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ORIGINAL ARTICLE

# Activation of the transcription factor Nrf2 in macrophages, Caco-2 cells and intact human gut tissue by Maillard reaction products and coffee

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**Abstract** In addition to direct antioxidative effects, Maillard reaction products (MRPs) could increase the antioxidative capacity of cells through the induction of cytoprotective enzymes. Since many of those enzymes are regulated by the transcription factor Nrf2, the effect of MRPs on nuclear translocation of Nrf2 in macrophages and Caco-2 cells was investigated. Stimulation of both cell types by MRPs showed a concentration-dependent significant increase in nuclear translocation of Nrf2 up to fivefold after short-term (2 h) and up to 50-fold after long-term treatment (24 h). In intact human gut tissue, nuclear translocation of Nrf2 was significantly twofold increased after short-term incubation. To study the activation mechanisms, macrophages and Caco-2 cells were stimulated with MRPs in the presence of catalase, which significantly suppressed Nrf2 activation. Thus, activation was related to extracellular $H_2O_2$ continuously formed from MRPs. Short-term incubation with coffee, a MRP-rich beverage, led to a trend towards Nrf2 activation in macrophages, but not in Caco-2 cells or intact human gut tissue. Long-term incubation with coffee (1–4 mg/mL) significantly increased nuclear Nrf2 up to 17-fold. Since raw coffee was inactive under the tested conditions, the effect was related to roasting products. Coffee-induced Nrf2 translocation was, however, only slightly reversed by catalase. Therefore, the Nrf2 activity of coffee can only partially be explained by MRP-induced, $H_2O_2$-dependent mechanisms. Thus, it can be concluded that MRPs may increase the antioxidative capacity inside the cell by inducing Nrf2-regulated signalling pathways not only in different cell types, but also in intact gut tissue.

**Keywords** Nrf2 · Maillard products · Coffee · Roasting products · Hydrogen peroxide · Intact human gut tissue

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## Introduction

Antioxidants are currently considered as one of the major classes of functional food components. In addition to natural antioxidants such as polyphenols or carotenoids, secondary antioxidants formed during food processing have attracted growing attention (Urquiaga and Leighton 2000; Rousseau et al. 1992; van Boekel et al. 2010). A diet rich in thermally processed food, for example, increased resistance of plasma and serum LDL against oxidative stress (Seiquer et al. 2008; Dittrich et al. 2009). In particular, Maillard reaction products (MRPs) and melanoidins, which are formed during food processing by the reaction of amino acids with sugars, show potent oxygen and radical scavenging activity (Dittrich et al. 2003; Samaras et al. 2005; Borelli et al. 2002). In addition to direct antioxidative effects, MRPs and melanoidins may also exert indirect antioxidative activity by inducing the expression of cytoprotective proteins in cells. Pre-incubation of HepG2 cells with melanoidins, for example, increased the

concentration of intracellular reduced glutathione (GSH) and protected cells efficiently against oxidative stress (Goya et al. 2007; Martin et al. 2009). GSH is synthesised enzymatically by γ-glutamylcysteine synthetase and glutathione synthetase; both enzymes are regulated by the nuclear factor-erythroid-2-related factor 2 (Nrf2). This redox-sensitive transcription factor is ubiquitously expressed in tissue with particularly high levels in the intestine, lung and kidney (McMahon et al. 2001; Moi et al. 1994). In non-activated cells, Nrf2 is bound to the repressor Kelch-like ECH-associated protein 1 (Keap1) causing ubiquitination and degradation of Nrf2. Since Keap1 itself is anchored to the actin cytoskeleton, the inactive complex is restricted to the cytoplasm. Upon activation, Nrf2 is released from Keap1, translocates into the nucleus and binds to the antioxidant response element (ARE)/electrophile response element (EpRE) of certain genes. Genes that possess the respective elements in their promoter regions are thus regulated by Nrf2. Besides γ-glutamylcysteine synthetase and glutathione synthetase, phase II target genes such as NAD(P)H: quinone oxidoreductase 1, catalase and thioredoxin are up-regulated by Nrf2 (Surh et al. 2008). The proteins encoded by those genes are responsible for cellular detoxification and maintain antioxidant capacities, which may counteract unbalanced redox homoeostasis. Thus, dietary activation of Nrf2 could reverse biochemical dysfunction resulting from diseases related to oxidative stress and/or hyperglycaemia (Xue et al. 2008).

Activation of Nrf2 in the gut appeared to have several beneficial effects. Inflammatory bowel diseases, namely Crohn's disease and ulcerative colitis, are characterised by an imbalance between reactive oxygen species (ROS) and mucosal antioxidant response (Kruidenier et al. 2003). Indeed, as shown in a mouse model, activators of Nrf2 such as prohibitin can improve colitis (Theiss et al. 2009). Enhanced intestinal Nrf2 signalling was further associated with a beneficial effect on gut barrier dysfunction (Jin et al. 2008), intestinal mucosal injury (Jin et al. 2009) and intestinal inflammation other than colitis (Jin et al. 2008). Moreover, there is growing evidence that Nrf2 is also a promising target for the prevention of colorectal cancer (Saw and Kong 2011; Pool-Zobel et al. 2005).

To date, defined phytochemicals such as sulphoraphane (broccoli), carnosol (rosemary) and curcumin (turmeric) as well as dietary plant extracts from broccoli, coffee and spices (turmeric, rosemary) have been identified as potent Nrf2 activators. They induce EpRE activity in liver HepG2 cells indicating a beneficial impact on the pathogenesis of oxidative stress associated disorders (Paur et al. 2008). Indeed, the consumption of cruciferous vegetables such as broccoli was shown to reduce the risk of cancer such as colon and prostate cancer in humans (Clarke et al. 2008).

The present study investigated the impact of MRPs on Nrf2 translocation in vitro in intestinal Caco-2 cells and macrophages, which are important intestinal cell types. Furthermore, Nrf2 activation was studied ex vivo in intact human gut tissue samples. The second part of this study focused on the involvement of ROS in Nrf2 activation. These effects were compared with investigations of coffee, a beverage rich in MRPs.

## Materials and methods

### Chemicals and cell culture

D-(-)-Ribose, L-lysine, catalase (from bovine liver), albumin, bovine serum albumin (BSA), lipopolysaccharide (LPS) (from *Escherichia coli* 055:B5), magnesium chloride, trypan blue, phenylmethylsulphonylfluoride (PMSF), dithiothreitol (DTT), Nonidet P-40 substitute (NP-40), and horseradish peroxidase (HRP)-conjugated anti-rabbit antibody (A6154) were purchased from Fluka-Sigma-Aldrich (Buchs, Switzerland). Rabbit polyclonal anti-Nrf2 antibody (AP06252 PU-N) was from Acris Antibody GmbH (Herford, Germany). Hydrogen peroxide ($H_2O_2$) (30%), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), and potassium chloride were supplied by Merck KgaA (Darmstadt, Germany). Roasted coffee beans and raw coffee beans (both 100% *Coffea arabica*) were obtained from a local retailer. Bio-Rad DC-Protein Assay Reagents Package was obtained from BioRad Laboratories GmbH (Munich, Germany), protease inhibitor tablet complete (PIS) from Roche Diagnostics GmbH (Mannheim, Germany) and enhanced chemiluminescence reagent (ECL) Western blotting detection reagents from Amersham Biosciences (Munich, Germany). Cell lines were purchased from ATCC (Wesel, Germany). HAM's F12 (1,176 g/L $NaHCO_3$, stable glutamine), trypsin (0.25%)/EDTA (0.02%), foetal calf serum (FCS), penicillin (10,000 U/mL)/streptomycin (10,000 µg/mL), phosphate buffered saline (PBS) Dulbecco and nonessential amino acids were supplied by Biochrom AG (Berlin, Germany) and minimum essential media (MEM) by Invitrogen (Darmstadt, Germany). Endo-paractol was obtained from Temmler Pharma GmbH & CO. KG (Marburg, Germany) and dimethylsulphoxide (DMSO) from Acros Organics (Nidderau, Germany). Nitrocellulose transfer membrane (pore size: 0.45 µm) was supplied by Schleicher & Schuell GmbH (Dassel, Germany).

### Instruments

Universal grinder (Type A 10) and Ultra Turrax homogeniser were obtained from IKA®-Werke GmbH & CO. KG (Staufen, Germany). Mucosa oxygenator was purchased

from Intestino-Diagnostics (Erlangen, Germany) and VersaDoc™ Imaging System from BioRad Laboratories GmbH (Munich, Germany). The counting chamber (Neubauer, improved) was from Carl Roth GmbH + Co. KG (Karlsruhe, Germany).

