# The impact of organic diet intervention on antioxidant capacity and biomarkers of inflammation and oxidative stress: A systematic review

ORGANIKO LIFE+ project

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# Executive summary

## Purpose

The purpose of this report is to review existing literature regarding the effects of organic diet intervention on antioxidant capacity and biomarkers of inflammation and oxidative stress to support preparatory and ongoing work for designing a sound methodology for the data analysis of the children's health intervention trial (field trial) which is conducted under the Activity C3.1 of the ORGANIKO LIFE+ project named "Organic diet and children's health".

## Outcomes

The following outcomes have been reviewed with regards to an organic diet intervention treatment: antioxidant capacity, biomarkers of oxidative stress, antioxidants and biochemical markers associated with inflammation and oxidative stress.

## Results

The existing evidence from human intervention trials is not sufficient to support an association between the systematic organic food consumption and beneficial effects to human health.

## Conclusions

The lack of scientific evidence for a strong linkage between organic diet and health biomarkers of clinical or subclinical symptoms calls for the implementation of human studies with the following characteristics: (a) randomized controlled trial (b) sufficient sample size (c) exposure to organic diet as a whole (d) biomonitoring of selected pesticides and health biomarkers (e) long enough window of intervention and (f) adherence to standardized reporting of randomized controlled trials.

# Σύνοψη

## Σκοπός

Ο σκοπός αυτής της αναφοράς είναι η ανασκόπηση της υπάρχουσας βιβλιογραφίας σχετικά με τις επιπτώσεις των παρεμβάσεων βιολογικής διατροφής στην αντιοξειδωτική ικανότητα και σε βιοδείκτες φλεγμονής και οξειδωτικού στρες, έτσι ώστε να στηρίξει τις προπαρασκευαστικές και συνεχιζόμενες εργασίες για το σχεδιασμό μιας ορθής μεθοδολογίας για την ανάλυση των δεδομένων της παρέμβασης για την παιδική υγεία (δοκιμή πεδίου) που θα διεξαχθεί στο πλαίσιο της Δράσης C3.1 του έργου ORGANIKO LIFE + με τίτλο "Βιολογική διατροφή και υγεία παιδιών".

## Αντίκτυπος

Οι παρεμβάσεις βιολογικής διατροφής έχουν δείξει να επιδρούν στα ακόλουθα: αντιοξειδωτική ικανότητα, βιοδείκτες οξειδωτικού στρες, αντιοξειδωτικά, βιοχημικούς δείκτες που σχετίζονται με φλεγμονή και οξειδωτικό στρες.

## Αποτελέσματα

Τα υπάρχοντα στοιχεία δεν είναι επαρκή για να δείξουν ότι υπάρχει σχέση μεταξύ της συστηματικής κατανάλωσης βιολογικών τροφίμων και θετικών επιδράσεων στην υγεία.

## Συμπεράσματα

Η έλλειψη επιστημονικών στοιχείων που να αναδεικνύουν τη σχέση μεταξύ βιολογικών τροφίμων και βιοδεικτών υγείας απαιτεί την διεξαγωγή μελετών με τα ακόλουθα χαρακτηριστικά: α) τυχαιοποιημένη ελεγχόμενη δοκιμή β) επαρκές μέγεθος δείγματος γ) έκθεση σε βιολογικά τρόφιμα ως σύνολο διατροφής δ) παρακολούθηση επιλεγμένων φυτοφαρμάκων και βιοδεικτών υγείας ε) επαρκή διάρκεια παρέμβασης και στ) τήρηση γενικών κατευθυντήριων οδηγιών για την τυποποιημένη αναφορά τυχαιοποιημένων ελεγχόμενων δοκιμών.

## Introduction

Organic food consumption has increased during the last years, despite of the economic crisis and the higher price of the organic products (Katsarova 2015). Over the past 30 years, worldwide sales of organic foods have grown to over €66 billion in 2013. It is generally accepted that organic farming benefits the environmental sustainability; however, there is a scarcity of publications showing that consumption of organic food is associated with better health.

Organic farming is a farm management system that respects the nature's systems and cycles and promotes biodiversity. One of the basic principles in which organic production relies upon is the severe restriction of chemical pesticides. Exposure to pesticides was shown to be associated with genotoxicity, neurologic effects, reproductive effects and human chronic diseases, including cancer, diabetes, cardiovascular and kidney diseases (Sanborn et al. 2007; Bassil et al. 2007; Mostafalou & Abdollahi 2013). A proposed mechanism through which pesticides are implicated in human disease is by the induction of oxidative stress, which can lead to the production of pro-inflammatory enzymes thus resulting to increased inflammation (Abdollahi et al. 2004; Mostafalou & Abdollahi 2013; Soltaninejad & Abdollahi 2009).

A number of studies have shown decreased urinary pesticides metabolites after consumption of organic diet (Curl et al. 2003; Lu et al. 2006; Lu et al. 2008; Oates et al. 2014; Bradman et al. 2015). It is expected that an organic diet with low or no pesticides residues would affect the biomarkers of antioxidant capacity, oxidative stress and inflammation. The current systematic review aims to synthesize and assess the published literature on the effect of organic diet interventions on antioxidant capacity and markers of inflammation and oxidative stress in humans. We focused on intervention studies because of their ability to evaluate direct impact of exposure on specific outcomes (Thiese 2014). There are four main types of interventional study designs and these are pre-post study design, non-randomized trial, randomized controlled trial and crossover randomized controlled trial with randomized controlled trial being the strongest interventional study design because allocation bias and confounding of unknown variables are minimized.

## Methods

### Protocol

A review protocol exists in the PROSPERO International prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016041451).

### Search method

A preliminary electronic search using the PubMed database was performed to gather published work on the association between organic diet and markers of oxidative stress and inflammation and antioxidant capacity. The search strategy was developed using Medical Subject Heading (MeSH) terms. The complete search on PubMed was between 1 January 1958 and 10 June 2016 and the search terms were the following: organic[All Fields] AND ("humans"[MeSH Terms] OR "humans"[All Fields] OR "human"[All Fields]) AND (("food"[MeSH Terms] OR "food"[All Fields]) OR ("diet"[MeSH Terms] OR "diet"[All Fields]) OR ("nutritional status"[MeSH Terms] OR ("nutritional"[All Fields] AND "status"[All Fields]) OR "nutritional status"[All Fields] OR "nutrition"[All Fields] OR "nutritional sciences"[MeSH Terms] OR ("nutritional"[All Fields] AND "sciences"[All Fields]) OR "nutritional sciences"[All Fields])) AND (("health"[MeSH Terms] OR "health"[All Fields]) OR oxidation[All Fields] OR oxidative[All Fields] OR ("antioxidants"[Pharmacological Action] OR "antioxidants"[MeSH Terms] OR "antioxidants"[All Fields] OR "antioxidant"[All Fields])). Titles and abstracts were scanned for relevance and relevant articles were screened for eligibility. Bibliography lists and authors of relevant articles were searched to identify further eligible studies.

