjects.
2- To evaluate CoQ10 supplementation efficacy on the changes in serum adiponectin concentrations in comparison to the control group in unhealthy subjects.
3- To assess CoQ10 supplementation efficacy on the changes in serum high-sensitivity C-Reactive Protein (hs-CRP) levels in comparison to the control group in unhealthy subjects.
4- To determine and summarize the evidence about the efficacy of CoQ10 supplementation to reduce the lipid profile (Low-density lipoprotein (LDL), cholesterol, and triglyceride (TG)) in comparison to the control group in unhealthy subjects.
5- To investigate the heterogeneity assessment of primary studies and other sources.

Method and analysis

The design of this systematic review has been developed according to the recommended details presented on the 2015 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines checklist (19). Moreover, the flowchart of PRISMA will be applied to explain the number of included and excluded primary studies in the different stages of this systematic review (Supplementary Appendix 1). This systematic review protocol has been prepared in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses for Protocols 2015 (PRISMA-P 2015) guidelines (19). This protocol is registered in the international prospective register of systematic reviews (PROSPERO 2016: CRD42016052200; website: http://www.crd.york. ac.uk/PROSPERO).

Eligibility criteria

Study design/characteristics: Primary studies will be included in the systematic review if they contain Randomized Clinical Trials (RCTs) at least single-blind with either parallel or crossover designs, comprising concurrent control groups, which were published in any language between January 1990 and January 2017. Reviewers will exclude methods other than RCTs conducted on humans, such as review articles, case studies, case series, observational studies (cross-sectional, case-control, and cohort), experimental studies with animals or in vitro, and studies on healthy subjects, proceedings, editorials/commentaries, letters as well as reports comprising insufficient data on baseline or follow-up TNF-α and IL-6 in each group. We will exclude studies reporting CoQ10 in combination with other substances.

Subject types: In order to be eligible for inclusion, primary studies will be considered if the tested intervention targets non-athlete adult subjects (≥ 18 years) or at least one of the population subgroups, with various chronic inflammatory conditions or non-communicable diseases (such as diabetes, hypertension, obesity, etc.), and at least one of the two sexes.

Intervention(s): Our target will be RCT studies that used oral CoQ10 supplementation (in capsule form or in any other form) daily in divided dosage or single doses (amount/day) for any intervention duration.

Comparator(s)/control: Studies will be eligible that compared oral CoQ10 supplementation versus control that placebo, standard therapy alone, no intervention and other natural or pharmacological agents will be accepted as controls**.**

Outcome(s): Studies will be included in the review if they report the effect of the intervention on the primary outcomes in terms of serum/plasma TNF-α and IL-6 levels at the baseline and at the end of treatment duration in the study groups.

Secondary outcomes of project: We will also record the results of intervention effectiveness on one or several of the following outcomes if available:

- BMI
- Serum adiponectin and levels
- Serum hs-CRP levels
- Lipid profile measurements (TG, cholesterol, and LDL)
- The heterogeneity assessment of primary studies and other sources

Data sources

Searching electronic bibliographic databases for this systematic review will be conducted by two reviewers between January1990 and January 2017. Bibliographic and electronic databases will be searched using the following assess words within topics and abstracts:

1. Cochrane Central Register of Controlled Trials (CENTRAL)

2. Web of Science
3. Medline (http://www.ncbi.nlm.nih.gov/pubmed)
4. SCOPUS
5. EMBASE

Database of ongoing clinical trials

Registers of clinical trials will be searched for in the following databases:

- www.clinicaltrials.gov
- ISRCTN registry
- www.who.int/trialsearch/

Other resources

To ensure research saturation, the other resources will be manually reviewed to find additional eligible studies such as key journals and the reference list of all the included relevant research articles, meta-analyses, and review publications on CoQ10. In regard to the recommendations of the Institute of Medicine Standards and the Cochrane Handbook for Systematic Reviews of Interventions, we will evaluate some of informally published content in academic sources (the grey literature), including thesis data and abstracts of papers presented at different conferences. For further comprehensiveness, we will contact the authors of these papers by email on the basis of the available abstracts; we will request for the full text if necessary.

