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ORIGINAL CONTRIBUTION

Dietary oxidized fat activates the oxidative stress-responsive transcription factors NF-κB and Nrf2 in intestinal mucosa of mice

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Abstract
Purpose Oxidized fats are known to induce oxidative stress resulting in the up-regulation of antioxidant enzymes, with the underlying mechanism being unclear. It is known, however, that the response of tissues to oxidative stress is mediated by redox-sensitive transcription factors such as NF-κB and Nrf2. The aim of this study, therefore, was to test the hypothesis that ingestion of an oxidized fat causes activation of these transcription factors in the small intestinal mucosa.
Methods Female mice were randomly assigned to 2 groups of 12 mice each and administered orally by gavage either oxidized or fresh fat once per day.
Results After 6 days of treatment, mice were killed, intestinal mucosa was isolated, and nuclear concentration of NF-κB and Nrf2 and expression of NF-κB- and Nrf2-regulated oxidative stress-responsive genes were determined. Oxidized fat markedly increased nuclear concentration of NF-κB and Nrf2 and transcript levels of oxidative stress-responsive genes, like aldo–keto reductase 1B8, vanin-1, glutathione peroxidase 1, and superoxide dismutase-1. In addition, oxidized fat increased the concentrations of PPAR-regulated genes.
Conclusions The activation of oxidative stress-sensitive pathways likely reflects an adaptive response of the intestinal mucosa to prevent oxidative damage to the intestinal mucosa.

Keywords Oxidized fat · Mucosa · Nrf2 · NF-κB · Mouse

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Introduction

Oxidized lipids as components of heated or fried foods play an important role in human nutrition in industrialized countries. Animal experiments revealed that ingestion of heated fats containing lipid peroxidation products provokes a wide array of biological effects. One of the most frequently reported effects of oxidized fat is induction of oxidative stress and redox imbalances [1–8]. This has been evidenced by elevated concentrations of lipid peroxidation products, reduced concentrations of exogenous (e.g., tocopherols, ascorbic acid) and endogenous antioxidants (e.g., glutathione), and a decreased glutathione/glutathione disulfide ratio in tissues of animals fed oxidized fat [1–8]. Because the intestinal mucosa is directly exposed to oxidized fats during intestinal passage after ingestion of such fats, it is not surprising that oxidative stress and redox imbalances are especially induced by oxidized fat in the intestinal mucosa [7, 8].

It is generally accepted that reactive oxygen species (ROS) generated during oxidative stress are important mediators in the expression of genes involved in antioxidant defense and inflammatory processes [9, 10]. This is explained by the activation of redox-sensitive transcription factors, such as nuclear factor-κB (NF-κB), acting as regulators of genes involved in inflammation and the adaptation to oxidative stress [11]. Intestinal NF-κB has long been considered to be specifically associated with gut-associated lymphoid tissue. However, it has now been established that NF-κB is also present in intestinal epithelial cells, which are the main cellular component of the intestinal mucosa. Epithelial NF-κB is critical for maintaining the function and integrity of the gut epithelial barrier by up-regulating genes involved in antimicrobial defense and attenuation of oxidative stress [12].

Transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) is another important redox-sensitive transcription factor that protects tissues including the intestine by inducing antioxidant and detoxifying genes in response to oxidative or xenobiotic stress [13].