### Selection of studies

Peer-reviewed English-language human intervention studies were eligible for inclusion if they reported results on antioxidant capacity or markers of oxidative stress or markers of inflammation resulting from intervention with organic diet or organic food items in comparison with conventional diet. *In vitro* and animal studies were excluded.

### Data extraction

One author extracted data from the included studies for assessment of study quality and evidence synthesis and another author independently repeated the search and the extracted information was checked. Extracted information included study design; study population and participant demographics; details of the intervention/exposure and control conditions; study methodology; markers; outcomes and times of measurement.

### Data synthesis

Due to the range of different outcomes measured across the small number of existing field trials, a systematic meta-analysis was not justified. A narrative synthesis of the findings from the included studies is provided, structured around the intervention type, intervention duration, exposure details, study population, outcome type, samples number and main results.

## Results

The search strategy in PubMed identified 4068 unique records, of which 4054 were excluded because they were irrelevant (n=3999), including animal studies, *in vitro* studies (n=36) and reviews (n=19) (Figure 1). The potentially relevant articles (n=14) were assessed for eligibility; Four articles were excluded because they were not interventions (Prado et al. 2010; Hoefkens et al. 2010), did not studied relevant biomarkers of oxidative stress and inflammation or antioxidant capacity (Mark et al. 2013) and compared the consumption of organic food items (Whittaker et al. 2015). The search of reference lists and authors of the selected ten articles identified two additional relevant studies (Di Daniele et al. 2014; Akçay et al. 2004). A total of twelve articles were included in this review (Table 1).

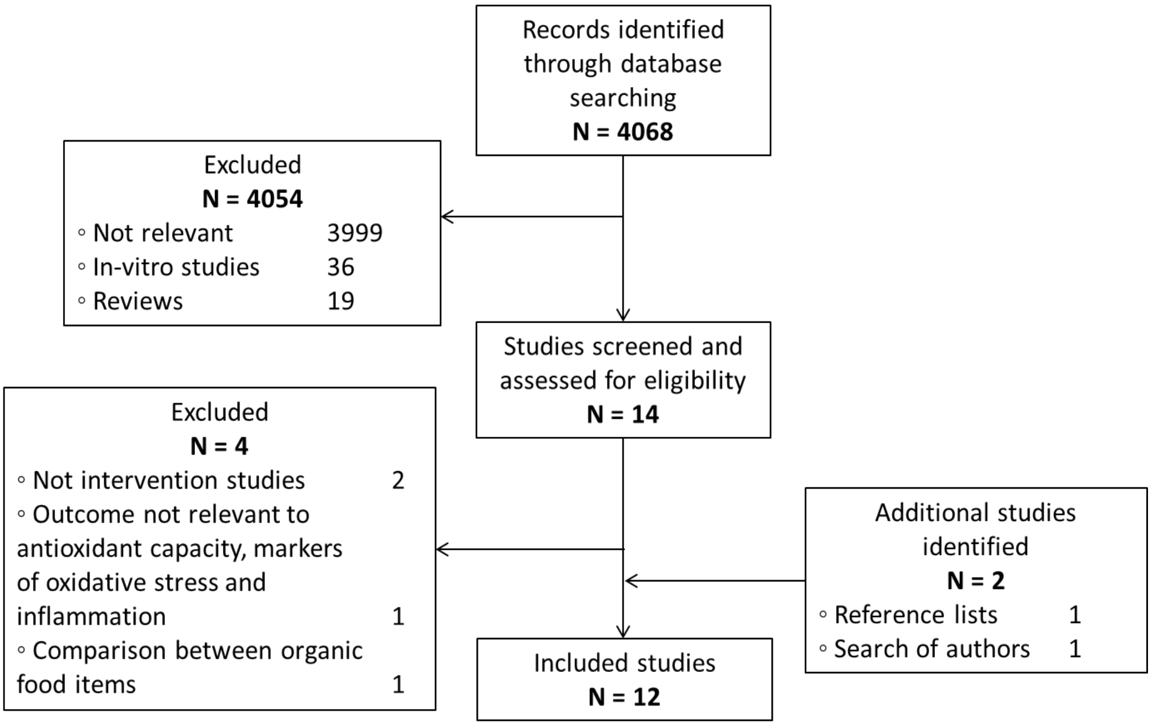


**Figure 1** Flow chart of the study selection process of available articles of organic diet intervention effects on human antioxidant capacity and markers of inflammation and oxidative stress

### Overview of studies characteristics

The included studies were both randomized and non-randomized trials (five randomized & seven non-randomized studies) and their designs included cross-over trials (Grinder-Pedersen et al. 2003; Briviba et al. 2007; Caris-Veyrat et al. 2004; Søltoft et al. 2011; Akçay et al. 2004; Di Renzo et al. 2007; De Lorenzo et al. 2010; Di Daniele et al. 2014), controlled trials (Stracke et al. 2010; Stracke et al. 2009) and intervention (pre-post) trials (Gonçalves et al. 2011; Alleva et al. 2016).

The number of participants in the studies was relatively small; in all studies, the sample size was less than 50 adults with the exception of one study, where there were 130 participants (De Lorenzo et al. 2010). In most of the studies, the study population was healthy whereas in 2 articles, chronic kidney disease patients were also included (De Lorenzo et al. 2010; Di Daniele et al. 2014). The majority of studies examined the effect of specific organic food products, such as apples (Briviba et al. 2007; Stracke et al. 2010), tomato puree (Caris-

Veyrat et al. 2004), wine (Akçay et al. 2004), grape juice (Gonçalves et al. 2011), honey (Alleva et al. 2016) and carrots (Stracke et al. 2009). A total of only five studies examined the effect of organic diet as a whole (Grinder-Pedersen et al. 2003; Søltoft et al. 2011; De Lorenzo et al. 2010; Di Renzo et al. 2007; Di Daniele et al. 2014).

