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TOCOTRIENOLS, HEALTH AND AGEING: A SYSTEMATIC REVIEW

Tocotrienols, ageing and well-being

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ABSTRACT

Objectives: A systematic review of studies was undertaken to evaluate the potential effect of tocotrienols intake or its circulating levels on parameters associated with successful ageing (i.e., cognitive function, osteoporosis and DNA damage). **Methods**: Following PRISMA guidelines a systematic review of epidemiological observational studies and clinical trials was undertaken. Inclusion criteria included all English language publications in the databases PubMed and Scopus, until end of July 2016.

Results: Evidence from prospective and case-control studies suggested that increased blood tocotrienols levels were associated with favorable cognitive function outcomes. Clinical trial with tocotrienols supplementation for 6 months suggested a beneficial role of tocotrienols intake on DNA damage rate, but only in elderly. Regarding osteoporosis, only *in vitro* studies with human bone cells cultures were identified, demonstrating significant inhibition of osteoclasts and promotion of osteoblasts activity. **Conclusions**: Research in middle-aged and elderly humans suggests potential beneficial anti-ageing action with respect to cognitive impairment and DNA damage, with other data limited to laboratory studies. Therefore, clinical trials are required to further elucidate the potential beneficial effects of tocotrienols on aspects associated with healthy ageing.

Key words: tocotrienols; ageing; osteoporosis; cognitive function; systematic review.

1. INTRODUCTION

The technical and biological definition of ageing initially was set as a time-dependent functional decline that affects almost all living organisms and gradually leads to death. However, the ageing phenomenon and phenotype in humans has been proved to be far more complex (1). Therefore, despite the fact that specific biological processes have been recognized to promote ageing, there is still a lack of widely accepted ageing factors and markers (2). Academic interest in ageing has grown as a necessary response to the doubling in the global population of the elderly over the past 50 years. Public health frameworks have also identified well-being, active and healthy ageing as their primary goals, aiming to reduce the burden of age-related morbidity, including osteoporosis, Alzheimer's Disease (AD) and dementia (3). Relatively recent scientific advances in biomedical field have allowed for a range of biomarkers to be proposed to assess biological age including DNA damage (methylation), renal function, growth hormones and inflammatory markers (4).

It has been proposed that a number of nutrients that can interact in a potentially synergistic manner to improve biomarkers associated with ageing, and as such these nutrients have been suggested as potential ageing inhibitors (5). Among several of these, vitamin E was considered one of the potent antioxidants especially in lipid systems, and as such via this mechanism had the potential to delay the ageing process (6). Apart from tocopherols (a vitamin E subtype), tocotrienols also have evoked research interest linked to the potential with respect to cellular protection (7, 8), neuroprotective actions (9, 10), anti-inflammatory effects (11) and also as antiosteoporotic agents per se (12, 13) or combined with statins (14). Unfortunately, the majority of the studies to date, have been limited to animal models and cell

cultures (15, 16), impeding the validity of applying any recommendations with respect to tocotrienols for humans with respect to healthy ageing.

The aim of this systematic review was to provide an analysis of the published data to assess the effect of tocotrienols on age-related conditions including cognitive function impairment, osteoporosis and DNA damage rates in middle-aged men and women.

2. METHODS

The literature search, data extraction and systematic review were undertaken following the principles of the PRISMA 2009 guidelines (17).

2.1 Eligibility Criteria

Studies with samples consisting of men and/or women aged over 50 years that had either received tocotrienols' supplementation or the intake of tocotrienols had been measured either as blood circulating levels or as dietary intake of tocotrienols.

2.2 Information sources and search strategies

Original research studies published in English until July 31, 2016 were selected following a computer-assisted literature search of two online databases (PubMed, Scopus). Computer searches used combinations key words relating to the aim of the paper, i.e. "tocotrienol", "ageing", "aging" and "human". In addition, the reference list of the retrieved articles was used to find relevant to the present articles that were not allocated through the searching procedure. The initial search resulted in 67 unique entries across both databases. The relevance of studies was assessed by using a hierarchical approach based on: title, abstract, and the full manuscript.

