Acceptability And Nutritional, Metabolic, Functional Impact Of An Italian-Mediterranean, Sustainable, Plant-Based Dietary Pattern

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Activities carried out during the first two years of the PhD are related to a pilot dietary intervention study aiming to evaluate the effects of a plant-based diet, called EAT-IT. Participants metabolic and functional markers were analysed to assess health-related outcomes. Being a critical point of these dietary patterns, the acceptability was evaluated; in addition, food intake, consumption of ultra-processed foods and adherence to Mediterranean diet was evaluated to understand the characteristics of the dietary patters. These results will help to find out new strategies to facilitate and increase the adoption of plant-based diets for the Italian population.

Accettabilità e impatto nutrizionale, metabolico e funzionale di un modello alimentare italo-mediterraneo, sostenibile, vegetale

Durante i primi due anni del dottorato, le attività svolte sono state relative a uno studio pilota di intervento dietetico volto a studiare gli effetti di una dieta plant-based, chiamata EAT-IT. L’effetto sullo stato di salute è stato analizzato tramite la valutazione di marcatori metabolici e funzionali dei partecipanti mentre la determinazione delle caratteristiche del pattern alimentare è stata ottenuta attraverso l’analisi dell'assunzione dietetica, del consumo di alimenti ultra-processati e dell'aderenza alla dieta mediterranea; infine, è stata valutata l’accettabilità essendo questa un punto critico di tali modelli dietetici. Questi risultati aiuteranno a trovare nuove strategie per facilitare la popolazione italiana ad aumentare l'adozione di diete plant-based.

**Key words**: Dietary patterns; Sustainability; Health-related markers

# **Introduction**

In accordance with the PhD thesis project previously described (Vinelli, 2021), this poster reports the main results of the first activities concerning:

(A1) analysis of nutritional, metabolic, functional parameters from biological samples collection;

(A2) evaluation of dietary intake and nutritional characteristics of the EAT-IT dietary pattern.

# **Materials and Methods**

* 1. **Study design**

A single-blind, randomized controlled, crossover trial was performed. Subjects eligible to be enrolled had to be healthy, aged >18 years old and not following a specific diet (e.g. vegetarian or vegan diet). Participants were randomly allocated to follow the EAT-IT diet or an IDG-based control diet; diets were personalized based on individual energy and nutrient requirements. The two phases were separated by a washout period and the three phases lasted 6 weeks each. Before and after each intervention, weighted food records and biological samples were collected, such as blood, fecal and urine samples to assess health-related parameters.

* 1. **Nutritional assessment**

During each intervention, nutrient intake was assessed through 7-day weighted food records, while acceptability, NOVA ultra-processed foods consumption, level of adherence to the Mediterranean Diet were determined through validated questionnaires.

* 1. **Biomarkers**

Biochemical biomarkers assessed were: creatinine Jaffe (mg/dl); uric acid (mg/dl); AST/GOT (U/L); ALT/GPT (U/L); GGT (U/L); triglycerides (mg/dl); fasting glycemia (mg/dl); C-reactive protein hs (mg/L); cholesterol (mg/dl); HDL (mg/dl); LDL (mg/dl); insulin (uU/ml). All these parameters were determined using standard laboratory methods. In addition, serum samples will be used for future analysis in estimating dietary exposure to substances present in food like chemicals and contaminants, intentionally added or naturally present.

* 1. **Gut microbiota composition and SCFAs**

Fecal samples were used to analyze gut microbiota composition by 16SrRNA gene quantification and taxonomic profiling. SCFAs, in particular lactic, acetic, succinic, propionic, butyric, isobutyric, isovaleric, valeric acid were evaluated by ultrahigh-performance liquid chromatographic-Orbitrap mass spectrometry (LC-HR-MS).

* 1. **Inflammatory parameters and DNA damage**

Serum TNF-alfa, adiponectin, IL-6, LBP and endothelin were evaluated by enzyme-linked immunosorbent assay (ELISA) kits. DNA damage was assessed by Comet Assay through an enzymatic treatment with FPG.

**2.6 Statistical analysis**

SPSS software was used to evaluate the significance of the results obtained.

# **Results and Discussion**

Baseline characteristics of the 9 subjects who completed the study were as follow. Age: 25 ± 2 (23 to 30 years); gender (n, %): female 5 (50), male 4 (40); education (n, %): high education (university level) 10 (100); n household component 2.1 ± 1.2; height (m): 1.7 ± 0.1; BMI 21.4 ± 1.5.

**3.1 Nutritional assessment**

The evaluation of dietary intake showed no differences in total energy and other nutrients intake despite a statistically significant increase of vegetal protein/total protein (p<0.001) and dietary fibers (p<0.001) intake compared to the control group.

Regarding the acceptability, it was reported “sufficiently good” and tolerable in terms of preparation (not easy, but not difficult), only one subject evaluated the diet as “extremely unappealing” and “extremely difficult” to be prepared. On average, the habitual diet has been judged easier than the EAT-IT diet which was considered quite acceptable and potentially feasible in the long-term. In conclusion, the diet was easily accepted and consumed on average except for the preparation of some products such as legumes. In this context, useful strategies and recipes are necessary to help and facilitate the adoption of plant-based diets and will be integrated in a more acceptable and feasible Mediterranean healthy and sustainable dietary pattern.

Furthermore, the consumption of ultra-processed foods has been assessed with NFFQ while the level of adherence to Mediterranean diet by MEDI-LITE questionnaire; both questionnaires showed not differences between EAT-IT and IDG diets.

## **Biomarkers**

Among biochemical parameters, a statistically significant decrease of insulin (8,44 ± 2,18; 6,53 ± 2,24 uU/ml; p<0.05) and a reduction of cholesterol (181,2 ± 42,5; 166,1 ± 39,4 mg/dl) close to significance (p=0.067) were found after the EAT-IT pattern intervention.

**Figure** **1** Principal coordinates analysis of weighted UniFrac distance

## **Gut microbiota composition and SCFAs**

## In addition, gut microbiota composition and total SCFAs did not change overtime following the EAT-IT diet probably due to the high inter-individual variability amongst the participants and the small sample size. However, a positive correlation between total fibre intake and valeric and isovaleric acid was found. Furthermore, gut microbiota β-diversity resulted very variable and was greater between subjects than within the same subject as reported in figure 1.

* 1. **Inflammatory parameters and DNA damage**

Serum TNF-alfa, adiponectin, IL-6, LBP and endothelin levels were evaluated by ELISA kits.

No significant effect of treatment, nor treatment x time interaction was found between the EAT-IT and IDG for adiponectin and endothelin; results from other markers are still under elaboration.

This pilot study represents the first step on the pathway towards the optimization of the EAT-IT diet to a more feasible pattern improving adherence to an Italian-Mediterranean diet. In fact, it is important to underline that the EAT-IT recommendations were quite far from the current dietary habits of the Italian population in terms of portion sizes and frequencies of consumption, especially for legumes and nuts. The results of this study showed difficulty in preparing legumes-based meals. For this reason, future steps will focus on elaborating strategies able to facilitate the preparations of plant-based meals, such as choosing different food sources and increasing different cooking methods that could also potentially impact on the final nutrient intake. Future analysis on health-related parameters will help exploring the potential beneficial effects of optimized diets in different target of populations.

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