### Preparation of Maillard reaction mixtures and a heated ribose solution

A heated Maillard reaction mixture ($MRM_h$) was prepared from an equimolar solution of D-(-)-ribose and L-lysine (each 0.5 M), dissolved in PBS and adjusted to pH 9.4 with HCl (9.7 M). Filtered aliquots of 20 mL were heated in capped screw neck bottles for 30 min at 120°C in a drying oven. After cooling in an ice bath, the pH of $MRM_h$ was adjusted from pH 8.6 to 8.0 with HCl (9.7 M), and $MRM_h$ was filter-sterilised (0.22 µm). Aliquots were stored at −20°C. An unheated Maillard reaction mixture ($MRM_u$) was prepared simultaneously, but without the heating step. For the heated ribose solution ($rib_h$), the ribose was heated alone without addition of lysine as described for $MRM_h$. Since the concentration of the reaction products in the final mixtures is not known, the given concentration reflects the initial concentration of the reactants. Assuming that about 30% of ribose and lysine were converted into Maillard products under the applied conditions, the content of MRP of 50 mM $MRM_h$ is equivalent to the concentration of melanoidins in a coffee brew (0.45 g melanoidins per 100 mL) (Fogliano and Morales 2011).

### Preparation of roasted and raw coffee extract

Using a standardised preparation method, 15 g of roasted coffee beans was ground for 30 s in a universal grinder. Raw coffee beans were subjected first to three freeze/thaw cycles in liquid nitrogen followed by grinding for 60 s. A quantity of 3.75 g ground coffee powder was filter-brewed with hot tap water (Ø 85°C) to a total volume of 48 mL (coffee temperature: Ø 60°C). The coffee was cooled on ice for 1 h. Thereafter, the pH was adjusted with NaOH (2 N) from pH 5.6 (Ø) to pH 7.4. The volume of the coffee brew was filled up to 50 mL with hot water and filter-sterilised (0.22 µm). In all experiments, the coffee extract was freshly prepared and used exactly 1 h after brewing. The coffee concentration used in the experiments refers to the dry weight, which was analysed in at least three independent preparations per coffee lot.

### Cell culture

Cells of various passages were cultured at 37°C in a humidified atmosphere with 5% $CO_2$. Each cell culture medium was supplemented with penicillin (100 U/mL) and streptomycin (100 µg/mL). Rat macrophages (NR8383) were maintained in HAM's F12 medium supplemented with 15% (v/v) heat-inactivated FCS. Human epithelial colorectal adenocarcinoma cells (Caco-2) were grown in MEM supplemented with 20% (v/v) FCS and nonessential amino acid solution (0.1 mM). NR8383 macrophages were harvested by scratching; Caco-2 cells were detached by trypsin (0.25%)/EDTA (0.02%).

### Tissue culture of intact human gut tissue

The study was approved by the ethics committee of the University Medical Centre in Erlangen (Germany). Patients gave their informed consent to this local study. Mucosal gut specimens (3–15 mg) were taken from patients during gastrointestinal endoscopies because of various gastrointestinal complaints and to check up for manifestations of inflammation, neoplasm or food allergy. Samples were taken from two defined locations in the lower gastrointestinal tract, the terminal ileum of the small intestine and the ascending colon of the large intestine (Ø 6–7 biopsies per location per patient). In all patients, macroscopic as well as microscopic investigation of the biopsies did not show any chronic inflammatory gut diseases such as Crohn's disease. The human gut tissue samples were kept in PBS transport media at 37°C and bubbled constantly with room air providing an adequate oxygen supply at a $pO_2$ of 85–95 mm Hg (mucosa oxygenation) to avoid ischaemia (Raithel et al. 2006). The wet weight was determined prior to stimulation.

### Analysis of Nrf2 translocation in cell lines—in vitro stimulation and nuclear protein extraction

Cells were stimulated with MRMs, ribose solution, coffee extracts (roasted and raw) and $H_2O_2$. In some experiments, cells were co-treated with catalase (150 U/mL) or heat-inactivated (5 min; 95°C) catalase (150 U/mL), which was added to the cells 10 min prior to stimulation. As negative control, cells were incubated with water/PBS instead of the stimulant. More specifically, NR8383 macrophages ($3 \times 10^6$ cells) were grown for 4 days followed by stimulation in serum-free media for 2 h in short-term experiments and up to 24 h in the long-term experiments. To change the incubation medium, floating cells were collected by centrifugation (1,500 rpm, 2 min) and both floating and adherent cells were washed separately with serum-free media and unified thereafter. Caco-2 cells ($1 \times 10^6$ cells) were grown for 5 d. Prior to stimulation, the medium was removed and adherent cells were washed with PBS and thereafter stimulated for 2 h in PBS.

The cellular nuclear extracts were prepared on ice directly after stimulation unless stated otherwise, according

to Andrews and Faller (1991) with slight modifications. Briefly, adherent cells were detached by scraping. Both floating cells (macrophages) and adherent cells were collected by centrifugation (1,500 rpm, 4 min, 4°C). Floating and adherent cells were combined and washed three times with ice-cold PBS. Next, the cell pellet was re-suspended in 1 mL of ice-cold hypotonic buffer $A_{cell}$, which was freshly supplemented with PIS (1%), PMSF (2 mM) and DTT (0.5 mM). After incubation for 15 min on ice, 65 µL of 10% NP-40 was added and cells were mechanically lysed by vortexing for 15 s. Cell nuclei were collected by centrifugation and washed with buffer $A_{cell}$ to completely remove cytoplasmic proteins. Finally, nuclear proteins were extracted with 52 µL of ice-cold high salt extraction buffer B, which was freshly supplemented with PIS (1%), PMSF (2 mM) and DTT (0.5 mM). The suspension was vortexed every 20 min. After 1 h, the supernatant containing the nuclear proteins was collected by centrifugation. Protein concentration of the nuclear extract was measured directly according to the DC-Protein protocol using the manufacturer's recommendations (Bio-Rad). BSA, which was dissolved in supplemented buffer B, was used as standard. Nuclear cell extracts were stored at −80°C until use for Western blotting.

### Analysis of Nrf2 translocation in intact human gut tissue—ex vivo stimulation and nuclear protein extraction

Human gut tissue samples were incubated and stimulated using a mucosa oxygenation method (Raithel et al. 2006). Briefly, two mucosal gut specimens (terminal ileum/ascending colon) were exposed to $MRM_h$ or roasted coffee extract for 2 h in a supplemented incubation medium (PBS, 3% albumin, 2.4% HEPES buffer and 0.0025% endoparactol as defoaming agent). The negative control specimens were exposed to PBS or water. In some experiments, catalase (150 U/mL) was added to the human gut tissue samples 10 min prior to stimulation. During stimulation at 37°C, human gut tissue samples were continuously oxygenated (mucosa oxygenation) to prevent ischaemia and to support the diffusion of the medium dissolved stimuli into the tissue (Raithel et al. 2006).

After stimulation, the nuclear proteins were extracted according to a modified method of Thiele et al. (1999). In detail, human gut tissue samples were washed in a 50-mL tube with ice-cold PBS for 2 min while shaking at 400 rpm. To lyse the cells, the human gut tissue samples were re-suspended in ice-cold hypotonic buffer $A_{tissue}$ (10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl) which was freshly supplemented with DTT (0.5 mM), PMSF (0.2 mM), PIS (1%) and NP-40 (0.56%), followed by three freeze–thaw cycles in liquid nitrogen. Afterwards, the specimens were mechanically disrupted with an Ultra Turrax homogeniser for 1 min on ice and allowed to stand for 30 min before the cell nuclei were collected by centrifugation. The subsequent nuclear protein extraction was carried out as described above using 25 µL instead of 52 µL of the high salt extraction buffer B including supplements.

### Nrf2 analysis by Western blotting

After in vitro or ex vivo stimulation, the nuclear Nrf2 content was determined in the isolated nuclear protein extracts. The nuclear Nrf2 was detected immunochemically via Western blotting according to a modified method described by Muscat et al. (2007) for Western blot analysis of NF-$\kappa$B. Briefly, nuclear proteins were denatured at 95°C for 7 min. For normalisation, an aliquot of the nuclear extract was applied to reach a constant protein load (10 µg for cell experiments; 5 µg for tissue experiments) per lane. In some experiments, equal protein load was controlled by analysing in parallel nucleoplasmic $\beta$-actin by Western blotting (RSD $< 16\%$). The proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 10% SDS-polyacrylamide gel) and transferred to a nitrocellulose membrane by Western blotting. After blocking, the membranes were incubated with the primary rabbit anti-Nrf2 antibody (NR8383 macrophages: diluted 1:2,000 in blocking buffer; Caco-2 cells and human gut tissue: diluted 1:1,000 in blocking buffer) overnight at 4°C. Thereafter, the membranes were exposed to the HRP-conjugated second anti-rabbit antibody (1:2,000 diluted in blocking buffer) for 1 h at room temperature. After a washing step, the membranes were incubated with an ECL reagent to visualise the protein bands. The data was analysed densitometrically using a Versa-Doc™ Imaging System. The apparent size of Nrf2 was ~43 kDa. Nrf2 activation in stimulated cells was expressed as n-fold increase of nuclear Nrf2 amounts compared to untreated control cells exposed only to the solvent PBS/water.