The duration of intervention period was relatively short; it varied from short periods of 1 day (Briviba et al. 2007; Akçay et al. 2004) to 12-15 days (Søltoft et al. 2011; Di Renzo et al. 2007; De Lorenzo et al. 2010; Di Daniele et al. 2014; Stracke et al. 2009; Alleva et al. 2016) and to 20-28 days (Grinder-Pedersen et al. 2003; Caris-Veyrat et al. 2004; Stracke et al. 2010; Gonçalves et al. 2011).

In most studies, there were two intervention periods with either having a wash-out period in between (Grinder-Pedersen et al. 2003; Briviba et al. 2007; Akçay et al. 2004), or without a wash-out period (De Lorenzo et al. 2010; Di Renzo et al. 2007; Di Daniele et al. 2014). Five studies included one intervention period (Caris-Veyrat et al. 2004; Gonçalves et al. 2011; Alleva et al. 2016; Stracke et al. 2009; Stracke et al. 2010) and only one of them included a wash-out period after the intervention (Caris-Veyrat et al. 2004). Only in one study, there were 3 intervention periods (Søltoft et al. 2011). Most studies conducted one trial except two studies that conducted two trials in different periods (Søltoft et al. 2011; Alleva et al. 2016).

The biospecimen collected in all studies was blood and three studies collected urine samples, as well (Grinder-Pedersen et al. 2003; De Lorenzo et al. 2010; Di Daniele et al. 2014). In most studies, two samples were collected for each participant in each intervention period (at the beginning and end of the intervention); in some studies more than two samples were collected, ranging from three to nine samples per intervention period (Briviba et al. 2007; Akçay et al. 2004; Caris-Veyrat et al. 2004; Grinder-Pedersen et al. 2003; Stracke et al. 2009). The included studies measured a number of parameters, i.e., markers of antioxidant capacity (TEAC, ORAC, FRAP), markers of oxidative stress and damage (MDA, CAT, SOD, LDL oxidation, ROS), antioxidants (carotenoids, flavonoids, vitamins), markers of inflammation (CRP, TNF-α, interleukins, T lymphocytes) and biochemical markers associated with oxidative stress and inflammation (glucose, cholesterol, triacylglycerol, albuminuria, uric acid). Half of the studies didn't observe a significant ($p>0.05$) difference resulting from the organic diet intervention and no markers of inflammation were found to be significantly affected. In the following section, the results of the studies that reported significant differences are summarized based on the types of outcomes.

## Antioxidant capacity

In three studies, markers of antioxidant capacity showed a significant change after the consumption of organic diet (Grinder-Pedersen et al. 2003; Di Renzo et al. 2007; Alleva et al. 2016). The markers that changed in these studies were Trolox Equivalent Antioxidant Capacity (TEAC), Oxygen Radical Absorbance Capacity (ORAC) and DNA repair activity. TEAC decreased ($p < 0.05$) after the systematic intake of organically produced diet for 22 days by 16 adults when compared to the baseline (Grinder-Pedersen et al. 2003). This study included two intervention periods of 22 days separated by a washout period of three weeks. However, because TEAC showed carry-over effects in the second intervention period, its values were determined from results produced during the first intervention period. The decrease in TEAC seemed contradictory due to the higher levels of flavonoids in the organic diet. ORAC increased by 21% ($p \leq 0.05$) after the consumption of a 14-day organic diet by 10 men compared to a 14-day conventional diet (Di Renzo et al. 2007). Similarly, the DNA repair activity increased after a 15-day organic honey supplementation to 34 chronically exposed to pesticides adults during a low pesticides exposure period ($p = 0.001$) and a high pesticides exposure period ($p = 0.006$) (Alleva et al. 2016).

**Table 1** Characteristics of intervention trials examining the effect of organic diet on biomarkers (continues on next page)

| Reference | Study design | Participants | Exposure | Duration | Outcome | Total no of samples | Significant Results* |
|---|---|---|---|---|---|---|---|
| Grinder-Pedersen et al., 2003 | Randomized, crossover, double-blind trial | 6 men and 10 women | Controlled organic or conventional diets with 4 different menus | **Total:** 65 days **Intervention period:** 2 X 22 days | SOD, GSH-Px, GR, CAT, TEAC, FRAP, MDA, 2-AAS, flavonoids, flavanones | 8 blood samples (days -1, 0, 22, and 23) and 4 24h-urine samples (days 0 and 22) | (+) quercetin, kaempferol<br>(-) TEAC |
| Di Renzo et al., 2007 | Cross-over trial | 10 men | Conventional and organic diets | **Total:** 28 days **Intervention period:** 14 days | ORAC | 2 blood samples (days 15 and 29) | (+) ORAC |
| De Lorenzo et al., 2010 | Cross-over trial | 130 healthy men and male CKD patients | Conventional and organic products | **Total:** 28 days **Intervention period:** 14 days | Urinary albumin excretion, vitamin B12, glucose, homocysteine, triglycerides, total cholesterol, HDL cholesterol, calcium, phosphorus, sodium, potassium, hs-CRP, TNF-α, IFN-γ, IL-1α, IL-1β, IL-6 | 2 blood and 2 urine samples (days 15 and 29) | (-) Homocysteine, phosphorus (healthy and CKD subjects)<br>(-) Total cholesterol, calcium, urinary albumin excretion (CKD patients) |
| Di Daniele et al., 2014 | Cross-over trial | 40 CKD patients | Controlled Italian Mediterranean conventional and organic diets | **Total:** 28 days **Intervention period:** 14 days | MTHFR genotype, homocysteine, urea, total cholesterol, calcium, phosphate, potassium, vitamin B12, urinary albumin excretion | 2 blood and 2 urine samples (days 15 and 29) | (-) Homocysteine, urea, total cholesterol, calcium, phosphorus, potassium, vitamin B12 and urinary albumin excretion |
| Gonçalves et al., 2011 | Intervention pilot trial | 10 male triathletes | 300 ml/d organic grape juice | **Total:** 20 days **Intervention period:** 20 days | Insulin, glucose, uric acid, total polyphenols, e-SOD, HOMA2-IR, microvascular parameters (FCD, diameters of capillaries, RBCV, $RBCV_{max}$, $TRBCV_{max}$) | 2 blood samples (after 20h of exercise on days 0 and 21) | (+) Total polyphenols, insulin, uric acid, FCD, $RBCV_{max}$<br>(-) Glucose, e-SOD, $TRBCV_{max}$ |
| Alleva et al., 2016 | Intervention trials | 34 adults chronically exposed to pesticides | 50 g/d organic honey in two periods (low and high pesticide exposure) | **Total:** 30 days (2 trials) **Intervention period:** 15 days per trial | DNA damage response parameters (FPG, ENDO III, DNA repair activity) | 4 blood samples (days 0 and 16) | (+) DNA repair activity<br>(-) FPG and ENDOIII lesion accumulation |

