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ชีวเคมีโภชนาการ
ภาคปลาย 2560
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Body Fluids and Water Balance

- น้ำจัดเป็น essential nutrient มีอยู่ทั้งในเซลล์และรอบ ๆ เซลล์ หลอดเลือด
- หล่อลื่นข้อต่อต่าง ๆ และสร้างความชุ่มชื้นให้เนื้อเยื่อ เช่น ตา จมูกและปาก
- สร้างความเต่งให้เซลล์
- ละลายสารอาหาร แร่ธาตุ แก๊สและเอนไซม์
- เป็นตัวกลางในการขนส่งสารอาหารและออกซิเจนไปยังเซลล์และกำจัดน้ำออกจากเซลล์ผ่านตับและไต
- ควบคุมอุณหภูมิร่างกาย

- ปริมาณน้ำในร่างกาย
- ขึ้นอยู่กับอายุ เพศ ความอ้วน
- เด็กเกิดใหม่ มีน้ำประมาณ 75%
- วัยรุ่น 60%
- อายุ 50 ปีมีน้ำประมาณ 50%
- ผู้ใหญ่ ผู้หญิงมีน้ำน้อยกว่าผู้ชาย
- คนอ้วนมีน้ำน้อยกว่าคนผอม

- สมดุลน้ำ

TABLE 35-1 Daily Water Balance in a 65-kg Man Calculated to Illustrate Minimal Required Drinking Water Intake

Water Intake		Water Loss	
SOURCE	LITERS	ROUTE	LITERS
Preformed water	0.85	Insensible—lungs	0.30
Metabolic water	0.37	Insensible—skin	0.40
Drinking—minimum	0.22	Feces	0.10
		Urine	0.64
Total	1.44	Total	1.44

- ชีวเคมีของปัสสาวะ

ปริมาณ 600-2500 cc/วัน (เฉลี่ย 1200 cc/วัน)

กลางวัน:กลางคืน = 2:1 – 4:1

Polyuria > 2500 cc/วัน เกิดจากเบาหวาน หรือ diuretic และดื่มแอลกอฮอล์

Oliguria < 400 cc/วัน เกิดจาก ไตวาย ท้องเสีย หรือท่อปัสสาวะอุดตันบางส่วน

Anuria < 50 cc/วัน

- สีและกลิ่น

ปกติสีเหลืองใส

สีที่ผิดปกติขึ้นอยู่กับโรค พยาธิสภาพ อาหารและยาบางชนิดที่รับประทานเข้าไป

เบาหวาน ใส ไม่มีสี

ถ้ามีกลิ่น acetone แสดงว่าเป็นเบาหวาน

ถ้ามีความขุ่นอาจมีฟอสเฟต คาร์บอเนต urate oxalate หนอง เลือด เซลล์เยื่อบุ

- pH

ปกติ 5.5-6.5

ถ้าเป็นกรด เกิดจากกินโปรตีนสูง อดอาหาร หรือติดเชื้อ *E.coli*

ปัสสาวะเป็นด่าง เกิดจากกินมังสวิรัติ หรือติดเชื้อ *Pseudomonas*

- ความถ่วงจำเพาะ 1.008-1.030

ถ้าสูง เกิดจากมีไข้ ท้องเสีย อาเจียน เสียเหงื่อมาก มีกลูโคส โปรตีนในปัสสาวะ

ถ้าต่ำ เกิดจากไตผิดปกติ หรือแค่ดื่มน้ำมากเกินไป

- องค์ประกอบของ urine

- 95% เป็นน้ำ อีก 2% เป็น urea
- ที่เหลือเป็น uric acid, creatinine, Na, K, Cl, Ca, P, Mg, phosphate, sulfate และ ammonia

