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# Metabolic changes of the blood metabolome after a date fruit challenge

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ABSTRACT

Date fruits are rich in phytochemicals that have anti-oxidative properties and are therefore considered as functional foods. However, it is unclear which part of the date metabolites actually enter the blood stream and remain bioavailable to exert any beneficial action. To answer this question, we conducted a nutritional challenge study in which we monitored plasma metabolome of 21 healthy volunteers after intake of Khlas, Deglet Nour, and glucose at five time points. Among the 1089 identified blood circulating metabolites, we found molecules that were specific to date consumption, including metabolites of the polyphenols ferulic-, caffeic-, and vanillic acid. Consumption of the sucrose-rich *Deglet Nour* led to a substantial increase in blood sucrose levels. Interestingly, consumption of serotonin-rich dates did not alter serotonin blood levels, but resulted in a sharp increase in its breakdown product 5-hydroxyindolacetate. We elucidated metabolites present in the blood after date consumption with potential health beneficial effect.

## 1. Introduction

Positive changes in lifestyle and healthy diet choices can potentially improve the outcomes of common disorders. Recently, functional foods attracted much attention in this area. They are defined as foods that contain biologically active compounds that have clinically established benefits on health in the context of prevention, management, and treatment of chronic diseases (Lang, 2007). For instance, functional foods are considered in the management of type 2 diabetes (T2D), dyslipidemia and cardiovascular disease (CVD), osteoporosis, as well as different types of cancer (Arts & Hollman, 2005; Hasler, 2002; Hunter & Hegele, 2017; Mirmiran, 2014). Especially the molecules of plant origin (phytochemicals) including phytosterol, soluble fiber from psyllium, soy protein, and β-glucagon from whole oat products, were associated with reduced risk of chronic diseases (Boeing et al., 2012).

Date fruits are a major food component in Arab culture and many health benefits have been attributed to their consumption. Date fruits are rich in macronutrients and micronutrients, including carbohydrates, fats, dietary fibers, minerals, proteins, phytochemicals and vitamins, many of which are associated with health benefits (Al-Farsi & Lee, 2008; Al-Shahib & Marshall, 2003). Primarily phenolic acids including benzoic or cinnamic acid derivatives, including protocatechuic, p-hydrobenzoic, vanillic, caffeic, p-coumaric, ferulic, and sinapic acids (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005; Regnault-Roger, Hadidane, Biard, & Boukef, 1987), as well as flavonoids have been reported to confer certain health protective effects, due to their anti-oxidative, anti-mutagenic, anti-cancer, anti-inflammatory, anti-obesogenic and neuroprotective activities (Karasawa et al., 2011; Mohamed & Al-Okbi, 2004; Pujari, Vyawahare, & Kagathara, 2011; Thaiss et al., 2016; Vayalil, 2002). Ferulic acid, the most commonly known phenolic derivative of cinnamic acid (Rosazza, Huang, Dostal, Volm, & Rousseau, 1995), has therapeutic potential in the treatment of complex diseases like diabetes, Alzheimer's disease, and cancer (Chang et al., 2006; Kawabata et al., 2000; Murakami et al., 2002; Ono, Hirohata, & Yamada, 2005; Sri Balasubashini, Rukkumani, & Menon, 2003).

To have a therapeutic potential, the active molecules of functional food have to enter circulation to be bioavailable after oral administration. Hence, molecules that are present in the food need to be taken up by the digestive tract and remain in blood circulation long enough to be available for targeted tissue. However, only some phytochemicals are absorbed directly by the small intestine and a large portion of the phenolic compounds is modified either by the gut microbiota or in the

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liver (Boeing et al., 2012). Frequently the phenolic compounds are modified by conjugation e.g. methylation, sulfation, and glucuronidation, to facilitate their biliary and urinary elimination (Boeing et al., 2012). Therefore, the presence of many phytochemicals has low bioavailability, which could be described as transient (D'Archivio, Filesi, Varì, Scazzocchio, & Masella, 2010).

Given that date fruits are rich in molecules with supposed health benefits (Al-Farsi et al., 2005; Al-Shahib & Marshall, 2003), it would be of interest to determine the actual compound bioavailability *in vivo* to support the suggested health effects of date fruits. In our previous studies we found that two commercially important date fruit varieties namely *Deglet Nour* and *Khlas*, are rich in phytochemicals and showed distinct genetic and metabolic profiles (Diboun et al., 2015; Mathew et al., 2015, 2014). Here, our goal was to assess the overall impact of *Deglet Nour* and *Khlas* date fruits uptake on the human metabolism and describe the actual bioavailability of phenolic compounds.

## 2. Methods

### 2.1. Study design

The ethics committee of Weill Cornell Medicine-Qatar approved this study under protocol number 15-00073. All experiments and procedures conform to the 1975 Declaration of Helsinki. The volunteers were enrolled between March and August 2016. Written informed consent was obtained from all study participants. All experiments were conducted at the Medical Laboratories Clinic, a private primary health care provider in Doha (Qatar).

Twenty-one subjects, 13 females and 8 males, were enrolled in the present study (Fig. 1). The study participants underwent a physical examination of baseline measurements, including height, weight, blood pressure and pulse rate. Elevated blood glucose (> 120 mg/dL) levels prior to the challenge was an exclusion criterion. The body mass index of enrolled individuals ranged between 19.59 and 31.37 (average 26.03; ± 3.64). One subject had elevated blood glucose (> 120 mg/dL; measured using a blood glucometer, Omron HEA-230, Japan) at the third intervention and did not participate in this experiment. All other subjects had normal fasting blood glucose levels (80–120 mg/dL).

