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Coffee inhibits the reactivation of glucocorticoids by 11β-hydroxysteroid dehydrogenase type 1: A glucocorticoid connection in the anti-diabetic action of coffee?

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Abstract **Recent epidemiological studies demonstrated a beneficial effect of coffee consumption for the prevention of type 2 diabetes, however, the underlying mechanisms remained unknown. We demonstrate that coffee extract, corresponding to an Italian Espresso, inhibits recombinant and endogenous 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity. The inhibitory component is heat-stable with considerable polarity. Coffee extract blocked 11β-HSD1-dependent cortisol formation, prevented the subsequent nuclear translocation of the glucocorticoid receptor and abolished glucocorticoid-induced expression of the key gluconeogenic enzyme phosphoenolpyruvate carboxykinase. We suggest that at least part of the anti-diabetic effects of coffee consumption is due to inhibition of 11β-HSD1-dependent glucocorticoid reactivation.**

Keywords: 11β-Hydroxysteroid dehydrogenase; Glucocorticoid; Coffee; Cortisol; Diabetes

1. Introduction

Coffee is one of the most often consumed beverages worldwide. Recently, several population studies demonstrated an association of coffee consumption with improved glucose tolerance and insulin sensitivity and a lower risk of type 2 diabetes [1,2]. Constituents other than caffeine seem to be responsible for the anti-diabetic effects of coffee, however, neither the active compound nor the responsible target have been identified so far.

Based on a series of animal experiments and observations from studies with humans, it became evident that excessive glucocorticoid action plays a causal role in the pathogenesis of type 2 diabetes and metabolic syndrome [3,4]. In particular, the enhanced local conversion of inactive 11-ketoglucocorticoids (cortisone, 11-dehydrocorticosterone) to active 11β-hydroxyglucocorticoids (cortisol, corticosterone) by 11β-HSD1 in metabolically active tissues such as liver, adipose tissue and skeletal muscle has been implicated in these disorders [5–7]. Whereas 11β-HSD1 knock-out mice were resistant to high fat diet-induced metabolic syndrome [8,9], mice specifically overexpressing 11β-HSD1 in adipose tissue showed all typical features of the metabolic syndrome [10,11]. Mice overexpressing 11β-HSD1 in the liver developed insulin resistance, dyslipidemia, and hypertension, but they were not obese [12]. Moreover, several studies reported an elevated 11β-HSD1 expression in adipose tissue of obese patients [5].

Treatment of obese and diabetic mouse strains with selective 11β-HSD1 inhibitors resulted in a significant improvement of glucose tolerance and insulin sensitivity, accompanied with reduced visceral fat deposits [13–15]. These studies suggest that inhibition or downregulation of 11β-HSD1 provides beneficial effects opposing the onset of metabolic syndrome [16].

We previously demonstrated the existence of natural compounds selectively inhibiting 11β-HSD1, e.g. flavanone and 2′-hydroxyflavanone present in red and yellow fruits and vegetables [17]. Because coffee is a rich source of biologically active compounds [18] and because of its anti-diabetic effect, we tested the hypothesis whether coffee contains compounds inhibiting 11β-HSD1 activity.

2. Materials and methods

2.1. Preparation of coffee extract

To prepare a coffee extract with a composition corresponding to that of an Italian Espresso, 10 g of freshly grinded roasted beans of *Coffea Arabica* L. were extracted with 30 ml of water for 15 s at 100 °C and a pressure of 15 bar. After sterile filtration, aliquots of the filtrate were frozen at −20 °C and stored until further analysis. To assess the effect of charcoal treatment on the inhibitory effect of the coffee extract, 10 ml of extract was incubated with 50 mg of activated charcoal for 10 min under stirring, followed by filtration. This procedure was repeated three times and the inhibitory potential of the stripped extract on 11β-HSD1 activity was measured. For the liquid/liquid extraction, 500 μl extract was mixed with 500 μl of the corresponding organic solvent (*n*-hexane, dichloromethane or ethyl acetate), followed by vigorous mixing for 10 s and incubation for 10 min. The water and organic phase were separated after centrifugation for 10 min at 10000 × g.

