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One-week administration of hydroxytyrosol to humans does not activate Phase II enzymes

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ABSTRACT

The notion that (poly)phenols act as direct free radical scavengers is being challenged by mere chemical and biochemical considerations such as bioavailability and intracellular concentrations. An alternative hypothesis that is gaining considerable traction is that (poly)phenols are processed by the body as xenobiotics via the Keap1/Nrf2/ARE signaling axis, leading to the induction of Phase II enzymes. However, there are no solid human data to confirm this interesting supposition. In this study, we tested the activities of hydroxytyrosol (HT) on Phase II enzymes' expression in a double-blind, randomized, placebo-controlled study. We tested two HT doses, i.e. 5 and 25 mg/d, vs. placebo following a Latin square design. We report that HT is well tolerated but does not significantly modify Phase II enzyme expression in peripheral blood mononuclear cells. Moreover, we were unable to record significant effects on a variety of surrogate markers of cardiovascular disease such as lipid profile and inflammation and oxidation markers. Available evidence indicates that the "hormesis hypothesis" that (poly)phenols activate Phase II enzymes requires solid human confirmation that might be provided by future trials.

This study is registered at ClinicalTrials.gov (identifier: NCT02273622).

1. Introduction

(Poly)phenols are the products of plants' secondary metabolism and are endowed with several botanical activities [1]. Notable examples include roles in pollination, color, insect repelling, and cellular signal transduction. In addition, since the Zutphen Study [2] (poly)phenol consumption by humans is being consistently associated with better cardiovascular prognosis and chemoprevention. Pharma-nutritionists are trying to explain the molecular mechanisms responsible for the purported healthful activities of (poly)phenols; major emphasis is being placed on their antioxidant actions, which would counteract the noxious effects of reactive oxygen species and free radicals. However, the widespread notion that (poly)phenols act as direct free radical scavengers is challenged by mere chemical and biochemical considerations such as bioavailability and intracellular concentrations (namely, as compared with endogenous antioxidants), reaction kinetics, etc. [3].

An alternative hypothesis that is gaining considerable traction is that (poly)phenols are processed by the body as xenobiotics [3,4]. Therefore, they stimulate stress-related cell signaling pathways that result in increased expression of genes encoding cytoprotective genes. In particular, Nrf2 (NF-E2-related factor 2) is a transcription factor which binds to the Antioxidant Response Element (ARE) in cells and thus regulates enzymes involved in antioxidant functions or detoxification such as thioredoxin reductase-1 and glutathione peroxidases [3]. According to the hormesis theory, (poly)phenols paradoxically act on the Keap1/Nrf2/ARE signaling axis to produce additive increases in electrophilic signaling that results in the induction of Phase II enzymes and increased nucleophilic substrates, such as glutathione, thioredoxin, and NADPH. In brief, (poly)phenols likely exert indirect rather than direct antioxidant actions. However, there are no solid human data to confirm this interesting supposition.

