The Effects of Italian Mediterranean Organic Diet (IMOD) on Health Status

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Abstract: *Objective:* The aim of this study was to verify the effects of Italian Mediterranean Diet (IMD), consisting of organic versus conventional foods, on body composition, and biochemical parameters in a healthy individuals and in Chronic Kidney Disease (CKD) patients, in order to decrease cardiovascular diseases (CVD) risk factor and the progression of renal diseases.

Design: After providing a written fully informed consent to the study, 150 Caucasian Italian men were recruited: 100 healthy male individuals (mean age 44,66±13,98 years; range 30-65 years) and 50 male CKD patients (mean age 46,25±5,97 years; range 42-54 years). These patients were affected by stage 2 and 3 of Chronic Renal Failure according to the K-DOQI 2003. Usual dietary intake and physical activity, during the previous 12 months were estimated by a semiquantitative food-frequency questionnaire. The following were measured at baseline and after consumption of conventional/organic 14 days IMD: Body mass index (BMI), Body composition, by Dual-X absorptiometry (DXA) scanner, total plasma homocysteine (tHcy), serum phosphorus, glycemia concentrations, lipid profile, and microalbuminuria.

Results: A significant reduction of total homocysteine (tHcy) and phosphorus blood values were observed in the studied subjects**.** Body composition analysis by DXA highlighted high significant differences between conventional (T_0) and organic diet (T_1) for fat mass parameter, expressed as kilograms and as percentage ($p<0.001$). Improvement of lean body mass was observed in CDK patients ($p=0.004$).

Conclusions: Our study clearly demonstrates that the Italian Mediterranean Organic Diet (IMOD), according to the "Nicotera diet", was able to reduce tHcy, phosphorus, microalbuminuria levels and CVD risk in healthy individuals and in CDK patients.

Keywords: Mediterranean diet, chronic inflammatory disease, body composition, homocysteine, organic and conventional food.

INTRODUCTION

Several observational studies have provided scientific evidence that diets rich in fruit, vegetables, legumes, whole grains, fish, and low-fat dairy products are associated with lower incidence of various chronic diseases, as atherosclerosis, cardiovascular diseases, and cancer [1-14].

In addition, there are several data associating the Mediterranean Diet (MD) with a lower risk of coronary heart disease (CHD) morbidity and mortality [15-23].

The characteristics of the MD were high intake of cereals, grains, vegetables, dried beans, olive oil, garlic, fresh herbs, seafood, and fruit. Wine was taken with food in moderation. Meat and poultry were also eaten in moderation, with poultry more frequently served than red meat. Animal fats included in butter, cream and lard were not included in the diet. Since the 1950s, Ancel Keys and his co-workers studied the diets of the Mediterranean basin. The people of Greece, particularly Crete, had the longest life expectancy in the world until the 1960s, followed by southern Italy, Spain, and France [24].

Subsequent studies among the elderly in Greece, and other European countries showed that the overall Mediterranean dietary pattern was more important for longevity than the single nutrients, and it was associated with increased survival among older people [25,26].

Moreover, Scarmeas and colleagues reported the results of a community-based study involving 2258 non-demented individuals in New York in which adherence to a traditional Mediterranean diet (MeDi) was associated with significant reduction in risk for Alzheimer incidence [27].

The mechanisms through which the Mediterranean diet exerts its favourable effects on the cardiovascular system are diverse and a number of plausible explanations have been provided, including management of arterial blood pressure levels, body weight, blood lipid concentrations, inflammation and coagulation process, and endothelial function [28,29].

As consumers are aware of their health and more conscious of environmental conditions, there is an increasing demand for food obtained from alternative cultural practices limiting the use of mineral soluble fertilizers and synthetic pesticides.

According to European Community Regulations (2092/91/ECC and updatings), "organic" plant foods are those produced without the use of synthetic chemical pesticides and largely without the addition of readily soluble mineral fertilizers. It is thought that in the absence of pesticides, the plants could contain higher levels of antioxidant components as a result of enhanced synthesis of active phytochemicals produced in defence against biotic and abiotic stress [30].

To date, there has been few studies on the comparison of organic and conventional food products in nutritional intervention studies in humans [31,32].

The present study was undertaken to explore whether the consumption of Italian Mediterranean Diet (IMD), according to the so called "Nicotera Diet" [33-36], based on conventional or organic foods, could affect the body composition and chemical-clinical

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parameters, in selected healthy consumers and in Chronic Kidney Disease (CKD) age-matched patients, in order to decrease cardiovascular diseases (CVD) risk factor and the progression of renal diseases, independently of ethiopathogenesis.

SUBJECTS AND METHODS

Study Population Subjects

In order to avoid potential confounding factors following the menstrual cycle hormone fluctuations, women were excluded from the study. A total of 150 Caucasian Italian males were invited to join this study from May 2006 to March 2008. Exclusion criteria included smoking, alcohol abuse, type II diabetes, recent cardiovascular events (<6 month), and cancer.

A total of 100 Caucasian Italian healthy subjects (mean age 44.66±13.98 years; range 30-65 years) were enrolled from a religious community in Rome; according to clinical examination and disease history, all of them were free from hypertension, cardiovascular diseases and none smoked or took any other drug.

