Coffee enhances the expression of chaperones and antioxidant proteins in rats with nonalcoholic fatty liver disease

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Coffee consumption is inversely related to the degree of liver injury in patients with nonalcoholic fatty liver disease (NAFLD). Molecular mediators contributing to coffee's beneficial effects in NAFLD remain to be elucidated. In this study, we administrated decaffeinated espresso coffee or vehicle to rats fed an high-fat diet (HFD) for 12 weeks and examined the effects of coffee on liver injury by using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) proteomic analysis combined with mass spectrometry. Rats fed an HFD and water developed panacinar steatosis, lobular inflammation, and mild fibrosis, whereas rats fed an HFD and coffee exhibited only mild steatosis. Coffee consumption increased liver expression of the endoplasmic reticulum chaperones glucose-related protein 78 and protein disulfide-isomerase A3; similarly, coffee drinking enhanced the expression of the mitochondrial chaperones heat stress protein 70 and DJ-1. Furthermore, in agreement with reduced hepatic levels of 8-isoprostanes and 8-hydroxy-2′-deoxyguanosine, proteomic analysis showed that coffee consumption induces the expression of master regulators of redox status (i.e., peroxiredoxin 1, glutathione *S*-transferase α2, and D-dopachrome tautomerase). Last, proteomics revealed an association of coffee intake with decreased expression of electron transfer flavoprotein subunit α, a component of the mitochondrial respiratory chain, involved in *de novo* lipogenesis. In this study, we were able to identify by proteomic analysis the stress proteins mediating the antioxidant effects of coffee; moreover, we establish for the first time the contribution of specific coffee-induced endoplasmic reticulum and mitochondrial chaperones ensuring correct protein folding and degradation in the liver. (Translational Research 2014;163:593–602)

Abbreviations: ALT = alanine aminotransferase; ER = endoplasmic reticulum; FFA = free fatty acid; HFD = high-fat diet; HSP70 = heat shock protein 70; mtHSP70 = mitochondrial heat shock protein 70; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; PAGE = polyacrylamide gel electrophoresis; PCR = polymerase chain reaction; PRDX1 = peroxiredoxin 1; ROS = reactive oxygen species

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Submitted for publication June 26, 2013; revision submitted November 1, 2013; accepted for publication December 1, 2013.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2013.12.001

AT A GLANCE COMMENTARY

Salomone F, et al.

Background

Coffee consumption is associated with decreased overall and liver-related mortality. Coffee drinking has been shown to prevent liver injury progression both in humans and animal models of nonalcoholic fatty liver disease (NAFLD); however, the molecular mediators contributing to coffee's beneficial effect have been not identified.

Translational Significance

We demonstrate that coffee improves liver injury in rats with NAFLD by enhancing the expression of chaperones, located in mitochondria and endoplasmic reticulum, ensuring correct protein folding, and by increasing the expression of antioxidant proteins. On the basis of these findings, there is a significant molecular rationale for supporting the consumption of coffee as a component for a healthy diet in patients with NAFLD.

Nonalcoholic fatty liver disease (NAFLD) ranges from simple fatty liver to nonalcoholic steatohepatitis (NASH), which is associated with increased liver-related mortality resulting from cirrhotic and tumorigenic evolution.[1] Steatogenesis in NAFLD is caused by an increased supply of free fatty acids (FFAs) to the liver, which, combined with *de novo* lipogenesis, leads to triglyceride accumulation.[2] Different from patients with simple fatty liver, in patients with NASH, metabolic FFA handling triggers hepatocyte damage and activation of fibrogenesis through different cellular events: an increase in the production of reactive oxygen species (ROS), prevalent in the mitochondria[3]; impairment of protein folding in the endoplasmic reticulum (ER)[4]; and impairment of lysosomal degradation of proteins and lipids.[5] Both genetic and environmental factors, especially dietary habits,[6] have been implicated in modulating this complex network of molecular signals finally leading to fibrogenesis and clinical outcomes.

Coffee is the most consumed beverage worldwide; recently, a large, prospective study demonstrated that consumption of both caffeinated and decaffeinated coffee is associated with decreased all-cause mortality.[7] Coffee consumption has been reported to reduce the risk of advanced liver disease and its complications[8-10] as well as hepatocellular carcinoma independent of etiology.[11-13] In patients with NAFLD, coffee consumption is an independent protective factor for fibrosis.[14,15] Despite

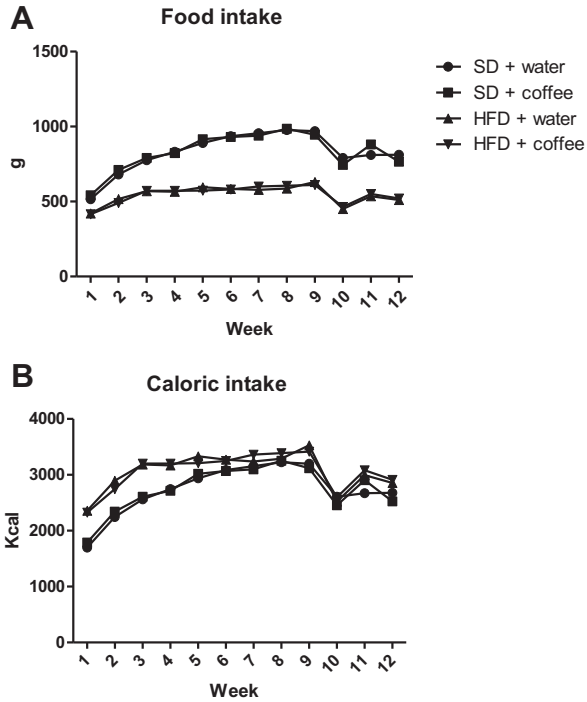

Fig 1. (**A, B**) Effects of coffee on food and caloric intake. Coffee-containing beverages were prepared by filtering via a paper filter a mix of boiling water and decaffeinated coffee powder. The average daily consumption of liquid (water or the coffee solution) was about 30 mL per rat. Coffee preparation was diluted in 20 mL water to afford rats a daily dose of 1.5 mL, which is the dose corresponding to 6 cups of espresso coffee or 2 cups of filtered coffee for a person weighing 70 kg. Coffee administration influenced neither food intake (**A**) or caloric intake (**B**), recorded weekly. HFD, high-fat diet; SD, standard diet.

such evidence, we know the molecular events involved in the beneficial effects of coffee in NAFLD in part only; recently, decaffeinated coffee administration in rats with diet-induced obesity was shown to be associated with decreased liver oxidative stress and inflammatory cytokines levels.[16] However, the molecular mediators contributing to the protective effects of coffee remain to be elucidated. To this aim, we administrated decaffeinated espresso coffee or vehicle to rats fed a high-fat diet (HFD), a physiological model of NAFLD, and assessed changes in liver proteomic profiles by using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) proteomic analysis combined with mass spectrometry.

MATERIALS AND METHODS

Animals and treatments. The experimental protocol was approved by the ethics committee for animal experiments at the Federico II University of Naples. Twenty-four male Wistar rats weighing 180–220 g were housed randomly in wire-bottomed cages. Animals were obtained from Harlan Italy (Udine, Italy) and were maintained under controlled temperature conditions of