A recent study clearly demonstrated that administration of a thermally oxidized oil strongly induces the expression of antioxidant enzymes such as glutathione peroxidase 1 (GPX-1) and Co/Zn-superoxide dismutase (SOD1) in the small intestine of rats being indicative of an adaptive response of the intestine to the oxidative stress induced by the ingested lipid peroxidation products [14]. The molecular mechanisms underlying this adaptive response, however, have not been studied yet. In light of the role of NF-κB and Nrf2 in mediating the adaptive response to oxidative stress, we hypothesized that ingestion of an oxidized fats causes activation of these transcription factors in the small intestinal mucosa. To test this hypothesis, we performed a feeding experiment with mice that were treated orally with an oxidized fat prepared under deep-frying conditions. As parameters, we considered the nuclear concentrations of the NF-κB subunit p50 and Nrf2 and the transcript levels of genes that are involved in oxidative stress response and known to be regulated by either NF-κB or Nrf2 or both of them, such as GPX-1, SOD1, heme-oxygenase 1 (HO-1), aldo–keto reductase 1B8 (AKR1B8), and vanin-1 [15–18]. Because the peroxidation products of oxidized fats, such as hydroxy and hydroperoxy fatty acids, were shown to be ligands and activators of peroxisome proliferator-activated receptor (PPAR) α and γ [19, 20], which are known to interfere with oxidative stress signaling pathways including NF-κB, we also studied the effect of oxidized fat on the activation of PPARα and γ.

Methods

Animals and diets

Female mice (129S4/SvImJ) supplied by Charles River with an initial body weight of 21.8 ± 1.9 g (mean ± SD) were randomly assigned to 2 groups of 12 mice each. They were kept individually in Macrolon cages in a room controlled for temperature (22 ± 2 °C), relative humidity (50–60%), and light (12-h-light/-dark cycle). All experimental procedures described followed established guidelines for the care and handling of laboratory animals [21] and were approved by the council of Saxony-Anhalt. All mice were orally administered 0.25 mL fresh or oxidized sunflower oil by gavage once per day on 6 consecutive days 2 h after the beginning of the light cycle. All mice were fed a commercial standard basal diet (Altromin 1324, Altromin, Lage, Germany). According to the declaration of the manufacturer, this diet contained (per kilogram) 11.9 MJ metabolizable energy, 190 g crude protein, 60 g crude fiber, 40 g crude fat, and 70 g crude ash. To standardize food intake, the diets were fed daily in restricted amounts of 2.5 g/d equivalent to an intake of 29.8 kJ metabolizable energy per day. Water was available ad libitum from nipple drinkers during the entire experiment.

Preparation and chemical analysis of the oxidized fat

The thermo-oxidized oil was prepared by heating sunflower oil (obtained from a local supermarket) in a domestic fryer from Bartscher GmbH (GF-8SE, Salzkotten, Germany) at 190 °C for 48 h. The extent of lipid peroxidation was determined by assaying the peroxide value [22], concentration of thiobarbituric acid substances (TBARS) [23], concentration of conjugated dienes [24], acid value [22], and the percentage of total polar compounds [25]. The fatty acid composition of the dietary fats was determined by gas chromatography (GC). For that purpose, fats were methylated with trimethylsulfonium hydroxide to fatty acid methyl esters (FAME) [26]. FAME were separated by GC using a Chrompack 9000 system (Fa. Agilent Technologies, Waldbronn, Germany) fitted with an automatic on-column injector, a polar capillary column (60 m FFAP, 0.25 mm internal diameter, 0.25 μm particle size, Macherey and Nagel, Düren, Germany), and a flame ionization detector [27].

Sample collection

At day 6, mice received the last dose of fresh or oxidized oil and 0.5 g of the diet 2 h after the beginning of the light cycle and were killed 4 h later by decapitation under light anesthesia with CO_2. Blood was collected into heparinized polyethylene tubes. Plasma was obtained by centrifugation of the blood (1,100 g, 10 min, 4 °C) and stored at −20 °C. The small intestine was rapidly excised, flushed with ice-cold 0.9% NaCl (w/v), and opened length-wide. The mucosa was scraped off and snap-frozen for RNA isolation. All tissue samples were stored at −80 °C pending further analysis.

