ORGANIKO LIFE+ PROJECT

Children's Biomonitoring Report

Action C3 – Organic diet and children's health

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Executive summary

Purpose

This report focuses on oxidative stress and inflammation (OSI) biomarkers analysis, based on the results of the cluster-randomized crossover trial conducted in primary school children in Cyprus, within the framework of the ORGANIKO LIFE+ project.

Outcomes

We measured three OSI biomarkers in urine samples: 8-hydroxy-2'-deoxyguanosine (8-OHdG), 8-iso-prostaglandin F2α (8-iso-PGF2a) and malondialdehyde (MDA).

Results

In regression models, significantly lower levels of the OSI biomarker 8-OHdG (GMR=0.888; 95% CI: 0.808, 0.976; Q=0.035) were observed during the organic period. A significant negative interaction between days of treatment and the dietary organic intervention was observed for 8-iso-PGF2a (GMR=0.984; 95% CI: 0.977, 0.99; Q<0.001) and MDA (GMR=0.995; 95% CI: 0.990, 0.999; Q=0.028), indicating a time-dependent reduction during the intervention period.

Conclusions

In this cluster-randomized crossover trial, data showed that there was an immediate and sustained reduction in 8-OHdG during the organic period, and, after an initial increase, a gradual reduction during the organic period of 8-iso-PGF2a and MDA.

Σύνοψη

Σκοπός

Η παρούσα έκθεση επικεντρώνεται στην ανάλυση των επιπέδων βιοδεικτών οξειδωτικού στρες και φλεγμονής (OSI), βάσει των αποτελεσμάτων της τυχαιοποιημένης διασταυρούμενης μελέτης που πραγματοποιήθηκε σε παιδιά πρωτοβάθμιας εκπαίδευσης στην Κύπρο, στο πλαίσιο του έργου ORGANIKO LIFE+.

Αντίκτυπος

Μετρήθηκαν 3 βιοδείκτες OSI: 8-υδροξυ-2'-δεοξυγουανοσίνη (8-OHdG), 8-ισο-προσταγλανδίνη F2α (8-iso-PGF2a) and μαλονδιαλδεΰδη (MDA).

Αποτελέσματα

Στατιστικά μοντέλα έδειξαν ότι, κατά τη διάρκεια της παρέμβασης με βιολογική διατροφή, παρατηρήθηκαν σημαντικά χαμηλότερα επίπεδα του βιοδείκτη 8-OHdG (GMR=0.888; 95% CI: 0.808, 0.976; Q=0.035). Μια σημαντική αρνητική αλληλεπίδραση παρατηρήθηκε μεταξύ των ημερών παρέμβασης και της βιολογικής διατροφής στα επίπεδα του 8-ισο-PGF2a (GMR = 0.984, 95% CI: 0.977, 0.99, Q <0.001) και του MDA (GMR = 0.995, 95% CI: , 0.999, Q = 0.028), υποδεικνύοντας μία χρονικά εξαρτώμενη μείωση των βιοδεικτών αυτών κατά τη διάρκεια της περιόδου παρέμβασης.

Συμπεράσματα

Σε αυτή την τυχαιοποιημένη διασταυρούμενη μελέτη, τα δεδομένα έδειξαν ότι υπήρξε άμεση και παρατεταμένη μείωση των επιπέδων του 8-OHdG κατά τη διάρκεια της βιολογικής περιόδου και, έπειτα από μια αρχική αύξηση, μια σταδιακή μείωση και στα επίπεδα του 8-ισο-PGF2α και MDA.

Introduction

The aim of the C3 Action of the ORGANIKO LIFE+ project was to evaluate the effectiveness of an organic diet intervention in decreasing the body burden of pesticides metabolites and reducing the concentration of oxidative stress and inflammation (OSI) biomarkers. This report will focus only on the results of the OSI biomarkers analysis.

Methods

Trial oversight

The ORGANIKO LIFE+ study was an investigator-initiated 2 x 2 cluster (school)-based, randomized, crossover trial. The trial was conducted in six primary schools with two periods (40-days organic diet vs. 40-days of conventional diet) in Limassol, Cyprus during October 2016-April 2017. The full trial design can be found in the Appendix (see Study Protocol). The trial protocol was approved by the Cyprus National Bioethics Committee (ΕΕΒΚ/ΕΠ/2016/25) and the Cyprus Ministry of Education and Culture (7.15.06.15/2). The trial was performed in accordance with the principles of the Declaration of Helsinki. This trial is registered with ClinicalTrials.gov, number: NCT02998203.

Trial population

The following eligibility criteria were set for the clusters (schools): i) being a public primary school, and ii) being located in the urban area of Limassol, Cyprus. Eligible participants were healthy 10-12-year old primary school children (5th and 6th grade), who had been living in Cyprus for at least the previous five years and were systematically consuming conventional food (>80% of a week's meals) prior to the study recruitment. Eligible participants with any self-reported chronic disease conditions (e.g., asthma, type I diabetes or other chronic disease) or food allergies (e.g., gluten or lactose tolerance) were excluded. Informed consent was obtained from the school headmaster, a written informed consent was provided by the children's parents or legal guardians, and a verbal assent was obtained from the children.

