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Julia Baudry, Véronique Ducros, Nathalie Druesne Pecollo, Pilar Galan, Serge Hercberg, et al.. Some differences in nutritional biomarkers are detected between consumers and non-consumers of organic foods: findings from the BioNutriNet Project. *Current Developments in Nutrition*, 2019, 15 (3), pp.3. 10.1093/cdn/nzy090 . hal-01930384

HAL Id: hal-01930384

<https://hal.science/hal-01930384v1>

Submitted on 21 Nov 2018

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Some differences in nutritional biomarkers are detected between consumers and non-consumers of organic foods: findings from the BioNutriNet Project

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Abbreviations: BMI: Body Mass Index, DBP: diastolic blood pressure, DHA:

docosahexaenoic acid, EPA: eicosapentaenoic acid, PUFA: polyunsaturated fatty acid,

MUFA: monounsaturated fatty acid, mPNNS-GS: modified Programme National Nutrition

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Santé-guidelines score, Org-FFQ: organic food frequency questionnaire, SBP: systolic blood pressure, SFA: saturated fatty acid

Source of support: The BioNutriNet project was supported by the French National Research Agency (Agence Nationale de la Recherche) in the context of the 2013 Programme de Recherche “Systèmes Alimentaires Durables” (ANR-13-ALID-0001).

The NutriNet-Santé cohort study is funded by the following public institutions: Ministère de la Santé, Santé Publique France, Institut National de la Santé et de la Recherche Médicale (INSERM), Institut National de la Recherche Agronomique (INRA), Conservatoire National des Arts et Métiers (CNAM) and Paris 13 University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors’ disclosures: DL has acted as a scientific expert in 2018, with no honoraria or personal funding, in two non-for-profit French foundations (“Fondation Bjorg, Bonneterre et citoyens” and “Fond de dotation Institut de l’alimentation bio”).

Word count: 4,581

Number of Tables: 4 + 3 supplemental tables

Number of Figures: 0

Running Title: nutritional biomarkers and organic consumption

Abstract

Background: Meta-analyses have compared the nutrient content of both organic and non-organic foods. However, the impacts of such variations on human nutritional biomarkers still need to be assessed.

Objective: In a nested clinical study from the NutriNet-Santé study, we aimed to compare the nutritional status of “organic” and “non-organic” food consumers matched on a propensity score.

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Methods: Based on self-reported organic food consumption assessed through a food frequency questionnaire, 150 low and 150 high organic food consumers were selected with <10% or >50% of organic food in their diet respectively (expressed as the proportion of organic food in the whole diet in g/d). Participants were matched using a propensity score derived from sociodemographic, food and health variables. Fasting plasma samples were analyzed using acknowledged laboratory methods for measurements of iron status, magnesium, copper, cadmium, carotenoids, vitamins A and E, and fatty acids.

Results: We found significant differences between low and high organic food consumers with similar dietary patterns, with respect to plasma levels of magnesium, fat-soluble micronutrients (α -carotene, β -carotene, lutein and zeaxanthin), fatty acids (linoleic, palmitoleic, γ -linolenic and docosapentanoic acids), and some fatty acid desaturase indexes. No differences between the two groups were detected for plasma levels of iron, copper, cadmium, lycopene, β -cryptoxanthin, vitamins A and E.

Conclusion: If confirmed by other studies, our data suggest that a high consumption of organic foods, compared to very low consumption, modulates, to some extent, the nutritional status of individuals with similar dietary patterns. Further research including prospective cohort studies is needed to evaluate the clinical relevance of such differences.

Keywords: epidemiological study, vitamins, minerals, carotenoids, fatty acids, organic food, biomarkers

Introduction

In the past few years, a considerable development of the organic food market (1) has been observed, especially in Europe. This growth has been largely driven by consumer concerns for food safety, healthy diet and environment (2,3). Organic products are considered healthier than conventional (non-organic) products by consumers mainly because of their absence of synthetic pesticide residues (4–9). This is supported by findings from human trials which

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reveal that replacing conventional foods with organic foods leads to a drastic reduction in urinary pesticide residues and metabolites in various population subgroups (10–13).

Observational data from cohorts conducted in USA (14) and France (15) also confirm differences in exposure based on urinary pesticide metabolites.

Differences of practices between the production systems (organic/conventional) may also lead to differences in the contents of several bioactive compounds and nutrients in both animal and plant-based foods. These differences may be related to changes in feeding regime and lifespans for animals and to fertilization and weed/pest control for crops. These factors could modulate the levels of some nutrients and/or defense secondary metabolites (16).

Organic foods, compared to conventional foods, overall show better nutritional content, that include higher levels of specific antioxidants in plant foods and more beneficial fatty acid profiles in dairy products and meats, according to recent meta-analyses (17–19).

