Food Research International 63 (2014) 428–438

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Modulation of inflammatory gene transcription after long-term coffee consumption

Swantje Winkler [a], Natalie Dieminger [b], Volker Blust [a], Annett Riedel [c], Tamara Bakuradze [d], Gina Montoya [d], Ute Hassmann [a], Roman Lang [b], Thomas Hofmann [b], Veronika Somoza [c], Elke Richling [d], Gerhard Bytof [e], Herbert Stiebitz [e], Ingo Lantz [e], Dorothea Schipp [f], Jochen Raedle [g], Doris Marko [a,*]

[a] University of Vienna, Department of Food Chemistry and Toxicology, Währingerstr. 38, A-1090 Vienna, Austria
[b] Chair of Food Chemistry and Molecular Sensory Science, Technical University Munich, Lise-Meitner-Strasse 34, 85354 Freising, Germany
[c] University of Vienna, Department of Nutritional and Physiological Chemistry, Althanstrasse 14 (UZA II), A-1090 Vienna, Austria
[d] Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany
[e] Tchibo GmbH, Überseering 18, D-22297 Hamburg, Germany
[f] www.ds-statistik.de, Rosenthal-Bielatal, Germany
[g] Innere Medizin III, Westpfalzklinikum, Kaiserslautern, Germany

ARTICLE INFO

Article history:
Received 7 January 2014
Received in revised form 22 April 2014
Accepted 26 May 2014
Available online 10 June 2014

Keywords:
Nrf2
Inflammation
Obesity
Coffee
Chlorogenic acid
Polymorphism

ABSTRACT

Scope: Obesity has been found to be associated with low grade inflammation accompanied by chronic oxidative stress. The transcription factor Nrf2 is likely involved in lipid metabolism and inflammation processes, possibly mediated by an antioxidant response element (ARE)-similar region located in the promoter of lipogenic genes like peroxisome-proliferator activated receptor γ (PPARγ) and pro-inflammatory interleukin 6 (IL6). The present study investigates the influence of coffee consumption on the transcription of obesity-associated genes in human peripheral blood lymphocytes (PBL). Two different coffee blends with comparable caffeine concentrations were provided, rich either in chlorogenic acids and trigonelline (market blend, MB) or in *N*-methylpyridinium (NMP, study blend, SB).
Methods and results: In a cross-over randomized double blind intervention study 84 volunteers (male and female, 25.6 ± 5.8 years, BMI 22.9 ± 1.9 kg/m^2, healthy, nonsmokers, regular coffee drinker) daily consumed 750 mL of the respective coffee over a period of 4 weeks, respectively. Transcription of IL6 in PBL was found to be positively associated with body fat. In the first intervention period consumption of MB decreased significantly the transcription of Nrf2, PPARγ and IL6 while concomitantly an enhanced level of PPARα mRNA was found. Due to carry-over effects for Nrf2 and PPARα, data of both intervention periods could only be pooled for PPARγ and IL6. Pooled data from both intervention periods showed a significant decrease of IL6 transcripts for SB consumption only. The changes in gene transcription appear to correlate with the level of different CGA metabolites in the plasma of the volunteers. Initial results further indicate a potential contribution of genetic polymorphisms in the *nrf2* promoter and the *pparγ*-gene to the influence of coffee consumption on PPARγ transcription.
Conclusion: Regular coffee consumption affects the transcription of genes associated with obesity and/or inflammation. Metabolites of chlorogenic acids as well as genetic polymorphisms may be relevant influencing factors.

1. Introduction

In recent years the prevalence of adults being overweight has alarmingly increased to 1.4 billion people worldwide. Overweight is ranked as the fifth leading risk of global deaths through sequels such as cardiovascular diseases, diabetes mellitus, musculoskeletal disorders and some types of cancer (Fujioka, 2002; Kang & Park, 2012; Visscher & Seidell, 2001).

Hence, it is aimed to identify substances of our daily nutrition that bear weight losing properties. After water coffee is the most consumed beverage worldwide with USA, Brazil and Germany as leading countries

Abbreviations: ARE, antioxidant response element; BC, blood collection; BMI, body mass index; CGA, chlorogenic acids; CRP, C-reactive protein; DHFAglcd, dihydroferulic acid glucuronide; FA, ferulic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HO1, heme oxygenase; IL6, interleukin 6; LPS, lipopolysaccharide; Nf-κB, nuclear factor 'kappa-light-chain-enhancer' of activated B-cells; NMP, *N*-methylpyridinium; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PPAR, peroxisome proliferator-activated receptor; RPL13, ribosomal protein L13a; SD, standard deviation; TNFα, tumor necrosis factor α.