### MTT assay

The viability of NR8383 macrophages stimulated with $MRM_h$ was investigated according to a modified method of Mosmann (1983). The method is based on the ability of active cells to metabolise the MTT into dark blue insoluble formazan. Specifically, NR8383 macrophages ($1 \times 10^6$ cells) were allowed to settle overnight at 37°C in a 12-well plate. Next, cells were stimulated with $MRM_h$ (10–100 mM) for 24 h at 37°C in supplement-free cell culture medium. In the case of catalase co-treatment, catalase (150 U/mL) was added to the cells 10 min prior to

stimulation. Negative control cells were incubated with PBS without stimulant. As positive control, cells were treated with 10% DMSO instead. After stimulation, cells were incubated with MTT (1 mg/mL in PBS) for 3 h at 37°C. The developing dark blue formazan crystals were dissolved in isopropanol/1 N HCl (25:1) solution under agitation. Finally, the absorbance was read after 10 min at 595 nm. The absorbance of control cells was considered 100% viable cells. The cell viability of stimulated cells was expressed as percent of control. For media replacement during the assay, the cell suspension was centrifuged at 1,500 rpm for 2 min at room temperature.

### Determination of cell viability by the trypan blue dye exclusion test

The cell viability of NR8383 macrophages exposed to coffee extract was further determined by the trypan blue dye exclusion test. Depending on cellular membrane integrity, which is one main characteristic of vital cells, the anionic diazo dye trypan blue is absorbed into the cell and appears blue. Briefly, NR8383 cells ($1 \times 10^6$ cells) were grown for 4d in the incubator before being exposed to coffee extract (1–4 mg/mL) in PBS for up to 6 h. After stimulation, cells were treated with trypan blue dye solution (5 mg/mL PBS) and viable as well as dead cells were counted under the microscope via a counting chamber. Cell viability was calculated as the ratio between living cells to total cell number and expressed as a percentage of the values obtained for control cells, which was set as 100%.

### Statistical analysis

Statistical significance of the data was calculated using Graphpad prism 5. The results were expressed as the mean ± SD from n independent experiments for $n > 2$ and mean ± range for $n = 2$. The significance of differences was calculated by a Student's $t$ test and levels of confidence were defined as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

## Results

### Induction of nuclear translocation of Nrf2 by MRPs in different cell types and intact human gut tissue

The purpose of the study was to investigate the activity and mechanisms of MRPs to induce nuclear translocation of Nrf2 in different cell lines and in intact human gut tissue. For this purpose, MRPs were generated by heating ribose and lysine for 30 min at 120°C. Similar mixtures have been previously applied to determine physiological effects of MRPs (Muscat et al. 2007; Hegele et al. 2009). In order to investigate whether Maillard reaction products activate Nrf2 in vitro, NR8383 macrophages were incubated for 2 h (short-term incubation) with a heated MRM. The activation of Nrf2 was defined as the translocation of cytoplasmic Nrf2 into the nucleus. Nuclear Nrf2 was detected by Western blotting. $MRM_h$ significantly induced Nrf2 activation in macrophages in a concentration-dependent manner reaching a fivefold up-regulation at 100 mM $MRM_h$ (Fig. 1).

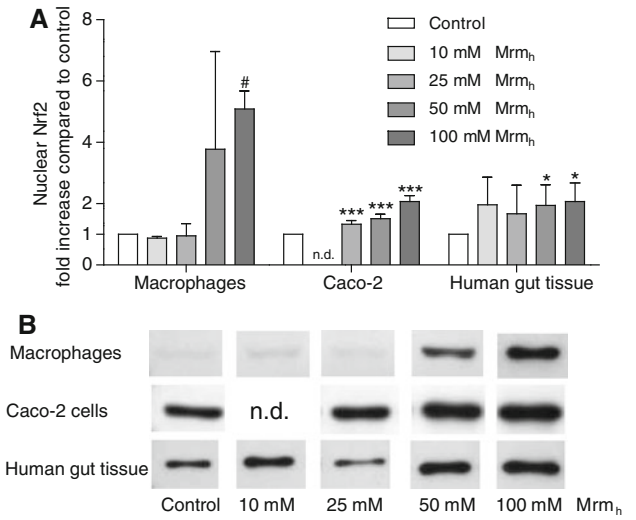


**Fig. 1** Nrf2 activation by $MRM_h$ in NR8383 macrophages, Caco-2 cells and intact human gut tissue samples during short-term incubation. Cells and tissue were incubated with $MRM_h$ (10–100 mM) for 2 h. **a** The intensity of nuclear Nrf2 is expressed as n-fold increase compared to PBS-treated control cells. Data are mean ± SD for $n > 2$; mean ± range for $n = 2$ (#) (macrophages: $n = 2$–5; Caco-2: $n \geq 5$; gut tissue: $n \geq 5$). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. (n.d. = not determined), **b** Representative Western blot of Nrf2 in NR8383 macrophages, Caco-2 cells and human gut tissue

For long-term incubation experiments, macrophages were incubated with different concentrations of $MRM_h$ for up to 24 h (Fig. 2a). The nuclear Nrf2 level increased consistently in a time- and concentration-dependent manner. No influence on Nrf2 translocation was measured over an incubation period of 24 h in the case of 10 mM $MRM_h$. However, exposure of macrophages to ≥25 mM $MRM_h$ significantly enhanced Nrf2 translocation, reaching 50-fold higher Nrf2 levels after stimulation for 24 h with 50 mM $MRM_h$ compared to PBS-treated control cells. It was confirmed by the MTT assay that incubation with ≤25 mM $MRM_h$ for up to 24 h did not exert cytotoxic effects. Incubation with 50 mM $Mrm_h$ for 24 h resulted in a cell viability of 60 ± 21%. As a positive control, 50 µM *tert*-butylhydroquinone was applied under the same conditions, leading to a 1.9-fold increased nuclear Nrf2 content compared to the control. *tert*-Butylhydroquinone is an established inducer of nuclear Nrf2 translocation (Cheung et al. 2011).