Randomization and masking

Schools were randomized a priori to two groups that differed in the sequence of the treatments; organic diet followed by conventional diet (Group 1) or conventional diet followed by organic diet (Group 2). The participant ratio of Group 1: Group 2 was 1: 2.5. Details on the reasons for the cluster randomization and the recruitment process are available in the Supplementary Appendix (see Study Protocol). Briefly, the blinding of the participants to group assignment was not possible, since participants knew which diet they were following. The blinding of the researchers to the participants' identity was achieved by the coding of all study

materials (urine containers, questionnaires, and diaries). The study personnel who performed the samples analyses were kept masked to the allocation.

Trial Procedures

The recruitment process started with contacting randomly selected schools and then organizing meetings with parents and children to inform them about the study. After expression of interest to participate in the study, eligibility criteria were checked by the research team. Upon signing of informed consents, study materials (i.e., first morning urine void sample collection instruction, coded vials for urine collection, and food diaries) were provided. During the conventional period, participants were asked to maintain their usual dietary choices (>80% conventional diet) for a total of 40 days (Figure 1). During the organic period, participants were asked to follow strictly the two 20-day sequential organic dietary menus provided to them for 40±3 days. The organic dietary menus were prepared by a registered dietitian based on the European Food Safety Agency (EFSA) guidelines for energy intake of 10-12 years old children,[1] and included five meals per day (breakfast, morning snack, lunch, afternoon snack, and dinner). The fully prepared meals were delivered daily by the organic restaurant to the schools where participating children were collecting them (see Study Protocol for details). Participants crossed over to the alternate diet on the following day after the first period was completed. A washout period was not required as it was intrinsically included in the two periods, since the first urine sample of the second period was collected about 12 days after the beginning of the second period and the pesticide half-lives are short (half-lives ranging 6.4-16.5 hours for pyrethroids and 5-33 hours for neonicotinoids), so no carryover effect was expected.[2,3]

Each participant provided up to six first morning urine samples during the whole duration of the 2-period study; one baseline sample, two samples in the conventional period, and three samples in the organic period. Standardized methods were adopted for the anthropometric measurements (weight, height, and waist circumference), which were taken at the beginning of the study, at the end of the organic period, and at the end of the study (for Group 2, the end of study and end of organic period was the same time point) by trained researchers (see Supplementary Appendix).[4] Besides the baseline questionnaire, a food frequency questionnaire was also administered to the parents at the end of the conventional period through a telephone interview in order to collect information about the food habits of the children during the conventional period. All parents were asked to complete a food diary during the organic period for compliance assessment of non-organic food consumption incidences.