The authors observed a higher amount of some phenolic compounds, including flavonoids and phenolic acid (range +18-69%), as well as higher levels of some carotenoids (xanthophylls), vitamin C and lower protein levels in organic plant foods compared to conventional plant foods. Two reviews also reported trends towards higher magnesium levels in organic plant foods (20,21). Data concerning comparisons of copper levels in foods are scarce although a study conducted on vegetables showed higher copper contents in organic lettuce, lower contents in peppers and comparable levels in tomatoes (22).

Organic dairies were also shown to have higher contents of α -tocopherol and iron but by contrast lower iodine and selenium concentrations compared to conventional dairies (17).

The levels of minerals and heavy metals in organic and conventional meats were evaluated but the disparities in data did not allow to state in a definitive manner on differences between the two methods of production (19).

Regarding toxic heavy metals, the meta-analysis of Baranski et al. (18) reported that organic

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crops, especially cereals, contained significantly lower cadmium concentrations (- 48%) compared to conventional crops.

Concerning fatty acids contents, a pioneer review (23) and a recent meta-analysis of 170 studies (20,22) showed a higher content of polyunsaturated fatty acids (PUFAs), and more markedly of nutritionally-desirable omega-3 fatty acids (+ 56%) in milk and dairies.

Moreover, a meta-analysis based on 67 comparative studies (20) showed that organic meats had significantly higher contents in total PUFAs (+ 23%) and n-3 PUFAs (+ 47%) with significantly lower monounsaturated fatty acids (MUFAs) and slightly, but significantly, lower concentrations of saturated myristic and palmitic acids (20,22).

Clinical studies comparing the nutritional status of consumers of organic or conventional foods are scarce and are based on poor methodologies that include low number of subjects and short test durations, and have led to inconclusive findings (24). In a cross-over trial in patients, urinary excretion of quercetin and kaempferol (two main dietary phenolic compounds) were significantly increased after introduction of an organic diet (25).

In this context, the objective of the present observational study was to test for differences in nutritional biomarkers among adults with low and high self-reported organic food consumption. These biomarkers included plasma levels of vitamins A and E, carotenoids, minerals (iron, magnesium, and copper), heavy metal (cadmium) as well as fatty acid profiles. We hypothesized that reported differences in food nutrient (or compound) contents observed between the two farming systems (organic and conventional) could lead to higher plasma concentrations of the above-mentioned nutrients, vitamins, and minerals, but also to lower plasma levels of cadmium in organic food consumers compared to their conventional counterparts, independently of the diet composition. We, therefore, matched the two groups on numerous traits, including food group consumption, in order to isolate the specific effect of

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the production system (organic or conventional), beyond the dietary pattern structure, on some selected nutritional status biomarkers.

Material and methods

Study population

The NutriNet-Santé study is a web-based observational prospective study conducted on a large sample of adult volunteers. The overall objective is to study the link between nutrition and health (26). Adults (aged 18y or older) are recruited in the general population through multimedia campaigns. All questionnaires are filled in online using a dedicated secured web-platform.

In addition, participants were also invited, on a voluntary basis, to join health centers for biological sampling and clinical examination (2011-2013). A total of about 20,000 volunteers were included in this subsample. Electronic and paper written informed consents were signed by all subjects attending the visit.

The NutriNet-Santé study is conducted according to the Declaration of Helsinki guidelines and was approved by the Institutional Review Board of the French Institute for Health and Medical Research (IRB Inserm n°0000388FWA00005831) and the "Commission Nationale de l'Informatique et des Libertés" (CNIL n°908450/n°909216/n°1460707). Electronic informed consent is obtained from each participant. All procedures of the clinical study were approved by the Consultation Committee for the Protection of Participants in Biomedical Research" (C09-42 on May 5th 2010). The NutriNet-Santé study is registered in ClinicalTrials.gov (#NCT03335644).

Data collection

At baseline and yearly thereafter, participants were asked to complete questionnaires providing sociodemographic (age, sex, educational level, employment status, place of

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residence) and lifestyle (smoking status, physical activity) information and health data (menopausal status for women, medical history and medication).

During the clinical visits, clinical outcomes were measured (systolic and diastolic blood pressure (SBP/DBP), weight and height) using standardized procedures (27).

Hypertension, hypercholesterolemia, hypertriglyceridemia and type II diabetes status were defined using standardized cut-offs: high BP (SBP/DBP \geq 140/90 mmHg), hypertriglyceridemia (\geq 150 mg/dL), low HDL-cholesterolemia (<40 mg/dL for men or <50mg/dL for women) and hyperglycemia (fasting blood glucose <1.01 g/L).

Assessment of total and organic food consumption

To estimate total and organic food consumption, subjects were asked to complete an optional 264 item-organic semi-quantitative food frequency questionnaire (Org-FFQ) in June 2014.

Extensive details have been provided elsewhere (28). For each item, subjects were asked to report the frequency of consumption and the quantity consumed during the last year. For each food item, a five-point Likert-type scale (corresponding to the following modalities: never, rarely, half of the time, often and always) was also provided, to estimate the frequency of organic food consumption. Organic food intake for each item was obtained by applying a weighting of 0, 0.25, 0.5, 0.75 and 1 to the respective frequency modalities.