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2.3 Study selection

Predefined research methodology was used by two authors (ENG and NN) who screened all the abstracts that were identified following the application the search criteria described before. The full-texts of the articles that matched the eligibility criteria presented above, were read in order to reach the final number of the study that met the inclusion criteria.

2.4 Study quality

Exclusion criteria for the clinical trials included lack of randomization, lack of control group and non-human studies. Observational studies and clinical trials not published in English and studies that performed a post-hoc analysis of previous published studies, already selected for this review, were also excluded.

2.5 Data items

The recorded aspects included the study design, the number of the participants, the exposure and the outcome for prospective studies, the duration of follow-up, the intervention and the placebo for clinical trials and the measuring way of tocotrienols intake.

2.6 Data collection process

As presented in *Figure 1*, of the initial 67 studies that were extracted from the electronic databases, only 7 studies (3 observational studies, 1 clinical trial and 3 in vitro studies with human bone cells) were included on the basis of the title or abstract (i.e., relevant research hypothesis studied).

[Figure 1]

3. RESULTS

Cognitive function

Case-control studies

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A cross-sectional study, that derived a sub-group consisting of 168 patients with AD, 166 with Mild Cognitive Impairment and 187 controls from a multicenter European study, assessed the cognitive function among elderly using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and other validated tools and revealed that the plasma tocotrienols levels are inversely linked with cognitive function. More specifically, serum total tocotrienols were significantly higher in controls as compared to AD patients (118 vs. 91 nM/mmol cholesterol, $p<0.05$). When this data was further analyzed using a multivariable analysis adjusted for age, gender, educational status, APOE genetic profile, residential location and disease duration, being in the highest tertile of tocotrienols levels was associated with almost 95% lower odds of having AD as compared with lowest tocotrienols tertiles (Odds Ratio (OR)=0.06, 95% Confidence Intervals (CI): 0.02-0.21). The authors suggested that α-tocotrienol prevents regulates cell death and prevents neurodegeneration. Strengths of this study were the assessment of several types of vitamin E, which provides safe findings results for the effect of tocotrienols, but its cross-sectional design cannot provide causal associations (18).

Prospective studies

The first prospective study which evaluated the impact of circulating tocotrienols' levels in a Swedish cohort at risk of developing AD risk was reported in 2010. The study sample consisted of 232 AD-free older adults (aged >80 years of age) who were part of the Kungsholmen Project. At baseline examination, all the circulating vitamin-E subtypes were measured, including tocotrienols. During the 6-year follow-up period the individuals who were in the highest tertile of circulating total tocotrienols had reduced risk of developing AD as compared to those in the lowest tertile (Hazard Ratio (HR)=0.46, 95% CI: 0.23-0.92), even after adjusting for

potential confounders. However, when the different vitamin E subtypes were separately evaluated, no significant association was detected between different tocotrienol types and 6-year AD risk (HR for α-tocotrienol=0.70, 95% CI: 0.44-1.11, HR for beta-tocotrienol=0.69, 95% CI: 0.45-1.06). The authors suggested that it is the total amount and combination of tocotrienol types that affect the cell metabolism, which is linked to cognitive function, not the specific type of tocotrienols. The latter raised important questions concerning the translation of those findings in dietetic advice and supplementation guidance, which still remain unknown. Unfortunately, no information regarding vitamin E dietary intake was obtained, thus, there is no information regarding the link between serum tocotrienol levels, dietary intake and AD risk (19).