* Corresponding author at: University of Vienna, Department of Food Chemistry and Toxicology, Währingerstr. 38, 1090 Vienna, Austria. Tel.: +43 1 4277 70800.
E-mail address: doris.marko@univie.ac.at (D. Marko).

http://dx.doi.org/10.1016/j.foodres.2014.05.073
0963-9969/

(ICO, 2011). Obesity is accompanied by low-grade inflammation increasing oxidative stress, since adipocytes attract macrophages that infiltrate into the adipose tissue (Medzhitov, 2008). The key regulator tumor necrosis factor α (TNFα) subsequently enhances nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (Nf-κB) action that in turn initiates an immune reaction by binding to the DNA and starting transcription of various cytokines. Thus, during weight loss decreased levels of the proinflammatory factors like TNFα, IL6 and hepatic C-reactive protein (CRP) can be observed (Higdon & Frei, 2003; Stienstra, Duval, Muller, & Kersten, 2007).

Furthermore, oxidative stress activates the transcription factor Nrf2 by detaching it from its cytosolic anchor Keap1. After release from Keap1, Nrf2 translocates into the nucleus where it binds jointly with co-factors to the *cis*-acting antioxidant response element (ARE). Consequently, ARE-regulated transcription of phase II biotransformation enzymes such as glutathione *S*-transferases (GST), UDP-glucuronyltransferases (UGT) or heme oxygenase 1 (HO1) is activated (Boettler, Sommerfeld, et al., 2011; Boettler, Volz, et al., 2011; Cho et al., 2010). Besides, Nrf2 is likely involved in lipid metabolism and inflammation processes, since adipose tissue mass is decreased in Nrf2-knockout models and these animals are protected against weight gain and obesity from a high fat diet (Hou et al., 2012; Huang, Tabbi-Anneni, Gunda, & Wang, 2010; Pi et al., 2010). In Nrf2-deficient 3T3-L1 cells differentiation is repressed (Hou et al., 2012; Pi et al., 2010). In contrast, Keap1-knockdown and -deficiency animal models lead to accelerated differentiation of preadipocytes, higher body mass, fat mass and hepatic triacylglycerides (Pi et al., 2010; Xu, Kulkarni, Donepudi, More, & Slitt, 2012). This process is possibly mediated by an ARE-similar region located in the promoter of lipogenic genes like peroxisome-proliferator activated receptor γ (PPARγ) and C/EBPα that are therefore up-regulated after Nrf2-activation (Pi et al., 2010). PPARs are ligand-activated transcription factors belonging to a superfamily of nuclear receptors controlling mainly lipid metabolism *via* regulation of related enzymes (Huang et al., 2010; Pi et al., 2010).

Nrf2 may also be responsible for down-regulation of PPARα, a lipolytic gene that is involved in the oxidation of fatty acids due to enhancing the transcription of enzymes (Huang et al., 2010) like carnitine palmitoyltransferase-I and medium-chain acyl-CoA dehydrogenase (Sugden, Caton, & Holness, 2010). Besides enhancing enzymes required for fatty acid oxidation this latter receptor moreover reduces inflammation possibly *via* accelerated expression of IκB that controls Nf-κB and therefore down-regulates the expression of proinflammatory cytokines (Stienstra et al., 2007). Furthermore Nrf2 is directly able to influence inflammatory processes by binding to ARE in the promoter of IL6 thereby up-regulating its expression (Wruck et al., 2011).