RNA isolation and real-time detection RT-PCR analysis

Total RNA was isolated from mucosa samples using Trizol™ reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Total RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. Synthesis of cDNA and determination of mRNA abundance by real-time detection PCR

was performed as recently described in detail [28]. The mRNA abundance of genes was measured by real-time detection PCR using SYBR Green I and the Rotor Gene 2000 system (Corbett Research). Real-time detection PCR was performed with 1.25 units Taq DNA polymerase, 500 mmol desoxyribonucleotide triphosphates, and 26.7 pmol of the gene-specific primers (Operon, Köln, Germany). For determination of mRNA concentration, a threshold cycle was obtained from each amplification curve using the software RotorGene 5.0 (Corbett Research). Calculation of the relative mRNA concentration was made using the $2^{-\Delta\Delta Ct}$-method with GAPDH as reference gene as previously described [29]. The relative mRNA concentration of the genes investigated is expressed as fold change in the oxidized fat group compared to the fresh fat group. Characteristics of gene-specific primers were as follows (forward 5′-3′, reverse 5′-3′; NCBI GenBank): Adipophilin (CCCTGTCTACCAAGCTCTGC, CGATGCTTCTCTTC CACTCC; NM_007408.3), AKR1B8 (CAAACCAAGTC AAAGAAG CTG, CTTGCTGACAATGAAGAGGTC; NM_008012.1), GAPDH (TGTTCCTACCCCCA ATGT GT, GTCATTGAGAGCAATGCCAG; NT_109320.4), GPX-1 (CTCTTTACCTTCCT GCGGAA, GGACAGCA GGGTTTCTATGT; NM_008160.5), HO-1 (GATTT GTCTGAGG CCTTGAAG, CTTAAAGCCTTCTCT GGACAC; NM_010442.1), PPARα (AGGCAGATG AC CTGGAAAGTC, ATGCGTGAACTCCGTAGTGG; NM_011144.3), PPARγ (CCAGTT TCGATCCGTAGAAG, CCATAAAGTCACCAAAGGGC; NM_011146.2), SOD1 (GATG ACTTGGGCAAAGGTGG, CTGCGCAATCCCA ATCACTC; NM_011434.1), Vanin-1 (GA AGTGGT ATCTATGCACCC, CTGTAGGTAGTACTGCCCTT; NM_011704.1).

Immunoblot analysis

Nuclear extracts were prepared from scraped mouse mucosa with a Nuclear Extract Kit (Active Motif, Rixensart, Belgium) according to the manufacturer's protocol. Protein concentrations in the nuclear extracts were determined by the bicinchoninic acid protein assay kit (Interchim, Montluçon, France) with BSA as standard. Four nuclear extract pools of each group were prepared with three animals contributing equally to each pool. From each nuclear extract pool, 30 µg protein was separated on 10% SDS–PAGE for NF-κB/p50 and 12.5% SDS–PAGE for Nrf2 and electrotransferred to a nitrocellulose membrane (Pall Corporation, Pensacola, FL, USA). Loading of equal amounts of protein in each line was verified by Panceau S staining. After incubating the membranes overnight at 4 °C in blocking solution, membranes were incubated with primary antibodies against Nrf2 (polyclonal anti-Nrf2 antibody; Abcam, Cambridge, UK), NF-κB/p50 (polyclonal anti-NF-κB/p50 antibody; Santa Cruz, Heidelberg, Germany), and β-actin (monoclonal anti-β-actin antibody, Abcam, Cambridge, UK) as a housekeeping protein to control for adequate normalization at room temperature. The membranes were washed and then incubated with a horseradish peroxidase-conjugated secondary anti-rabbit-IgG antibody (Sigma–Aldrich, Steinheim, Germany) at room temperature. Afterward, blots were developed using ECL Plus (GE Healthcare, München, Germany). The signal intensities of specific bands were detected with a Bio-Imaging system (Syngene, Cambridge, UK) and quantified using Syngene GeneTools software (nonlinear dynamics).

Statistical analysis

Treatment effects were analyzed with one-way ANOVA using the Minitab Statistical software Rel. 13.0 (Minitab). For significant *F*-values, means were compared by Fisher's multiple range test. Values in the text are means ± SD. Means were considered significantly different at $p < 0.05$.