| Reference | Study design | Participants | Exposure | Duration | Outcome | Total no of samples | Significant Results* |
|---|---|---|---|---|---|---|---|
| Briviba et al., 2007 | Randomized, cross-over, double-blind trial | 6 men | 1kg of organic or conventional apples (Golden Delicious) | **Total:** 9 days **Intervention period:** 2 X 1 day | LDL oxidation, glucose, triacylglycerol, uric acid, DNA damage | 18 blood samples (day 0 and 1h, 2h, 3h, 4.5h, 6h, 9h, 12h and 24 h) | No significant differences |
| Caris-Veyrat et al., 2004 | Randomized, cross-over, single-blind trial | 20 women | 96 g/day of organic or conventional tomato puree | **Total:** 42 days **Intervention period:** 21 days | Vitamin C, carotenoids | 3 blood samples (days 0, 22 and 42) | No significant differences |
| Søltoft et al., 2011 | Double-blind, cross-over trials | 18 men in each trial | Controlled organic or conventional diets | **Total:** 128 days (2 trials in 2 years) **Intervention period:** 3 X 12 days per trial | Carotenoids | 12 blood samples (days 1 and 13) | No significant differences |
| Akçay et al., 2004 | Cross-over trial | 6 men and 2 women | 200 mL and 100 mL of organic or conventional red wine (Cabernet Sauvignon) for men and women | **Total:** 44 days **Intervention period:** 2 X 1-day | Total cholesterol, triglycerides, HDL-cholesterol, AOA, total phenols, e-SOD, e-CAT, e-TBARS, LDL-TBARS, Cu-LDL-TBARS | 6 blood samples (days 0 and 1h and 3h) | No significant differences |
| Stracke et al., 2009 | Randomized, controlled, double-blind trial | 36 men | 200 g/d organic or conventional carrots | **Total:** 14 days **Intervention period:** 14 days | Glucose, triacylglycerol, cholesterol, uric acid, carotenoids, vitamins E and C, FRAP, ORAC, TEAC, LDL oxidation | 4 blood samples (days 0, 2, 7 and 14) | No significant differences |
| Stracke et al., 2010 | Randomized, controlled, double-blind trial | 43 men | 500 g/d organic or conventional apples (Golden Delicious) | **Total:** 28 days **Intervention period:** 28 days | Glucose, triacylglycerol, cholesterol, uric acid, polyphenols, carotenoids, vitamins E and C, PBMC proliferation, cytokine secretion, NK cells, NKT cells, T lymphocytes, FRAP, ORAC, TEAC | 2 blood samples (days 0 and 28) | No significant differences |

* $p \leq 0.05$ (+) significant increase (-) significant decrease. Abbreviations meaning can be found on page 12.

## Biomarkers of oxidative stress

A significant decrease in biomarkers of oxidative stress was shown in two studies that had the same study design; intervention (pre-post) trials (Gonçalves et al. 2011; Alleva et al. 2016). After 20 days of organic grape juice intake (300 ml/day) from ten male triathletes, the erythrocyte superoxide dismutase activity (e-SOD) decreased ($p=0.04$) (Gonçalves et al. 2011). Similarly, an organic honey supplementation for 15 days to 34 adults with a chronic exposure to pesticides led to decreased Formamido Pyrimidine Glycosylase (FPG) and Endonuclease III (ENDO III) sites during both periods of pesticides exposure ($p = 0.028$, $p = 0.024$ for FPG and $p = 0.039$, $p = 0.0005$ for ENDO III) (Alleva et al. 2016).

## Antioxidants

In three studies, parameters belonging in the class of antioxidants were significantly affected (Grinder-Pedersen et al. 2003; Di Daniele et al. 2014; Gonçalves et al. 2011). The urinary excretion of flavonoids quercetin and kaempferol ($p < 0.05$) increased after consumption of the organic diet compared to the conventional diet in two 22-day intervention periods (Grinder-Pedersen et al. 2003). Similarly, total plasma polyphenols were increased ($p = 0.048$) after 20 days of organic grape juice intake (Gonçalves et al. 2011). On the contrary, vitamin B12 levels decreased after a 14-day intervention period with Italian Mediterranean organic diet compared to low protein diet and Italian Mediterranean diet in 40 patients with chronic kidney disease (CKD) (Di Daniele et al. 2014).

## Biochemical markers associated with inflammation and oxidative stress

In three studies, biochemical markers associated with inflammation or oxidative stress were significantly ($p<0.05$) affected (Di Daniele et al. 2014; De Lorenzo et al. 2010; Gonçalves et al. 2011). Homocysteine levels decreased in healthy adults ($p = 0.011$) and CKD patients ($p = 0.003$, $p < 0.05$) after a 14-day organic diet intervention (De Lorenzo et al. 2010; Di Daniele et al. 2014). Moreover, total cholesterol ($p = 0.037$, $p < 0.05$) and albuminuria ($p = 0.003$, $p < 0.05$) decreased in CKD patients after a 14-day organic diet intervention. Differently, the fasting serum insulin and plasma uric acid increased ($p = 0.03$, $p = 0.01$) and the fasting plasma glucose concentration decreased ($p < 0.001$) after a 20-day organic grape juice intake by ten male triathletes (Gonçalves et al. 2011).

## Discussion

The review analysed the available published literature on the impact of organic diet intervention on human antioxidant capacity and biomarkers of inflammation and oxidative stress. This systematic review identified a small number of relevant articles with various study designs, exposures and outcomes. It was evident that the research in this field is new and gaining renewed interest, since all articles were published after 2000 and most articles were published after 2010.