A total of 50 male patients (mean age 46.25±5.97 years; range 42-54 years), with CKD and stable renal function (stage 2 and 3 according to the K-DOQI 2003) were recruited from Day-Hospital of Policlinico "Tor Vergata" University Hospital (Rome), according with the level of kidney function. The Glomerular Filtration Rate (GFR) of the patients affected by Stage 2 of CKD was between 89-60 ml/min and in stage 3 was 59-30 ml/min. We selected 40 patients with GFR <70 and > 45 ml/min (corresponding to the stages 2 and 3 CDK of K-DOQI Guidelines 2003). The cause of primary renal failure was chronic glomerulonephritis: 25 patients with IgA glomerulonephritis, 15 patients with membranoproliferative glomerulonephritis, and 10 patients with membranosous glomerulonephritis. All were managed with standard conservative treatment. None of these patients were on dialysis.

Participation in the study included a complete medical history to gather information about health status, current medications including supplements of vitamins and minerals, alcohol drinking, smoking, physical activity (PA) and family history for chronic diseases.

Subjects were classified on the basis of Body mass index (BMI), and body fat mass percentage (FM%) as the followed: non-obese, with BMI<25 kg/m^2 and FM%<30%; preobese-obese, with BMI >25 kg/m^2 and FM%>30%.

All the volunteers provided a written consent at the enrolment. The protocol was approved by the "Tor Vergata" University Medical Ethical Committee, Rome, Italy.

Physical Activity Questionnaire (PAQ)

Data on Physical Activity (PA) were collected using a simple questionnaire, to assess levels of physical activity in diverse domains, such as working activity, leisure time activity and sedentary activities, participation in organised sport, in a usual week. The questionnaire grades the level of PA into three categories (sedentary, moderate and vigorous) based on the time spent on activity of life on programmed physical exercise.

To estimate vigorous PA we considered time/week spent on 20 min of intense PA. To estimate moderate PA we considered time/week spent on 60 min of moderate PA. To estimate sedentary PA we considered h/day spent on sedentary behaviours. Participants were asked to maintain their usual exercise habits.

Diet Assessment

An Italian Mediterranean Diet was used. Although there are many forms of the Mediterranean diet, we used the original Nicotera Mediterranean diet. For more detailed information about this type of Mediterranean diet, refer to Fidanza and Fidanza-Alberti [33,34], De Lorenzo *et al.* [35], Fidanza *et al.* [36], and De Lorgeril and Salen [37]. Total daily energy content of the diet was determined on an individual basis, being equal to 71% of the resting metabolic rate (RMR), calculated using De Lorenzo *et al.* [38], prediction equation for the Italian population. Initial caloric levels were adjusted, when necessary, to maintain the body weight.

The recommended composition of the dietary regimen was as follows: carbohydrates, 50% to 60%; proteins, 15% to 20% (of which about 50% was comprised of vegetable proteins); total fat, less than 30% (saturated fat, less than 10%; and cholesterol consumption, less than 300 mg per day), and 30 g of fibers. No alcoholic beverages were allowed except 100 ml/day of red wine. The composition of the diet in terms of foods and food combinations was planned to obtain an animal to vegetable protein ratio as close to 1:1 as possible. The Italian Recommended Dietary Allowances were incorporated to ensure proper vitamin and mineral intake [39]. In CKD patients the potassium was according the Dietary reference intakes of Institute of Medicine of National Academy, Washington.

The IMD was evaluated by a dietetic software package (DS Medigroup, Milan, Italy).

Usual dietary intakes over the past 12 months were collected by a semiquantitative food-frequency questionnaire. The questionnaire classifies the average food intake according to 9 frequency categories ranging from "almost never or less than once per month" to ">6 times/day" using standardized portion sizes for each dietary item, including beverages and nutritional supplements. The alimentary diary and nutrient intake were analysed using diet analyser software INDALI. Daily and weekly food intake in grams was calculated from food intake frequency and portion sizes. The weekly frequency of composition of animal foods was as follows: four for fish, two for meat and two for cheese.

All selected subjects consumed for 14 days conventional products and for other 14 days organic products. These subjects followed, for the first 14 days, an adequate nutritional diet and used only foods obtained by conventional agriculture techniques. Successively, for the other 14 days, these subjects had an exclusively "organic" diet, based on the same prescriptions used in "conventional" days; these prescriptions were the same for all studied subjects.

No change of total energy intake (kcal/die) was required during the experimental time. The physical activity of the subjects was not different during the time course of the study, and no change in Resting Metabolic Rate (RMR) was expected.

Anthropometric Measurements

Anthropometric parameters of all the participants were measured according to standard methods [40]. Before taking data, the subjects were instructed to take off their clothes and shoes before performing all the measurements. Body weight (kg) was measured to the nearest 0.01 kg, using a balance scale (Invernizzi, Rome, Italy). Height (m) was measured using a stadiometer to the nearest 0.1 cm (Invernizzi, Rome, Italy). Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Dual X-ray Absorptiometry (DXA)

The body composition was determined by means of Dual-X absorptiometry (DXA) (Lunar model DPX-IQ Lunar Corp., Madison) fan beam scanner [41,42]. The subjects were instructed not to exercise within 24 h from the test. The subjects received complete instructions on the testing procedure. They wore a standard cotton t-shirt, shorts and socks. They laid supine on the DXA, without moving for 20 min while the DXA scan recorded their results. The coefficient of variation (CV%=100xSD/mean) intra and inter subject ranging from 2% to 5%. Radiation exposure was equivalent to 0.01 mSv.

Resting Metabolic Rate (RMR) Measurement

RMR was measured by indirect calorimetric method. The oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured for a 30 min period by an open circuit in direct calorimeter using a face mask (Sensormedic 2900, California, USA). The gas analysers were calibrate daily for pressure and gas concentrations following the instruction of the manufacturer.