Results

Characterization of the experimental fats

Palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic acid [18:2 (n-6)] were the major fatty acids in both fats (Table 1). Due to oxidation-dependent loss of PUFA during heat treatment of the fat, the oxidized fat had a lower proportion of linoleic acid and slightly higher proportions of saturated fatty acids and oleic acid than the fresh fat (Table 1). The oxidized fat had much higher concentrations of peroxides (2.9-fold), conjugated dienes (19-fold),

Table 1 Characteristics of the experimental oils

	Fresh oil	Oxidized oil
Major fatty acids, g/100 g total fatty acids		
14:0	0.62	0.78
16:0	5.95	7.03
18:0	3.83	4.51
18:1	23.5	26.2
18:2 (n-6)	64.0	59.0
18:3 (n-3)	0.13	0.09
22:0	0.91	1.02
Peroxidation products		
Peroxide value, mEq O2/kg	2.47	7.16
Conjugated dienes, mmol/kg	6.91	131
TBARS, mmol/kg	<0.1	422
Total polar compounds, %	2.87	31.0
Acid value, g KOH/kg	0.20	0.53

TBARS (422-fold), polar compounds (10.8-fold), and a higher acid value (2.7-fold) than the fresh fat (Table 1).

Initial and final body weights

Initial (fresh fat, 21.8 ± 1.98 g; oxidized fat, 21.7 ± 1.94 g; $n = 12$; $p < 0.05$) and final (fresh fat, 21.1 ± 1.68 g; oxidized fat, 21.4 ± 1.63 g; $n = 12$; $p < 0.05$) body weights did not differ between both groups of mice.

Nuclear concentrations of NF-κB/p50 and Nrf2 in small intestinal mucosa

Under basal conditions, NF-κB/p50 and Nrf2 are sequestered in the cytoplasm by cytosolic inhibitory proteins in an inactive state, whereas activation causes dissociation from inhibitory proteins and translocation into the nucleus and binding of the active protein to specific sequences in the regulatory region of target genes. We therefore determined concentrations of NF-κB/p50 and Nrf2 in the nuclear fraction to evaluate activation of NF-κB/p50 and Nrf2 by the oxidized fat. Mice treated with the oxidized fat had 2.2- and 4.0-fold higher nuclear concentrations of NF-κB/p50 and Nrf2, respectively, in the small intestinal mucosa than mice treated with the fresh fat ($p < 0.05$, Fig. 1).

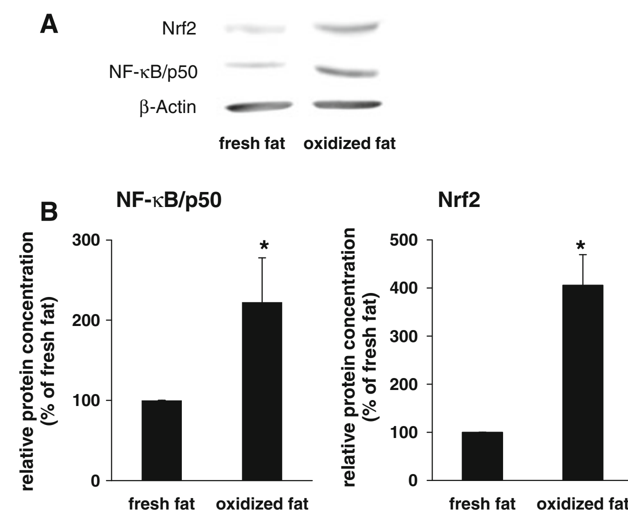

Fig. 1 Nuclear concentrations of NF-κB/p50 and Nrf2 in small intestinal mucosa of mice administered either fresh fat or oxidized fat. **a** Representative immunoblots specific to Nrf2 and NF-κB/p50 are shown for one of 4 pools per group. Immunoblots for the other pools revealed similar results. **b** *Bars* represent data from densitometric analysis and represent means ± SD ($n = 4$ pools/group). *Bars* are expressed as the percentage of the protein concentration of the fresh fat group. *Different from mice administered fresh fat, $p < 0.05$