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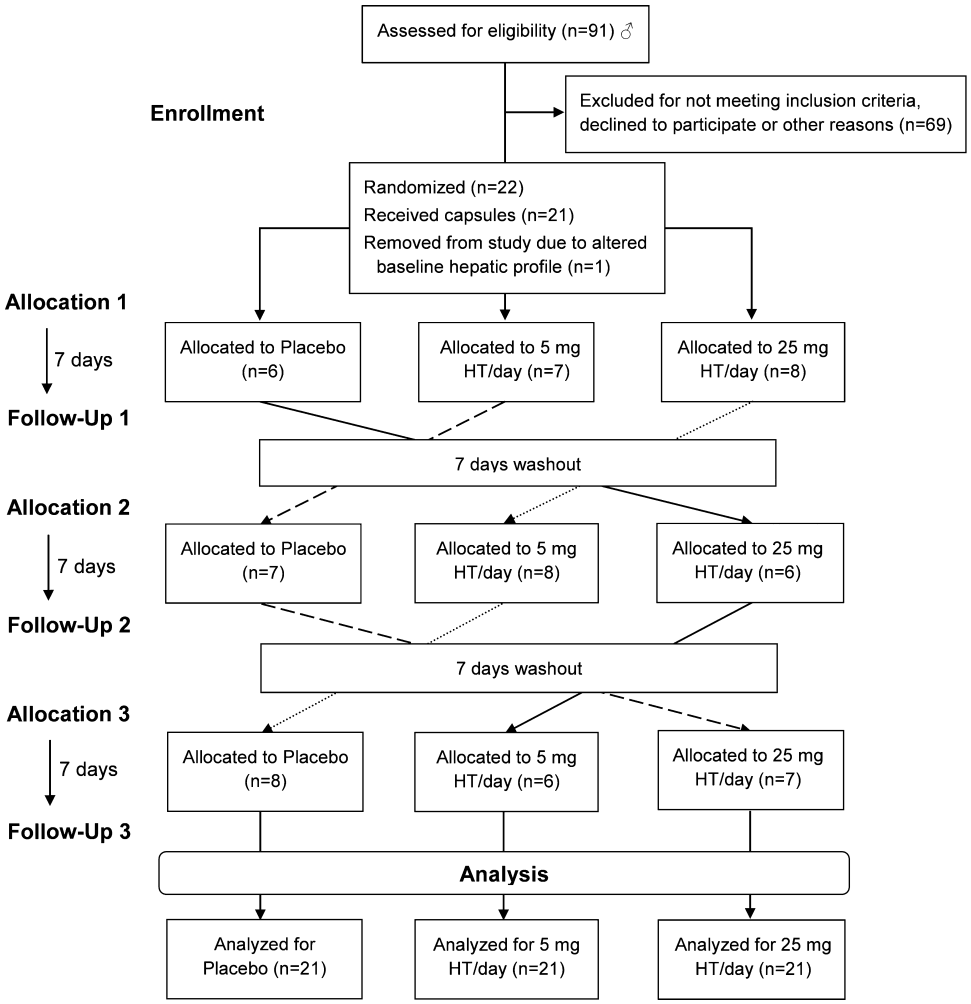

Fig. 1. Study flowchart.

In this study, we tested the activities of hydroxytyrosol (HT) on Phase II enzymes' expression in a double-blind, randomized, placebo-controlled study.

2. Materials and methods

2.1. Subjects and study design

The study protocol was approved by the local Ethics committee and was fully explained to the participants. Written informed consent was obtained by all subjects prior to starting the trial. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and is registered at ClinicalTrials.gov (identifier: NCT02273622).

This was a double-blind, randomized, placebo-controlled study. We tested two HT doses, i.e. 5 and 25 mg/d provided via administration of Hytolive® (Genosa, Madrid, Spain), an olive mill waste water (OMWW) extract selectively enriched in HT, i.e. devoid of oleuropein or other HT-containing secoiridoids as assessed by HPLC [5], via anion-exchange chromatography. Twenty-two apparently healthy volunteers were recruited from within the IMDEA-Food Genyal platform database. Inclusion criteria were: age between 20 and 40 years; adequate understanding of the study; willingness to complete the entire treatment. Exclusion criteria were: body mass index <19 or >26; diagnosis of diabetes mellitus, hypertension, dyslipidemia or other cardiometabolic disorders; impaired cognitive function; diagnosed hepatic, renal, or cardiovascular disease; allergy to olives and their derivatives; pharmacological therapies; and habitual smoking. To our knowledge, there are no previous studies testing the activities of HT on Phase II enzymes' expression in humans. Therefore, we designed a pilot study and we could not calculate power. However, the use of repeated measures increases the power to detect treatment differences in mean levels of the outcome measure over time.

We followed a Latin square design: after one-week washout, i.e. olive-free diet, subjects were randomly assigned to either the placebo (maltodextrin), 5 mg/d HT, or 25 mg/d HT group (Fig. 1). The complete capsule composition is described in Supplementary Table 1. Administration of each treatment was carried out for one week, followed by a one-week washout after which treatments were switched. Volunteers were instructed not to consume any olive-based products or medication throughout the study and were instructed to write down any occurrences (consumption of prohibited foods, medication intake, etc.) and intolerance issues (diarrhea, acidity, nausea, abdominal distension, or halitosis) in questionnaires. Capsules were provided in bottles labeled A, B, or C and their contents were unknown to both the volunteers and the nurse who administered the bottles. Compliance was assessed by capsule count.

Blood samples were drawn and anthropometric data (height, weight, body mass index (BMI), bioelectric impedance analysis (BIA)) and vital constants (systolic (SBP) and diastolic blood pressure (DBP), and heart rate) were monitored at each visit. Morning urine was also collected at each time point.