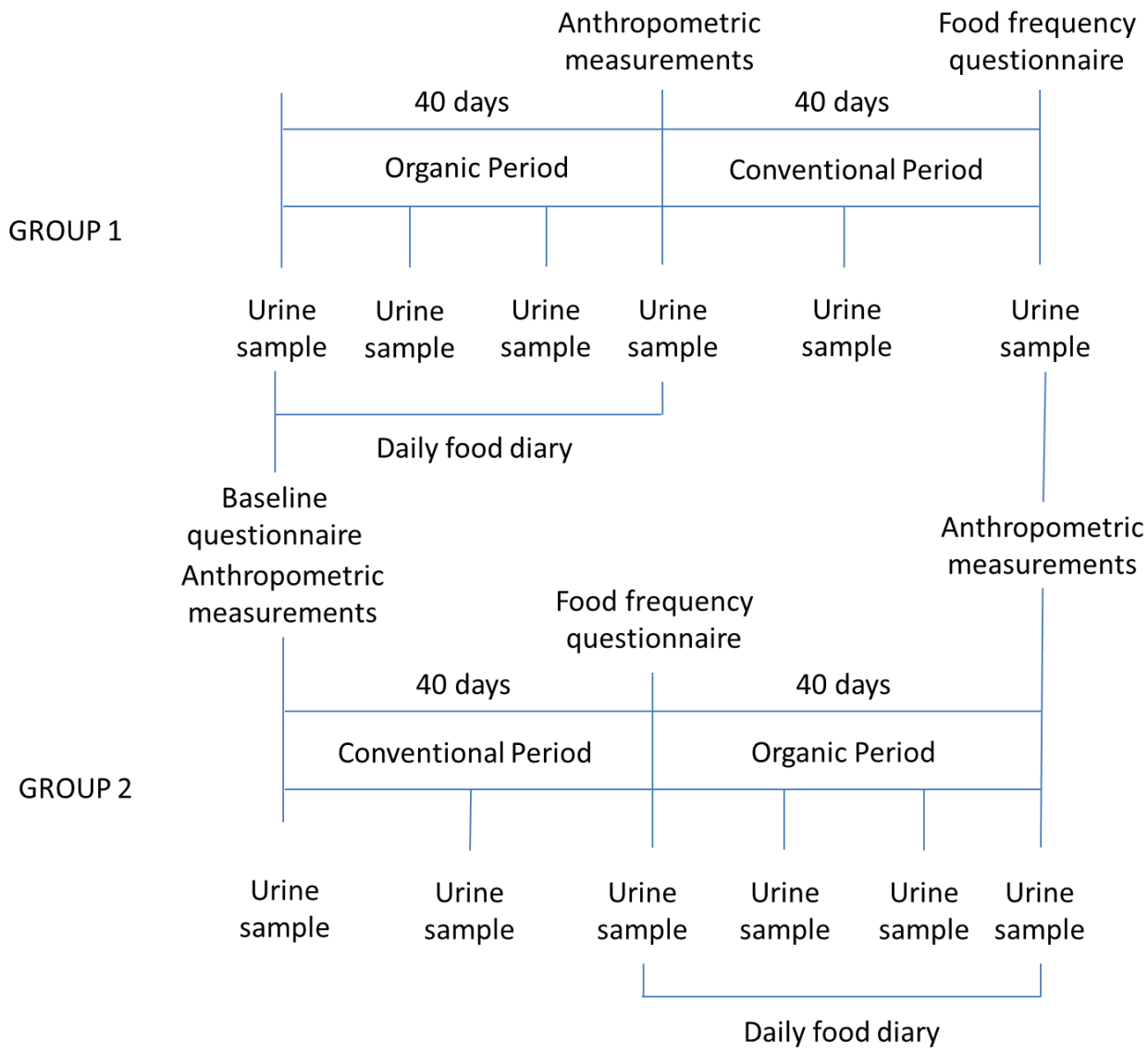

Figure 1 Study timeline and data collection procedure for the two groups of the study.

Urine sample collection

On specific sampling dates, first morning urine voids were obtained at home in polypropylene, sterilized urine vials and collected at school by the research team. Urine vials were temporarily stored in a school/home fridge (4°C) until transferred to laboratory facilities for storage at -80°C.

OSI biomarkers analysis

Competitive ELISA kits were used to determine urinary concentrations of 8-iso-PGF2α (Catalog no: STA-337; Cell Biolabs, Inc, California, USA) and 8-OHdG (Catalog no: ABIN2964843; antibodies-online, Aachen, Germany). The analyses were performed according to the manufacturer's instructions. Detection limits for 8-iso-PGF2α and 8-OHdG were 49 pg/mL and 0.59 ng/mL, respectively. MDA was measured using a spectrophotometric method, as previously described,[5] with an LOD of 0.28 μmol/L. Creatinine-corrected

biomarker values were calculated after measurements of urinary creatinine using the colorimetric Jaffé method.[6]

Statistical analysis

Participants who followed the organic treatment for at least 12 days and provided at least one urine sample during the organic period were included in the main analysis. The baseline characteristics were compared between the study groups (Group 1 vs Group 2) and between dropouts (i.e., enrolled students but did not participate for at least 12 days). Categorical variables were described with sample size and percentages and compared by chi-square test. Approximately normally-distributed continuous variables were described with means and standard deviations (SD), and compared by t-test, and non-normal continuous variables with medians and interquartile ranges (25th–75th percentiles) and compared by Wilcoxon test. The mean daily energy intake for the conventional diets was calculated based on the calories of each item of the food frequency questionnaire. Box plots were used to visually inspect the biomarker data.

For OSI biomarkers values <LOD, they were deterministically imputed as LOD/2 since <20% of the values were below detection limit. All biomarker data were corrected for urine dilution (biomarker mass per gram of urinary creatinine) prior to statistical analysis.

Changes in biomarkers between the conventional and organic treatments were assessed with: (i) the percent change between the last sample of the conventional treatment period (before the start of the organic treatment) and the last urine sample of organic treatment period, and (ii) the overall difference in median levels of biomarkers concentrations between the conventional and organic phase. The percent change was estimated only for the participants who completed the full course of the organic treatment, using the log-transformed, creatinine-adjusted biomarker levels. A one-sample t-test was used to assess whether the percent change was different than zero. The overall differences in the medians of biomarkers between the conventional and the organic phase were assessed with the non-parametric Wilcoxon test on the creatinine-adjusted concentrations pooling all conventional samples (including the baseline) and the organic samples for all participants, regardless of the duration for which they followed the organic treatment.

Linear mixed-effect regression models were used to account for the duration and the effect of treatment (organic or conventional diet) where the OSI biomarkers were the main outcomes. All models included student-level (repeated measures within person) and school-level (multiple students clustered within each school) random intercepts with an unstructured covariance matrix. Continuous variables, other than time (days of treatment), were centered at the population means. Linear models were fitted for the OSI biomarkers (log-transformed, creatinine-corrected). A first set of models included fixed effects for treatment condition (organic or conventional) and time (days of treatment, where time=0 was used for the start of the treatment. The models were adjusted for the baseline value of the outcome to account for the background participant levels. An interaction term for the day of treatment and the treatment was considered and subsequently dropped if did not meet the threshold of p-value=0.05.