Search strategy

The aims of literature search strategies will be to find all the relevant RCTs conducted on humans using an appropriate set of key search terms to delimit the concepts 'Coenzyme Q10', 'IL-6', 'TNF-α' and 'Inflammation'. The search terms of any component of this study were found in the medical subject headings (MeSH) tags of the PubMed database, EMTREE, and a free text word; a combination of these were used to create a proper electronic search strategy. In regard to each database, a search strategy will be adopted. The search strategy and the syntax of the PubMed database are presented in detail in the online supplementary Appendix 2.

Study records

Data management

Two primary researchers (FF and JH) will perform the initial search of the electronic databases using the strategy search and guided by the PRISMA-P statement. FF and JH will also manually review the reference lists of all the included studies. In order to conduct data management, the Endnote X7 software will be used. The main reviewer will import the results of literature searches into an EndNote library and then delete the duplicate records.

Selection process

The selection phase of this systematic review is compliant with the PRISMA guidelines. The selection of relevant studies for inclusion in the review will be performed in a three-step process. The two independent reviewers (FF and PI) will first screen the titles and the abstracts of all the records identified by the database searches in line with the inclusion/exclusion criteria in order to identify a subset of potentially eligible articles. Any discrepancies relating to inclusion in each step of the screen process (title/abstract, and then, full text review) will be resolved by discussion and/or consultation with a third researcher with specific expertise in chronic diseases and CoQ10.

We will then obtain the full texts of potentially eligible articles that appear to meet our inclusion criteria on the basis of their title/abstract. The full-text screening process of the included abstracts will be carried out by two independent reviewers (FF and PI). At each step of the selection process, a record of the reasons behind excluding certain studies will be maintained.

Data extraction

A standard data extraction form designed by the primary reviewer will be used for data extraction from all the selected studies (see online Supplementary Appendix 2). The data extraction form will be pilot tested by our team on three selected studies and will be refined as necessary in order to ensure the reliability of the data extraction process. The two reviewers (FF and NM) will independently extract the information from the included studies. Data extraction will be completed using the full text of the published reports or via correspondence with the study authors if the data provided in the published articles was inadequate to complete the extraction process. The principal investigator involved in the process of data extraction will have practice using the form and will receive appropriate training if deemed necessary. Similarly, any disagreement in the extracted data process which cannot be resolved through consultation will be referred to a third reviewer with specific expertise in order to reach a consensus.

Data items

The Participants, Interventions, Comparisons, Outcomes, Study characteristics (PICOS) criteria will be applied to systematize our information extraction.

- Study characteristics: name of the first author, study design, place and time of the study, country of origin, year of publication, and size of the sample divided into separate groups.
- Participant sociodemographic characteristics: age, ethnicity, sex, number of participants, disease type, and initial healthy status.
- Intervention and their specific: dosage, length of follow-up, type of administration, treatment group sample size, blinding procedure, withdrawals, and dropouts.
- Outcomes: definition and measures of primary (TNF-α and IL-6 levels) and secondary outcomes (BMI, serum adiponectin, hs-CRP levels, lipid profile, heterogeneity assessment in primary studies, and other miscellaneous points).

Outcomes and prioritization

We will consider pooling studies that include TNF-α and IL-6 levels in unhealthy subjects as our primary clinical outcomes and other outcomes as secondary outcomes. Our prioritization will also be given to studies with a RCT design, which examined the effect of CoQ10 supplementation alone under inflammation conditions. Article presentation in the review will be ultimately improved by prioritization of the search strategy items.

Missing data

In order to perform data management in certain conditions set by the Cochrane Institute, our investigators will contact the corresponding authors of the studies by email to obtain clarification if the data provided are incomplete in the study reports. We will have to send reminder emails (up to three times) if the authors do not reply to the initial email. Reviewers will consider the incomplete information as missing data if a response is not received after three emails.