2.2. 11β-HSD1 activity assays

Recombinant 11β-HSD1 was expressed in HEK-293 cells and enzymatic activities were measured in cell lysates and in intact cells as described previously [17,19]. Briefly, frozen cell pellets were resuspended in ice-cold buffer TS2 (100 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM $MgCl_2$, 250 mM sucrose, 20 mM Tris–HCl, pH 7.4) and sonicated. Reactions were carried out for 10 min at 37 °C in a final volume

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of 20 μl containing 10 nCi [1,2,6,7-^{3}H]-cortisone (American Radiolabeled Chemicals, St. Louis, MO, USA), 250 nM unlabeled cortisone, 400 μM NADPH and different amounts of coffee extract. For measurements in intact cells, 50 000 transfected cells per well were seeded in poly-D-lysine coated 96-well Biocoat plates (Becton–Dickinson, Basel, Switzerland). The medium was replaced 24 h later by 80 μl fresh medium, 10 μl medium containing various concentrations of coffee extract and 10 μl medium containing 10 nCi [1,2,6,7-^{3}H]-cortisone and 250 nM unlabeled cortisone. Cells were incubated for 2 h at 37 °C under 5% CO_2, reactions stopped by adding an excess of unlabeled cortisone and cortisol in methanol, followed by separation of steroids by TLC and determination of the conversion of radiolabeled substrate by scintillation counting. Data (mean ± S.D.) were obtained from at least four independent experiments.

2.3. Glucocorticoid receptor translocation assay

HEK-293 cells (300 000 cells/well) were grown on poly(L-lysine)-coated glass slides in six-well plates containing 2 ml DMEM medium. Cells were transfected by calcium phosphate precipitation, with 1 μg/well of a plasmid for green-fluorescent (GFP)-tagged human GR [20] and 1 μg/well of a plasmid for human 11β-HSD1 [21]. To remove steroids, cells were washed twice with charcoal-treated DMEM 6 h post-transfection, followed by incubation for another 16 h. Cells were then incubated for 40 min with cortisone, cortisol, coffee extract or vehicle as indicated. After rinsing the cells with phosphate-buffered saline and fixation for 10 min with 4% paraformaldehyde, the subcellular localization of GFP-GR was detected by fluorescence microscopy.

2.4. Analysis of phosphoenolpyruvate carboxykinase mRNA expression

Currently, there is no suitable liver cell line available that expresses 11β-HSD1, and 11β-HSD1 activity disappears upon cultivation of primary hepatocytes. To study the effect of hepatic 11β-HSD1-dependent glucocorticoid reactivation on the regulation of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK), we applied rat H4EII hepatoma cells stably expressing mouse 11β-HSD1 [22]. These cells were kindly provided by Dr. Kurt Amrein, Hoffmann LaRoche, Basel. Cells were cultured in MEM medium, followed by incubation in serum-free MEM. Coffee extract at concentrations indicated were added 15 min prior addition of 50 nM cortisone, and cells were harvested after incubation for 3 h at 37 °C. The mRNA levels were determined by real-time RT-PCR using TaqMan technology as described previously [23]. The relative expression of PEPCK mRNA was determined versus β-actin as control. Data represent mean ± S.D. determined from triplicates.

3. Results

3.1. Coffee extract inhibits 11β-HSD1 activity

To test our hypothesis that coffee beverage contains compounds with anti-diabetic effects due to decreased local glucocorticoid reactivation, we prepared a coffee extract with a composition similar to that of an Italian Espresso and tested its effect on 11β-HSD1-dependent conversion of cortisone to cortisol. The presence of coffee extract at a final concentration of 1% almost completely inhibited the 11β-HSD1-dependent oxoreduction of cortisone in cell lysates (Fig. 1). Upon incubation with various concentrations of coffee extract, a dose-dependent inhibition curve with an IC_{50} of approximately 0.25% was observed in cell lysates and of 0.7% in intact cells. Coffee extract similarly inhibited 11β-HSD1 activity in fully differentiated mouse 3T3-L1 adipocytes and in mouse C2C12 myotubes (not shown), two metabolically relevant endogenous cell models [24]. Inhibition of 11β-HSD1 was 7–10-fold more efficient than that of 11β-HSD2 and 17β-HSD1 (not shown), indicating that coffee preferentially inhibits glucocorticoid reactivation. Comparable results were obtained with five different commercially available coffee brands, while extracts from cocoa powder, which is also a rich source of polyphenolic compounds, did not inhibit 11β-HSD1.