Multiple testing was accounted for using the Benjamini-Hochberg method,[7] considering 75 regression parameter tests of the aforementioned models; Q-value<0.05 indicates statistical significance of an association after controlling the false discovery rate at 5%. Geometric mean ratios (GMR) and 95% CI were estimated exponentiating the regression parameters from the log-transformed outcome variable models.

Odds ratios (OR) for detection of 6-CN and 95% CI were estimated exponentiating the regression parameters from the logistic regression model. All analyses were performed in R (v.3.5) with RStudio (v.1.1.423).

Results

Participant characteristics

Between October and December 2016, 12 public schools in the urban area of Limassol city were assessed for eligibility in the study and their headmasters were contacted (Figure 2). Six schools agreed to participate; three schools were randomly allocated to Group 1 (67 children) and the other three to Group 2 (124 children). There were 24 children in Group 1 and 18 children in Group 2 who withdrew from the study 1–11 days after the beginning of the organic period and did not provide an organic period urine sample, who were excluded from the data analysis. A total of 149 children were included in the main analysis with 43 children in Group 1 and 106 children in Group 2.

Overall, the sex distribution of the children was balanced (51% males), though a higher percentage of females was allocated in Group 2 (Table 1). The mean age was 11 years old and the majority of children (89%) completed 29–40 days of organic diet. A high level of education was reported for the participants' parents, with the majority holding at least a university/college degree (82% for mothers and 65% for fathers). At baseline, most children had a normal weight (61%) with 38% being overweight or obese. The two groups differed in their consumption of specific foods during the conventional period. Specifically, children in Group 1 reported a lower consumption of meat, fish, eggs, nuts, and legumes (11 vs 17 portions per week), vegetables (4 vs 6 portions per week) and fats, sweets, and oils (25 vs 35 portions per week) than those in Group 2. The mean energy intake of the participants during the conventional period (2229 kcal) was estimated to be higher than the reference dietary guidance value [average=1976 kcal (2043 kcal for boys and 1908 kcal for girls)] calculated based on the EFSA average requirements for children of age 11 with moderate physical activity lifestyle.[19]

Follow-up

The % change between the baseline and after 40 days of organic diet was highest for 8-OHdG (-1.7%), followed by 8-iso-PGF2a (-1.6%), and MDA (-0.1%) (Table 2). Median biomarker differences by treatment were not significant for the OSI biomarkers (Table 3). In Table 4, the percentiles of the pesticide metabolites for the whole study period, the organic period and the conventional period can be found.

In regression models, significantly lower levels of the OSI biomarker 8-OHdG (GMR=0.888; 95% CI: 0.808, 0.976; Q=0.035) were also observed during the organic period (Table 5). The 8-OHdG reduction during the organic period is evident in both study groups (Figure 3); however, it is more prominent for the last sample of the organic period for Group 1 (First organic) and for the 1st sample of the organic period for Group 2 (First Conventional). A significant negative interaction between days of treatment and the dietary organic intervention was observed for 8-iso-PGF2a (GMR=0.984; 95% CI: 0.977, 0.99; Q<0.001) and MDA (GMR=0.995; 95% CI: 0.990, 0.999; Q=0.028), indicating a time-dependent reduction during the intervention period (Table 5). This time-dependent reduction can be seen from clearly for Group 2 (Figures 4 & 5).