Subjects were instructed to drink only water, consume no alcohol, no proteins for 12 h before testing and refrain from smoking and sport activity for 24 hrs before testing. Prior to the RMR measurements, the subjects layed supine for 25-30 min in a quiet room. All tests were performed in a supine position of the subjects. The room temperature was fixed at an average of 22 °C. For additional quality control two different certified oxygen/carbon dioxide gas mixtures (SIAD Ltd Co, Rome, Italy) were used.
RMR was calculated from oxygen consumption and carbon dioxide production according to the formula of Weir [43]:

$RMR=1.44x[3.91x\ VO_2\ (ml)+1.106x\ VCO_2\ (ml]$

For the calculation of RMR, only data of subjects in apparently steady-state conditions (i. e.,VO_2 and VCO_2 did not vary more than 5% from the mean value of the 30 min measurement period) were used.

Bioelectrical Impedance Analysis (BIA)

Resistance, reactance, impedance and phase angle at 50 kHz frequency were measured using a Bioelectrical Impedance Analysis (BIA 101S, by Akern/RIL System-Florence). Body Composition Analysis was assessed to estimates total body water (TBW), intracellular body water (ICW), extracellular body water (ECW) and body cell mass (BCM), using manufacturer's equations.

Analysis of Blood Samples

Blood samples (10 mL) were collected into sterile tubes containing EDTA (Vacutainer®), *via* venipuncture early in the morning (07.00-09.00 *a.m.*) after an overnight fast (12 hrs). All materials were immediately placed on ice and plasma was separated by centrifugation at 1600 x g for 10 min at 4° C. Fasting plasma glucose concentrations were measured using the glucose oxidase method with an automated glucose analyzer (COBAS INTEGRA 400, Roche Diagnostics, Indianapolis, IN, USA), with reagents provided by the same company. Serum lipid profile components and mindless tests were determined by modular Analitics SWA (Roche Diagnostics GmbH, D-68298 Mannhein, Germany). Haemoglobin dosage was performed by XE 2100 (Sysmex Corporation, Japan). Triglyceride (TG) concentrations were determined through standard enzymatic colorimetric techniques (Roche Modular P800, Roche Diagnostics, Indianapolis, IN, USA), with reagents provided by the same company. Vitamin B12 concentration was measured by an automated chemiluminescence system (Centaur, Bayer); serum total Homocysteine concentration was determined by a fully automated HPLC method, using reversed-phase separation and fluorescence detection, with reagents provided by the same company. For the determination of the C-reactive protein, a highly sensitive method based on polystyrene particle coated with monoclonal antibodies specific to human CRP was used (CardioPhase hsCRP).

Urinary albumin excretion (UAE) was measured on a morning urinary sample and values defining microalbuminuria are 20-200 mg/ml. Analyses were carried out by the accredited Clinical Chemical Laboratories of the "Tor Vergata" Polyclinic (PTV) of Rome, Italy.

Immunological Assay

Early morning blood samples were taken from each individual for biochemical screening tests after a 12 hour overnight fast. All materials were immediately placed on ice. The plasma was obtained by centrifugation at 1600 x g for 10 min at 4° C.

Plasma concentrations of TNF-α, IFN–γ, IL-1 and IL-6 were determined in duplicate using a high sensitivity commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (Mabtech, Italy). All assay procedures were performed as described by the manufacturer. The lower limit of cytokine's detection was 0.02 pg/mL for IL-6, 0.06 pg/mL for IL-6 and for TNF-α, and 0.03 pg/mL for IFN-γ.

Oxygen Radical Absorbance Capacity Assay

The ORAC methodology is arguable the most accepted and accurate indicator of antioxidant status, mainly because it is based on measurements of fluorescence rather than absorbance.

This increases sensitivity and so permits a much lower molar ratio of antioxidant sample: reagents, thus minimizing the likelihood of cross-reactions between sample and reagents. In addition, the ORAC methodology measures "total radical scavenging ability", since it is unique in that it takes reactions to completion, permitting a calculation of "total area under curve". The ORAC assay works by the following principle. A sample is added to a free radical generating system, the inhibition of the free radical action is measured and the results calculated are related to the antioxidant capacity of the sample. AAPH is used as the free radical generator and b-PE is used as a target for free radical attack. Free radicals cause a conformational changes in the protein structure of b-PE leading to fluorescence quenching in a dose and time-dependant manner.

The ORAC method was the followed. The final reaction mixture for the assay (2 ml) was prepared as follows: 1.750 ml of 75 μM phosphate buffer pH 7.0 + 0.100 ml of 20 μM Trolox used as standard, or 0.100 ml of sample, or 0.100 ml of buffer alone used as blank; + 0.100 ml of 34 mg/l β-PE was added in each well. The oxidant reaction was started by the addition of 0.050 ml AAPH 160 mM to each well. The quenching of PE was measured using a Varian Cary Eclipse Fluorescence Spectrofotometer at λ = 546 nm (λ exicitation) and λ = 573 nm (λ emission) and it was monitored every 2.5 min at 37°C for 1 hour or until the fluorescence's variation was less than 2%.

The ORAC value is calculated according to the formula:

ORAC (Micromol Trolox Equivalents/g) = [(As – Ab)/(At – Ab)] kah

where As is the area under the curve (AUC) of β- PE in the sample, calculated with the Origin 2·8 integrating program (Microcal Software). At is the AUC of the Trolox, Ab is the AUC of the control, k is the dilution factor, a is the concentration of the Trolox in mmol/l, and h is the ratio between the litres of extract and the grams of vegetable or oil used for the extraction.