The proportion of organic food in whole diet was calculated by dividing the total organic food consumption out of the total food consumption (excluding water).

We also computed the mPNNS-GS (modified Programme National Nutrition Santé-guidelines score), an *a priori* dietary score reflecting the level of adherence to the French nutritional guidelines; higher scores (max=13.5) reflect higher dietary quality (29).

Selection of the subsample and matching procedure

Of the 33,384 subjects who had completed the Org-FFQ, we selected those who had attended the clinical visits with valid dietary data and no missing covariates (N=5,746). Then, we

selected subjects with fasting at least 6 hours before the visit, with no history of type I diabetes, Crohn's disease, all types of cancer, neurological diseases, cardiovascular diseases, digestive system diseases (including cirrhosis, hepatitis, celiac disease and colitis), lupus, spondylolisthesis and sclerosis (N=4,598). A propensity matching procedure without replacement was applied among subjects with high or low organic food consumption in their diet (N=2,351). We obtained two propensity score matched groups of 150 subjects differing on the organic valence of their diet.

Blood collection and biomarkers analysis

During the clinical visit, participants underwent a 43 ml fasting blood sampling using Vacutainer® tube to be distributed into 5 vacutainers containing different kinds of anti-coagulants and separators (9 ml tubes containing EDTA K2, one 9 ml tube containing lithium-heparin and two 8 ml plastic tubes containing an inert gel). These vacutainers were used to obtain plasma, serum, buffy coat (for DNA extraction) and red blood cells. The tubes were then fractionated into 28 aliquots per subject and stored at a temperature of -80°C at the central laboratory of the biobank (in Paris 13 University). For a given analyte, all measurements were performed in the same laboratory.

Vitamin and mineral biomarkers analysis

Plasma vitamin A and E as well as 6 carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, zeaxanthin, and lycopene) were analyzed by reverse-phase high performance liquid chromatography (HPLC) coupled to diode-array detection (30). Spectra of retinol ($\lambda=325$ nm), α -tocopherol ($\lambda=292$ nm), and carotenoids ($\lambda=450$ nm) were recorded. The quantification of retinol, α tocopherol and β -carotene was based on calibration curves with each primary standard. For other carotenoids, the extinction coefficients as measured in the solvent mixture, in which the compounds were eluted, was used to establish the corresponding calibration curve. Quality controls were purchased from Chromsystems (vitamins A and E

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serum control bi-level and β -carotene serum control bi-level). Between-run precision was 12% for retinol, 13% for α -tocopherol and 12% for carotenoids.

Plasma copper and cadmium were determined by inductively coupled plasma to mass spectrometry (ICP-MS) (31). Plasma was diluted to 1:25 with 1% (w/v) HNO₃ prior to ICP-MS determination. Gallium (Ga at 650 nmol/L) and Rhodium (Rh at 50 nmol/L) were used as internal standards for copper and cadmium determination, respectively. Quality controls were purchased from Recipe (ClinChek serum level 1 and 2). Between-run precision was less than 2.3% for copper and 6.3% for cadmium. Plasma magnesium, and iron as well as transferrin, and ferritin were determined on Dimension Vista® 1500 Analyser (Siemens Healthcare Diagnostics). Iron and magnesium were measured by the photometric method. Transferrin was determined by the nephelometric technique and ferritin by the chemiluminescent technology (LOCI®). Between-run precision was 1.8% at 12.9 μ mol/L for iron (level 1) and 3.3% at 1.12 mmol/L for magnesium (level 2) (Liquichek Multiqual, 3 levels, Biorad). Between-run precision was 2.5 % at 29 μ g/L for ferritin (Immunoassay Plus, level 2, Biorad) and 2.75% at 2.6 g/L for transferrin (Immunology, level 2, Biorad).

Fatty acid analysis

Plasma fatty acid composition (14:0 to 22:6n-3) was determined. Fatty acids were extracted from fasting plasma and transmethylated with acetyl chloride in methanol. (1/19 : v/v). Plasma (200 μ L) was diluted in 1.9 mL of acetyl chloride/methanol and the mixture was heated at 100°C during 60 minutes. After cooling the mixture, 1 mL hexane and 1 mL distilled water were added and centrifuged at 2 000 rpm during 3 min under 10°C. The hexanic phase was taken and the extraction was conducted twice. Hexanic phases were pooled, diluted with methanol (200 μ L of methanol to facilitate drying) and dried under nitrogen. Dried samples were diluted in 200 μ L hexane. Fatty acid methyl esters were analyzed by fast GC (Gas Chromatograph Clarus 600, Perkin Elmer) performed from a 0.5- μ L