Relative mRNA concentrations of NF-κB- and Nrf2-regulated genes involved in oxidative stress-response in small intestinal mucosa

To further evaluate activation of NF-κB and Nrf2 by the oxidized fat, we determined relative mRNA concentrations of known target genes such as GPX-1, SOD1, HO-1, vanin-1, and AKR1B8. Relative mRNA concentrations of GPX-1, SOD1, vanin-1, and AKR1B8 in small intestinal mucosa were about 1.5-, 1.6-, 1.9-, and 7.8-fold, respectively, higher in mice administered the oxidized fat than in those administered the fresh fat ($p < 0.05$, Fig. 2). Relative mRNA concentration of GPX-1 in small intestinal mucosa tended to be higher (1.3-fold) in mice of the oxidized fat group than in those of the fresh fat group ($p < 0.1$, Fig. 2). Relative mRNA concentration of HO-1 in small intestinal mucosa did not differ between both groups of mice ($p > 0.05$, Fig. 2).

Relative mRNA concentrations of PPARα and PPARγ and PPARα/PPARγ-regulated genes in small intestinal mucosa

To evaluate activation of the PPARα and PPARγ pathways by the oxidized fat, we determined relative mRNA concentrations of the receptors and selected PPARα/PPARγ-target genes. Mice administered the oxidized fat had 3.6-, 2.6-, 5.6-, and 1.5-fold higher relative mRNA concentrations of PPARα, FATP, CYP4A10, and adipophilin in small intestinal mucosa than mice administered the fresh fat ($p < 0.05$, Fig. 3). Relative mRNA concentration of PPARγ in small intestinal mucosa did not differ between both groups of mice ($p > 0.05$, Fig. 3).

Discussion

The present study tested the hypothesis that ingestion of an oxidized fat causes activation of oxidative stress-sensitive transcription factors, such as NF-κB and Nrf2, in the small intestinal mucosa. The oxidized fat used in this study was prepared by heating sunflower oil at a relatively high temperature over a short period. This reflects a fat that has been prepared under conventional deep-frying conditions. The relatively high concentrations of lipid peroxidation products (conjugated dienes, TBARS, peroxides, and carbonyls) in the oxidized fat indicate that this fat was strongly oxidized. Such fats generally contain high concentrations of secondary lipid peroxidation products such as aldehydes or ketones and rather low concentrations of primary lipid peroxidation products such as hydroxy and hydroperoxy fatty acids.