ถ้ามี uric acid มาก เป็นโรค gout และอาจตะกอนเกิดนิ่ว

Creatinine เกิดจากการทำลายกล้ามเนื้อ

ยูเรีย 15-25 กรัมต่อวัน

Bilirubin 0-0.02 mg/วัน ถ้ามากแสดงว่าตับอักเสบ

ถ้ามีโปรตีนมากกว่า 150 mg/วัน แสดงว่าออกกำลังกายหนัก มีไข้ หรือเป็นโรคไต

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Original Article

Urine metabolomics shows an induction of fatty acids metabolism in healthy adult volunteers after supplementation with green coffee (*Coffea robusta* L.) bean extract

Gregorio Peron[a], Davide Santarossa[b], Dario Voinovich[b], Stefano Dall'Acqua[a,*], Stefania Sut[a]

[a] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Via Marzolo 5, Padova 35131, Italy
[b] Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.le Europa 1, Trieste 34127, Italy

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ABSTRACT

Background and objective: Green coffee bean extract is used as herbal medicine or supplement for weight reduction and obesity. The active constituents are considered caffeine and chlorogenic acid (CGA) derivatives. The mode of action of CGA is still unclear and can be related to peroxisome proliferator-activated receptor α (PPAR-α) and liver X receptor Rα (LXR-α). Metabolomics may be an innovative tool for the description and discovery of the multiple target nature of such phytocomplex.

Methods: 24 h urine samples were collected once a week from ten healthy adult volunteers consuming daily 400 mg of dry Green coffee bean extract (GCBE, 4.9% of chlorogenic acid) each day for 30 days (5 harvesting days, considering also the first day of supplementation). Urine samples were analyzed by LC-QTOF using both untargeted and targeted approaches. The latter was used to monitor two urinary markers of oxidative stress (allantoin, 8-OHdG).

Results: Metabolomics analysis (PLS-DA) revealed changes in urine composition before and during the treatment with GCBE. Markers related to treatment were metabolites related to polyphenol administration as hippuric acid, benzoic acid derivatives, dihydroferulic and dihydrosinapic acid sulphate, but also carnitine derivatives and dicarboxylic acids. On the other hand, no changes in the levels of allantoin and 8-OHdG were observed.

Conclusion: This preliminary study showed the possible usefulness of metabolomics approach in the evaluation of GCBE consumption in healthy subjects. The observed changes in urinary composition can be related to the catabolism of GCBE constituents and to induced fatty acid metabolism, mainly related to carnitine derivatives. This latter result could be considered, at least in part, as a further proof of the mode of action of green coffee extract.

Introduction

Nowadays, overweight and obesity are considered important health concern, especially in the western countries (Nguyen and El-Serag, 2010), being amongst the biggest medical problems of the 21st century (Kużbicka and Rachoń, 2013). In fact, obesity is known to contribute to comorbid conditions that may become life threatening such as diabetes, hypertension, dyslipidemia or respiratory problems (Buchanan and Beckett, 2013). Multiple factors contribute to obesity, including genetics, bad dietary habits and lack of sufficient physical activity (Kużbicka and Rachoń, 2013; Booth et al., 2012). Moreover, excess weight can lead to metabolic syndrome, a combination of obesity, dyslipidemia, impaired glucose tolerance and hypertension (Deen, 2004; Quick and Kiefer, 2013), which could lead to other severe health problems such type 2 diabetes and cardiovascular disease (CVD) (Eckel et al., 2005). Several weight management strategies can be used. As an example, moderate overweight could be overcome starting from changing dietary habits and increasing physical activity. The use of herbal medicines and food supplements associated to physical activity and a balanced diet is also frequently adopted as weight management strategy (Yang et al., 2017; Gaullier et al., 2005), and the so called nutraceuticals are largely used for their reported health-promoting

Abbreviations: GCBE, Green coffee bean extract; CGA, Chlorogenic acid; PPAR-α, Peroxisome proliferator-activated receptors α; 8-OHdG, 8-Hydroxydeoxyguanosine; LC, Liquid Chromatography; MS, Mass Spectrometry; UPLC, Ultra Performance Liquid Chromatography; LOD, Limit of Detection; LOQ, Limit of Quantification; DAD, Diode Array Detector; ESI, Electrospray Ionization; PCA, Principal Component Analysis; PLS-DA, Partial Least Squares – Discriminant Analysis; QC, Quality Control; QTOF, Quadrupole time-of-flight mass spectrometer; RT, Retention Time; SRM, Single reaction monitoring; TQD, Tandem quadrupole mass spectrometer; USP, United States Pharmacopeia; VIP, Variable Importance on Projection

* Corresponding author.
E-mail address: stefano.dallacqua@unipd.it (S. Dall'Acqua).