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sample injected in split mode (split ratio 200:1) with a hydrogen flow rate of 10 ml/min (61.4 cm/s and a constant head pressure of 206.8 kPa). The column was a capillary column (BP×70, 10 m×0.1 mm ID×0.2 µm film thickness) (SGE International Pty. Ltd., Australia). The temperature program was as follows: initial, 60°C with a 0.5-min hold; ramp, 20°C/min to 200°C, 7°C/min to 225°C with a 1-min hold and then 160°C/min to 250°C with a 1-min hold. The instrumental conditions were as follows: flame ionization detector was set at 280°C; the air and nitrogen make-up gas flow rates were 450 ml/min and 45 ml/min, respectively; the detector sampling frequency was 50 Hz; the run time for a single sample was 13.23 min with a sample injection-to-injection time of 16 min. Between-run precisions were: <2% for C14:0, C16:0, C16:1n-7, C18:1n-9 and C18:2n-6, C18:3 n-3, from 2 to 7% for C20:3n-6, C20:4n-6, C20:5n-3, C22:5n-6 and C22:6n-3, from 7 to 9% for C18:0, C18:3n-6 and C24:1n-9.

Statistical analysis

Selection of the participants (namely, selection of two groups of 150 subjects with organic or non-organic diet) and matching method (based on propensity score) are described in

Supplemental Material 1. A balance diagnostic of the matching method was performed (using SAS macro %*pmdiag*) to provide standardized differences for variables included in the propensity score model as recommended by Ali et al. (32). Characteristics of the participants are presented by group of consumers (organic vs non-organic) and compared using Wilcoxon signed-rank test for matched samples for continuous variables, McNemar test for binary variables and conditional logistic regression for categorical variables (>2 modalities variables). Distribution indicators, frequency of detection and of quantification are also presented.

To test for residual confounding in food group consumption between organic and conventional consumers, for biomarkers for which corresponding nutrient value was available in the conventionally-grown food composition database (β -carotene, retinol, iron, magnesium

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and some fatty acids), a supplementary model controlled for the nutrient intake was performed using pair-adjusted ANCOVA. For fatty acids, intakes were considered as percentage of energy intake.

Thus, in case of similar content for a given nutrient in both organic and conventional food, this adjustment should reduce observed differences due to disparities in food patterns.

As the methodological approach to test for differences between ANCOVA and matched paired Wilcoxon test do not rely on the same hypothesis, the p-values may differ between the two methods. Therefore such findings should be interpreted in the light of possible modification of the effect by the adjustment. All analyses were performed using 9.4 version of the SAS software (SAS Institute Inc., Cary, NC, USA).

Results

The findings of the balance diagnostic of the matching process are presented in **Supplemental Table 1**. As expected, overall, no differences in health, sociodemographic and diet factors were detected between organic and non-organic groups, except for consumption of mixed dishes (p-value <0.05). The mean proportion of organic food in the diet was markedly different between groups with an average of 3 % of total food intake in the non-organic group and of 67% in the organic group. Participants' characteristics are shown in **Table 1**.

Participants were approximately 58y, 30% were men and more than 60% held a high school diploma. Average BMI was 24 kg/m² and the overall nutritional quality of the diet, reflected by the mPNNS-GS, was relatively high (8.7 vs 13.5 max).

No significant differences were found between the two groups for α -tocopherol and retinol fasting plasma levels as shown in **Table 2**. Organic consumers exhibited higher plasma concentrations of α -carotene, β -carotene, lutein and zeaxanthin while no differences were found for other carotenoids (β -cryptoxanthin and lycopene) (**Table 2**).

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The means of plasma concentrations in minerals and iron-related proteins in organic and conventional groups are shown in **Table 3**. Organic consumers displayed a higher plasma concentration of magnesium and a lower plasma concentration of iron while ferritin and transferrin levels as well as saturation coefficient were comparable. No difference between groups was observed for the other minerals tested i.e. cadmium and copper.

Plasma fatty acid profiles in both groups are presented in **Table 4**. Organic consumers had lower relative plasma levels of palmitoleic acid, γ -linolenic acid and docosapentaenoic acid and higher linoleic acid levels. No differences were found for other individual fatty acid moieties as well as for total SFAs, MUFAs and total PUFAs. Organic consumers showed a significantly lower C18:3n-6/C18:2n6 ratio and a borderline statistically significant lower C16:1n-7/C16:0 ratio, compared to non-organic consumers

The supplementary models adjusted for corresponding nutrient intake are presented in **Supplemental Table 3**. Overall, findings were unchanged except the iron concentration for which there was no difference detected between organic and conventional groups after adjustment for iron intake. In addition, the difference in α -linoleic and EPA concentrations became statistically significant with lower concentrations in the organic group compared to the conventional one.

Discussion

In the present observational study, we compared various biomarkers of the nutritional status in two groups of consumers with discriminant organic food consumption (representing either <10% or >50% of the diet) while controlling for numerous variables including food group consumption through propensity score matching. Significantly higher concentrations of β -carotene, lutein and zeaxanthin, magnesium, and some differences in fatty acid profile were observed in the plasma of high organic consumers.