It is of note that only half of the identified studies reported significant differences after the consumption of organic diet or specific organic food items while a small number of biomarkers was shown to be affected. None of the markers of inflammation examined in these studies was shown to be affected by the organic diet intervention (CRP, TNF-α, IFN-γ, IL-1α, IL-1β, IL-6, PBMC proliferation, cytokine secretion, NK cells, NKT cells, T lymphocytes). Markers of antioxidant capacity, such as ORAC and DNA repair activity, increased after exposure to organic diet as a whole and as a supplement whereas TEAC decreased (Di Renzo et al. 2007; Alleva et al. 2016; Grinder-Pedersen et al. 2003). A decrease in e-SOD, FPG and ENDO III, which are markers of oxidative stress, was shown after a short organic grape juice or organic honey supplementation (Gonçalves et al. 2011; Alleva et al. 2016). Antioxidants like flavonoids and total polyphenols increased after intake of organic diet whereas vitamin B12 decreased (Grinder-Pedersen et al. 2003; Gonçalves et al. 2011; Di Daniele et al. 2014). Other parameters associated with inflammation and oxidative stress, that were found to be affected by the organic diet interventions were total cholesterol, insulin, glucose, uric acid, homocysteine and albuminuria (Di Daniele et al. 2014; De Lorenzo et al. 2010; Gonçalves et al. 2011).

Due to the small number and large variability on the study design of the articles, the existing evidence doesn't show that organic diet can affect antioxidant capacity and biomarkers of oxidative stress and inflammation. However, the fact that other biochemical markers associated with oxidative stress and inflammation were affected after consumption of organic diet or items, might imply that the direct effects of an organic diet intervention can be evident in precursors of inflammation and oxidative stress.

In terms of quality, the identified articles presented some drawbacks. None of the articles were reported in accordance with recognized guidelines provided by the EQUATOR network, such as the CONSORT guidelines for randomised controlled trials and specifically the reporting guidelines laid out in the CONSORT statement to randomized trials of nonpharmacologic treatments (http://annals.org/aim/article/2633220/consort-statement-randomized-trials-nonpharmacologic-treatments-2017-update-consort-extension). Most of the studies didn't report sufficient information regarding the randomization and blinding methods. All studies had small sample size (≤ 43 participants) except one study with 130 participants (De Lorenzo et al. 2010) and the sample size calculations are not reported in the articles. The duration of the organic diet intervention was relatively small in all studies (≤ 28 days) and no explanation about the reason the specific duration was selected was reported in the studies. The fact that most of the studies assessed the effect of organic food items instead of full organic diets is a limitation since the rest of the diet isn't controlled and hence it was more difficult to determine the effect of organic diet on specific biomarkers.

This review has several advantages such as the systematic approach, the methodology and the broad search terms. A potential limitation of the review is that the search was conducted in one database and hence it is possible that not all of the relevant articles were identified. Moreover, it is possible that we missed some relevant studies due to the fact that we excluded foreign language articles and grey literature.

Further research in the field is recommended, using randomized controlled trials in humans with sufficient sample size, exposure to organic diet as a whole and long enough window of monitoring exposures that can capture more effectively the effect of organic diet. Furthermore, the reporting of future trials should follow the international guidelines provided of the CONSORT Statement for trials of nonpharmacologic treatments.

This review was necessary in order to provide the basis for the study design for the children's health study which is implemented under the Activity C3.1 of the ORGANIKO LIFE+ project. A complementary report with information on the effect of organic diet on health outcomes based on cohort studies and on pesticides metabolites, on the association of pesticides and other environmental pollutants with health biomarkers and on the effect of short-term diet/exercise interventions on health biomarkers was prepared in order to assist further in the study design development (see Appendix).

In order for the study to have a well-designed methodology for the assessment of the organic diet effect on health biomarkers in children, it needs to have the following characteristics: (a) randomized controlled trial (b) sufficient sample size (c) exposure to organic diet as a whole (d) biomonitoring of selected pesticides and health biomarkers and (e) long enough window of intervention. The results of this study will help in elucidating the role of organic food consumption in specific health biomarkers of effect.

# Abbreviations

2-AAS: 2-aminoadipic semialdehyde

AOA: total antioxidant activity

CAT: catalase

CKD: chronic kidney disease

CRP: C-reactive protein

Cu-: copper

e-: erythrocyte

ENDO III: endonuclease III

FCD: functional capillary density

FPG: formamido pyrimidine glycosylase

FRAP: ferric reducing ability of plasma

GSH-Px: glutathione peroxidase

GR: glutathione reductase

HDL: high-density lipoproteins

HOMA2-IR: homeostasis model assessment for the insulin resistance

IFN-γ: interferon gamma

IMD: Italian Mediterranean diet

IMOD: Italian Mediterranean organic diet

IL: interleukin

LDL: low-density lipoproteins

MDA: malondialdehyde

MTHFR: methylenetetrahydrofolate reductase

NK: natural killer cells

ORAC: oxygen radical absorbance capacity

PBMC: peripheral blood mononuclear cells

RBCV: red blood cell velocity at rest

$RBCV_{max}$: red blood cell velocity peak after an one-min arterial occlusion

SOD: superoxide dismutase

TEAC: Trolox equivalent antioxidant capacity

TBARS: thiobarbituric acid-reactive substance

TNF-α: tumor necrosis factor alpha

$TRBCV_{max}$: time taken to reach $RBCV_{max}$

# Appendix

## Introduction

Due to the fact that only a small number of human studies investigating the association of organic diet with health exist, other associations between organic diet components and health outcomes are also reported here such as the effect of organic diet on pesticides metabolites, the association of pesticides and other environmental pollutants with health biomarkers and the effect of short-term diet/exercise interventions on health biomarkers. The results of this report will further assist in the proper data analysis of the children's intervention study which will be conducted under the Activity C3.1 of the project.

## Methods

The PubMed database was used for the search of studies. Search strategies were developed using title/abstract terms. Peer-reviewed, English-language human studies were included; In-vitro and animal studies were excluded.

- ***Effect of organic diet on health outcomes based on cohort studies:*** The search terms included relevant exposures and outcomes i.e. organic, conventional, health, food, diet, cohort, prospective. Eligible studies were human studies that examined health outcomes.
- ***Effect of organic diet on pesticides metabolites:*** The search terms included relevant exposures and outcomes i.e. organic, conventional, pesticides, food, diet, chlorpyrifos, malathion and organophosphates.
- ***Association of pesticides and other environmental pollutants with health biomarkers:*** The search terms included relevant exposures and outcomes i.e. pesticides, environmental pollutants, oxidative stress and inflammation.
- ***Effect of short-term diet/exercise interventions on health biomarkers:*** The search terms included relevant exposures and outcomes i.e. diet, exercise, intervention, oxidative stress and inflammation.