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and/or disease-preventing properties (Sut et al., 2016). However, concerning such products literature data are contradictory, showing both their efficacy (Quick and Kiefer, 2013; Shimoda et al., 2006; Onakpoya et al., 2011) and inefficacy (Pittler and Ernst, 2004). Natural products as fucoxantines and carotenoids have been recently considered as possible treatments for obesity (Miyashita and Hosokawa, 2014; Maeda, 2015), along with other classes of natural products, such as polyphenols (Poddar et al., 2011; Ríos-Hoyo et al., 2015).

One popular natural product used in the recent years for moderate overweight treatment is the green coffee beans extract (GCBE, *Coffea robusta* L.), which is produced from coffee beans that have not been roasted (Thom, 2007). Roasting can cause partial degradation of the phenylpropanoid esters of quinic acid (as chlorogenic acids, CGA) during high temperature treatments (Farah and Donangelo, 2006). CGA are considered the main constituents of GCBE (Farah and Donangelo, 2006). Although the content of CGA may vary according to several factors as species and cultivar, degree of maturation, agricultural practices, climate and soil, the amounts of total CGA in regular green coffee beans, on dry matter basis, may vary from 4 to 8.4 % for *Coffea arabica*, and from 7 to 14.4 % for *Coffea robusta*, with some hybrids presenting intermediate levels (Farah and Donangelo, 2006). The extract also contains caffeine, that together with CGA are considered to exert potential weight loss activity (Shimoda et al., 2006). The effects of consumption of GCBE observed in animal models and in humans are related to modulation of glucose metabolism, inhibition of fat accumulation, weight reduction and alteration of body fat distribution, exerted by CGA (Shimoda et al., 2006). Moreover, other proposed mechanisms are the reduction of the intestinal absorption of glucose (Shimoda et al., 2006) and the inhibition of the enzymatic activity of hepatic glucose-6-phosphatase (Shimoda et al., 2006; Arion et al., 1997). More recently, in an *in vivo* study CGA of green coffee were demonstrated to improve the blood lipid metabolism in rats by alleviating the levels of fatty acids and triglycerides and modulating the multiple factors in liver through AMP-activated protein kinase (AMPK) pathway, showing a possible mode of action of this ingredient in the management of obesity (Sudeep et al., 2016). The presence of caffeine can be considered useful because of its stimulant effect, hence it increases energy expenditure in humans, contributing to weight loss effects of coffee (Dulloo et al., 1989). Furthermore, other proposed mechanisms for caffeine effect on weight loss include increased thermogenesis, which consequently enhances lipolysis and lipid metabolism (Dulloo et al., 1989; Greenberg et al., 2006).

Food supplements containing GCBE are increasing in use to control weight gain, but the efficacy of these products is still under debate, due to contradictory data published in literature. In a review of 2013, Buchnan and Beckett indicated that a limited number of clinical studies were published related to the efficacy of GCBE, and that in most of them the clinical significance of the weight loss was minimal (Buchanan and Beckett, 2013). The same authors underlined the significant limitation of the reviewed studies as lack of blinding, direct comparisons, safety assessment, lack of comprehensive endpoints, very low sample size, and not inclusion of lifestyle modifications (Buchanan and Beckett, 2013). On the other hand, other papers reported moderate effects of GCBE on weight loss (Quick and Kiefer, 2013; Shimoda et al., 2006; Onakpoya et al., 2011). Revuelta–Iniesta and coll. compared the effects of a 2-weeks green coffee and black coffee consumption in 20 healthy subjects, observing that systolic blood pressure were significantly reduced after green coffee, as well as body mass index and abdominal fat, with no changes in energy intake. Furthermore, cortisol/cortisone ratio in urine was reduced after green coffee, suggesting that green coffee can play a role in reducing cardiovascular risk factors overall (Revuelta-Iniesta and Al-Dujaili, 2014).