The second prospective study in this area, conducted by the same research group in 140 Finnish older adults, over an 8-year follow-up period and assessed the role of several vitamin E types on the development of cognitive impairment in AD-free participants. At baseline, the individuals who developed cognitive impairment has significantly lower levels of circulating beta-tocotrienol (adjusted for cholesterol levels) (mean standard ± deviation=8.3 ± 2.0 vs. 9.1 ± 2.3, p=0.028), as well as lower levels of γ-tocotrienol (46.8 ± 7.8 vs. 51.8 ± 9.6, p=0.001). However, after adjusting for age, gender, education level, APOE status, Body Mass Index, smoking, alcohol intake, and cardiovascular disease history, being in the highest tertile of circulating tocotrienols was not associated with 8-year cognitive impairment risk (all p-values>0.05). With only the γ-tocopherol levels being inversely associated with cognitive impairment risk (adjusted HR=0.27, 95% CI: 0.10-0.78). The authors concluded that there could be a potential association between vitamin E and cognitive function in elderly individuals, however, the study sample was small and no power

calculation (either a priori or post hoc) was undertaken performed. Although adjustments for alcohol and smoking were undertaken to attempt to control for confounders, no dietary habits were taken into account so this could be seen to be a significant risk of bias(20).

DNA damage

Clinical trials

A small number of in vitro studies have been conducted to investigate several roles of vitamin E on cell metabolism (i.e., angiogenesis, apoptosis, immuno-suppression, etc) with these providing positive data (21). However, only one randomized, double-blinded clinical trial has been performed in elderly humans, which was undertaken in Malaysia. Sixty-four individuals were randomly assigned to receive either a 74% tocotrienol vitamin E supplement (160 mg/day), or a placebo capsule for six months. The study excluded smokers, those with significant disease, those taking medication, alcohol and/or other supplements. Information on the socio-economic characteristics was obtained together with their physical activity levels and dietary habits. DNA damage was measured through the total DNA damage score as proposed by Kobayashi et al. (22). Total DNA damage decreased significantly among the intervention group ($p<0.001$), but not in the placebo group ($p>0.05$). Additionally, the decrease in DNA damage appeared to be greater in the sub-group of older individuals (>50 years of age), $p<0.001$. The authors suggested that the protective effect of tocotrienols supplementation could be mainly attributed to its antioxidant properties and signaling properties, which lead to either lower DNA damage rate, or higher DNA repair rate. Despite the apparent efforts that the study researchers took to assess a range of several lifestyle confounders, no information was given on whether the two

groups differed in terms of lifestyle characteristics, such as physical activity, overall diet quality or vitamin E intake from food, were not taken into account (23).

Osteoporosis

The majority of the published research studying the effect of tocotrienol on bone metabolism has been undertaken in animal models (21, 24, 25), thus, it is out of the scope of the present review. To date are only three studies that investigated the impact of tocotrienol on human bone cell metabolism were found, with all of them being reports of in vitro studies. Brooks et al. in 2011 investigated the potential of vitamin E to prevent bone loss. Specifically, they compared different vitamin E homologues (tocotrienols isomers) to clinically used medication (biophosphonate and pamidronate) on human osteoclasts cultures. This demonstrated that osteoclastic resorption and γ-tocotrienol was found to inhibit osteoclast activity but not osteoclasts formation. Contrarily, α-tocotrienol was found to affect both activity and formation and thus could be defined toxic. The authors suggested that there is a potential anti-resorptive action of γ-tocotrienol that warrants further investigation (26). A further study assessed the role of tocotrienols on human osteoclasts cultures was published in the same year and suggested biochemical pathways through which these vitamin E homologues act. Specifically, α-tocotrienol when added in osteoclasts cultures inhibited the ability of Receptor of NF-kB ligand (RANKL) to induce osteoclast differentiation. This suggested that α-tocotrienol can inhibit RANKL expression in osteoblasts probably through Prostagladin-E2 downstream molecules. Finally, α-tocotrienol inhibits bone-resorbing activity of mature osteoclasts, which is attributed to its antioxidants capacity, which is unlikely to be reproducible in in vivo human studies. All those actions were only described for tocotrienols, but not for tocopherols, suggesting that tocotrienols could be in a pivotal point for bone metabolism (27).