No differences were detected for vitamin E (α -tocopherol) and vitamin A (retinol) between

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the two groups, while carotenoid plasma levels were mostly higher in high organic consumers than in low-organic consumers. The status of fat-soluble micronutrients depends on many factors affecting their absorption and metabolism, involving (i) the state of the food (raw, processed or cooked), (ii) the composition of the meal and (iii) the individual genetic factors (33). Although higher α -tocopherol levels were detected in few number of organic foods, such as organic milk (17) and olive oil (34), these sources of intake remained minor compared to the main sources, i.e. vegetable oils and wheat germ/wholegrain cereals. Regarding retinol (vitamin A) levels, the main food sources are offal and meat. Vitamin A can also be provided by plant foods as they are good sources of provitamin A-carotenoids (including mainly β -carotene, α -carotene and β -cryptoxanthin). However, the absence of difference in the overall vitamin A status between the two groups of consumers could be explained by the large variation in the inter-individual metabolism of provitamin A carotenoid. The latter relies on many factors, such as lifestyle, gender and age as well as genetic variants (35). Interestingly, the higher plasma carotenoid concentration (α -carotene, β -carotene, lutein and zeaxanthin) evidenced herein in organic consumers could likely be related to higher intakes of such carotenoids notably provided by organic vegetables (16,18,20). In addition, as regards β -carotene, further adjustment for intake (calculated from conventionally grown food composition) did not modify the findings that argue for difference in contents related to the farming system. In our study, among the fat-soluble micronutrients, the strongest differences were for lutein and zeaxanthin. Two hypotheses may explain such marked differences: (i) lutein and zeaxanthin are xanthophylls (having an oxygen within their structures) known to be better absorbed than carotenes (33), (ii) lutein and zeaxanthin are non-provitamin A carotenoid that are not enzymatically-centred cleaved in comparison with the provitamin A xanthophyll β -cryptoxanthin. Thus, the higher plasma level of lutein/zeaxanthin in organic consumers may likely reflect the higher intake of these two xanthophylls elicited by the

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consumption of organic vegetables which may be richer in these compounds than conventional vegetables (18). This observation of higher concentrations in plasma lutein in organic consumers is in agreement with data of an experimental study conducted in rats given an organic crop-regimen (36).

To date, data comparing organic and non-organic consumers as regards minerals status are scarce. Our study showed a significant slightly higher concentration of plasma magnesium in organic consumers compared to non-organic consumers. Such observations are in line with some reviews (20,21) reporting higher magnesium contents in organic plant foods but to the best of our knowledge, no data in humans are available. Although differences found between groups in the plasma magnesium levels are small, they might have some long-term clinical relevance in terms of risk of metabolic syndrome (37) or pre-diabetes/type 2 diabetes (38).

We did not find any differences concerning the iron status markers between the two groups of consumers. This is in line with the limited or no difference in iron contents in foods from organic or conventional agriculture (39). Regarding plasma copper levels, no difference was found when comparing the two groups of individuals. This is concordant with a cross-over intervention trials using stable isotope which did not report differences in copper metabolism after consumption of organic vs conventional diet (40). Evidence regarding differences in copper content between organic and non-organic foods is scarce while copper-based sanitary preparations are classically used both in conventional and organic agricultural practices. However, factors such as the type of soil could be more significant than the cropping system to predict the mineral content of plants.

Of note, we did not observe any differences in plasma cadmium concentrations between organic and non-organic consumers. Although data on comparison between contents of heavy metals in organic and conventional consumers are scarce, the cadmium content of organic crops, compared to conventional ones, has been reported to be noticeably lower, especially in

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cereals (15) due to differences in fertilizer use for plant growing in the two agricultural systems (18,41). Plant foods are an important source of toxic cadmium for humans but several other exposure routes exist (42) which may explain at least partly the absence of differences found herein in plasma cadmium levels in the two groups of food consumers.

It is well known that the plasma fatty acids composition is the result of both the fatty acid intakes and the body metabolic capacity to metabolize, elongate and desaturate various fatty acids (43). The plasma fatty acid status observed in organic consumers was mostly comparable to matched non-organic-consumers for main fatty acids (SFAs, MUFAs, PUFAs) with a few exceptions such as higher levels of linoleic acid and lower levels of palmitoleic, γ -linolenic and docosapentanoic acids. It is noteworthy that we did not observe any difference between groups for long-chain n-3 fatty acids (EPA and DHA) despite the documented higher levels of n-3 fatty acids in organic dairies and meat (17,19). In the model adjusted for respective intake from conventionally grown food composition data, EPA concentration was even lower among organic consumers than in conventional ones. It is possible that the relatively small levels of n-3 fatty acids present in milk or meat cannot be sufficiently elevated to modify the fatty acid profile at the body level. Another explanation may rely on the low consumption of animal products in the selected sample (with matching based on organic consumers). The higher plasma level of linoleic acid could likely result from some higher intake of vegetable oils rich in linoleic acid in organic consumers and/or a lower rate of conversion into other n-6 moieties. Interestingly, a low-linoleic acid factor identified through principal component analysis has been shown to predict the metabolic syndrome development over 20 y, independent of smoking habits, physical activity and BMI (44). The other lower plasma fatty acids found in organic consumers (palmitoleic, γ -linolenic and docosapentanoic acids) are metabolic intermediates produced by elongation and/or desaturation of fatty acids from different families; despite their low plasma concentration levels, such differences can