The ORAC Unit was calculated according to the formula:

1 ORAC Unit = 1μM Trolox equivalent

Chemicals

AAPH (2,2'-Azobis(2-aminopropane)dihydrochloride) was purchased from Polyscience (Warrington, PA, USA). A working solution of 160 mM was prepared fresh by adding 5 ml phosphate buffer to 217 mg AAPH and was stored on ice until used for analyses. Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) A stock solution (100 microM) was prepared by dissolving 5.0 mg Trolox in 200 ml of phosphate buffer. This was further diluted 1:5 v/v to give a working solution of 20 μM. β-phycoerythrin (β-PE; Sigma-Aldrich) A stock solution was prepared by dissolving 1 ml of PE in 14.7 ml of phosphate buffer. This as diluted further 1:2 to give a working solution.

Food Sampling and Extraction

All vegetables and fruits were obtained fresh from a local greengrocer in the spring and summer of 2008. In some cases, more than one cultivar was tested. For each food product, at least four samples were tested, each in duplicate. The following fresh vegetables were analysed: lettuces (Lactuga romana, var. Romana); tomatoes (Solanum lycopersicum L. var. Miroo a grappolo); garlics (Allium sativum var. Bianco); carrots (Daucus carota var. Tancar), beans (Faseolus vulgaris var. Borlotti), potatoes (Solanum tuberosum), celeries (Apium graveolens L. var. dulce), peas (Pisum sativum), courgettes (Cucurbita pepo, var. verde di Milano). The following fresh fruits were analysed: apples (Pirus malus, var. deliciuos), pears (Pyrus communis L. var. Williams), lemons (Citrus limon), strawberries (Fragaria vesca), bananas (Musa sp.). All tested foods were both from conventional and organic agricultural practices.

Food samples were processed as follows: sample of the pooled fresh edible part of each lot of foods were homogenized using an Ultra-Turrax T8 under nitrogen atmosphere (to preserve oxidation) for 5 min. After homogenizing 5 g of samples were extracted twice in 20 ml of water, and centrifuged at 1800 rpm for 10 minutes. The extracted was stored at –20°C always under nitrogen atmosphere until was analyzed.

Statistical Analysis.

All continuous variables were checked for normality using the Kolmogorov-Smirnov test.

Data are reported as means and standard deviations for normally distributed variables. Differences between the baseline and final values were tested using Paired samples t-test, and Mann-Whitney test. The minimal level of significance of the differences was fixed at $P \leq 0.05$ for all the procedures.

RESULTS

Baseline Characteristics

Among 150 Caucasian Italian males subjects recruited for the study, 17 were excluded at the screening (two had prostate cancer, five had an OGTT compatible with diabetes, and ten were diabetic), and 3 were withdrawn for missing data in any variables considered. Thus, a total of 130 subjects completed the study, and their data were eligible for data analysis.

Table **1** shows the body composition and laboratory parameters in healthy subjects and in CKD patients at baseline (T_0).

No significant differences were observed with regard to studied parameters between T_0 and T1.

Regarding inflammation parameters, in healthy individuals and CDK patients no differences between T_0 and T_1 were observed.

Regarding physical activity, at baseline the majority of the subjects were classified as sedentary or, at least, with moderate PA. The frequency of a vigorous (20 min of intense PA time/week) PA was 5.6 ± 0.8 %; the frequency of a moderate (60 min of intense PA time/week) PA was 29 ± 1.1 %; the frequency of a sedentary behaviour (h/week) PA was 65.4 ± 1.2 %.

According to study design, no significant changes of PA during experimental time were observed.

Daily and weekly food intake in grams was calculated from food intake frequency and portion sizes. At baseline, the healthy subject's daily intake of carbohydrates was derived mainly from pasta (80-120 g), bread (70-150 g) and fresh legumes (50 g). The daily intake of fruit was 400-650 g and vegetables 350-500 g. Daily intake of meat was 150-300 g. Daily intake of fish was 15-30 g. Extra-virgin olive oil was consumed daily in the amount of 20-50 g. The dietary regimen was as follows: carbohydrates, 62%; proteins, 23%; total fat, 15%, and 27 g of fibers. The composition of the diet in terms of foods and food combinations was nearest to the Italian Recommended Dietary Allowances [39].

Effects on Body Weight and Body Composition

Subjects were analysed after a consumption for 14 days of a conventional diet (T_1) and after a consumption for 14 days of an organic diet (T_2). In general, compliance with the diets was excellent, and all subjects tolerated the organic foods well.

At T_1 the RMR value was 1777.1±373.85 Kcal/die; the VO_2 consumption was 263.33±56.63 l/min; the VCO_2 production was 191.33±37.55 l/min; the respiratory ratio (RR= VCO_2/ VO_2) 0.74±0.07, according with protein substrate consumption.

No significant differences were observed with regard to these calorimetric values between T_0 vs. T_1 and T_2 for all the subjects.

Body composition characteristics at T_1 and T_2 of healthy individuals are given in Table **2**.

No significant differences were observed with regard to weight (T_1= 90.66±17.50 vs T_2= 91.05±18.33 kg), and Body Mass Index (BMI T_1= 31.69±6.62 vs T_2= 31.83±7.01 kg/m^2), after organic and conventional diet, with the methods applied.

Body compositions by DXA highlighted high significant differences between conventional (T_1) and organic diet (T_2) for fat and lean mass parameters, expressed as kilograms ($p<0.001$), and as percentage ($p<0.001$, $p<0.004$, respectively).