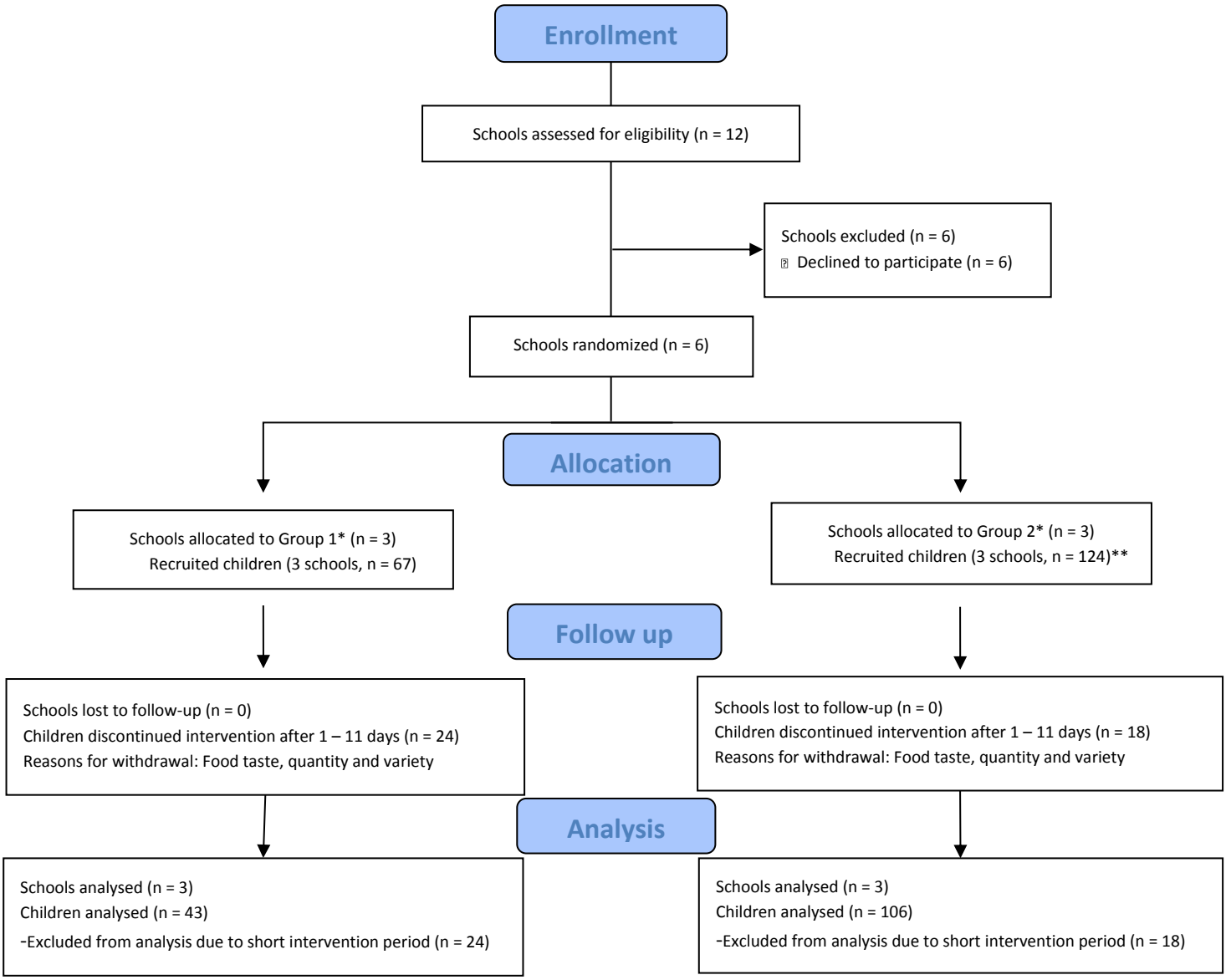

Figure 2. Flow diagram of participants included in the analysis.

*Group 1: First organic period that was followed by the conventional period, Group 2: First conventional period that was followed by the organic period

**Two children followed the opposite design compared to the rest children because they decided to participate after the trial had already started, at the end of the conventional period. These two children started with the second leg of the trial (organic diet) and then continued with the conventional diet.

Table 1. Demographics and baseline characteristics of the study population (overall and by group).

	Overall		Group 1		Group 2		
	Mean (SD) / Median [IQR]	**N (%)**	**Mean (SD) / Median [IQR]**	**N (%)**	**Mean (SD) / Median [IQR]**	**N (%)**	**p-value^**
n		149		43		106	
Sex							0.018
Female		73 (49.0)		14 (32.6)		59 (55.7)	
Male		76 (51.0)		29 (67.4)		47 (44.3)	
Age (years)	11.16 (0.59)		11·03 (0·53)		11.21 (0.61)		0.101
Mother's education level							0.871
Master/PhD		41 (27.9)		13 (31.0)		28 (26.7)	
University/college		80 (54.4)		22 (52.4)		58 (55.2)	
Secondary		26 (17.7)		7 (16.7)		19 (18.1)	
Father's education level							0.447
Master/PhD		41 (27.5)		15 (36.6)		26 (25.7)	
University/college		56 (37.6)		16 (39.0)		40 (39.6)	
Secondary		43 (28.9)		10 (24.4)		33 (32.7)	
Primary		2 (1.3)		0 (0.0)		2 (2.0)	
Weight status*							0.114
Thinness		2 (1.4)		0 (0.0)		2 (1.9)	
Normal weight		90 (60.8)		23 (54.8)		67 (63.2)	
Overweight		36 (24.3)		9 (21.4)		27 (25.5)	
Obese		20 (13.5)		10 (23.8)		10 (9.4)	
Waist circumference (cm)	69.00 [63.00, 77.00]		69·00 [66·50, 81·50]		69.00 [62.00, 76.00]		0.153
Days in organic period							0.589
12-21 days		12 (8.1)		4 (9.3)		8 (7.5)	
22-28 days		4 (2.7)		2 (4.7)		2 (1.9)	
29-40 days		133 (89.3)		37 (86.0)		96 (90.6)	
Number of samples provided							0.001
2		3 (2.0)		3 (7.0)		0 (0.0)	
3		3 (2.0)		3 (7.0)		0 (0.0)	
4		8 (5.4)		0 (0.0)		8 (7.5)	
5		3 (2.0)		1 (2.3)		2 (1.9)	

6	132 (88.6)	36 (83.7)	96 (90.6)	
Physical activity time** (hours/week)	4.00 [2.00, 6.00]	3.50 [1.75, 5.50]	4.00 [2.00, 6.00]	0.291
Sedentary activity time*** (hours/week)	19·00 [13.00, 28.00]	20.50 [14.50, 26.50]	16.40 [12.00, 28·00]	0.258
Milk products**** (portions/week)	15.85 (8.82)	15.85 (8.64)	15.85 (8.93)	0.999
Meat, fish, eggs, nuts, legumes**** (portions/week)	15.05 (7.31)	10.72 (4.46)	16.69 (7.52)	<0.001
Vegetables**** (portions/week)	5.38 (3.84)	3.78 (3.06)	5.98 (3.95)	0.003
Fruits**** (portions/week)	9.70 (6.69)	8.32 (5.33)	10.22 (7.09)	0.143
Cereals**** (portions/week)	21.78 (9.52)	19.45 (11.57)	22.66 (8.52)	0.081
Fats, sweets, oils**** (portions/week)	32.47 (17.70)	25.43 (11.27)	35.13 (18.96)	0.004