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have some relevance when considering the metabolic pathways involved. Indeed, higher levels of palmitoleic acid have been associated with hypertriglyceridemia (45). This is also illustrated by the calculated ratios of precursor/metabolite as representative of specific desaturase enzymatic activities (46). Interestingly, we also found that organic consumers displayed a significantly lower C18:3n6/C18:2n6 ratio and a borderline significantly lower C16:1:n7/ C16:0 ratio. The first ratio reflects the activity of the delta 6 desaturase enzyme. The second one reflects the activity of the delta 9 desaturase enzyme (also called SCD1). Several publications have reported that estimated increases in such enzyme activities are associated with the metabolic syndrome (44), a condition with central obesity and several metabolic imbalances considered as a major risk factor for type 2 diabetes and cardiovascular diseases (47). Despite the strong matching performed herein, the data obtained suggest that the organic consumers exhibit some different fatty acid profiles that are associated with a potentially lower risk for the above mentioned pathological condition. This appears consistent with our previous observations. We found that, in the NutriNet-santé cohort, regular organic consumers compared to non-consumers, have a odds of overweight and obesity reduced by about half in both men and women (2), a risk of developing obesity reduced by 31% (48) and a probability of having a metabolic syndrome reduced by 31% (49).

The current study has some limitations. Similarly to other cohort's participants, volunteers involved in the NutriNet-Santé study are more likely to be more concerned by nutritional and health-related issues than the general population. Thus, the external validation of these findings should be made with caution. Specifically, the participants exhibited particular profiles with regard to sociodemographics and dietary patterns (1).

In addition, food consumption was evaluated using a self-administered food frequency questionnaire that is susceptible to measurement errors leading to a probable overestimation of the consumption in particular for organic food (2). Another limitation relies on the fact that

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among the organic consumers, the total mean share of organic foods was 67% only, i.e. diet is not entirely composed of organic foods. This could limit the possible differences in the observed impacts of food production practices as organic consumers also partly consumed conventional foods. Blood vitamin C concentration was not estimated due to methodological constraint related to biological collection.

Finally, despite a wide range of variables accounted for in the propensity score matching procedure, we cannot rule out possible residual confounding between groups of consumers (organic and non-organic) in particular as we are focusing on food group consumption in the matching procedure and not on specific food.

We also did not consider genetic factors that can play a role in nutrient absorption and metabolism.

“Important strengths should also be mentioned. We used accurate biological measures obtained in expert laboratories. The use of the Org-FFQ permitted to obtain detailed data on food consumption, including information on the usual proportion coming from organic sources of several food groups, within the overall diet. The use of a wide range of covariates, including sociodemographic, health conditions, and lifestyle variables, enabled us to precisely characterize the individuals selected in our study. Furthermore, the reasonable size of the sample enabled us to conduct analyses with a sufficient statistical power. The extensive matching made to compare the non-organic consumers with organic consumers allowed us to evaluate the impact of food composition elicited by the agriculture practices on some traits of the nutritional status of human adults, independently of numerous variables including food pattern. It is the first comparative study performed based on such an approach that considers the actual level of consumption. Nevertheless, this matching procedure that blunts numerous differences in lifestyles, dietary patterns and socio-economic traits of non-organic consumers vs organic ones does not allow to extrapolate the present results to the actual characteristics of the non-organic consumers exhibiting different dietary patterns (2).

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In conclusion, the present study allowed the estimation of the possible impacts of food composition variations due to agricultural practices on some traits of the nutritional status. We found that high consumption of organic foods, compared with low, within the same dietary pattern, could modulate the plasma levels of magnesium but not iron, copper or the heavy metal cadmium. Some differences were detected for some carotenoids such as α -carotene, β -carotene, lutein and zeaxanthin but not vitamins A and E, lycopene or β -cryptoxanthin and for some fatty acid relative levels such as linoleic acid, palmitoleic, γ -linolenic and docosapentanoic acids and some fatty acid desaturase indexes. The clinical effects of such differences need further investigation by evaluating long term health effects.

Acknowledgements:

The authors thank Catherine Tardivel for lipid profile identification. They also thank Cédric Agaesse and Anne-Elise Dussoulier (dietitians); Frédéric Coffinières, Thi Hong Van Duong, Younes Esseddik (IT manager), Paul Flanzky, Régis Gatibelza, Jagatjit Mohinder and Maithyly Sivapalan (computer scientists); and Julien Allègre, Nathalie Arnault, Laurent Bourhis, Véronique Gourlet, PhD and Fabien Szabo de Edelenyi, PhD (supervisor) (data-manager/statisticians) for their technical contribution to the NutriNet-Santé study and all the volunteers of the NutriNet-Santé cohort.