Risk of bias assessment (in individual studies)

Risk of bias assessment of the individual studies will be carried out by two independent review authors (FF, JH) using the guidelines of a tool developed by Cochrane Collaboration to assess and report the risk of bias in the following seven criteria:

1. Sequence generation
2. Allocation concealment
3. Blinding of participants and personnel
4. Blinding of outcome assessment
5. Incomplete outcome data
6. Selective outcome reporting
7. Other potential sources of biases.

Two principal investigators (FF, JH) will first test pilot the Cochrane tool items on three primary articles. In case of disagreement on the risk of bias assessment, consultation with the third person will be employed to reach a resolution. The options of yes, no, or unclear will be used for each component of our chosen domains, and then, the risk of bias will be documented as a risk of bias category from the following: low, unclear, or high. We will then describe reasons for each assessment.

Data synthesis

If the number of eligible studies is an adequate, we will carry out a meta-analysis according to both outcomes. Based on the conditions of the primary studies in terms of methodology, one of the two models (fixed or random effects model) will be used.

A narrative data synthesis of all the included studies after a systemic review will be performed and it will be assigned in a text as well as separate tables. For the purposes of data synthesis, all the data from continuous outcomes analyses will be presented as mean difference or

Standardized Mean Difference (SMD), both with 95% Confidence Interval (CI); the Risk Ratio (RR) index will be calculated for the qualitative and the categorical data.

To investigate clinical and methodological heterogeneity, we will undertake the determination of the feasibility of meta-analysis. For this purpose, we will consider the main sources of heterogeneity including different study design (crossover or parallel and year of publication), population characteristics (gender, ethnicity, age, disease types and stage distribution), duration of follow-up, sampling interval, and test characteristics. The Q Cochrane test will be applied to statistically check the extent of heterogeneity among primary studies as recommended by the Cochrane Handbook for Systematic Reviews of RCT. The I^2 statistic will also be used to determine the extent of heterogeneity between studies and to guide our choice of the model (fixed or random effects model). $I^2 > 50\%$ will be considered as severe heterogeneity. However, if we find substantial heterogeneity and a sufficient number of eligible studies, meta-regression, and subgroup analyses according to gender, BMI, and the sample size, SMD will be conducted in order to identify the heterogeneity of the sources. If some studies are at high risk of bias, we will conduct a sensitivity analysis in order to assess the impact of the methodological quality and the effect of studies with a lower sample size on the power of review conclusions. All data from sensitivity analyses will be presented and summarized in tables. The forest plots will be used for the graphical representation and the final synthesis of primary studies. The Stata (Version 12) software (Stata Corp LP, College Station, Texas, USA) will be used for all the mentioned analyses.

Assessment of possible reporting bias

We will investigate the likelihood of outcome reporting bias (publication and other reporting biases) using funnel plots (i.e., plots of study results against precision) if the number of studies included in a meta-analysis is sufficient (~10 studies). Begg's test and Egger's test will also be used in order to assess for asymmetry. When this is non-negligible, we will use the trim and fill method to correct the results. The statistically significant correlation will be indicated using a p-value of ≤ 0.05.

Ethics and dissemination

This research is a protocol for a systematic review and no ethics approval is needed. The findings from the full systematic review will be published in a peer-reviewed journal and will also be exhibited at national/international academic and clinical conferences.

As previously mentioned, inflammation is recognized as a common cause of numerous chronic disease pathogeneses in humans (17, 18). Among the constituents of pharmacotherapy, as anti-inflammatory medicines or inflammatory blockers are currently being applied in extended ranges, these blockers are highly expensive and have more side effects (1, 18). Hence, a natural component such as CoQ10 is needed, which is not only safe but also is cost-effective and readily

available (17). CoQ10 can also be considered as an important agent for the conservative management of this condition.

The purpose of this systematic review will be comprehensively identified and summarized in studies reporting whether CoQ10 supplementation effectively can reduce serum TNF-α and IL-6 levels in unhealthy subjects. The results of this study will assist future research that studies the anti-inflammatory effect of CoQ10 as a treatment approach in many human chronic diseases. Furthermore, the results of this systematic review with meta-analysis will be important to create awareness among clinicians, therapists, and patients on the topic of CoQ10 effectiveness.