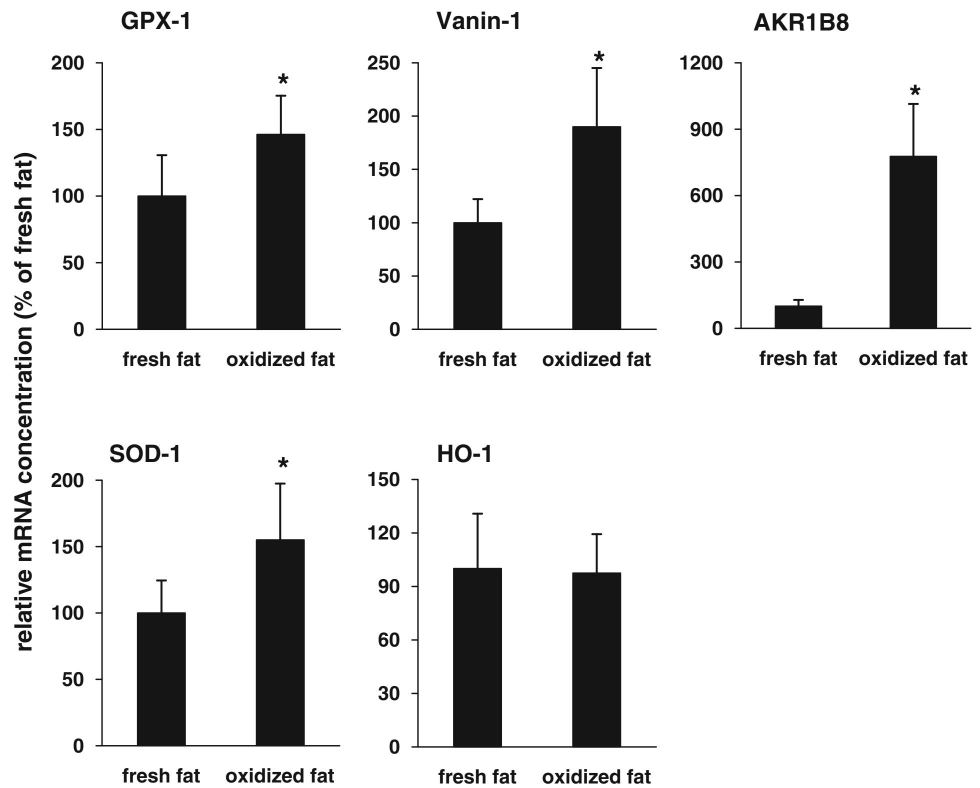

Fig. 2 Relative mRNA concentrations of NF-κB- and Nrf2-regulated genes involved in oxidative stress response in the intestinal mucosa of mice administered either fresh fat or oxidized fat. *Bars* represent means ± SD ($n = 12$/group) and are expressed as the percentage of the relative mRNA concentration of the fresh fat group. *Different from mice administered fresh fat, $p < 0.05$

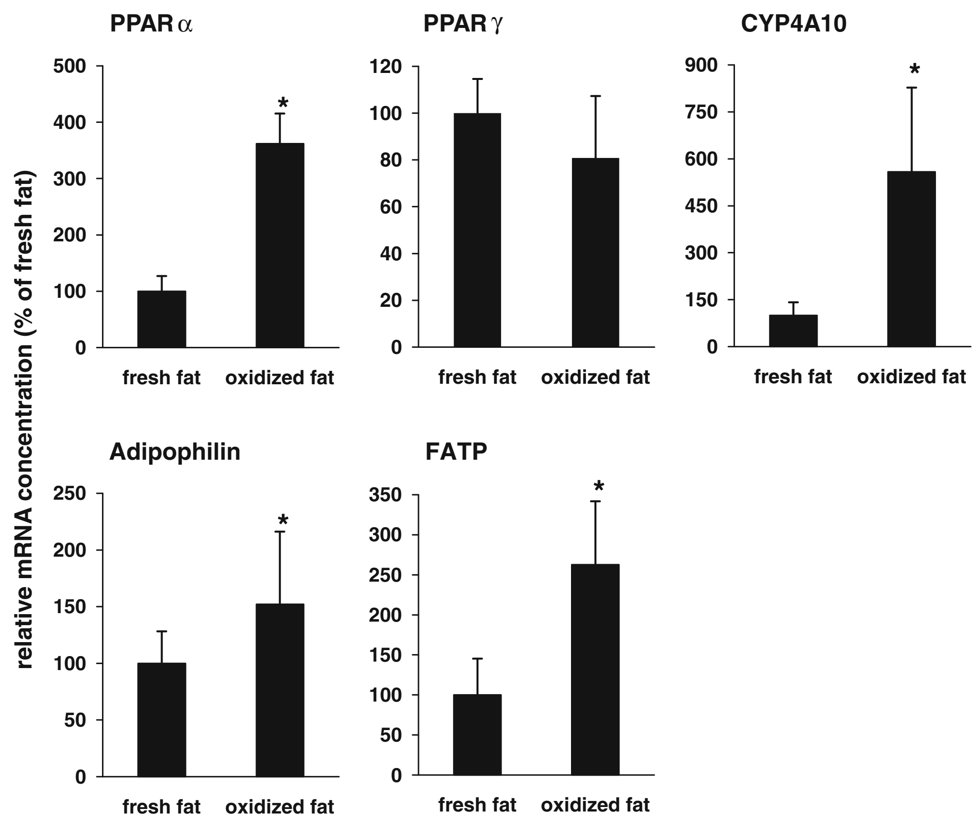

Fig. 3 Relative mRNA concentrations of PPARα and PPARγ and PPARα/PPARγ-regulated genes in the intestinal mucosa of mice administered either fresh fat or oxidized fat. *Bars* represent means ± SD ($n = 12$/group) and are expressed as the percentage of the relative mRNA concentration of the fresh fat group. *Different from mice administered fresh fat, $p < 0.05$