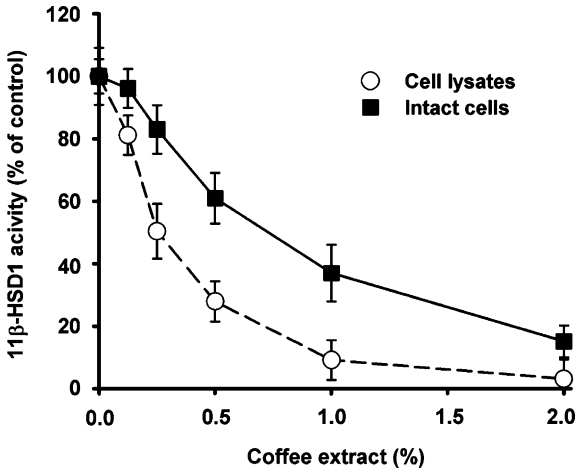

Fig. 1. Inhibition of 11β-HSD1 activity by coffee extracts. Inhibition of 11β-HSD1-dependent conversion of cortisone to cortisol by various concentrations of coffee extract was measured in cell lysates (open circles) and intact HEK-293 cells (filled squares) expressing recombinant 11β-HSD1. Data represent mean ± S.D. from at least four independent experiments.

3.2. Exclusion of caffeine as the 11β-HSD1 inhibitor

Next, we measured the effect of extracts from decaffeinated coffee on 11β-HSD1 activity and obtained an inhibition comparable to that of normal coffee extract. Moreover, 2 mM of pure caffeine did not affect 11β-HSD1 activity. We also found no inhibitory effect with 200 μM of caffeic acid, chlorogenic acid or trigonelline, three well-known biologically active substances present in coffee beverage (not shown).

3.3. Chemical properties of the inhibitory compound

We performed experiments to obtain initial information on the stability and polarity of the inhibitory compound. Boiling of the aqueous coffee extract for 30 min did not affect its effect,

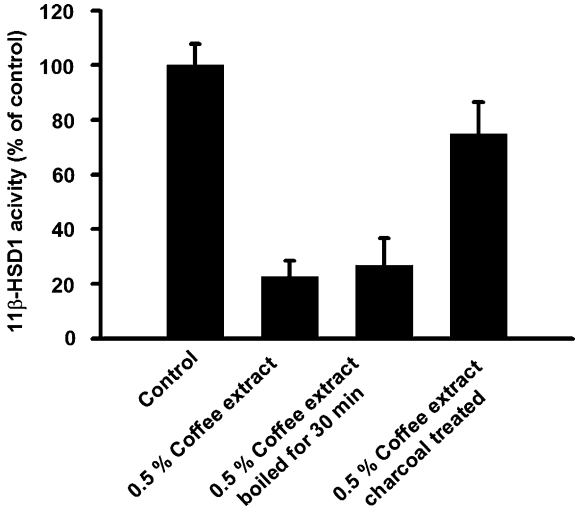

Fig. 2. The active compound is heat-stable and binds to charcoal. Prior to subjection to the 11β-HSD1 activity assay, the coffee extract was boiled for 30 min or pretreated with charcoal.

demonstrating that the inhibitor is thermo-stable (Fig. 2.). The inhibitory effect of the extract was abolished, however, upon charcoal treatment. To further assess the solubility of the inhibitor, the aqueous coffee extract was mixed with an equal volume of the organic solvents *n*-hexane, dichloromethane, or ethyl acetate, followed by separation of the two phases and determination of the presence of the inhibitory substance in both phases (Fig. 3). An equal partition was obtained with water/ethyl acetate, whereas highly hydrophobic solvents such as *n*-hexane extracted only small amounts of the inhibitor. These observations and the fact that it is solubilized from coffee beans with water suggest that the inhibitor is a fairly polar compound.

3.4. Blockade of 11β-HSD1-mediated GR activation by coffee extract

We tested the effect of coffee extract on the 11β-HSD1-dependent conversion of inactive cortisone to active cortisol and the subsequent induction of nuclear translocation of the GR. In the inactive state the GR resides in the cytoplasm. Upon binding cortisol it undergoes a conformational activation and translocates into the nucleus where it regulates genes with a GR-response element (GRE). As shown in Fig. 4, incubation of HEK-293 cells expressing GFP-GR and 11β-HSD1 with cortisone led to almost complete translocation of the receptor into the nucleus. Simultaneous incubation with coffee extract abolished nuclear receptor translocation, indicating inhibition of 11β-HSD1-dependent conversion of cortisone to cortisol. That the effect of coffee extract was due to inhibition of 11β-HSD1 is indicated by the lack of a direct effect of the extract to prevent cortisol-induced GR activation.