Confidence in cumulative evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) (20) guidelines will be applied in order to assess the quality of the evidence in relation to the effect of CoQ10 on the primary and secondary outcomes. The quality assessment of evidence will be performed based on the following domains: design and risk of bias, consistency, directness, precision, and publication bias.

Contributors: AA, MV, AK, and LJ were responsible for the systematic review protocol design process and the formulation the research question for this work. FF and JH searched the electronic databases and reviewed the collected data. NM and PI participated in the assessment of the full text papers and data collection.

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Competing interests: None declared.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: Results from the completed work will be disseminated through peer-reviewed publications and social networks.

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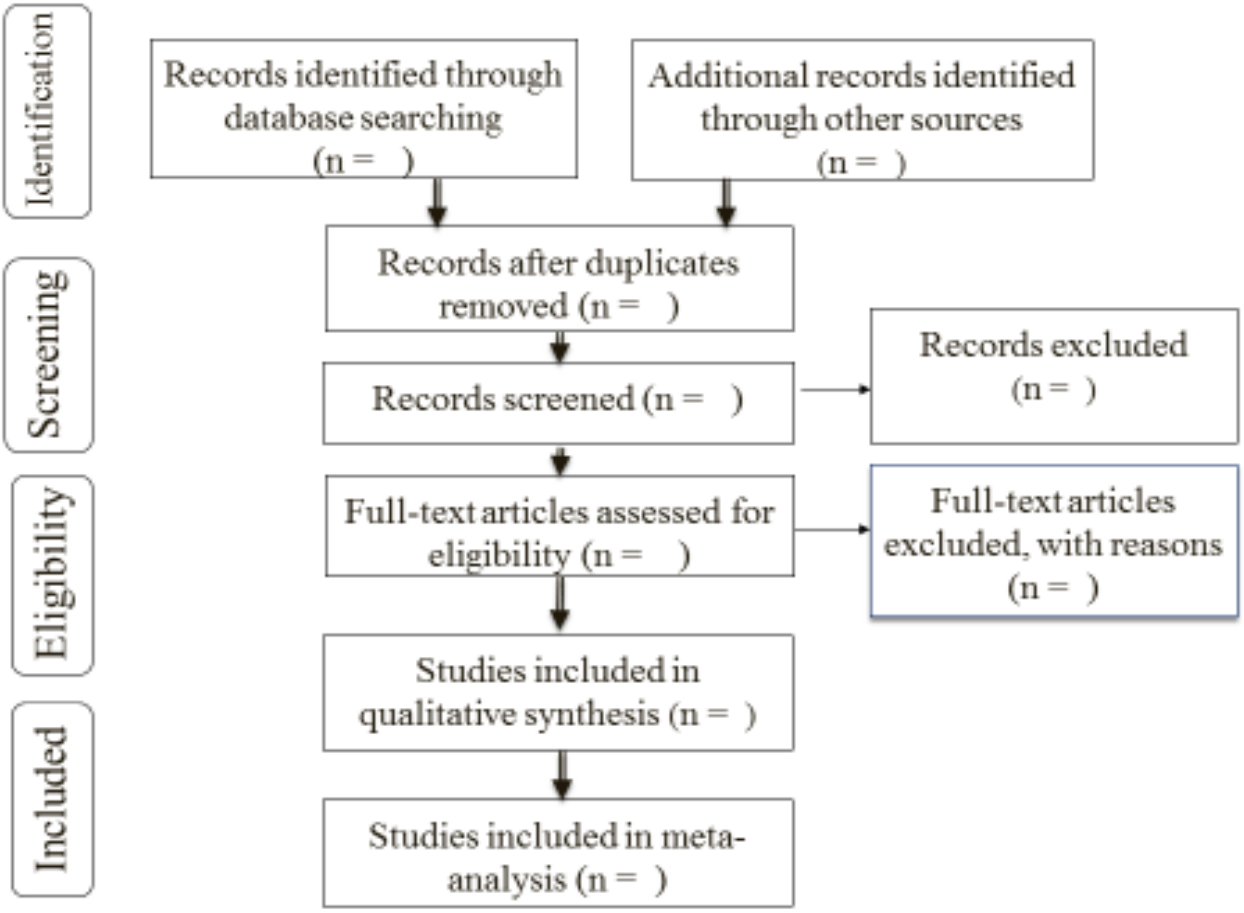
Supplementary 1. PRISMA Flow Diagram



Supplementary appendix 2.