The present study shows for the first time that administration of an oxidized fat activates the transcription factors NF-κB and Nrf2 in the intestinal mucosa of mice. It is well known that peroxidation products from oxidized fats are efficiently taken up by the intestinal epithelial cells [30, 31], and that the cellular exposure to oxidized fats leads to the generation of ROS [32]. Regarding that NF-κB and Nrf2 are activated by various stimuli including ROS [13, 33], it is therefore very likely that the ingestion of lipid peroxidation products and the resulting oxidative stress in the intestinal mucosa have led to the activation of NF-κB and Nrf2. This finding in mice is in contrast to a recent observation in pigs, in which oxidized fat did not activate NF-κB in the small intestinal mucosa [34]. Although it cannot be excluded that the effect in mice is a species-specific phenomenon, it is more likely that differences in the administration of the oxidized fat are responsible for the divergent effect between mice and pigs. In the pig study, the oxidized fat was fed as part of a normal diet, whereas in the mice study, the oxidized fat was administered by gavage. In the latter case, the intestinal mucosa is probably more directly exposed to the ingested oxidized fat than in the case that the oxidized fat is virtually diluted by the other feed components. A further reason for the lack of response in the pig study might be that the amount of fat administered to the animals when related to their body weights was clearly higher in the mice study than in the pig study. Our study, moreover, showed that administration of oxidized fat caused an increase in the transcript levels of several oxidative stress-responsive genes, including SOD1, AKR1B8, vanin-1, and GPX-1, in the intestinal mucosa. Up-regulation of SOD1 and GPX-1 in the intestine and other tissues by oxidized lipids has been already observed in previous studies [14, 35], and up-regulation of these genes has been generally interpreted as an adaptive, defensive response of tissues to prevent possible harmful effects of ROS [36, 37]. A recent study demonstrated that the feeding of oxidized fat also results in increased activities of antioxidant enzymes such as catalase and selen-dependent GPX in the intestine [14] indicating that transcriptional up-regulation of antioxidant genes is also translated to the metabolic level. To the best of our knowledge, up-regulation of AKR1B8 and vanin-1 by oxidized fat has not been described yet. AKRs like AKR1B8 catalyze the reduction of both environmental aldehydes and aldehydes generated endogenously from lipid peroxidation such as 4-hydroxynonenal or core aldehydes [38]. Due to these properties, it has been suggested that AKR1B8, like GPX-1 and SOD1, is an important component of the cellular antioxidant defense mechanisms that protect against injurious lipid peroxidation products [39]. Induction of the epithelial cell-specific vanin-1 is also indicative of the induction of oxidative stress, because cysteamine provided by the pantetheinase activity of this ectoenzyme acts pro-oxidative in glutathione metabolism and activates stress pathways. In addition, vanin-1 facilitates the intestinal epithelial cells to produce inflammatory mediators. This likely explains that vanin-1-deficient mice, which lack tissue cysteamine, show a remarkably increased resistance to stress and acute intestinal inflammation [40, 41]. We, therefore, postulate that the induction of AKR1B8 and vanin-1 by oxidized fats is probably also part of the adaptive response of the intestinal mucosa to cope with the oxidative stress induced by the lipid peroxidation products.