Each participant underwent three food challenges: (1) 75 g of glucose drink (Trutol 75®, Glucose Tolerance Beverage, Virginia, U.S.A.), (2) ten date fruits of the *Deglet Nour* variety, and (3) ten date fruits of the *Khlas* variety. There was at least one week time interval between each challenge.

All dates used in this study were purchased as a single batch of packaged units from a local supermarket in Doha, Qatar. *Deglet Nour* date fruits were produced in Tunisia and packaged by Bayara, Tunisia; *Khlas* date fruits were produced in the U.A.E and packaged by Date Crown, U.A.E.

All subjects were instructed to observe an overnight fast of 12 h prior to the experiment. On the day of the challenge, the subjects received either the glucose drink or one of the date varieties and were instructed to ingest this within 5 min. The participants were then allowed to drink mineral water ad libitum. Blood samples were collected by intravenous puncture at five time points: prior to food intake, and 30, 60, 90, and 120 min after food intake. The subjects stayed at the clinic throughout the test period and did not have access to any other food.

### 2.2. Sample collection

Blood samples were collected by venipuncture into a 10 ml EDTA vacuum container (Sarstedt, Germany) and centrifuged immediately at 2500*g* for 10 min at 37 °C. Plasma aliquots were transported within 5 h on ice to the laboratory at Weill Cornell Medicine-Qatar and stored at −80 °C until shipped to Metabolon Inc. (Durham, NC) on dry ice.

### 2.3. Metabolomics analysis of blood samples

Samples were processed following standard protocols established by Metabolon Inc. (Durham, NC) (Evans, DeHaven, Barrett, Mitchell, & Milgram, 2009). Briefly, samples were deproteinised and precipitated with methanol, centrifuged and reconstituted in solvents specific to the chromatography platform systems. The reconstituted extracts were divided into five equal parts (one spare) for metabolomics analysis and subjected to four different chromatography applications. Three of the reconstituted extracts were subjected to reversed phase Ultrahigh Performance Liquid Chromatography (UPLC) coupled to an Orbitrap mass spectrometer interfaced with a heat electrospray ionization (ESI) source and mass analyzer. Two of the three chromatography systems were adjusted for ionization of positive ions and one chromatography system aligned for ionization of negative ions. The fourth extract was subjected to hydrophilic interaction ultrahigh-performance liquid chromatography (HILIC-UPLC) coupled to a mass spectrometer fitted with a heat electrospray ionization and mass analyzer optimized for ionization of negative ions. The metabolites were identified by matching the information on retention time, mass to charge ratio, and matching of the mass spectral data to a reference library that is based on chemical standards established by Metabolon Inc. (Dehaven, Evans, Dai, &

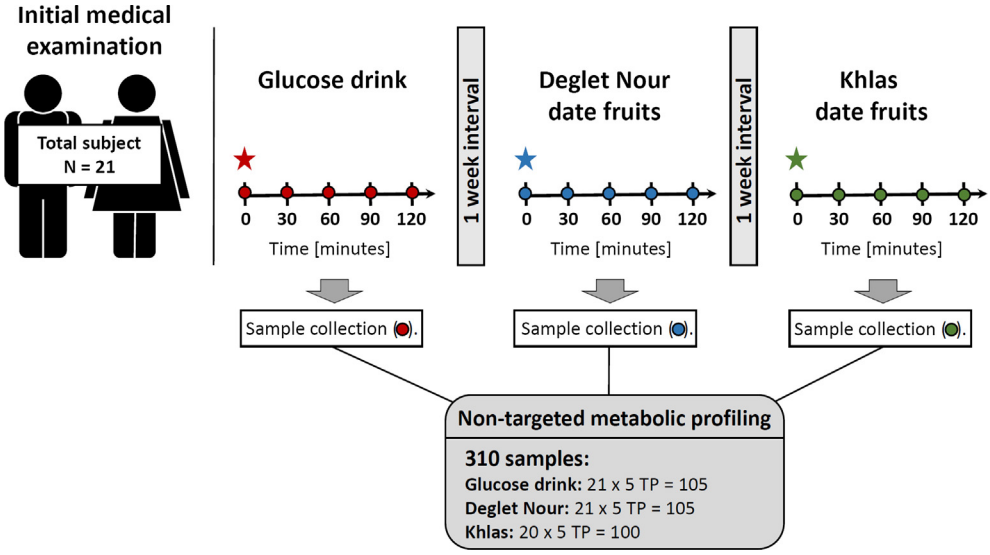


**Fig. 1.** Study design. Twenty one healthy subjects were enrolled for the study after initial medical examination. The subjects underwent the three challenges: (1) 75 g glucose drink; (2) ten *Deglet Nour* date fruits; (3) ten *Khlas* date fruits, with at least one week interval between each challenge. Participants were instructed to observe after overnight fasting of at least 12 h prior to each challenge. A blood sample was collected at base-line (time point 0) and the subject was then asked to ingest a glucose drink, depicted by red star, date fruits *Deglet Nour* on the second visit or *Khlas* on the third visit, as depicted by blue and green star, respectively. Blood samples were collected at 30, 60, 90, and 120 min after consumption. In total 310 samples were collected and analyzed using the Metabolon HD4 non-targeted metabolomics platform.