Table **3** shows the body composition characteristics at T_1 and T_2 of CKD patients. A significant decrease of weight (T_1= 85.17± 13.97 vs T_2= 79.52±10.41 kg, $p<0.0365$), and BMI (BMI T_1= 26.95±3.30 vs T_2= 25.36±2.60kg/m^2 $p<0.0059$) was observed.

As shown in Table **3** significant differences between T_1 and T_2 were obtained for all the variables with the exception of lean mass (kg).

Effects on Blood Biochemical Parameters and Systemic Inflammation

The blood median and range values of all groups are shown in Table **4**. As further indicated in Table **4**, organic diet period caused in all subjects, healthy and CKD subjects, a significant lowering of tHcy ($p=0.0106$, $p=0.0026$ respectively), and phosphorus blood values ($p<0.0001$, $p=0.0382$, respectively). Total cholesterol ($p=0.0369$), calcium ($p<0.0001$) and microalbuminuria ($p=0.00286$) were lower after the IMOD only in CDK patients. Furthermore, a significant increase of vitamin B12 plasma level in healthy individual was observed ($p=0.0019$).

A significant decrease ($P\leq0.05$) in systemic inflammation assessed by hs-CRP (mg/dL) were observed in both groups.

A significant decrease in TNF-α, IL-6 and IL-1 family serum concentration were highlighted in the healthy subjects between T_1 and T_2 ($P\leq0.05$). Furthermore, at T_1 in healthy subjects, a positive correlation, according Dunnett's multiple comparison, between hs-CRP and the level of IL-6 (0.48, $P\leq0.05$), and TNF-α (0.46, $P\leq0.05$), was observed. At T_2, a negative significant correlation between lean mass and Hcy (-0.73, $P\leq0.01$), IL-1α (-0.72, $P\leq0.01$), IL-1β (-0.75, $P\leq0.01$), and IL-6 (-0.72, $P\leq0.01$) was highlighted. However, in CDK patients any decrease in cytokine serum concentration was obtained.

Table **5** shows the ORAC Unit values of conventional and organic foods. The percentage of the increment of ORAC Unit of organic food *vs* conventional products highlighted a significant increase ($p < 0.005$, 0.001) of the antioxidant capacity: bananas (97.52%), apples (+333.33%), lemons (+1.2%), strawberries (+433.3%), orange (+78.66%), lettuces (+368.8%), tomatoes salsa (+81.31%), carrots (+560%), beans (+250%), celeries (+195.78%),

Table 1. Body Composition and Laboratory Parameters in Healthy Subjects and in CKD Patients at Baseline (T0)

Parameters	Healthy Subjects		CKD Patients	
	T0		T0	
	Mean	SD	Mean	SD
BMI (kg/m^2)	31.9	±6.7	32.0	±7.5
W (kg)	90.8	±18.2	92.0	±18.9
FM (%)	36.1	±2.5	25.3	±2.5
FM(kg)	27.7	±3.9	21.5	±3.8
LM (%)	64.8	±2.4	74.5	±2.3
LMM (kg)	54.9	±2.3	62.8	±2.1
Homocysteine (μmol/l)	24.2	±5.4	23.8	±5.3
Azotemia (mg/dl)	34.1	±12.4	85.3	±46.8
Creatinine (mg/dl)	0.91	±0.32	1.91	±0.70
Total Cholesterol (mg/dl)	173.1	±58.3	185.4	±18.3
HDL Cholesterol (mg/dl)	32.4	±13.1	29.8	±7.6
Triglycerides (mg/dl)	100.2	±45.3	170.1	±55.5
Calcium (mg/dl)	9.71	±0.12	9.84	±0.61
Phosphorus (mg/dl)	4.72	±0.13	4.30	±0,90
Sodium (meq/l)	141.9	±0.92	141.3	±1.3
Potassium (meq/l)	4.42	±0.16	4.93	0.38
Glucose (mg/dl)	99.2	±25.1	87.2	±6,8
Vitamin B_{12} (pg/ml)	215.4	±20.8	576.1	±248.3
Microalbuminuria (mg/l)	-	-	94.2	±120.3
hs-CRP(mg/dl)	0,53	±1,4	5,7	±4,8
TNF-α (pg/mL)	21.69	±4.7	150.1	±24.3
IL-1α (pg/mL)	17.34	±5.0	56.1	±8.3
IL-1β (pg/mL)	7.48	±3.94	76.2	±4.8
IL-6 (pg/mL)	5.95	±2.8	16.1	±2.3
INF-γ (pg/mL)	19.19	±6.9	57.1	±18.3

All values are the mean ± SD. *) $P \leq 0.05$ was considered statistically significant. BMI= Body mass index; W=Weight; FM, Fat Mass; LM, Lean Mass; hs-CRP= high sensitive C-Reactive Protein.

Table 2. Body Composition Parameters at T1 and T2 in Healthy Subjects

Parameters	T1		T2		*P* *
	Mean	SD	Mean	SD	
BMI (kg/m^2)	31.69	± 6.62	31.83	± 7.01	NS
W(kg)	90.66	± 17.50	91.05	± 18.72	NS
FM (%)	35.50	± 2.33	25.07	± 2.47	0.001
FM(kg)	27.42	± 3.79	21.31	± 3.91	0.001

(Table 2) Contd….

Parameters	T1		T2		*P* *
	Mean	SD	Mean	SD	
LM (%)	64.50	± 2.33	74.93	± 2.47	0.001
LM (kg)	55.27	± 2.41	63.69	± 2.09	0.004
BCM (kg)	53.90	± 3.70	58.69	± 2.59	0.01
TBW (%)	51.90	± 13.70	56.10	± 17	0.01
ECW (%)	41.50	± 14.20	44.91	± 12.2	0.01
ICW (%)	58.50	± 14.20	44.9	± 12.2	0.01

All values are the mean ± SD.