Contributions:

The author contributions were as follows: SH, EKG, DL and PG conceived and designed the experiments. VD, LD and MJA performed analytical measurements. JB, MJA, DL and EKG analyzed the data and wrote the paper. All the authors were involved in interpreting results and editing the manuscript. EKG had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1: Main characteristics of the sample, n=300, NutriNet-Santé¹

	Non-Organic group	Organic group	P-value ²
	N=150	N=150	
Proportion (%) of organic food in the diet	3 ± 3	67 ± 13	<0.0001
Age (y)	58.71 ± 12.78	58.35 ± 11.69	0.60
% Male	28%	32%	0.47
Energy intake (kcal/d)	1926.62 ± 561.27	1994.94 ± 601.57	0.37
mPNNS-GS³	8.73 ± 1.76	8.73 ± 1.67	0.59
Body mass index (BMI) (kg/m²)	24.18 ± 4.11	24.19 ± 4.02	0.93
Tobacco status (%)			0.38
never smoker	48.67	47.33	
former smoker	44.00	40.67	
current smoker	7.33	12	
Physical activity (%)			0.85
Missing	15	12	
low	25	29	
medium	55	52	
high	55	57	
Vegetarian or vegan diet (yes) (%)	1.33	2	0.65
Location (%)			0.75
rural community	24	25.33	
urban unit with a population smaller than 20 000 inhabitants	11.33	14	
urban unit with a population between 20 000 and 200 000 inhabitants	18.67	20.67	
urban unit with a population higher than 200 000 inhabitants	46	40	
Education			0.60

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< high school diploma	22	22	
high school diploma	12.67	16.67	
> high school diploma	65.34	61.34	
Income per household unit (€) (%)			0.77
refused to declare	9.33	7.33	
900-1200	6.67	8.67	
1200-1800	22.67	20	
1800-2700	22	26.67	
>2700	39.33	37.33	
Nutrient intakes			
β -carotene	4731.23 \pm 3136.39	4852.71 \pm 2689.19	0.36
Retinol (g/day)	512.84 \pm 397.83	506.76 \pm 446.20	0.61
Vitamin E (g/day)	14.66 \pm 8.24	14.35 \pm 5.45	0.82
Iron (g/day)	14.86 \pm 5.50	15.86 \pm 5.64	0.10
SFA (g/day)	30.34 \pm 11.37	32.86 \pm 12.45	0.13
MUFA (g/day)	33.45 \pm 15.63	37.59 \pm 14.45	0.004
PUFA (g/day)	14.51 \pm 8.15	15.06 \pm 6.21	0.26
Copper (mg/day)	1.98 \pm 0.82	2.12 \pm 0.75	0.05
Magnesium (mg/day)	480.86 \pm 168.42	470.20 \pm 161.79	0.46
α -linoleic acid (g/day)	1.46 \pm 1.19	1.67 \pm 1.09	0.01
DHA (g/day)	0.26 \pm 0.27	0.27 \pm 0.24	0.37
EPA (g/day)	0.21 \pm 0.23	0.21 \pm 0.21	0.41
Linoleic acid (g/day)	11.67 \pm 7.09	11.95 \pm 5.25	0.36
Arachidonic acid (g/day)	0.13 \pm .06	0.14 \pm 0.07	0.30

Abbreviations: DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid; SFAs: saturated fatty acid

¹Values are means \pm SD or % as appropriate

²P-value referred to Wilcoxon signed rank tests for continuous variables, Mc Nemar's test or conditional logistic regression for categorical variables

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³mPNNS-GS for modified Programme National Nutrition Santé-Guidelines score, a dietary index reflecting the level of adherence to French nutritional guidelines. Maximum score=13.5 points.

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Table 2 Plasma concentrations of α -tocopherol, retinol and pro-vitamin A carotenoids (α and β -carotene, β -cryptoxanthin) and non-provitamin A carotenoids (lycopene, lutein and zeaxanthin) in organic and non-organic consumers¹

Concentrations ($\mu\text{mol/L}$)	Non-Organic group	Organic group	P-value ²
α -tocopherol	29.33 (28.27, 30.40)	30.34 (29.28, 31.40)	0.22
Retinol	1.66 (1.60, 1.73)	1.69 (1.63, 1.76)	0.52
α -carotene	0.30 (0.26, 0.34)	0.33 (0.29, 0.37)	0.04
β -carotene	0.96 (0.86, 1.06)	1.09 (1.00, 1.19)	0.04
β -cryptoxanthin	0.27 (0.24, 0.31)	0.30 (0.6, 0.33)	0.50
Lycopene	0.43 (0.39, 0.46)	0.47 (0.44, 0.50)	0.12
Lutein	0.41 (0.38, 0.43)	0.48 (0.45, 0.50)	0.002
Zeaxanthin	0.096 (0.087, 0.105)	0.113 (0.105, 0.122)	0.003

¹Values are means (95% Confidence Interval)

²P-value referred to the Wilcoxon signed-rank test for paired data.