Coffee consumption is prevalently associated with lower risk of type II diabetes (T2D) in men and women (Fujioka, 2002; Greenberg, Boozer, & Geliebter, 2006; Higdon & Frei, 2003; Lopez-Garcia, van Dam, Qi, & Hu, 2006; Lopez-Garcia, van Dam, Rajpathak, et al., 2006; Olthof, van Dijk, Deacon, Heine, & van Dam, 2011; van Dam & Feskens, 2002). This may be due to decreased obesity and inflammation markers (Kempf & Martin, 2010; Lopez-Garcia et al., 2006) for which caffeine is mostly taken into account (Fujioka, 2002; Lopez-Garcia, van Dam, Qi, et al., 2006; Lopez-Garcia, van Dam, Rajpathak, et al., 2006). Caffeine is known to stimulate thermogenesis by up-regulating the expression of uncoupling proteins (UCP) in the mitochondria and to enhance lipolysis, fat oxidation and insulin secretion while decreasing blood glucose levels. Therefore, it may promote weight loss and improve diabetes (Butt & Sultan, 2011; Rudelle et al., 2007; Tunnicliffe & Shearer, 2008). However, decaffeinated coffee has been reported as well to have anti-diabetic or anti-obesity properties (Greenberg et al., 2006; Olthof et al., 2011), suggesting that other constituents than caffeine, *e.g.* chlorogenic acids (CGA) (Cho et al., 2010; Johnston, Clifford, & Morgan, 2003; Li, Chang, Ma, & Yu, 2009; Olthof et al., 2011), trigonelline (Greenberg et al., 2006; Johnston et al., 2003; Olthof et al., 2011) or other xanthines may contribute to the reported effects on lipid and glucose metabolism. CGA and their metabolites are already hypothesized to contribute to weight loss after consumption due to a decrease of body fat (Jin Son, Rico, Hyun Nam, & Young Kang, 2010; Tunnicliffe & Shearer, 2008) and to improve glucose and insulin metabolism (Butt & Sultan, 2011) mediated by reduced uptake of glucose from the gut (Greenberg et al., 2006) and extenuated activity of glucose-6-phosphatase in the liver (Thom, 2007).

In a recent human intervention study we observed a significant weight loss in lean subjects (BMI < 25 kg/m^2) after a 4 week consumption of a dark roasted coffee brew that contained high concentrations of NMP and CGA (Bakuradze et al., 2011). In the present study we addressed the question whether long-term consumption of coffee affects the transcription of genes involved in lipogenesis and inflammation, known to be associated with weight regulation and whether differences between two different coffee blends, reflected by the levels of CGA, trigonelline and NMP, result in differences in the transcriptional response pattern in PBL of the probands.

2. Materials and methods

2.1. Coffee brews

Two different coffee blends were used in this study. The CGA- and trigonelline-rich market blend (MB) was manufactured from five commercially available regular ground coffee. Quantified compounds were: 0.39 (±0.01) mg/g NMP, 6.27 (±0.12) mg/g trigonelline, 12.4 (±0.13) mg/g caffeine and 19.3 (±0.28) mg/g CGA. The NMP-rich study blend (SB) was obtained as a mixture from a dark roast coffee and a light roast coffee and contained 1.20 (±0.02) mg/g NMP, 3.42 (±0.20) mg/g trigonelline, 12.8 (±0.24) mg/g caffeine and 10.0 (±0.28) mg/g CGA.

2.2. Study design

84 healthy, male and female (25.6 ± 5.8 years, BMI 22.9 ± 1.9 kg/m2), nonsmoking, regularly coffee consuming volunteers participated in this study and were randomly divided into two groups (group A, n = 43, 24 males + 19 females; group B, n = 41, 22 males + 19 females). Overall the study took 20 weeks and was subdivided into 4 week-periods of alternating wash-outs and interventions as follows: wash-out 1, intervention 1, wash-out 2, intervention 2, and wash-out 3. Prior to and after every period blood of each subject was collected (BC) after an overnight fasting and analysis of body composition was performed. During the first intervention period group A daily consumed 750 mL of market blend, whereas group B obtained the same amount of study blend. This was reversed during the second intervention period. Compliance to coffee consumption was controlled by analysis of urinary NMP, a compound unique in its abundance to coffee and therefore a suitable biomarker for coffee consumption (Lang, Wahl, Stark, & Hofmann, 2012). (For details see Bakuradze et al., 2014).

2.3. Ethics

The study was approved by the Ethics Committee of the medical chamber Rhineland-Palatinate, Mainz, Germany (no. 837.414.10 (7423)) in December 2010. Signed letters of agreement were obtained from all subjects.

2.4. Quantitation of coffee constituents and metabolites in urine and plasma

Coffee constituents were quantified by HPLC-DAD and HPLC-SIDA-MS/MS techniques using literature methods (Lang et al., 2013b; Weiss et al., 2010). Compliance control was done by analysis of urinary NMP by HPLC-SIDA-MS/MS (Lang et al., 2012). Analysis of chlorogenic acid metabolites in human plasma was done as reported recently (Lang et al., 2013).

2.5. Isolation of human peripheral blood lymphocytes and RNA extraction

Performed as described previously (Volz et al., 2012).