## Results and Discussion

### Effect of organic diet on health outcomes

So far, five cohort studies have examined the association of organic food consumption with health biomarkers and outcomes. The focus of these studies are pregnant or breastfeeding or middle-aged women. One of the studies assessed the effect of the incorporation of organic dairy and meat products in the maternal diet on the contents of conjugated linoleic acid isomers (CLA) and trans-vaccenic acid (TVA) in human breast milk (Rist et al. 2007). Participants were 312 breast-feeding women with varying lifestyles and they were divided in three groups according to their responses about the intake of meat and dairy products in a food frequency questionnaire: conventional, 50-90% organic, >90% organic and other (combination of organic dairy and conventional meat products and vice versa). It was shown that the levels of rumenic acid and TVA in breast milk were higher in women who consumed organic meat and dairy products compared to the ones who followed a conventional diet. It is highlighted that in the case of rumenic acid, higher levels were even observed in the milk of mothers with an almost exclusive organic dairy and meat diet (>90% organic) compared to mothers with moderately organic diet (50-90% organic). Another study investigated the association of early-life organic food consumption with the development of atopic manifestations in the first 2 years of life (Kummeling et al. 2008). It was shown that consumption of strictly organic dairy products was associated with a reduced risk of eczema.

Two other studies examined associations between reported organic food consumption during pregnancy and two different outcomes – the risk of pre-eclampsia and the prevalence of hypospadias and cryptorchidism in male infants, based on the prospective Norwegian Mother and Child Cohort Study (Torjusen et al. 2014; Brantsæter et al. 2016). A lower risk of pre-eclampsia was observed in pregnant Norwegian women who reported frequent and consumption of organic vegetables compared with those with no or low consumption of these food items (Torjusen et al. 2014). Similarly, pregnant Norwegian women who reported "sometimes, often or mostly" consumption of organic food were less likely to give birth to a boy with hypospadias compared to women who reported rare or no consumption of organic food (Brantsæter et al. 2016). These findings are consistent with the results of a case-control study in Denmark in which an association between hypospadias in male infants and mothers not choosing the organic alternative and having a high intake of butter and cheese was found (Christensen et al. 2013).

Furthermore, through a large prospective study of women in the United Kingdom, the organic food consumption and cancer incidence has been examined (Bradbury et al. 2014). It was shown that women who reported that they usually or always consume organic didn't have a reduced risk of cancer overall but a significant reduction in the risk of non-Hodgkin lymphoma was shown.

### Effect of organic diet on pesticides metabolites

With regards to the association of organic diet and pesticides exposure, only five studies have been published that examine the effect of organic diet to urinary pesticide metabolites levels (Lu et al. 2008; Lu et al. 2006; Oates et al. 2014; Curl et al. 2003; Bradman et al. 2015). In four of them, the study population is children and in three of them, the pesticides analysed are organophosphate pesticides. It is observed that the metabolites of two organophosphate insecticides, malathion and chlorpyrifos (MDA and TCPy), decrease significantly in the urine of children consuming an organic diet compared to a conventional diet (Lu et al. 2008; Lu et al. 2006). Similar results have been shown in studies with children and adults since lower levels of total dimethyl metabolites of organophosphate pesticides have been found during consumption of an organic diet (Oates et al. 2014; Curl et al. 2003; Bradman et al. 2015). Moreover, total dialkylophosphates (ΣDAP) decreased during the organic diet phase in children and adults (Oates et al. 2014; Bradman et al. 2015). Another metabolite that was found to decrease significantly with the consumption of an organic diet in children was 2,4-D, a herbicide metabolite (Bradman et al. 2015). For dietary exposures of children to pesticides, it is better to analyse organophosphate pesticides due to the fact that children are exclusively exposed to them via their food (Lu et al. 2006).

### Association of pesticides and other environmental pollutants with health biomarkers

Due to the fact that we needed to understand how the pesticide exposure affects oxidative stress and inflammation biomarkers, we found some articles examining the association of pesticides and other environmental pollutants with health biomarkers. A common biomarker that was found to be associated with organophosphate pesticides and nonylphenol is 8-OHdG (Muniz et al. 2008; Lee et al. 2007; Wang et al. 2015).

More specifically, the 8-OHdG levels were significantly lower ($p \leq 0.01$) in the control population compared to applicators (Muniz et al. 2008; Lee et al. 2007) and urinary 8-OHdG significantly correlated with urinary DMP, DAP (Lee et al. 2007) and NP (Wang et al. 2015). Also, malondialdehyde (MDA) levels were found to be significantly lower in controls compared to farmers (Muniz et al. 2008; Madani et al. 2015). Another parameter of oxidative stress usually examined in these studies is C-reactive protein. Increased serum CRP levels were observed in farmers (Madani et al. 2015) and there was a positive association of organochlorine pesticides and CRP concentration (Kim et al. 2012). Furthermore, CRP was found to be strongly associated with homeostatic model assessment (HOMA-IR) among participants with high polychlorinated biphenyls or organochlorine pesticide concentrations (Kim et al. 2012).

Other markers which were significantly different in farmers than in controls were higher APE activity (Muniz et al. 2008), serum glucose, ALT, ALT/AST ratio, prothrombin, fibrinogen, plasma O2-levels, erythrocyte MDA and carbonyl protein contents and decreased plasma vitamin C and E, erythrocyte GSH amounts and erythrocyte antioxidant enzyme (catalase and SOD) activities (Madani et al. 2015). Other associations were also found between pollutants and antioxidant or inflammatory biomarkers. It was shown that a significant partial correlation of DEP, DMTP, DMDTP and the combined methyl sum and the tail length of the lymphocytes exists (Muniz et al. 2008). Moreover, higher prepubertal serum OCPs were associated with lower serum leptin concentrations over 4 years of follow-up (Burns et al. 2014), prenatal concentrations of DDE, HCB or ΣPCBs were associated with increasing levels of IL10 measured at the age of 4 years, with the strongest associations found for HCB (Gascon et al. 2014) and significant correlations were found between NP and 8-NO2Gua in pregnant women (Wang et al. 2015).

## Effect of short-term diet/exercise interventions on health biomarkers

In order to understand which biochemical markers can be easily affected by a short intervention, we searched for articles examining inflammatory and oxidation biomarkers in relation to short-term diet/exercise intervention. A common parameter that was examined in three studies and was decreased significantly in all three cases is the C-reactive protein. It was shown that CRP was significantly reduced after a 6-week diet and exercise intervention in children (Huang et al. 2015), after an 8-week orange juice supplementation trial in both normal and overweight adults (Dourado & Cesar 2015) and after a 1-month placebo controlled trial in adults (Xie et al. 2015). Also, total cholesterol and LDL seem to be affected significantly after a short-term intervention. Specifically, following a 14-day diet and exercise intervention in overweight/obese children, all serum lipids improved significantly, with the exception of HDL both the total cholesterol/HDL and LDL/HDL ratios decreased (Izadpanah et al. 2012). Moreover, total cholesterol and LDL levels were significantly reduced following an 8-week supplementation of orange juice in normal overweight adults (Dourado & Cesar 2015).