^the above variables were tested for differences between the two groups by chi-square tests for categorical variables, t-tests for normally distributed continuous variables and Wilcoxon tests for non-normally distributed continuous variables.
* Based on WHO 2007 cut-off points for BMI-for-age. BMI standard deviation scores taking in account age and sex were calculated and then based on the specific cut-offs, the BMI-for-age categories were created (<-2: Thinness; -2 < 1: Normal; >1: Overweight; >2: Obese)
** Summary of time spent in physical activities including hours per week spent on running, cycling, basketball, football, volleyball, swimming, dancing and other physical activities.
*** Summary of time spent in sedentary activities including hours per week spent on TV, computer, tablet, mobile phones, or other sedentary activities.
**** Food categories summarized based on consumption per week of food items belonging in each category as reported in the food frequency questionnaire for the conventional period of the study (food portion sizes were denoted in the questionnaire) – Food categories based on Children's Diet Pyramid for children aged 6-12 years (Ministry of Health, Cyprus)

Table 2 Percent change of last conventional sample to last organic sample

	% change [95% CI]	p-value
MDA (nmol/g creatinine)	0.1 [-1.1, 1.2]	0.913
8-OHdG (µg/g creatinine)	1.7 [-0.7, 4.0]	0.167
8-iso-PGF2a (ng/g creatinine)	1.6 [0.2, 2.9]	0.023

All variables are creatinine-adjusted and log-transformed. Based on the imputed data below LOD for MDA
Abbreviations: MDA: malondialdehyde; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2a: 8-iso-Prostaglandin F2a.

Table 3 Comparison of the medians of the biomarkers of oxidative stress/inflammation during the conventional treatment.

	Conventional	Organic	
	Median [25th-75th percentile]	Median [25th-75th percentile]	p-value
n	435	419	
MDA (µmol/g creatinine)	858.21 [691.40, 1016.46]	846.15 [724.58, 1029.20]	0.338
8-OHdG (µg/g creatinine)	322.60 [222.02, 471.06]	295.74 [202.04, 446.74]	0.070
8-iso-PGF2a (ng/g creatinine)	2963.74 [2112.35, 3871.45]	3028.44 [2299.91, 3955.47]	0.259

All variables are creatinine-adjusted. Based on the imputed data below LOD for MDA
Abbreviations: MDA: malondialdehyde; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2a: 8-iso-Prostaglandin F2a.

Table 4 Percentiles of the biomarkers of oxidative stress/inflammation for the whole study period, the organic period and the conventional period.

				Whole study period			
MDA (nmol/g creatinine)	189	526	708	852	1025	1410	3846
8-OHdG (µg/g creatinine)	69.4	116.7	212.3	309	456	801	2913
8-iso-PGF2a (ng/g creatinine)	261	1332	2229	2981	3912	6320	22508
				Organic period			
MDA (nmol/g creatinine)	237	553	725	846	1029	1485	3846
8-OHdG (µg/g creatinine)	69	108	202	296	447	800	1576
8-iso-PGF2a (ng/g creatinine)	447	1321	2300	3028	3955	6248	22508
				Conventional period			
MDA (nmol/g creatinine)	189	508	691	858	1016	1355	2462
8-OHdG (µg/g creatinine)	83.7	127.2	222	322.6	471	796	2913
8-iso-PGF2a (ng/g creatinine)	260.7	1339	2112	2964	3871	6320	21642

All variables are creatinine-adjusted. Based on the imputed data below LOD for MDA
Abbreviations: MDA: malondialdehyde; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2a: 8-iso-Prostaglandin F2a.

Table 5. Linear mixed-effect models of log-transformed OSI biomarkers (8-OHdG, 8-iso-PGF2a, MDA) as a function of time (# of days of treatment, time=0 is start of treatment day), organic diet treatment (in comparison to the conventional diet treatment) and their interaction terms (if p<0.05), adjusting for the baseline levels of the compounds, and accounting for the repeated measurements and clustering by school.