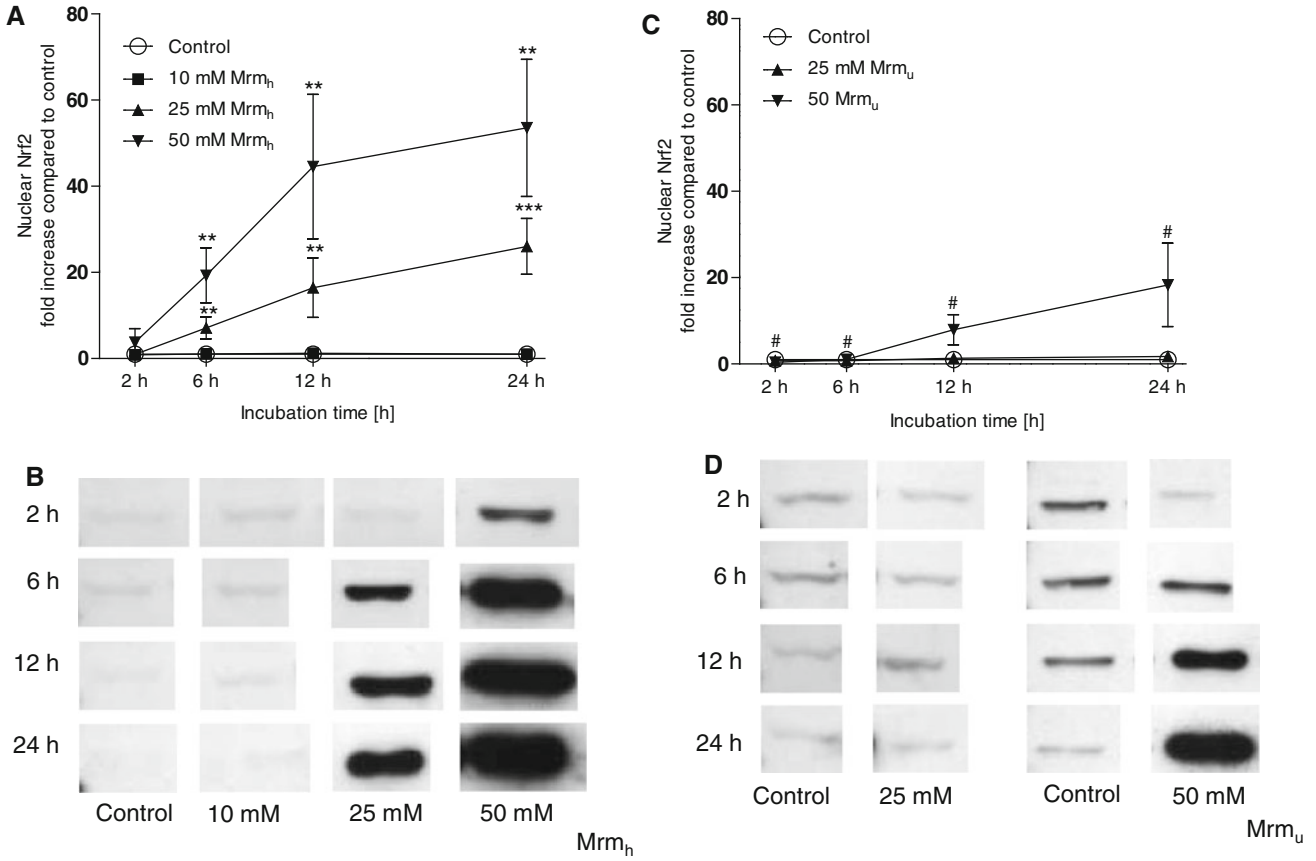


**Fig. 2** Nrf2 activation by **a** a heated Maillard reaction mixture ($MRM_h$) and **c** an unheated Maillard reaction mixture ($MRM_u$) in NR8383 macrophages during long-term incubation. Cells were stimulated with $MRM_h$ (10–50 mM) or $MRM_u$ (25–100 mM) for 2–24 h. The intensity of Nrf2 is expressed as n-fold increase compared to PBS-treated control cells. Data are mean ± SD for $n > 2$; mean ± range for $n = 2$ (#) ($MRM_h$: $n = 3$–5; $MRM_u$: $n = 2$–3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (**b/d**) Representative Western blot of Nrf2 in NR8383 macrophages incubated with **b** $MRM_h$ and **d** $MRM_u$

To study how MRPs induce the nuclear translocation of Nrf2, it was investigated whether long-term activation of Nrf2 depends on the continuous presence of the MRP stimulant. Thus, macrophages were incubated for 2 h with 25 mM $MRM_h$. On removal of the $MRM_h$ after 2 h, the cells were incubated for another 22 h and nuclear Nrf2 concentration was determined after a total incubation period of 24 h. Nuclear Nrf2 levels continued to increase even in the absence of the stimulus to about eightfold higher levels compared to short-term incubation alone (Fig. 3). Cell viability was not reduced after incubation with $MRM_h$ (25 mM) for 24 h.

To verify whether nuclear translocation of Nrf2 was indeed induced by MRPs and not by a high concentration of amino acids and sugars, an unheated mixture of ribose and lysine ($MRM_u$) was tested in the same way as $MRM_h$. Incubation with a 25 mM solution of ribose/lysine ($MRM_u$) for up to 24 h and with a 50 mM solution for up to 6 h did not lead to a detectable increase in nuclear Nrf2 signal intensity. Only after exposure to 50 mM $MRM_u$ for 12 and 24 h, the nuclear Nrf2 concentration was increased 8- and 18-fold, respectively, compared to control, which was, however, much lower than the induction by the heated mixture ($MRM_h$) (Fig. 2c).

The role of caramelisation products in Nrf2 activation was tested. As caramelisation products are formed by sugar degradation in the absence of amines from heat-treated ribose alone, macrophages were exposed to a heated ribose solution. Even when concentrations up to 100 mM were applied, no induction of nuclear translocation of Nrf2 could be detected (data not shown). Thus, it can be concluded that the observed effect was indeed induced by MRPs and not by caramelisation products.

To investigate if MRP-induced nuclear translocation of Nrf2 is unique in macrophages, intestinal Caco-2 cells were also short-term stimulated with $MRM_h$ containing MRPs. In all tested concentrations, $MRM_h$ led to a significant increase of nuclear Nrf2 concentration (Fig. 1). However, the induction was generally lower than the effect observed in macrophages (twofold vs. fivefold compared to control).

Macrophages and epithelial Caco-2 cells represent relevant cell types of the intestine. However, it is possible that

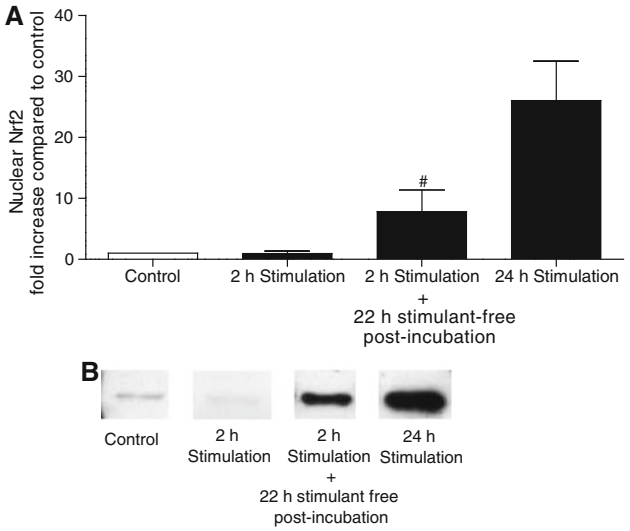


**Fig. 3** Nrf2 activation by $MRM_h$ in NR8383 macrophages. Cells were stimulated with the heated MRM (25 mM) for 2–24 h. Nuclear Nrf2 levels were analysed directly after stimulation or after a following post-incubation at 37°C without stimulant. **a** The intensity of Nrf2 is expressed as n-fold increase compared to PBS-treated control cells. Data are mean ± SD for $n > 2$; mean ± range for $n = 2$ (#) ($n = 2$–5). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, **b** Representative Western blot of Nrf2 in NR8383 macrophages

these cell lines show a different response than primary cells. Additionally, the interaction of different cell types in an intact tissue may have strong influence on cellular reactions. Therefore, ex vivo incubation of intact human gut tissue was introduced to assess these factors (Raithel et al. 2003). In the present study, intact human mucosal gut tissue samples obtained from the lower gastrointestinal tract of six patients were used. Depending on the number of biopsies available from each patient, each experiment was performed in five or six replicates. In all patients, macroscopic and microscopic investigation of the biopsies did not show any acute or chronic inflammatory gut diseases. As cell viability decreased by approximately 50% after mucosa oxygenation ex vivo for 6 h, the tissue samples were only subjected to short-term incubation for 2 h (Sauer et al. 2011). After an incubation time of 2 h, cell viability was not reduced. The tissue samples were stimulated ex vivo with different concentrations of $MRM_h$ and the nuclear translocation of Nrf2 was analysed. In the gut tissue samples, the amount of nuclear Nrf2 increased significantly after treatment with 50 mM and 100 mM MRPs (1.9-fold and 2.1-fold increase, respectively, compared to control) (Fig. 1).

## Role of extracellular $H_2O_2$ in the MRP-induced activation of Nrf2

To gain some insight into the mechanisms of how MRPs induce nuclear translocation of Nrf2, it was investigated if the observed Nrf2 activation was mediated by ROS, generated by MRPs (Mueller et al. 2011; Hegele et al. 2009). For this purpose, macrophages were simultaneously incubated with $MRM_h$ and catalase or heat-inactivated catalase. Nrf2 translocation was subsequently analysed at several time points. As illustrated in Fig. 4a, co-treatment with catalase, but not with heat-inactivated catalase, significantly blocked the $MRM_h$-induced translocation of Nrf2 in NR8383 macrophages. Cell viability was not reduced after incubation with $MRM_h$ (25 mM) ± catalase for 24 h. In the presence of NR8383 macrophages, the applied $MRM_h$ produced between 100 and 450 µM extracellular $H_2O_2$, depending on the $MRM_h$ concentration and incubation time. Co-incubation with catalase completely removed extracellular $H_2O_2$ (see (Sauer et al. 2011) for details).

Similarly, supplementation of catalase significantly reduced $MRM_h$-induced Nrf2 activation in Caco-2 cells (Fig. 4c) and also showed an inhibitory trend in human gut tissue samples ex vivo (Fig. 4e). Extracellular $H_2O_2$ ranged between 50 and 100 µM, when human gut tissue was incubated with $MRM_h$ depending on its concentration and incubation time (Sauer et al. 2011). The data indicate a similar mechanism of Nrf2 activation involving $H_2O_2$ across the tested cell types and tissue. The smaller effect of unheated MRM ($Mrm_u$) on Nrf2 translocation after long-term incubation was not blocked by co-treatment with catalase (data not shown) indicating that other mechanisms were involved.