Other papers focused their attention only on CGA, without studying the effects of a treatment with a complete green coffee extract. For example, Cho and coll. compared the effects of treatment with CGA and caffeic acid (0.02% w/w) in obese mice (Cho et al., 2010). Both caffeic acid and CGA significantly lowered body weight, visceral fat mass and plasma leptin and insulin levels, compared to the high-fat control group. They also reported lowered triglyceride (in plasma, liver and heart) and cholesterol (in plasma, adipose tissue and heart) levels. Both treatments significantly inhibited fatty acid synthase, 3 hydroxy-3-methylglutaryl CoA reductase and acyl-CoA: cholesterol acyltransferase activities, while they increased fatty acid β-oxidation activity and peroxisome proliferator-activated receptors α (PPAR-α) expression in the liver compared to the high-fat group (Cho et al., 2010). Hence, despite the large diffusion of GCBE, studies are still needed to fully understand the possible mode of action and the role that this plant extract may have as treatment of obesity and its clinical relevance.

The evaluation of the effects and of the possible mode of action of phytoconstituents is a challenge due to complex composition and possible multiple mode of action, and metabolomics may play a role as a new approach in the study of bioactive plant ingredients (Sut et al., 2016; Wolfender et al., 2013). Metabolomics-based studies may offer new opportunity in the evaluation of the possible efficacy, mode of action and safety related to the use of GCBE. Overall, urinary metabolomics can be a suitable approach, because sample collection is non-invasive and allows long-term studies, furthermore markers of oxidative status may be measured in urines offering the opportunity to study also the redox effect of GCBE administration (Il'yasova et al., 2012). The green coffee extract may present some effect in the weight management but the evaluation of its effects and mode of action may be difficult due to the complex phytochemical composition and due to the multiple possible molecular targets of its constituents. For this reason, a metabolomics approach was used in the present pilot study. Ten healthy adult with normal body mass index (BMI), no metabolic, cardiovascular overweight or obesity problems assumed 400 mg of dry GCBE daily for 30 days. The 24 h urinary samples were collected weekly, and analyzed by LC-MS. Multivariate data analysis approaches were applied and also targeted analysis were also performed to measure oxidative stress urinary biomarkers, namely allantoin and 8 hydroxydeoxyguanosine (8-OHdG), in order to assess the potential antioxidant activity of GCBE *in vivo*. To the best of our knowledge this is the first report describing the effects on healthy subjects of green coffee by metabolomics approach.

Materials and methods

Materials

The supplement containing a standardized dry GCBE (*Coffea robusta* L.) was purchased from a local market. The names of product and supplier are note reported to avoid any conflict of interests. The powder extract used in the experiment was a homogeneous batch and it was formulated in gelatin capsules, each containing 200 mg of product. Standard chlorogenic acid (product number: C3878), allantoin (product number: 05670) and 8-OHdG (product number: H5653) were purchased from Sigma Aldrich (Milan, Italy). HPLC-grade acetonitrile and formic acid were purchased from Sigma Aldrich (Milan, Italy), as well as deuterated methanol used in NMR analysis. Deionized water used in HPLC and UPLC analyses was filtered through a Milli-Q system equipped with a 0.22 μm cut-off filter (Millipore).

Chemical characterization of dry GCBE

Chemical characterization of dry GCBE was performed by ^{1}H NMR analysis and by HPLC-DAD-MS^{n}. For NMR exploratory analysis of GCBE, a Bruker Avance III spectrometer operating at 400 MHz was used. Briefly, 150 mg of dried extract were weighted in Eppendorf tube and 1 ml of deuterated methanol was added. The suspension was sonicated for 5 min then the tube was centrifuged at 13,000 rpm. The supernatant was transferred to a NMR tube and used for the ^{1}H NMR analysis. For the chromatographic analysis, the system was composed of an Agilent 1260 chromatographic system with 1260 autosampler,