	8-OHdG (ug/g) Coefficient (95% CI)	**8-iso-PGF2a (ng/g)** Coefficient (95% CI)	**MDA (nmol/g)** Coefficient (95% CI)
Time	0 (-0.005, 0.004)	0.011 (0.005, 0.016)	0.005 (0.002, 0.008)
Q-value	0.888	<0·001*	0·010*
Organic diet treatment	-0.119 (-0.213, -0.024)	0.408 (0.232, 0.584)	0·189 (0.083, 0.295)
Q-value	0.035*	<0.001*	0.004*
Interaction of Organic Diet * Time		-0.016 (-0.023, -0.010)	-0·005 (-0.01, -0.001)
Q-value		<0.001*	0.028*
Number of samples	534	649	705
Number of participants	114	144	149
Number of schools	6	6	6
Participant-level random intercept variance	0.030	0.026	0.006
School-level random intercept variance	<0.0001	0.005	0.004
Residual variance	0.295	0.219	0.087

Q-value: Benjamini-Hochberg (BH) adjusted p-value

*Q-value <0.05

Models details:

(a) 8-OHdG, 8-iso-PGF2a and MDA are creatinine adjusted and log-transformed

(b) Adjusted for baseline levels of the dependent variable.

(c) Random intercepts for the repeated visits within participants, and the participants nested within schools, with unstructured covariance matrix.

Abbreviations: 8-OHdG: 8-hydroxy-2'-deoxyguanosine; 8-iso-PGF2a: 8-iso-Prostaglandin F2a; MDA: malondialdehyde; CI: confidence interval; ORs: odds ratios

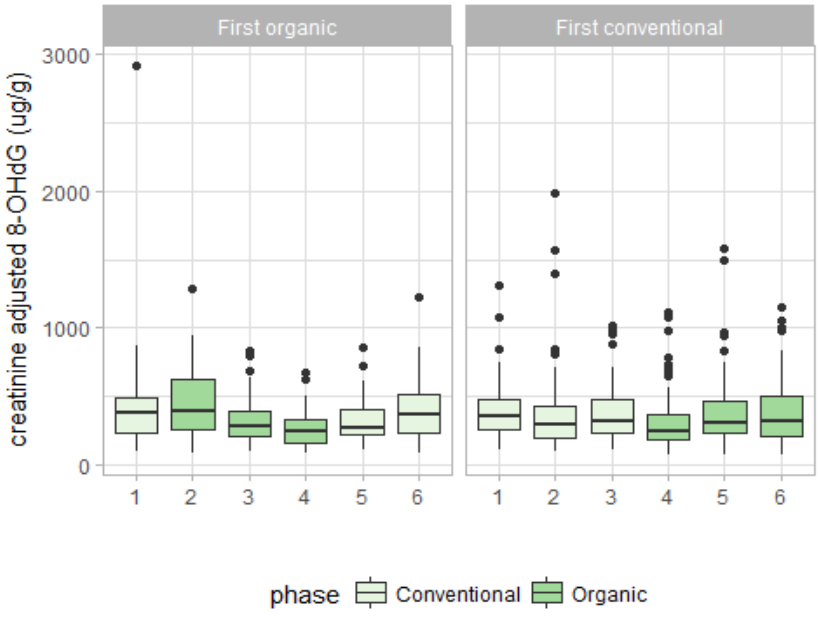

Figure 3 Boxplots of the creatinine adjusted 8-OHdG concentrations for each sample in both study groups

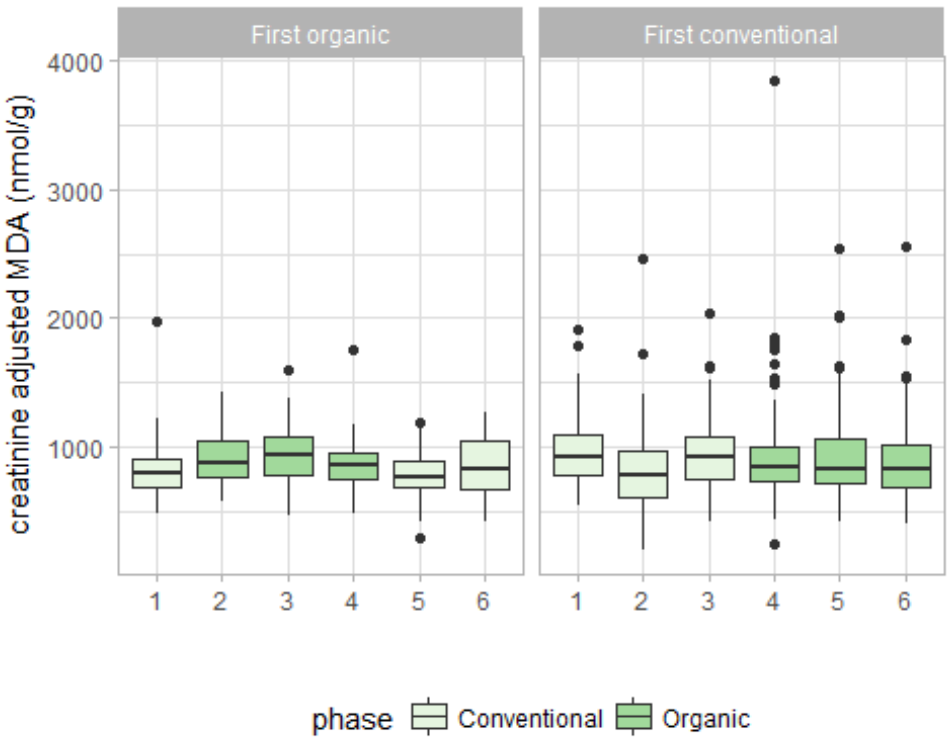

Figure 4 Boxplots of the creatinine adjusted MDA concentrations for each sample in both study groups