With regards to interleukins, IL-12 was significantly increased following an 8-week supplementation of orange juice in normal overweight adults (Dourado & Cesar 2015) and significant decreases were observed for IL-6, IL-8 and IL-1ra, following a 14-day diet and exercise intervention in overweight/obese children (Izadpanah et al. 2012). Also, leptin was significantly decreased following a 6-week and 46-week intervention trials in children (Huang et al. 2015) and a 14-day diet and exercise intervention in overweight/obese children (Izadpanah et al. 2012). Following an 8-week orange juice supplementation trial in both normal and overweight adults, other parameters that were significantly affected were increase in vitamin C and folate and decrease in lipid peroxidation and malondialdehyde levels (Dourado & Cesar 2015). Following a 14-day diet and exercise intervention in overweight/obese children, other parameters that were significantly affected were decreased fasting insulin, TNF, PAI-1, resistin and amylin, increased blood glucose and adiponectin and improved HOMA-IR and QUICKI (Izadpanah et al. 2012). In a placebo-controlled trial of overweight smokers examining the effect of anthocyanins, it was shown that urinary 8-iso-PGF2a levels and plasma Ox-LDL decreased significantly in the anthocyanin group (Davinelli et al. 2015). Following a 1-month placebo controlled trial in adults examining the effect of a mangosteen drink, ORAC decreased significantly in the supplementation group (Xie et al. 2015).

The gathered evidence from the prospective studies show association of reported organic diet with lower risk of eczema, non-Hodgkin lymphoma, pre-eclampsia, hypospadias and higher levels of TVA and rumenic acid in breast milk. However, the limitations of these studies are that the organic consumption is based on food frequency questionnaires and there is no biomonitoring of pesticide exposure. Organic food consumption is usually associated with a healthier lifestyle (exercise, higher consumption of fruits and vegetables and lower consumption of red and processed meat) so it is not possible to identify whether these associations are explained by the organic food consumption or these other lifestyle factors.

It is important that a number of studies have shown that exposure to pesticides affects a number of biomarkers of oxidative stress and inflammation since organic diet has been found to be associated with lower urinary pesticide levels. It is expected that organic food can have an impact on health biomarkers due to reduction of pesticides and future studies need to show this. The fact that short-term diet/exercise interventions may decrease significantly some biomarkers provides evidence that a well-designed organic diet intervention can be sufficient to affect health biomarkers.

## References

Abdollahi, M. et al., 2004. Pesticides and oxidative stress: a review. *Medical science monitor : international medical journal of experimental and clinical research*, 10(6), pp.RA141–A147. Available at: http://www.medscimonit.com/pub/vol_10/no_6/4163.pdf.

Akçay, Y.D. et al., 2004. The effects of consumption of organic and nonorganic red wine on low-density lipoprotein oxidation and antioxidant capacity in humans. *Nutrition Research*, 24(7), pp.541–554.

Alleva, R. et al., 2016. Organic honey supplemenation reverses pesticide-induced genotoxicity by modulating DNA damage response. *Molecular Nutrition and Food Research*, p.doi:10.1002/mnfr.201600005.

Bassil, K.L. et al., 2007. Cancer health effects of pesticides. *Canadian Family Physician*, 53(10), pp.1704–1711. Available at: http://www.hubmed.org/display.cgi?uids=17934034.

Bradbury, K.E. et al., 2014. Organic food consumption and the incidence of cancer in a large prospective study of women in the United Kingdom. *British Journal of Cancer*, 110(9), pp.2321–2326. Available at: http://dx.doi.org/10.1038/bjc.2014.148.

Bradman, A. et al., 2015. Effect of organic diet intervention on pesticide exposures in young children living in low-income urban and agricultural communities. *Environmental Health Perspectives*, 123(10), pp.1086–1093.

Brantsæter, A.L. et al., 2016. Organic food consumption during pregnancy and hypospadias and cryptorchidism at birth: The Norwegian mother and child cohort study (MoBa). *Environmental Health Perspectives*, 124(3), pp.357–364.

Briviba, K. et al., 2007. Effect of consumption of organically and conventionally produced apples on antioxidant activity and DNA damage in humans. *Journal of Agricultural and Food Chemistry*, 55(19), pp.7716–7721.

Burns, J.S. et al., 2014. Association between chlorinated pesticides in the serum of prepubertal russian boys and longitudinal biomarkers of metabolic function. *American Journal of Epidemiology*, 180(9), pp.909–919.

Caris-Veyrat, C. et al., 2004. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *Journal of Agricultural and Food Chemistry*, 52(21), pp.6503–6509.

Christensen, J.S. et al., 2013. Association Between Organic Dietary Choice During Pregnancy and Hypospadias in Offspring : A Study of Mothers of 306 Boys Operated on for Hypospadias. *JURO*, 189(3), pp.1077–1082. Available at: http://dx.doi.org/10.1016/j.juro.2012.09.116.

Curl, C.L., Fenske, R.A. & Elgethun, K., 2003. Organophosphorus pesticide exposure of urban and suburban preschool children with organic and conventional diets. *Environmental health perspectives*, 111(3), pp.377–82. Available at: /pmc/articles/PMC1241395/?report=abstract.

Davinelli, S. et al., 2015. A Randomized Clinical Trial Evaluating the Efficacy of an Anthocyanin–Maqui Berry Extract (Delphinol®) on Oxidative Stress Biomarkers. *Journal of the American College of Nutrition*, 34(sup1), pp.28–33. Available at:

http://www.tandfonline.com/doi/full/10.1080/07315724.2015.1080108.

De Lorenzo, a et al., 2010. The effects of Italian Mediterranean organic diet (IMOD) on health status. *Current pharmaceutical design*, 16(7), pp.814–24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20388092.

Di Daniele, N. et al., 2014. Effects of Italian Mediterranean organic diet vs. low-protein diet in nephropathic patients according to MTHFR genotypes. *Journal of Nephrology*, 27(5), pp.529–536.

Dourado, G.K.Z.S. & Cesar, T.B., 2015. Investigation of cytokines, oxidative stress, metabolic, and inflammatory biomarkers after orange juice consumption by normal and overweight subjects. *Food & Nutrition research*, 59(1), p.28147.