Since extracellular $H_2O_2$ played a crucial role in the $MRM_h$-induced Nrf2 activation, the impact of a pure $H_2O_2$ solution on Nrf2 activation was likewise investigated in macrophages. The nuclear Nrf2 level of macrophages did not significantly respond to either 100 or 500 µM $H_2O_2$ during incubation for 24 h (data not shown), suggesting that permanent generation of $H_2O_2$ from MRPs rather than the addition of a single bolus of $H_2O_2$ was important to induce nuclear Nrf2 translocation.

## Induction of nuclear translocation of Nrf2 by coffee in different cell types and intact human gut tissue

Coffee is a food product rich in MRPs, which are formed during roasting. Furthermore, Nrf2 induction by coffee has been observed in AREc32 cells, in EpRE-LUC transfected HepG2 cells and in human colon carcinoma cells (Boettler et al. 2010; Paur et al. 2010; Cavin et al. 2008). Therefore, this study investigated the effect of coffee on nuclear translocation of Nrf2 in different cell types present in intestinal tissue as well as on intact human gut tissue and compared the findings to the effect of MRPs.

Short-term stimulation with coffee extract for 2 h showed a non-significant trend in activating Nrf2 in NR8383 macrophages, but not in Caco-2 cells and human

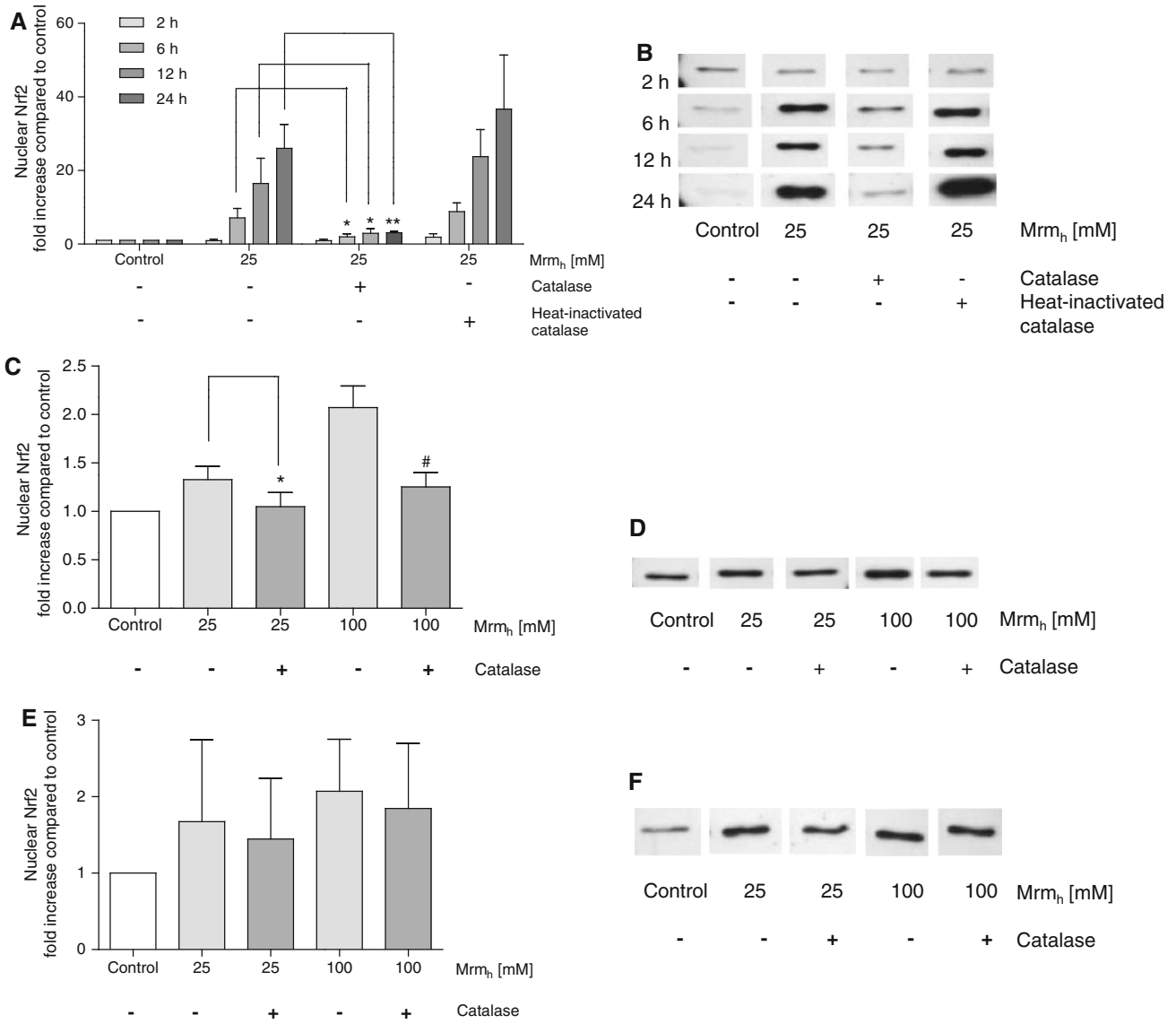


**Fig. 4** Nrf2 regulation by $MRM_h$ ± (heat-inactivated) catalase in **a** NR8383 macrophages, **c** Caco-2 cells and **e** human gut tissue. Cells and tissue samples were stimulated with $MRM_h$ (25 mM/100 mM) for 2–24 h. In some experiments, cells were co-treated with catalase (150 U/mL) or heat-inactivated catalase (150 U/mL). The intensity of Nrf2 is expressed as n-fold increase compared to PBS-treated control cells. Data are mean ± SD for $n > 2$; mean ± range for $n = 2$ (#) (NR8383 macrophages $n = 3$–5; Caco-2: $n = 2$–6; human gut tissue: $n = 3$–5). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Representative Western blot of Nrf2 in **b** NR8383 macrophages, **d** Caco-2 cells and **f** human gut tissue

gut tissue samples (Fig. 5). The maximal concentration of the tested coffee extracts (4 mg/mL) corresponds to a ~1:3 diluted (German) coffee beverage.

Additionally, Nrf2 translocation was monitored during prolonged stimulation of macrophages with coffee extract for up to 24 h. Indeed, coffee extract significantly activated Nrf2 in macrophages during prolonged stimulation (Fig. 6). The activation of Nrf2 increased gradually with sample concentration and incubation time. Whereas 1 mg/mL of coffee extract slightly elevated nuclear Nrf2 amounts twofold compared to water-treated control cells after incubation for 24 h, 4 mg/mL of coffee extract up-regulated nuclear Nrf2 levels nearly 20-fold. On the contrary, stimulation with raw coffee extract (4 mg/mL), which does not contain roasting products, did provoke only a minor Nrf2 activation in macrophages. The trypan blue dye exclusion test determined a viability exceeding 90% for all cells incubated for up to 6 h with coffee extracts (1–4 mg/mL).

The effect of catalase co-treatment on nuclear Nrf2 translocation in macrophages was monitored during stimulation with coffee extract. There was a slight trend towards reduced nuclear Nrf2 levels in the presence of catalase, which was, however, not significant (Fig. 7).