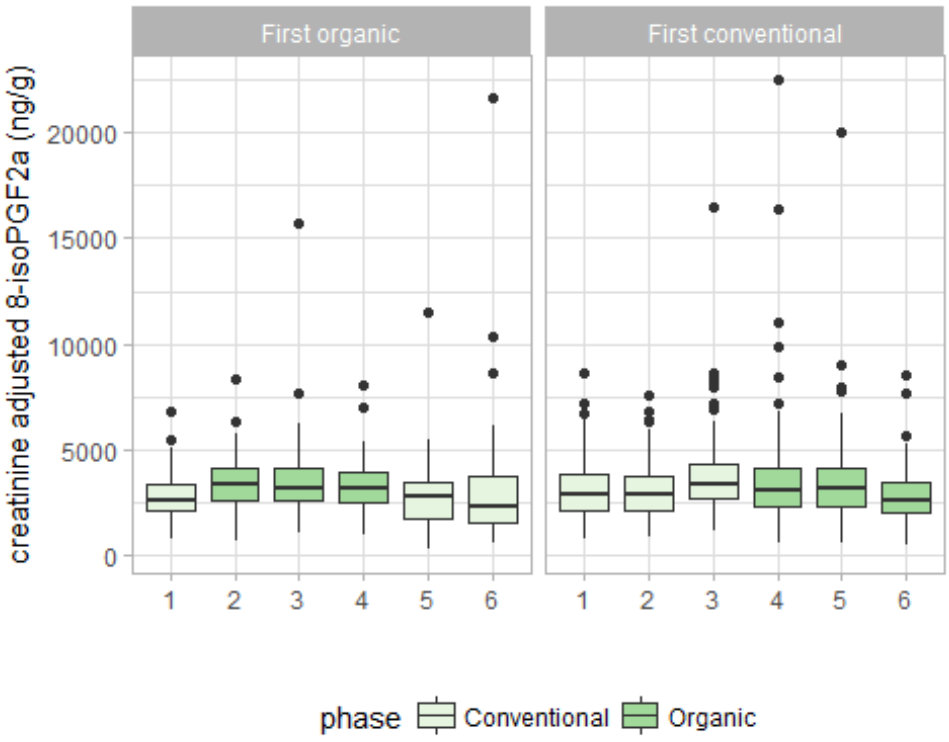

Figure 5 Boxplots of the creatinine adjusted 8-iso-PGF2a concentrations for each sample in both study groups

Discussion

In this cluster-randomized crossover trial, data showed that there was an immediate and sustained reduction in 8-OHdG during the organic period, and, after an initial increase, a gradual reduction during the organic period of 8-iso-PGF2a and MDA (Table 5, Figures 3-5).

Our decision to randomize the intervention at the school-cluster level was intended to avoid the transfer of knowledge about the organic diet intervention from children randomized in the intervention arm to children randomized in the conventional arm (contamination effect).[8] Compliance was reported to be 90% or higher (based on diaries), perhaps from a peer pressure effect as all children at a given school were assigned to follow the same intervention at the same time; however, we cannot exclude the participants' reporting bias. Our findings did not change when we excluded the two children who did not comply with their school's assigned randomization schedule but started the organic treatment with their classmates. A single organic foods-certified restaurant was responsible for the preparation and provision of organic meals to all schools during the organic period, eliminating differences in cooking preparation options and cooking quality. As such, the same raw products, preparation of foods, delivery and consumption of organic meals was followed by all participants. The risk of bias in intervention assignment was minimized by the use of central randomization. Thus, the results can be considered generalizable for the specific population (i.e. primary school children residing in an urban area following the Cypriot diet). Given that participating schools were randomly selected from various areas of the city of Limassol, we do not expect that background population characteristics such as neighborhoods' socioeconomic backgrounds have influenced the observed data.

A strength of the current trial is the larger sample size in comparison to previous trials on the effect of organic diet on OSI biomarkers and antioxidant capacity. Most studies included ≤ 40 participants and only one study had 130 participants.[9] This is the only study on this topic covering a large intervention duration of up to 40 days.

Limitations include the fact that the reported compliance may not reflect the actual compliance of the children to the organic diet, as children could either not consume all meal portion, or families could provide them with extra organic food items, if needed. The organic dietary treatment was a behavioral intervention that may have had other changes beyond the intended pesticide exposure reduction; one example is the caloric imbalance between the two periods. Another example is that, one fruit and three portions of vegetables per day were provided during the organic period which is higher than what the participants had mentioned they consumed regularly during the conventional period (28 portions vs 15 portions). Overall, participants might have changed their habits during their participation as they were initially informed about organic diet and the value of healthy lifestyle.

In conclusion, in our trial, a systematic organic dietary intervention program followed for up to 40 days by healthy children recruited from primary schools was found to reduce over time, all measured OSI biomarkers (8-iso-PGF2a, 8-OHdG and MDA).

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