Gascon, M. et al., 2014. Persistent organic pollutants and children's respiratory health: The role of cytokines and inflammatory biomarkers. *Environment International*, 69, pp.133–140. Available at: http://dx.doi.org/10.1016/j.envint.2014.04.021.

Gonçalves, M.C. et al., 2011. Organic grape juice intake improves functional capillary density and postocclusive reactive hyperemia in triathletes. *Clinics (São Paulo, Brazil)*, 66(9), pp.1537–41. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3164400&tool=pmcentrez&rendertype=abstract.

Grinder-Pedersen, L. et al., 2003. Effect of diets based on foods from conventional versus organic production on intake and excretion of flavonoids and markers of antioxidative defense in humans. *Journal of Agricultural and Food Chemistry*, 51(19), pp.5671–5676.

Hoefkens, C. et al., 2010. Consuming organic versus conventional vegetables: The effect on nutrient and contaminant intakes. *Food and Chemical Toxicology*, 48(11), pp.3058–3066. Available at: http://dx.doi.org/10.1016/j.fct.2010.07.044.

Huang, T. et al., 2015. Effects of a multi-component camp-based intervention on inflammatory markers and adipokines in children: A randomized controlled trial. *Preventive Medicine*, 81, pp.367–372. Available at: http://dx.doi.org/10.1016/j.ypmed.2015.09.014.

Izadpanah, a. et al., 2012. A short-term diet and exercise intervention ameliorates inflammation and markers of metabolic health in overweight/obese children. *AJP: Endocrinology and Metabolism*, 303(4), pp.E542–E550.

Katsarova, I., 2015. Organic food : Helping EU consumers make an informed choice. , (May), p.10.

Kim, K.S. et al., 2012. Interaction between persistent organic pollutants and C-reactive protein in estimating insulin resistance among non-diabetic adults. *Journal of Preventive Medicine and Public Health*, 45(2), pp.62–69.

Kummeling, I. et al., 2008. Consumption of organic foods and risk of atopic disease during the first 2 years of life in the Netherlands. *The British journal of nutrition*, 99(3), pp.598–605.

Lee, C.H. et al., 2007. 8-Hydroxydeoxyguanosine levels in human leukocyte and urine according to exposure to organophosphorus pesticides and paraoxonase 1 genotype. *International Archives of Occupational* *and Environmental Health*, 80(3), pp.217–227.

Lu, C. et al., 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. *Environmental Health Perspectives*, 116(4), pp.537–542.

Lu, C. et al., 2006. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. *Environmental Health Perspectives*, 114(2), pp.260–263.

Madani, F.Z. et al., 2015. Hemostatic, inflammatory, and oxidative markers in pesticide user farmers. *Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals*, 5804(January), pp.1–8. Available at: http://dx.doi.org/10.3109/1354750X.2015.1118545.

Mark, A.B. et al., 2013. Consumption of organic diets does not affect intake and absorption of zinc and copper in men - evidence from two cross-over trials. *Food & function*, 4(3), pp.409–19. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23192634.

Mostafalou, S. & Abdollahi, M., 2013. Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives. *Toxicology and Applied Pharmacology*, 268(2), pp.157–177. Available at: http://dx.doi.org/10.1016/j.taap.2013.01.025.

Muniz, J.F. et al., 2008. Biomarkers of oxidative stress and DNA damage in agricultural workers: A pilot study. *Toxicology and Applied Pharmacology*, 227(1), pp.97–107.

Oates, L. et al., 2014. Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. *Environmental Research*, 132, pp.105–111. Available at: http://dx.doi.org/10.1016/j.envres.2014.03.021.

Prado, R.P. et al., 2010. Influence of diet on oxidative DNA damage, uracil misincorporation and DNA repair capability. *Mutagenesis*, 25(5), pp.483–487.

Di Renzo, L. et al., 2007. Is antioxidant plasma status in humans a consequence of the antioxidant food content influence? *European Review for Medical and Pharmacological Sciences*, 11(3), pp.185–192.

Rist, L. et al., 2007. Influence of organic diet on the amount of conjugated linoleic acids in breast milk of lactating women in the Netherlands. *The British journal of nutrition*, 97(4), pp.735–743.

Sanborn, M. et al., 2007. Non-cancer health effects of pesticides. *Canadian Family Physician*, 53(10), pp.1712–1720.

Soltaninejad, K. & Abdollahi, M., 2009. Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. *Medical science monitor : international medical journal of experimental and clinical research*, 15(3), pp.RA75–A90.

Søltoft, M. et al., 2011. Effects of organic and conventional growth systems on the content of carotenoids in carrot roots, and on intake and plasma status of carotenoids in humans. *Journal of the Science of Food and Agriculture*, 91(4), pp.767–775.

Stracke, B. a et al., 2009. Bioavailability and nutritional effects of carotenoids from organically and conventionally produced carrots in healthy men. *The British journal of nutrition*, 101(11), pp.1664–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19021920.

Stracke, B.A. et al., 2010. No effect of the farming system (organic/conventional) on the bioavailability of apple (Malus domestica Bork., cultivar Golden Delicious) polyphenols in healthy men: A comparative study. *European Journal of Nutrition*, 49(5), pp.301–310.

Thiese, M.S., 2014. Observational and interventional study design types; an overview. *Biochemia Medica*, 24(2), pp.199–210.

Torjusen, H. et al., 2014. Reduced risk of pre-eclampsia with organic vegetable consumption: results from the prospective Norwegian Mother and Child Cohort Study. *BMJ open*, 4(9), p.e006143. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4160835&tool=pmcentrez&rendertype=abstract.

Wang, P.W. et al., 2015. Prenatal nonylphenol exposure, oxidative and nitrative stress, and birth outcomes: A cohort study in Taiwan. *Environmental Pollution*, 207, pp.145–151. Available at: http://dx.doi.org/10.1016/j.envpol.2015.08.044.

Whittaker, A. et al., 2015. An organic khorasan wheat-based replacement diet improves risk profile of patients with acute coronary syndrome: A randomized crossover trial. *Nutrients*, 7(5), pp.3401–3415.

Xie, Z. et al., 2015. Daily consumption of a mangosteen-based drink improves in vivo antioxidant and anti-inflammatory biomarkers in healthy adults: a randomized, double-blind, placebo-controlled clinical trial. *Food science & nutrition*, 3(4), pp.342–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4534161&tool=pmcentrez&rendertype=abstract.