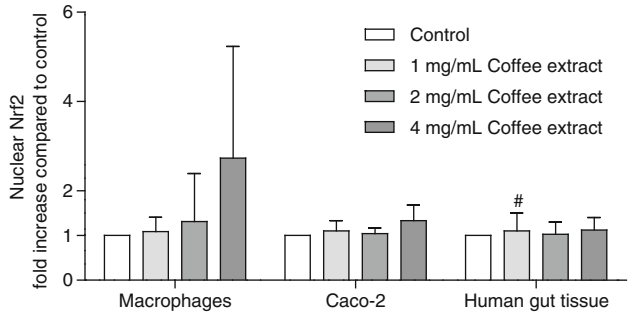


**Fig. 5** Nrf2 activation by short-term exposure of macrophages, Caco-2 cells and intact human gut tissue to coffee extract. Cells and tissue samples were incubated with coffee extract (1–4 mg/mL) for 2 h. The intensity of nuclear Nrf2 is expressed as n-fold increase compared to water-treated control cells. Data are mean ± SD for $n > 2$; mean ± range for $n = 2$ (#) (NR8383 macrophages: $n = 4$–8; Caco-2: $n = 3$; human gut tissue: $n = 2$–5)

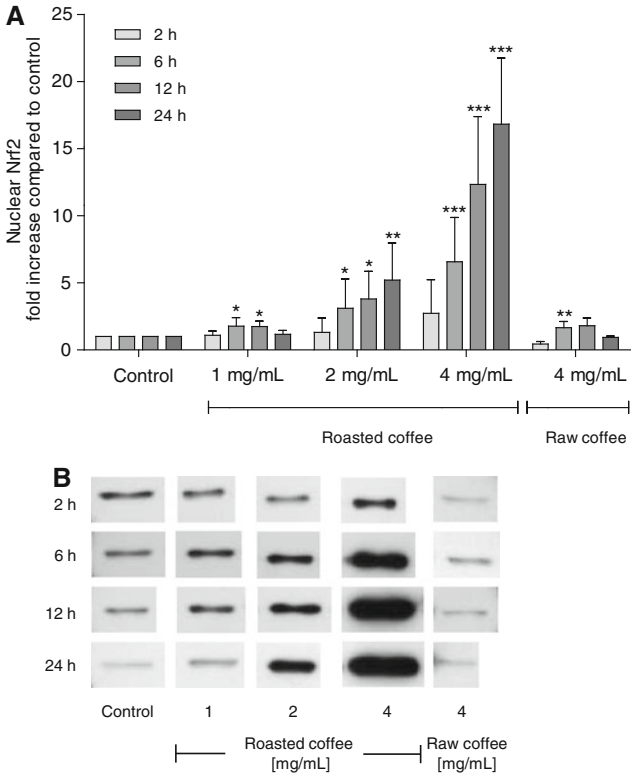


**Fig. 6** Nrf2 activation by roasted and raw coffee extract in NR8383 macrophages. Cells were stimulated with coffee extract (1–4 mg/mL) or raw coffee extract (4 mg/mL) for 2–24 h. **a** The intensity of Nrf2 is expressed as n-fold increase compared to water-treated control cells. Data are mean ± SD ($n = 3$–8). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ **b** Representative Western blot of Nrf2 in NR8383 macrophages

$H_2O_2$ concentration in the incubation solutions of macrophages with coffee ranged between 50 and 230 µM. No $H_2O_2$ could be detected after co-incubation with catalase (Sauer et al. 2011).

## Discussion

The goal of the present study was to investigate the influence of MRPs on the nuclear translocation of the redox-sensitive Nrf2 in different cell types present in intestinal tissue as well as in intact human gut tissue. Nrf2 is an inducible transcription factor, which is ubiquitously expressed in tissue (Moi et al. 1994). Activation of Nrf2 triggers a pathway regulating the expression of detoxifying and antioxidative active proteins such as glutathione S-transferase, heme oxygenase 1 and NAD(P)H: quinone oxidoreductase 1 (Surh et al. 2008; Itoh et al. 1997).

Activation of Nrf2 is classified as a therapeutic target in various diseases such as Parkinson's disease (Cuadrado et al. 2009), cardiovascular diseases (Li et al. 2009) and type 2 diabetes mellitus (Cheng et al. 2011). Moreover, the intake of dietary Nrf2 activators is of crucial relevance in intestinal dysfunction. Induction of Nrf2 is considered to reduce the risk of colorectal cancer (Saw and Kong 2011; Pool-Zobel et al. 2005) and has a beneficial impact on gut barrier dysfunction (Jin et al. 2008), intestinal mucosal injury (Jin et al. 2009) and intestinal inflammation (Jin et al. 2008) such as colitis (Khor et al. 2006).

During short-term stimulation (2 h), a heated MRM significantly activated Nrf2 in Caco-2 cells and particularly in macrophages in a concentration-dependent manner. Even intact human gut mucosal samples showed a significant nuclear translocation of Nrf2 within 2 h, indicating the biological relevance of this finding. Interestingly, short-term incubation of macrophages with $MRM_h$ followed by a stimulant-free post-incubation further enhanced Nrf2 activation compared to short-term incubation alone, suggesting that $MRM_h$ exhibits not only direct effects on different cell types, but possibly also long-lasting trophic and protective effects on intestinal tissue. To date, two Nrf2 activation mechanisms are proposed for the inhibition of Keap1-dependent Nrf2 degradation and subsequent translocation into the nucleus. Firstly, ROS and electrophiles, such as $H_2O_2$ and sulphoraphane, might stabilise cytoplasmic Nrf2 by modifying specific cysteine thiols of Keap1. Secondly, phosphorylation of serine and threonine residues of Nrf2 via protein kinases such as protein kinase C (PKC) and c-Jun N-terminal kinases (JNK) is also suggested to cause Nrf2 release from Keap1. Continuing activation of Nrf2 after removal of the stimulant points to Nrf2 activation via a signal transduction chain instead of direct interaction with the Nrf2-Keap1 complex (Surh et al. 2008). Alternatively, it cannot be completely ruled out that irreversible Keap1 cysteine modification is persistent over an extended period of 24 h and thus causes further up-regulation in the absence of the stimulant.

During long-term incubation of macrophages with $MRM_h$, the Nrf2 response increased over time up to 24 h.

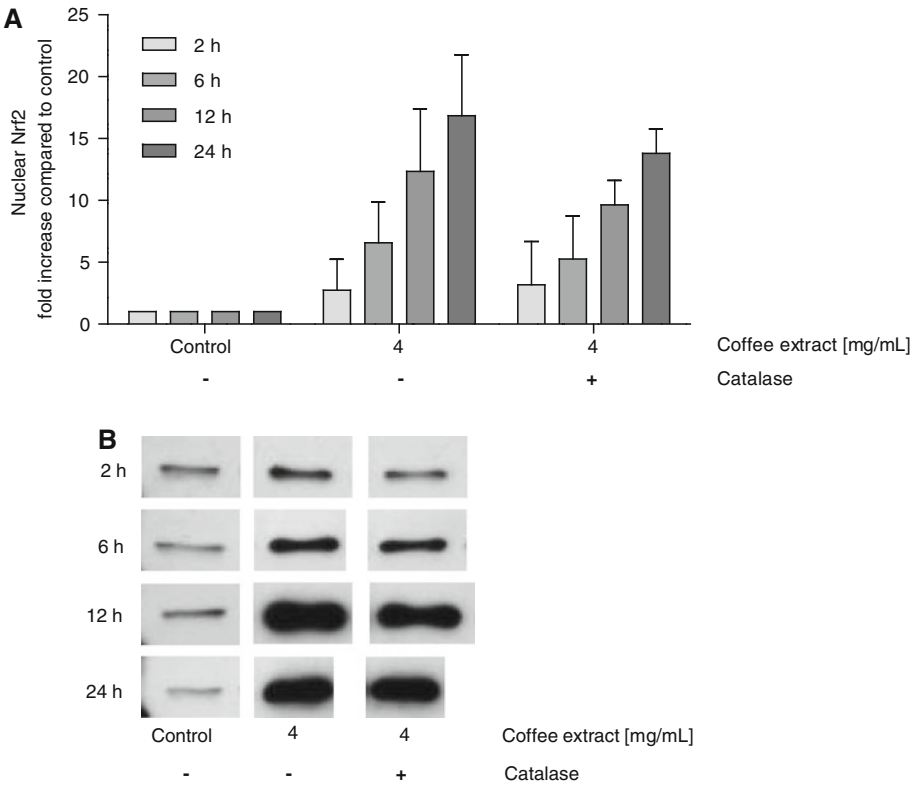


**Fig. 7** Nrf2 regulation by coffee extract ± catalase in NR8383 macrophages. Cells were stimulated with coffee extract (4 mg/mL) for 2–24 h. In some experiments, cells were co-treated with catalase (150 U/mL). **a** The intensity of Nrf2 is expressed as n-fold increase compared to water-treated control cells. Data are mean ± SD ($n = 5$–6) **b** Representative Western blot of Nrf2 in NR8383 macrophages

Very recently it was shown that a heated glucose-BSA solution (AGE-BSA) increased nuclear Nrf2 amounts in bovine aortic endothelial cells. Moreover, an enhanced protein expression of Nrf2-regulated proteins such as heme oxygenase 1 and NAD(P)H: quinone oxidoreductase 1 was detected (He et al. 2011). In vitro screening raised the question of how Nrf2 responded in human intestinal tissue consisting of a wide range of cell types. The human intestine represents a primary target for food-induced inter- and intracellular reactions independent of the resorption rate and systemic metabolism. The mucosa layer of the gut consists of a variety of cell types including immune cells such as macrophages, which were found to show the highest Nrf2 response. Therefore, Nrf2 activation by MRPs was studied ex vivo in the mucosa of the gut. Indeed, $MRM_h$ significantly activated Nrf2 not only in various cell lines, but also in human gut tissue ex vivo. It can be assumed that MRP-induced Nrf2 activation contributes to up-regulation of NAD(P)H: quinone oxidoreductase, thioredoxin and γ-glutamylcysteine synthetase caused by coffee roasting products (Paur et al. 2010).