Table 3 Plasma concentrations of minerals and proteins related to iron status in organic and non-organic consumers¹

Concentrations	Non-Organic group	Organic group	P-value ²
Iron (µmol/L)	16.21 (15.29, 17.17)	15.24 (14.39, 16.15)	0.05
Transferrin (g/L)	2.65 (2.58, 2.71)	2.62 (2.56, 2.68)	0.42
Ferritin (µg/L)	71.95 (61.25, 84.53)	73.67 (62.71, 86.55)	0.54
Saturation coefficient	24.50 (22.94 -26.16)	23.28 (21.81 -24.86)	0.30
Magnesium (mmol/L)	0.83 (0.82, 0.84)	0.84 (0.83, 0.85)	0.01
Copper (µmol/L)	16.74 (16.20, 17.29)	17.15 (16.60, 17.72)	0.64
Cadmium (nmol/L)	0.64 (0.61, 0.67)	0.63 (0.60, 0.66)	0.70

¹Values are expressed as means (95% Confidence Interval)

²P-value referred to the Wilcoxon signed-rank test for paired data.

Table 4 Plasma fatty acid levels (% total) in organic and non-organic consumers¹

% total fatty acids	Non-Organic group	Organic group	P-value ²
C14:0 (Myristic acid)	1.006 (0.959, 1.053)	0.975 (0.928, 1.022)	0.72
C16:0 (Palmitic acid)	24.895 (24.617, 25.174)	24.590 (24.311, 24.869)	0.24
C16:1n-7 (Palmitoleic acid)	2.008 (1.906, 2.110)	1.844 (1.742, 1.946)	0.04
C18:0 (Stearic acid)	6.393 (6.234, 6.553)	6.425 (6.265, 6.585)	0.83
C18:1 n-9 (Oleic acid)	18.679 (18.293, 19.065)	18.754 (18.368, 19.140)	0.66
C18:2 n-6 (Linoleic acid)	24.732 (24.203, 25.260)	25.650 (25.122, 26.179)	0.02
C18:3 n-3 (α-linolenic acid)	0.538 (0.510, 0.566)	0.506 (0.478, 0.533)	0.14
C18:3 n-6 (γ-linolenic acid)	0.399 (0.377, 0.422)	0.362 (0.340, 0.385)	0.06
C20:3 n-6 (Dihomo γ-linolenic acid)	1.903 (1.827, 1.978)	1.812 (1.737, 1.888)	0.12
C20:4 n-6 (Arachidonic acid)	7.869 (7.628, 8.111)	7.887 (7.646, 8.129)	0.97
C20:5 n-3 (Eicosapentaenoic acid, EPA)	1.452 (1.322, 1.583)	1.258 (1.128, 1.388)	0.10
C22:5 n-3 (Docosapentaenoic acid)	0.732 (0.705, 0.758)	0.683 (0.656, 0.709)	0.02
C22:6 n-3 (Docosahexaenoic acid, DHA)	3.153 (3.007, 3.299)	3.126 (2.980, 3.272)	0.93

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C24:1 n-9	1.324 (1.279, 1.369)	1.289 (1.244, 1.335)	0.41
Total SFAs, MUFAs, PUFAs			
SFAs	33.35 (32.98, 33.72)	33.07 (32.70, 33.44)	0.52
MUFAs	23.64 (23.21 - 24.07)	23.42 (22.98 - 23.85)	0.65
PUFAs	41.79 (41.26, 42.33)	42.29 (41.75, 42.82)	0.55
n-6 PUFAs	35.87 (35.35, 36.38)	36.67 (36.15, 37.18)	0.10
n-3 PUFAs	5.93 (5.65, 6.20)	5.62 (5.35, 5.89)	0.26
Fatty acid Ratios (desaturase indexes)			
C16:1n7/ C16:0	0.081 (0.077, 0.085)	0.075 (0.071, 0.079)	0.08
C18:1n9/18:0	3.015 (2.894, 3.136)	3.016 (2.895, 3.137)	0.95
C18:3n6/C18:2n6	0.017 (0.015, 0.018)	0.015 (0.013, 0.016)	0.02
C20:4n6/C20:3n6	4.406 (4.196, 4.616)	4.555 (4.344, 4.765)	0.28

Abbreviations: MUFA: mono-unsaturated fatty acid; PUFA: poly-unsaturated fatty acid;

SFAs: saturated fatty acid

¹Values are expressed as means (95%CI)

²P-value referred to the Wilcoxon signed-rank test for paired data

Fatty acids with plasma levels below 1% which were not statistically different between groups are not shown in Table 4 (C15:0, C16:1 n-9, C20:0 ; C20:1 ; C20 :2, C20:3 n-3, C21, C22 :0, C22:4 n-6, C22:5 n-6)

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