(“CoQ 10” OR CoQ10 OR Ubidecarenone OR “co-enzyme Q10” OR “ubiquinone Q10” OR “Bio-Quinone Q10” OR 2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone OR “ubisemiquinone radical” OR Q-ter OR ubisemiquinone OR (“coenzyme Q10” AND (Z,Z,Z,Z,Z,Z,E,E,E)-isomer) OR (“coenzyme Q10” AND “ion (1-)” AND (all-E)-isomer) OR “2, 3 dimethoxy 5 methyl 6 decaprenylbenzoquinone” OR caomet OR “coenzyme 910” OR “coenzyme q 10” OR “coenzyme Q10” OR decorenone OR mitocor OR neuquinone OR “quinone q 10” OR ubimaior OR “ubiquinone (10)” OR “ubiquinone 10” OR “ubiquinone 50” OR ubiten) AND ((“acute inflammation” OR “bacterial inflammation” OR “inflammation reaction” OR “inflammation response” OR “inflammatory condition” OR “inflammatory lesion” OR “inflammatory process” OR “inflammatory reaction” OR “inflammatory response” OR “inflammatory syndrome” OR (reaction AND inflammation) OR (response AND inflammatory) OR serositis OR “sterile inflammation” OR Inflammations) OR (“26 k protein” OR “b cell stimulating factor 2” OR “B cell stimulatory factor 2” OR “b lymphocyte stimulating factor 2” OR “beta 2 interferon” OR “beta2 interferon” OR “bsf 2” OR bsf2 OR “hepatocyte stimulating factor” OR “il 6” OR “interferon beta 2” OR “interferon beta2” OR “interleukin b” OR “interleukin hp1” OR interleukin-6 OR “liver cell stimulating factor” OR “plasmacytoma growth factor” OR “protein 26k” OR “Interleukin 6” OR “B-Cell Differentiation Factor” OR “B Cell Differentiation Factor” OR “B-Cell Differentiation Factor-2” OR “B-Cell Stimulatory Factor 2” OR “B-Cell Stimulatory Factor-2” OR BSF-2 OR (“Differentiation Factor” AND B-Cell) OR (“Differentiation Factor” AND “B Cell”) OR (“Differentiation Factor-2” AND B-Cell) OR (“Differentiation Factor 2” AND “B Cell”) OR “Hepatocyte-Stimulating Factor” OR “Hybridoma Growth Factor” OR (“Growth Factor” AND Hybridoma) OR “IFN-beta 2” OR IL-6 OR IL6 OR MGI-2 OR “Myeloid Differentiation-Inducing Protein” OR (“Differentiation-Inducing Protein” AND Myeloid) OR “Myeloid Differentiation Inducing Protein” OR (“Growth Factor” AND Plasmacytoma) OR “B Cell Stimulatory Factor-2”**)** OR (“mhr 24” OR “TNF alfa” OR “TNF alpha” OR “tumor necrosis factor alfa” OR “tumor necrosis factor-alpha” OR “tumour necrosis factor alfa” OR “tumour necrosis factor alpha” OR “tumour necrosis factor-alpha” OR “Tumor Necrosis Factor alpha” OR “Cachectin-Tumor Necrosis Factor” OR “Cachectin Tumor Necrosis Factor” OR TNFalpha OR TNF-alpha OR “Tumor Necrosis Factor” OR “Tumor Necrosis Factor Ligand Superfamily Member 2” OR (“TNF Superfamily” AND “Member 2”) OR Cachectin)) AND 1990/01/01:2017/01/15 [dp]

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	1
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	1
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	1
Support:			
Sources	5a	Indicate sources of financial or other support for the review	1
Sponsor	5b	Provide name for the review funder and/or sponsor	1
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	4,5
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5,6
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	6
Study records:			

Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	6
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	6
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7,8
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	8
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	10

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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