$MRM_h$-induced Nrf2 activation was reduced or fully suppressed in macrophages and Caco-2 cells after co-treatment with catalase. This observation indicates that extracellular $H_2O_2$ is involved in $MRM_h$-induced Nrf2 activation. Nrf2 activation by $H_2O_2$ has been previously reported in rat pulmonary microvascular endothelial cells (Ning et al. 2010). It is well known that MRPs generate $H_2O_2$ in various cell-free systems as well as in the presence of cells (Mueller et al. 2011; Hegele et al. 2009). Therefore, it can be assumed that $H_2O_2$ formation is not an artificial effect of cell culture in vitro, but occurs also in various settings such as food preparation or in the gastrointestinal tract. Also, it was previously shown that MRPs trigger nuclear NF-κB translocation via a $H_2O_2$-mediated mechanism (Muscat et al. 2007). Both NF-κB and Nrf2 are redox-sensitive transcription factors (Bellezza et al. 2010). Besides MRP-dependent $H_2O_2$ generation, NAD(P)H oxidase, a membrane-bound enzyme, can also liberate ROS into the extracellular space (Petry et al. 2010). It is hypothesised that extracellular $H_2O_2$, irrespective of its origin, was not completely detoxified by the antioxidant system of the cell including glutathione peroxidase, peroxiredoxins or catalase and numerous non-enzymatic antioxidants. Instead, extracellular $H_2O_2$ might penetrate the cell. The increasing intracellular oxidative stress level may then trigger cellular signalling such as the Nrf2 pathway. Interestingly, incubation of macrophages with a single bolus of $H_2O_2$ did not activate Nrf2. This may be due to complete cellular detoxification of a single dose of $H_2O_2$, whereas MRPs permanently generate baseline levels of $H_2O_2$. Moreover, Nrf2 activation by $MRM_h$ could also be enhanced by an interaction of $H_2O_2$ with MRPs. A similar effect was described for the DNA breaking ability of Maillard products (Iida et al. 2002). In the present study, heated ribose solution did not have any impact on Nrf2

translocation excluding any involvement of caramelisation products, which are generated in addition to MRPs during heat treatment of sugar-rich foods.

Since MRPs generated by heating an amino acid with a sugar induced nuclear Nrf2 translocation, the effect of coffee, a beverage rich in MRPs, was investigated in the same test system. During coffee roasting, amine and sugar components of the green coffee bean react extensively to give the so-called melanoidin fraction, which comprises about 25% of the dry matter of the coffee brew (Fogliano and Morales 2011). Thus, it can be assumed that MRPs in roasted coffee induce nuclear Nrf2 translocation in a similar way. Furthermore, other coffee ingredients or the more complex composition of coffee melanoidins may modulate MRP-induced Nrf2 activation likewise. After short-term incubation, coffee extracts did not influence the nuclear Nrf2 concentration either in Caco-2 cells or in human gut tissue, but showed a trend of increased Nrf2 activation in macrophages. Upon long-term incubation, however, coffee extracts significantly activated Nrf2 in macrophages in a concentration and time-dependent manner. This result was in alignment with studies on the effect of coffee on human colon carcinoma cells (Boettler et al. 2010), HepG2 cells transfected with an EpRE-LUC construct (Paur et al. 2010) and ARE-luciferase reporter cells (Cavin et al. 2008) as well as Nrf2-luciferase reporter mice (Paur et al. 2010). The incubation periods of these in vitro assays were 3, 17 and 24 h, respectively. Interestingly, the effect in human colon carcinoma cells did not show a clear relation to the coffee concentration; in contrast, the effect of 1 pg/mL coffee extract was even more pronounced than the effect of 1 or 500 μg/mL (Boettler et al. 2010).

On the contrary to roasted coffee extract, raw coffee extract did not up-regulate Nrf2 in macrophages. It was shown before that the level of Nrf2 activation was related to the degree of coffee roasting (Paur et al. 2010). Thus, the roasting products were considered to be the active compounds in coffee triggering Nrf2. Co-treatment with catalase showed only a slight trend to block the coffee-induced Nrf2 translocation indicating that coffee-induced Nrf2 activation cannot be fully explained by MRP-generated extracellular $H_2O_2$. These findings are consistent with the previous study on coffee-induced Nrf2 activation in human colon cells (Boettler et al. 2010). Besides the roasting products, several coffee compounds such as the diterpenes cafestol and kahweol, N-methylpyridinium, hydroxyhydroquinone and chlorogenic acid were identified as potential Nrf2 activators (Boettler et al. 2010; Paur et al. 2010; Higgins et al. 2008). However, none of these components could fully explain coffee-induced Nrf2 activation.

In summary, the effect of coffee extract and Maillard reaction products to activate Nrf2 in various cell lines and human gut tissue was studied. It was demonstrated that cellular effects of coffee and Maillard reaction products, which had been established in different immortalised cell lines, were also evident when intact human gut tissue was stimulated ex vivo. Moreover, this study provides growing evidence that the translocation of Nrf2 is triggered by the roasting products of the coffee extract, which are structurally similar to Maillard reaction products. The mechanism of Nrf2 activation by MRPs was dependent on their activity to generate $H_2O_2$. In contrast, coffee-induced Nrf2 translocation was only slightly reversed by catalase. Therefore, the activity of coffee can only be partially explained by MRP-induced mechanisms. The identification of MRPs as potent Nrf2 activators might explain, besides their direct antioxidative activity, how MRPs decrease the susceptibility towards oxidative stress in vitro and in vivo.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References


Andrews NC, Faller DV (1991) A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res 19(9):2499

Bellezza I, Mierla AL, Minelli A (2010) Nrf2 and NF-kB and their concerted modulation in cancer pathogenesis and progression. Cancers 2:483–497

Boettler U, Sommerfeld K, Volz N, Pahlke G, Teller N, Somoza V, Lang R, Hofmann T, Marko D (2010) Coffee constituents as modulators of Nrf2 nuclear translocation and ARE (EpRE)-dependent gene expression. J Nutr Biochem. doi:10.1016/j.jnutbio.2010.03.011

Borelli RC, Visconti A, Mennella C, Anese M, Fogliano V (2002) Chemical characterization and antioxidant properties of coffee melanoidins. J Agric Food Chem 50(22):6527–6533

Cavin C, Marin-Kuan M, Langouet S, Bezencon C, Guignard G, Verguet C, Piguet D, Holzhauser D, Cornaz R, Schilter B (2008) Induction of Nrf2-mediated cellular defenses and alteration of phase I activities as mechanisms of chemoprotective effects of coffee in the liver. Food Chem Toxicol 46(4):1239–1248. doi:10.1016/j.fct.2007.09.099

Cheng X, Siow RC, Mann GE (2011) Impaired redox signaling and antioxidant gene expression in endothelial cells in diabetes: a role for mitochondria and the nuclear factor-E2-related factor 2-Kelch-like ECH-associated protein 1 defense pathway. Antioxid Redox Signal 14(3):469–487. doi:10.1089/ars.2010.3283

Cheung KL, Yu S, Pan Z, Ma J, Wu TY, Kong AN (2011) tBHQ-induced HO-1 expression is mediated by calcium through regulation of Nrf2 binding to enhancer and polymerase II to promoter region of HO-1. Chem Res Toxicol 24(5):670–676. doi:10.1021/tx1004369

Clarke JD, Dashwood RH, Ho E (2008) Multi-targeted prevention of cancer by sulforaphane. Cancer Lett 269(2):291–304. doi:10.1016/j.canlet.2008.04.018

Cuadrado A, Moreno-Murciano P, Pedraza-Chaverri J (2009) The transcription factor Nrf2 as a new therapeutic target in Parkinson's disease. Expert Opin Ther Targets 13(3):319–329. doi:10.1517/13543780802716501

Dittrich R, El-Massry F, Kunz K, Rinaldi F, Peich CC, Beckmann MW, Pischetsrieder M (2003) Maillard reaction products inhibit

oxidation of human low-density lipoproteins in vitro. J Agric Food Chem 51(13):3900–3904. doi:10.1021/jf026172s

Dittrich R, Dragonas C, Kannenkeril D, Hoffmann I, Mueller A, Beckmann MW, Pischetsrieder M (2009) A diet rich in Maillard reaction products protects LDL against copper-induced oxidation ex vivo, a human intervention trial. Food Res Int 42:1315–1322