Furthermore, we assessed the effect of the inhibition of 11β-HSD1-dependent cortisol formation on the glucocorticoid-dependent expression of PEPCK (Fig. 5). Addition of 50 nM cortisone resulted in a 6-fold stimulation of PEPCK mRNA expression after a three hour incubation. Treatment of cells with increasing concentrations of coffee extract abolished glucocorticoid-induced PEPCK expression.

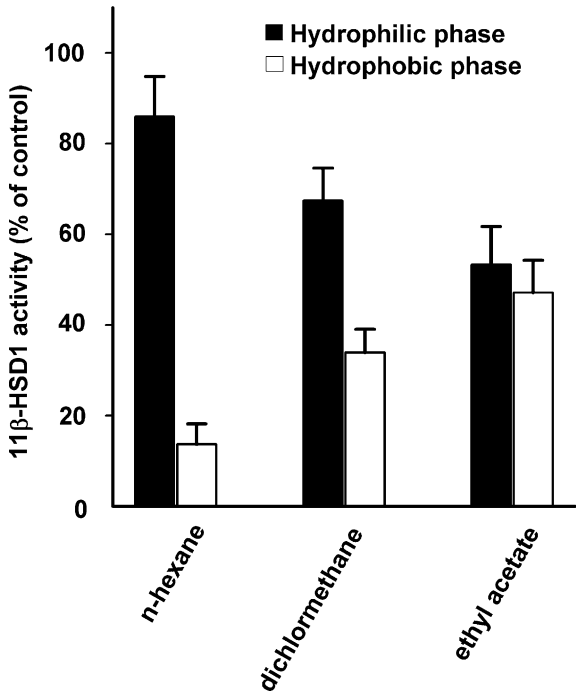

Fig. 3. Partition of the inhibitory compound in different solvent systems. The aqueous coffee extract was mixed thoroughly with an equal volume of *n*-hexane, dichloromethane or ethyl acetate. After separation of both phases, their inhibitory potential was determined by measuring 11β-HSD1 activity in cell lysates in the presence of 0.5% of the corresponding phase. The presence of 0.5% of any of the solvents tested did not affect the enzymatic reaction.

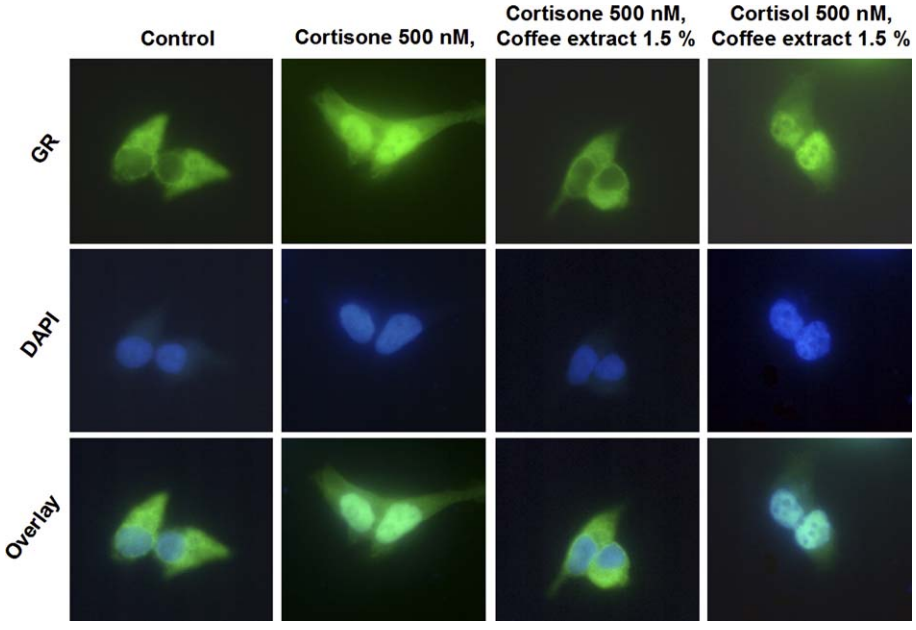

Fig. 4. Glucocorticoid receptor translocation assay. HEK-293 cells expressing GFP-GR and 11β-HSD1 were treated with vehicle (control panel), 500 nM cortisone, and with 1.5% of coffee extract and either 500 nM cortisone or cortisol, respectively, for 40 min. Cells were analyzed by fluorescence microscopy to detect GFP-GR (green fluorescence) and DAPI for nuclear staining (blue fluorescence).