Following the aforementioned suggestions for the antioxidant capacity of tocotrienols on bone metabolism, Nizar et al. evaluated the protection induced by tocotrienols against oxidative stress in bones' osteoblasts. The measuring outcome was cell viability after exposure to Hydrogen Hyperoxide. The study findings supported an important dose-related action, which could be toxic against osteoblasts in high γ-tocotrienols concentrations, whilst in moderate concentrations was proved to protect against oxidative stress and thus, protect against unfavorable bone metabolism (28).

4. DISCUSSION AND CONCLUSIONS

4.1 Main findings

There is growing evidence in the published literature to support the potential role of tocotrienols in healthy ageing. Specifically, case-control and prospective studies that evaluated the association between blood tocotrienols levels and cognitive function (Alzheimer's disease and cognitive impairment) have revealed that increased circulating tocotrienols' levels are associated with a protective effect against cognitive impairment and reduced risk of developing Alzheimer's disease. Beneficial effects on biomarkers, has been studied in a clinical trial which investigated the effect of 6-months tocotrienols' supplementation on DNA damage rate. This study found a significant decrease in DNA damage in the intervention group, but not for the control group. A potential explanation of these associations could be the strong antioxidant activity of tocotrienols, since oxidative stress (i.e., imbalance of reactive species generation and innate antioxidant defense) can lead to neurodegenerative disorders and DNA damage (10). However, a mediator that could possibly explain the reduced risk of cognitive impairment offered by tocotrienols, is the Nuclear Factor kappa-B. This factor regulates genes linked to inflammation, cell cycle and apoptosis and its

dysregulation is implicated in unfavorable inflammatory responses (11), including ageing.

With respect to osteoporosis, to date only bone cells cultures have been published for the role of tocotrienols in human osteoporosis, which showed a potential protective effect. To date, a clinical trial has been registered in 2014, is closed to reporting but has not yet been published (29). The in vitro studies the potential beneficial effects was proposed to be via the inhibition of osteoclasts with a simultaneous promotion of osteoblasts action (21). Potential in concordance with the data on reduced DNA damage, tocotrienol may be working in these in vitro experiments by the modification of the receptor activator of Nuclear Factor kappa-B against the genesis of osteoclasts, which is one half of the tocotrienols' antiosteoporotic action. The other half is the antioxidant capacity, which prevents oxidative damage and protects osteoblast from early apoptosis which is plausible in vitro studies(12). It has been suggested that tocotrienols, if co-administered with statins could have a synergistic effect against osteoporosis, without reaching intolerable dosage for both agents (14, 16, 25, 30).

4.2 Study limitations

The majority of the reviewed studies assessed tocotrienols' intake through the circulating tocotrienols levels rather than using supplementation dosage or dietary intake of the vitamin. To date, there is still a near complete lack of data to define what level of tocotrienols' intake could lead to the potentially protective circulating levels of this agent in order to translate this finding into a useful consumer or clinical recommendation. A key limitation of the data is that important confounders such as depressive symptomatology, physical activity, genetic predisposition, diet quality, smoking and other socio-economic parameters have not been taken into account.

4.3 Further research

Well designed, larger controlled studies, which are able to assess a wide variety of potential confounders, are needed to confirm the presented results. Future studies should also include pharmacokinetic trials of individual tocotrienols and their combination in order to provide insights into safe dosages and systemic absorption rates of these compounds. To gain a broad and full understanding a combination of further in vitro and epidemiological studies are needed to develop hypotheses and mechanisms, whilst clinical trials are needed to provide insights into the dosage needed to achieve the protective effects, as well as the best subtype tocotrienols.

Contributors

DBP and ENG wrote the paper. NN performed the research and reviewed the paper. DDM reviewed the paper.

Competing interests

None.