*) $P \leq 0.05$ was considered statistically significant.

BMI= Body mass index; W=Weight; FM, Fat Mass; LM, Lean Mass, by DXA; BCM=Body Cell mass; TBW= Total Body Water, ECW=Extra Cellular Water; ICW= Intra Cellular Water by BIA

Table 3. Body Composition Parameters at T1 and T2 in CKD Patients

Parameters	T1		T2		*P* *
	Mean	SD	Mean	SD	
Weight (Kg)	85.17	± 13.97	79.52	± 10.41	0.0365
BMI (kg/m^2)	26.95	±3.30	25.36	± 2.50	0.0059
FM (%)	26.06	±5.79	19.91	± 1.99	0.0007
FM (kg)	23.36	±8.88	16.18	± 3.34	0.0054
FT(%)	37.86	±3.57	34.38	± 4.53	0.0033
LM (%)	73.9	±5.83	80.08	± 1.99	0.001
LM (kg)	53.45	±6.69	54.63	± 6.76	NS
BCM (kg)	38.2	±3.25	45.42	±6.28	<0.001
TBW (%)	53.7	±3.56	56.47	±1.18	0.007
ECW (%)	38.82	±1.31	35.37	±3.49	0.0019
ICW (%)	61.17	±1.31	64.62	±3.49	0.019

All values are the mean ± SD.

*) $P \leq 0.05$ was considered statistically significant.

BMI= Body mass index; W=Weight; FM, Fat Mass; FT= Fat trunk; LM, Lean Mass, by DXA; BCM=Body Cell mass; TBW= Total Body Water, ECW=Extra Cellular Water; ICW= Intra Cellular Water by BIA

Table 4. Laboratory Parameters in Healthy Subjects and in CKD Patients at T1 and T2

	Healthy Subjects					CKD Patients				
	T1		T2			T1		T2		
	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	*P**
Homocysteine(μM/L)	23.06	± 5.17	12.71	± 6.15	0.0106	22.12	± 5.17	17.81	± 5.29	0.0026
Azotemia (mg/dl)	33.20	± 11.33	30.66	± 8.51	NS	83.21	± 47.49	80.76	± 50.92	NS•
Creatinine (mg/dl)	0.88	± 0.29	0.95	± 0.18	NS	1.75	± 0.61	1.67	± 0.27	NS
Total Cholesterol (mg/dl)	167.02	± 60.55	189.66	± 36.21	NS	181.57	± 14.84	165.57	± 27.71	0.0369
HDL cholesterol (mg/dl)	33.04	± 12.30	39	± 6.86	NS	30.92	± 7.41	32.07	± 6.76	NS

(Table 4) Contd….

	Healthy Subjects					CKD Patients				
	T1		T2			T1		T2		
	Mean	SD	Mean	SD	*P**	Mean	SD	Mean	SD	*P**
Triglycerides (mg/dl)	98.44	± 47.56	113.44	± 26.70	NS	168.71	± 54.53	156.85	± 37.88	NS
Calcium (mg/dl)	9.64	± 0.16	9.43	± 0.37	NS	9.93	± 0.57	9.33	0.44	<0.0001
Phosphorus (mg/dl)	4.64	± 0.15	3.01	± 0.13	<0,0001	4.10	± 0.88	3.54	± 0.26	0.0382
Sodium (mEq/L)	140.97	± 0.86	139.51	± 1.11	0,0141	140.85	± 1.09	140.57	± 0.85	NS
Potassium (mEq/L)	4.34	± 0.15	4.31	± 0.39	NS	4.90	± 0.34	4.67	±0.65	NS
Glucose (mg/dl)	98.91	± 24.28	92.66	± 22.02	NS	86.78	± 6.71	90.23	±8.55	NS
Vitamin B_{12} (pg/ml)	217.33	± 20.10	259.11	± 22.65	0.0019	574.92	± 247.49	516.42	±195.42	NS
Microalbuminuria (mg/L)	-	-	-	-	-	93.55	± 121.9	71.7	±100.48	0.00286
hs-CRP (mg/dl)	0.44	±0.64	0.05	±0.01	0.001	5.63	±4.82	4.51	±4.94	<0.001
TNFα (pg/mL)	20.17	±6.78	12.20	±2.20	0.05	142.1	±14.2	115.2	±10.2	NS
IL-1α (pg/mL)	15.81	±6.4	12.7	±8.64	0.05	50.1	±7.8	48.1	±7.3	NS
IL-1β (pg/mL)	7.12	±2.83	4.93	±2.56	0.05	66.8	±5.8	66.2	±5.8	NS
IL-6 (pg/mL)	4.81	±1.92	2.23	±2.2	0.05	15.2	±2.6	12.1	±5.3	NS
INF-γ (pg/mL)	20.98	±10.3	16.5	±1.1	0.05	47.2	±12.5	45.1	±16.7	NS

All values are the mean ± SD. •) Mann-Whitney test *) $P \leq 0{,}05$ was considered statistically significant