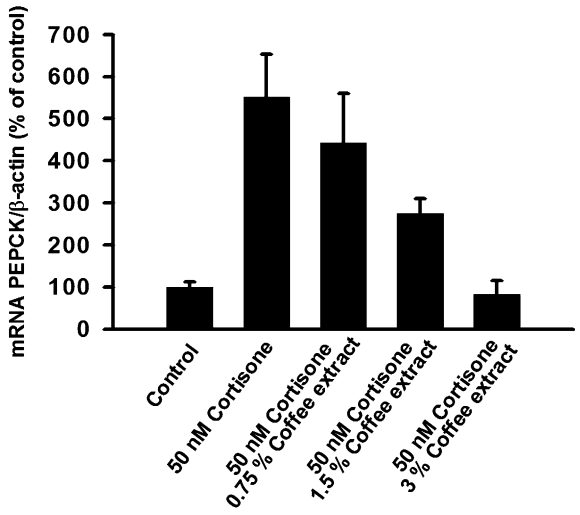

Fig. 5. Coffee extract prevents glucocorticoid-dependent stimulation of PEPCK mRNA expression by inhibition of 11β-HSD1. H4EII hepatoma cells stably expressing 11β-HSD1 were incubated with 50 nM cortisone in the presence or absence of various amounts of coffee extract for 3 h. The levels of mRNA were determined by real-time RT-PCR using TaqMan technology. Data represent mean ± S.D. from triplicates and are relative to the ratio of PEPCK mRNA to the β-actin control mRNA of untreated cells (control).

4. Discussion

A number of recent human population studies associated coffee consumption with a reduced risk for the development of type 2 diabetes [1], suggesting that coffee might be regarded as "functional food" for the prevention of metabolic disease [25]. However, little progress has been made so far in elucidating the mechanisms underlying the anti-diabetic effect of coffee drinking. It became clear that this effect is not related to caffeine itself, since consumption of decaffeinated coffee also exerted an anti-diabetic effect [26,27]. Moreover, although for a long time most of the biological effects of coffee beverage have been referred to as pure caffeine effects, a recent study indicated that acute caffeine ingestion impairs glucose tolerance while regular consumption of caffeinated or decaffeinated coffee beverage exerts a protective effect against type 2 diabetes [28].

Some biologically active ingredients of coffee that were suggested to contribute to its anti-diabetic actions are chlorogenic acid – through reduced glucose absorption and increased production of glucagon-like peptide 1 [29], lignans – through antioxidant and estrogenic activity [30], and trigonelline, which was shown to lower blood glucose levels by yet unknown mechanisms. However, none of these ingredients seems to be responsible for the anti-diabetic effects of regular coffee intake [2].

It is generally accepted that excessive glucocorticoid action plays an important role in the pathogenesis of obesity and type 2 diabetes. Many features of the metabolic syndrome are observed in patients with Cushing's syndrome due to excessive glucocorticoid production as a result of adrenal tumors or because of prolonged treatment with high pharmacologic doses [31]. In obese individuals, however, systemic glucocorticoid levels are not significantly elevated, but an increased local reactivation of glucocorticoids in adipose tissue and in skeletal muscle has been associated with the pathogenesis of the metabolic syndrome [32]. Importantly, recent studies demonstrated a significant improvement of glucose tolerance and insulin sensitivity in obese and diabetic animals treated with synthetic 11β-HSD1 inhibitors [13–15].

We previously reported the existence of natural compounds inhibiting 11β-HSD1 [17]. Flavanone and 2′-hydroxyflavanone, present in red and yellow fruits and vegetables, inhibited 11β-HSD1 oxoreductase activity in lysates and intact cells without inhibiting the related enzymes 11β-HSD2, 17β-HSD1 or 17β-HSD2. A recent in silico screening of a library of 114 000 natural compounds conducted by researchers at the Nestle Research Center yielded several flavanone derivatives, not specified further, that fitted into the substrate-binding pocket of 11β-HSD1 [33]. Coffee is an extraordinarily rich source of biologically active compounds, however, only very few compounds have been studied more extensively so far.

The present study suggests the presence of a thermo-stable substance with considerable polarity – a profile that suits a polyphenolic or flavonoid-like compound. Our results clearly demonstrate the presence of relevant amounts of compound(s) in coffee beverage that inhibit 11β-HSD1-dependent reactivation of glucocorticoids, thereby reducing the activation of the GR. Moreover, using a liver cell model, we provide evidence that coffee-induced inhibition of 11β-HSD1 decreases hepatic gluconeogenesis. Nevertheless, further experiments are required to identify the compound(s) responsible for the observed effects and to test their effects on glucose tolerance and insulin sensitivity in vivo.

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