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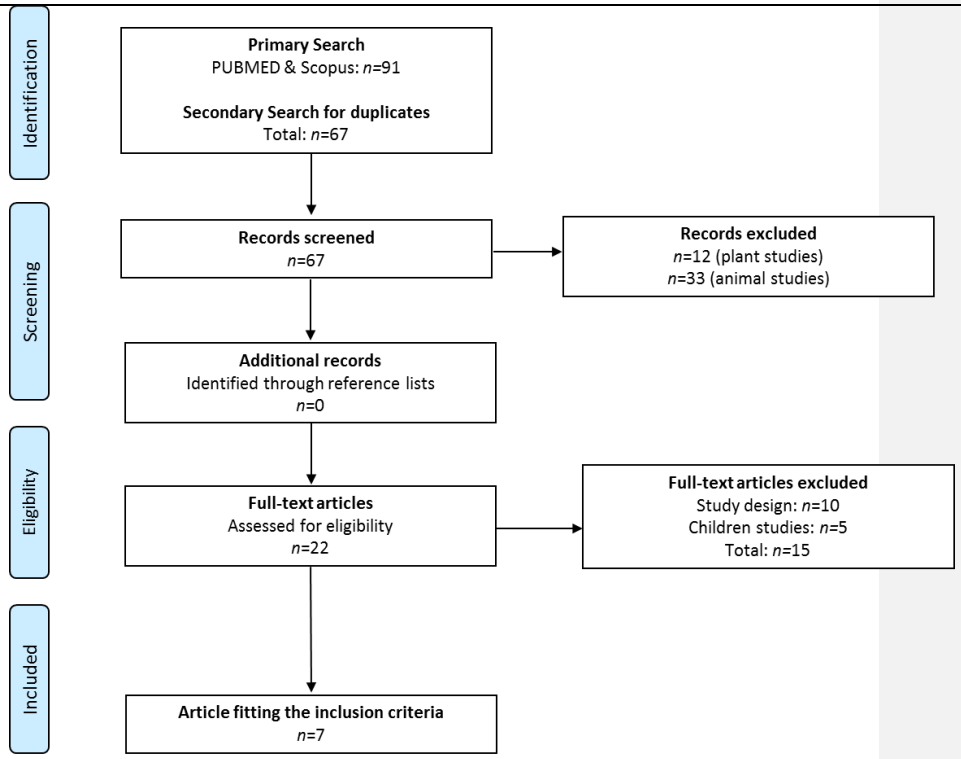

Figure 1. PRISMA flowchart showing the inclusion and exclusion of studies in the systematic review.

Table 1. Characteristics of epidemiological observational studies (n=10) and clinical trial (n=1) that evaluated the effect of tocotrienols intake diet on ageing (HR: Hazard Ratio; OR: Odds Ratio; CI: Confidence Interval).

Study	Country, year	N	Age, Sex	Exposure	Outcome	Results
Prospective studies (*n=2*)						
Mangialasche F. et al. (19).	Sweden, 2010	232	>80 years of age males and females	Blood tocotrienols	6-year Alzheimer's Disease risk	HR for alpha-tocotrienol=0.70, 95%CI: 0.44-1.11
Mangialasche F. et al.(20)	Finland, 2013	140	>80 years of age males and females	Blood tocotrienols	8-year cognitive impairment risk	HR for gamma-tocotrienol=0.27, 95%CI: 0.10-0.78
Cross-sectional studies (*n=1*)						
Mangialasche F. et al. (18).	Sweden, 2012	187 controls 166 Mild Cognitive impairment	>80 years of age males and females	Blood tocotrienols	Mild cognitive impairment	OR 3rd to 1st tertile=0.06, 95% CI: 0.02-0.21.
Clinical trial	**Country, year**	**Intervention**	**Age, Sex**	**Duration**		**Results**
Chin et al. (23).	Malaysia, 2008	74% tocotrienol supplement (160 mg/day) vs. placebo capsule	32/32 Males and females	6 months		For subjects >50 years of age: Total DNA damage decreased significantly among the intervention group ($p<0.001$), but not in the placebo group ($p>0.05$).