Table 5. Antioxidant Capacity in Conventional and Organic Products

Products	Conventional ORAC Unit		Organic ORAC Unit		-%
	Mean	SD	Mean	SD	
Lattuce	77	± 3.9	361	± 6.41	368.8**
Peas	12	±2.30	114	± 1.52	850**
Courgettes	1290	±5.79	1490	± 3.98	15.5*
Beans	14	±2.88	42	± 2.34	250*
Tomato	130	±3.57	220	± 4.23	69.23*
Tomato sauce	182	±5.83	330	± 5.89	81.31*
Carrot	15	±1.69	99	± 2.76	560**
Garlic	476	± 3.97	2120	± 3.20	345.37**
Celery	95	± 3.67	281	± 2.67	195.78*
Orange	750	± 3.17	1340	± 5.92	78.66*
Banana	121	±1.96	239	± 14.98	97.52*
Strawberry	6	± 1.91	32	± 1.17	433.3**
Lemon	1620	± 5.27	1640	± 1.27	1.2
Apple	6	± 0.97	26	± 3.14	333.33*
Pear	150	± 3.17	87	± 4.17	-42*

*P < 0.05; **P < 0.01, Mann Whitney Test.

peas (+850%), courgettes (+15.5%), garlic (+345.37%). Only pears (–42%) showed a lower antioxidant capacity respect to the conventional ones.

DISCUSSION

Mediterranean diet (MD) has been associated with a lowered incidence of cardiovascular diseases, metabolic disorders, Parkinson's and Alzheimer's diseases, and several types of cancer [44-51].

The protective effect has been attributed, at least in part, to the richness of MD in antioxidants [17,19]. Current evidence indicates oxidative damage as a promoter of pathophysiological changes occurring in oxidative stress–associated diseases, such as cardiovascular diseases (CVD), cancer, neurodegenerative disorders and also aging [52]. A wide range of evidences indicates the importance of total antioxidant capacity (TAC) in plasma and tissues, of its modification during the development of oxidative stress, and of its feasibility as a tool for investigating the association between diet and oxidative stress [53]. Recently, Martinez-Gonzalez and Estruch [54] underlined the need for randomized trials to use a whole-diet approach and not a simple antioxidant supplement to evaluate the role of the Mediterranean dietary pattern in human health. Adherence to a Mediterranean type diet has been shown to be associated with lower oxidized low-density lipoprotein (oxLDL) plasma level in a cross-sectional study and in a randomized controlled trials [55,56].

Moreover, it has been suggested [57] that organic products could contain 10-50% more phytochemicals than non-organic products. Previous data highlighted a possible impact on human health of a Mediterranean diet comprising the organic products versus conventional, due to the effect on the total plasma antioxidant capacity. In particular, an increase in the plasma antioxidant capacity was observed in the subjects receiving the organic diet [58].

Conscious that, regardless of its organic or conventional origin, a well-balanced diet is necessary to improve health and that the administration of a single or few organic foods would not evidence any possible beneficial effect, we decided to conduct our study by comparing the effectiveness of the Nicotera Mediterranean diet, based on conventional versus organic foods, in modulating body composition and biochemical parameters.

Our results demonstrated, for the first time, that the administration of the Italian Mediterranean organic diet (IMOD), according to the Nicotera diet guidelines, was associated with some benefits in healthy preobese/obese subjects and in Chronic Kidney Disease (CKD) age-matched patients.

As adipose tissue closely correlates with the possibility of developing type 2 diabetes and coronary heart diseases, due to a low-grade systemic inflammation [59,60], weight management and body composition changes can help to reduce the number of people at risk for cardiovascular diseases (CVD) and complications or premature mortality [45,61]. Although weight reduction remains a cornerstone of the therapy for obesity related diseases, from a public health perspective, the adoption of a diet similar to that investigated here may provide further benefits especially in patients who do not lose weight. The effect of the intervention of IMOD was associated with significant changes in body composition of all subjects, although they followed the usual diet and no change of total energy intake (kcal/day) was carried out, as well as the physical activity and life style did not differ during the time course of the study. In particular, the effect of the intervention diet was associated with significant changes in body composition, as a significant reduction of fat mass for all studied subjects. Furthermore, a significant increase of lean body mass percentage ($p<0.001$) was observed both in healthy individual than in CDK patients, suggesting a positive role on inflammation and risk of chronic diseases. These data are supported by the changing in extracellular and intracellular water body contents, and body cell mass observed in healthy and CDK subjects.

Adipose tissue is an important source of cytokines, and adiposity contributes to the proinflammatory milieu [62]. Three of the most important pro-inflammatory plasma markers are serum C-reactive protein (CRP), tumor necrosis factor (TNF)-α, and interleukin (IL)-6, all of which have been implicated in the pathophysiology of cardiovascular diseases as well as in diabetes mellitus [63-65]. In our study, organic food consumption induced a rapid clinical response, in healthy subjects, through the reduction of the serum concentration of pro-inflammatory cytokines and hs-CRP. However, in CDK patients only a significant reduction of hs-CRP was obtained.

Moreover, a significant reduction of total plasma homocysteine (tHcy), a marker of systemic inflammation associated to cardiovascular risk, was obtained for all studied individuals.

In our study, we observed, at baseline, a moderate value of tHcy (15-30 μM) in healthy subjects and elevated tHcy level in CDK patients. The IMOD led to a significant reduction in tHcy concentration, whereas the diet with conventional food did not. The pathogenesis of hyperhomocysteinaemia in patients with chronic renal failure is not fully understood. However, in patients with chronic renal failure, a progressive increase in Hcy levels (30-100 μM), has been reported with decreasing glomerular filtration rate [66,67]. Several intervention studies have provided evidences for the importance of B vitamins in Hcy metabolism [50,51].