Fogliano V, Morales FJ (2011) Estimation of dietary intake of melanoidins from coffee and bread. Food Funct 2:117–123

Goya L, Delgado-Andrade C, Rufian-Henares JA, Bravo L, Morales FJ (2007) Effect of coffee melanoidin on human hepatoma HepG2 cells. Protection against oxidative stress induced by tert-butylhydroperoxide. Mol Nutr Food Res 51(5):536–545. doi:10.1002/mnfr.200600228

He M, Siow RC, Sugden D, Gao L, Cheng X, Mann GE (2011) Induction of HO-1 and redox signaling in endothelial cells by advanced glycation end products: a role for Nrf2 in vascular protection in diabetes. Nutr Metab Cardiovasc Dis 21(4):277–285. doi:10.1016/j.numecd.2009.12.008

Hegele J, Munch G, Pischetsrieder M (2009) Identification of hydrogen peroxide as a major cytotoxic component in Maillard reaction mixtures and coffee. Mol Nutr Food Res 53(6):760–769. doi:10.1002/mnfr.200800221

Higgins LG, Cavin C, Itoh K, Yamamoto M, Hayes JD (2008) Induction of cancer chemopreventive enzymes by coffee is mediated by transcription factor Nrf2. Evidence that the coffee-specific diterpenes cafestol and kahweol confer protection against acrolein. Toxicol Appl Pharmacol 226(3):328–337. doi:10.1016/j.taap.2007.09.018

Iida T, Yoshiki Y, Someya S, Okubo K (2002) Generation of reactive oxygen species and photon emission from a browned product. Biosci Biotechnol Biochem 66(8):1641–1645

Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 236(2):313–322

Jin W, Wang H, Ji Y, Hu Q, Yan W, Chen G, Yin H (2008) Increased intestinal inflammatory response and gut barrier dysfunction in Nrf2-deficient mice after traumatic brain injury. Cytokine 44(1):135–140. doi:10.1016/j.cyto.2008.07.005

Jin W, Wang HD, Hu ZG, Yan W, Chen G, Yin HX (2009) Transcription factor Nrf2 plays a pivotal role in protection against traumatic brain injury-induced acute intestinal mucosal injury in mice. J Surg Res 157(2):251–260. doi:10.1016/j.jss.2008.08.003

Khor TO, Huang MT, Kwon KH, Chan JY, Reddy BS, Kong AN (2006) Nrf2-deficient mice have an increased susceptibility to dextran sulfate sodium-induced colitis. Cancer Res 66(24):11580–11584. doi:10.1158/0008-5472.CAN-06-3562

Kruidenier L, Kuiper I, Van Duijn W, Mieremet-Ooms MA, van Hogezand RA, Lamers CB, Verspaget HW (2003) Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. J Pathol 201(1):17–27. doi:10.1002/path.1408

Li J, Ichikawa T, Janicki JS, Cui T (2009) Targeting the Nrf2 pathway against cardiovascular disease. Expert Opin Ther Targets 13(7):785–794. doi:10.1517/14728220903025762

Martin MA, Ramos S, Mateos R, Rufian-Henares JA, Morales FJ, Bravo L, Goya L (2009) Biscuit melanoidins of different molecular masses protect human HepG2 cells against oxidative stress. J Agric Food Chem 57(16):7250–7258. doi:10.1021/jf9006032

McMahon M, Itoh K, Yamamoto M, Chanas SA, Henderson CJ, McLellan LI, Wolf CR, Cavin C, Hayes JD (2001) The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. Cancer Res 61(8):3299–3307

Moi P, Chan K, Asunis I, Cao A, Kan YW (1994) Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proc Natl Acad Sci USA 91(21):9926–9930

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1–2):55–63

Mueller U, Sauer T, Weigel I, Pichner R, Pischetsrieder M (2011) Identification of $H_2O_2$ as a major antimicrobial component in coffee. Food Funct 2(5):265–272. doi:10.1039/c0fo00180e

Muscat S, Pelka J, Hegele J, Weigle B, Munch G, Pischetsrieder M (2007) Coffee and Maillard products activate NF-kappaB in macrophages via $H_2O_2$ production. Mol Nutr Food Res 51(5):525–535. doi:10.1002/mnfr.200600254

Ning JL, Mo LW, Lai XN (2010) Low- and high-dose hydrogen peroxide regulation of transcription factor NF-E2-related factor 2. Chin Med J (Engl) 123(8):1063–1069

Paur I, Austenaa LM, Blomhoff R (2008) Extracts of dietary plants are efficient modulators of nuclear factor kappa B. Food Chem Toxicol 46(4):1288–1297. doi:10.1016/j.fct.2007.09.103

Paur I, Balstad TR, Blomhoff R (2010) Degree of roasting is the main determinant of the effects of coffee on NF-kappaB and EpRE. Free Radic Biol Med 48(9):1218–1227. doi:10.1016/j.freeradbiomed.2010.02.005

Petry A, Weitnauer M, Gorlach A (2010) Receptor activation of NADPH oxidases. Antioxid Redox Signal 13(4):467–487. doi:10.1089/ars.2009.3026

Pool-Zobel B, Veeriah S, Bohmer FD (2005) Modulation of xenobiotic metabolising enzymes by anticarcinogens—focus on glutathione S-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis. Mutat Res 591(1–2):74–92. doi:10.1016/j.mrfmmm.2005.04.020

Raithel M, Weidenhiller M, Shaban M, Abel R, Tuchbreiter H, Backhaus B, Donhauser N, Baenkler HW, Hahn EG (2003) Diagnostic use of mucosa oxygenation and histamine release experiments in patients with gastrointestinally mediated allergy (GMA). Inflamm Res 52(Suppl 1):13–14

Raithel M, Weidenhiller M, Abel R, Baenkler HW, Hahn EG (2006) Colorectal mucosal histamine release by mucosa oxygenation in comparison with other established clinical tests in patients with gastrointestinally mediated allergy. World J Gastroenterol 12(29):4699–4705

Rousseau EJ, Davison AJ, Dunn B (1992) Protection by beta-carotene and related compounds against oxygen-mediated cytotoxicity and genotoxicity: implications for carcinogenesis and anticarcinogenesis. Free Radic Biol Med 13(4):407–433

Samaras TS, Gordon MH, Ames JM (2005) Antioxidant properties of malt model systems. J Agric Food Chem 53(12):4938–4945. doi:10.1021/jf0501600

Sauer T, Raithel M, Kressel J, Muscat S, Munch G, Pischetsrieder M (2011) Nuclear translocation of NF-kappaB in intact human gut tissue upon stimulation with coffee and roasting products. Food Funct 2(9):529–540. doi:10.1039/c1fo10055f

Saw CL, Kong AN (2011) Nuclear factor-erythroid 2-related factor 2 as a chemopreventive target in colorectal cancer. Expert Opin Ther Targets 15(3):281–295. doi:10.1517/14728222.2011.553602

Seiquer I, Ruiz-Roca B, Mesias M, Munoz-Hoyos A, Galdo G, Ochoa JJ, Navarro MP (2008) The antioxidant effect of a diet rich in Maillard reaction products is attenuated after consumption by healthy male adolescents. In vitro and in vivo comparative study. J Sci Food Agr 88(7):1245–1252. doi:10.1002/Jsfa.3213

Surh YJ, Kundu JK, Na HK (2008) Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med 74(13):1526–1539. doi:10.1055/s-0028-1088302

Theiss AL, Vijay-Kumar M, Obertone TS, Jones DP, Hansen JM, Gewirtz AT, Merlin D, Sitaraman SV (2009) Prohibitin is a novel regulator of antioxidant response that attenuates colonic inflammation in mice. Gastroenterology 137(1):199–208, 208 e191–196. doi:10.1053/j.gastro.2009.03.033

Thiele K, Bierhaus A, Autschbach F, Hofmann M, Stremmel W, Thiele H, Ziegler R, Nawroth PP (1999) Cell-specific effects of glucocorticoid treatment on the NF-kappaBp65/IkappaBalpha system in patients with Crohn's disease. Gut 45(5):693–704

Urquiaga I, Leighton F (2000) Plant polyphenol antioxidants and oxidative stress. Biol Res 33(2):55–64

van Boekel M, Fogliano V, Pellegrini N, Stanton C, Scholz G, Lalljie S, Somoza V, Knorr D, Jasti PR, Eisenbrand G (2010) A review on the beneficial aspects of food processing. Mol Nutr Food Res 54(9):1215–1247. doi:10.1002/mnfr.200900608

Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Babaei-Jadidi R, Thornalley PJ (2008) Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. Diabetes 57(10):2809–2817. doi:10.2337/db06-1003