The observed association between the intake of organic food and tHcy concentrations may be explained by a biochemical link between homocysteine metabolism and vitamins metabolism. In particular, Berstad *et al.* [68] showed that tHcy appears to be a good marker of "adherence to dietary guidelines": subjects with high intake of fruit and vegetable, good sources of folate had lower tHcy concentrations. By the analysis of food intakes of all participants in our study, the meals habitually consumed provided amounts of total energy from proteins, carbohydrates, fats adequate to the Recommended Dietary Allowances (RDA). Micronutrients intakes (vitamins A, D, E, C, B6, B12, thiamin, niacin, riboflavin, folate, calcium, phosphorus, magnesium, iron, zinc and selenium) were according to the recommendations. In addition, a significant increase of vitamin B12 was observed in healthy subjects ($p= 0.0019$).

Moreover, the phosphorus-vitamin D-parathyroid axis should be monitored and, when need, corrected in Chronic Kidney Disease (CKD) patients, because derangements of these interacting measures can be associated with progression of cardiovascular diseases, in terms of Left Ventricular Hypertrophy (LVH) and vascular calcification, as well as progression of renal disease [69]. The restriction of phosphorus in the diet to 800-1200 mg/day is the keystone of control of serum phosphorus in CKD patients [70]. Phosphorus additives to foods now can contribute to reach an average of as much as 1000 mg/day of phosphorus. As absorption of these additives is almost 100% versus about 60% for phosphorus in grains, meat and dairy; avoidance of additive-containing foods is paramount. A source of phosphorus in the diet is the growing use of enhanced meats, where a variety of phosphorus-containing compounds are injected into meats for use as flavour enhancers and tenderizers [71]. Control of phosphorus lowers serum intact Parathyroid Hormone (PTHi) and likely inhibits parathyroid gland hyperplasia [72].

In our study, for the first time, we demonstrated a significant reduction of hyperphosphatemia both in healthy individuals than in CDK patients ($p=0,0382$), associated with an improvement of lipid profile, which suggests less need of lipids for endothelial cell repair, and a lower risk for CVD.

Furthermore, at T_2, in CKD patients, there was a significant reduction of Microalbuminuria (p=0,00286). Microalbuminuria reflects a generalized impairment of endothelium and represented a marker of increased cardiovascular risk and of progression of renal failure [73].

In summary, our data highlight a possible impact on human health of a Mediterranean diet comparing organic products versus conventional. The results of this study represent the first demonstration, to our knowledge, that a daily Mediterranean-style diet rich in organic foods intake might play a role in reducing the inflammatory state, fasting baseline tHcy, phosphourus, total cholesterol concentrations, microalbuminuria, increasing plasma vitamin B12 concentration, and in modulating body composition. This will lead to a lower incidence of CVD and this could be of particular significant in CDK patients. We suggest that the IMOD may play a role in longevity and quality of life of healthy and patients, directing the consumers towards the consumption of organic food, with higher nutritional quality, expressed as ORAC Units.

Several important directions can be highlighted for future research.

However, we realize that there are limitations to our study. First, our sample size was relatively small for population study, although large enough to provide us adequate statistical power. Second, although we did our best to control dietary intake of our participants, this was difficult to do because they were free-living. Intervention studies are need to clarify the nature and extent of association between dietary intake of organic foods, inflammation and other markers. In addition, future research prospectively, examining the relation between IMOD adherence and different patterns of weight gain and chronic diseases over longer time periods, may provide additional insights into the potential benefits of promoting this eating pattern.

Anyway, our data support the importance of behavioural interventions that encourage consumption of a healthier diet [74,75].

Furthermore, prescription of this Italian Mediterranean Organic diet (IMOD) by doctors may represent an appropriate primary therapeutic option for the global CVD risk prevention, in agreement with the Drug Italian Agency (Agenzia Italiana del Farmaco, AIFA) note n.13 guidelines (D.G.R. 1209/2002), the National Cholesterol Education Program (NCEP), the American Heart Association (AHA), and the Therapeutic Lifestyle Change (TLC). recommendations [76].The combination between the use of hypolipidemic drugs and IMOD dietary approach could amplify the efficacy of the treatment.

In conclusion, these results seem to be clinically relevant in terms of public health, particularly for reducing the risk for premature death in the general population, and are strictly concordant with current guidelines and recommendations from all the major scientific associations that strongly encourage a Therapeutic Lifestyle Change (TLC), and Mediterranean-like dietary pattern, for primary and secondary prevention of major chronic diseases [77, 78].

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ABBREVIATIONS

BIA	=	Bioelectrical Impedance Analysis
BMI	=	Body Mass Index
CHD	=	Coronary Heart Disease
CKD	=	Chronic Kidney Disease
CRP	=	C-reactive Protein
CVD	=	Cardiovascular Diseases
DXA	=	Dual X-ray Absorptiometry
FM	=	Fat Mass
GFR	=	Glomerular Filtration Rate
IMD	=	Italian Mediterranean Diet
IMOD	=	Italian Mediterranean Organic Diet
IL-2	=	Interleukin-2
IL-6	=	Interleukin-6
MD	=	Mediterranean Diet
ORAC	=	Oxygen Radical Absorbance Capacity
Ox-LDL	=	Oxidised Low Density Lipoprotein
PA	=	Physical Activity
PAQ	=	Physical Activity Questionnaire
RDA	=	Recommended Dietary Allowances
RMR	=	Resting Metabolic Rate
TAC	=	Total Antioxidant Capacity
TG	=	Triglyceride
tHcy	=	total plasma Homocysteine
TLC	=	Therapeutic Lifestyle Change
TNF-α	=	Tumour Necrosis Factor-alpha
UAE	=	Urinary Albumin Excretion

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