Induction of the above-mentioned oxidative stress-responsive genes in response to ROS is well known to be mediated by the binding of activated NF-κB/p50 and Nrf2 to specific DNA-sequences, called NF-κB response element (NF-κB-RE) and antioxidant response element (ARE), respectively, which are present in the 5′-flanking regulatory region of those genes. Although we did not perform gel shift experiments in this study to confirm increased binding to NF-κB-REs and AREs, we are confident about this because nuclear concentrations of NF-κB/p50 and Nrf2 were markedly elevated by the oxidized fat and the increased nuclear concentrations of these transcription factors correlated well with the increased levels of NF-κB-RE- and ARE-mediated gene products. It might be argued that nuclear protein concentrations of NF-κB/p50 and Nrf2 were elevated due to inadequate normalization with β-actin which is known to translocate between the cytosol and nucleus [42] and is therefore present in both cell fractions. However, β-actin is frequently used as a housekeeping protein for nuclear proteins [43, 44] and considered suitable for normalization of nuclear protein levels provided that it is constitutively and stably expressed under the specific experimental conditions. Since there is no evidence from the literature that oxidized fat influences the intracellular translocation of β-actin, the band intensities for β-actin were similar between both groups, and equal amounts of protein were separated by SDS–PAGE, we are confident that protein levels of NF-κB/p50 and Nrf2 were adequately normalized. Thus, we propose that the observed up-regulation of the above-mentioned genes in the intestinal mucosa of mice administered the oxidized fat is mediated by the activation of oxidative stress-sensitive transcription factors such as NF-κB and Nrf2. Since the above-mentioned genes are also regulated, at least partially, by other oxidative stress-responsive transcription factors such as activator protein (AP)-1 [45], it is not unlikely that activation of the above-mentioned genes by oxidized fat is mediated in cooperation of NF-κB and Nrf2 with other transcription factors. This has to be clarified in future studies.

In contrast to the present study, recent studies showed that Nrf2 activation causes inhibition of NF-κB [46–48].

The negative crosstalk between these two transcription factors has been explained by different mechanisms, such as competition between Nrf2 and NF-κB for the CREB-binding protein (CBP) and recruitment of histone deacetylase 3, a co-repressor of ARE, through the NF-κB subunit p65 and subsequent interaction with CBP or Maf kinases [49]. The fact that we did not find evidence for a negative cross-talk between Nrf2 and NF-κB may indicate that components of oxidized fat, unlike phytochemicals such as sulforaphane, stimulate a further yet unidentified pathway preventing inhibition of the NF-κB pathway by Nrf2.

In contrast to SOD1, GPX-1, AKR1B8, and vanin-1, HO-1, which is involved in heme catabolism, was not induced in the intestinal mucosa by the oxidized fat, although HO-1 is also regarded as a sensitive and reliable indicator of cellular oxidative stress [50], and the HO-1 gene promoter was shown to contain binding sites for both NF-κB and Nrf2 [51, 52]. Therefore, we cannot explain the observation that oxidized fat did not induce HO-1 in the intestinal mucosa, though the nuclear concentrations of NF-κB and Nrf2 were significantly elevated. However, one can speculate that in the present mice experiment, NF-κB has inhibited the binding of Nrf2 to the HO-1 promoter by specifically impeding binding of the above-mentioned CBP to Nrf2 at the HO-1 promoter.

In the present study, we also considered the PPARα- and PPARγ-signaling pathways, because it was shown that these pathways can negatively interfere with oxidative stress signaling pathways including NF-κB. Herein, we showed that the transcript levels of PPARα/γ-responsive genes, like FATP, CYP4A1, and adipophilin, were strongly increased by the oxidized fat in the intestinal mucosa of the mice. These findings are in good agreement with recent studies showing that oxidized fats cause activation of PPARα in liver and intestine of rats and pigs [53–55]. From the present findings, we cannot distinguish whether up-regulation of FATP, CYP4A1, and adipophilin was due to activation of PPARα, PPARγ or both of them, because the components of oxidized fat supposed to be responsible for PPARα activation are also ligands and activators of PPARγ and FATP, CYP4A1 and adipophilin are regulated by different PPAR isotypes [56–59]. In any case, the present findings clearly indicate that activation of PPAR-signaling by oxidized fat in the intestinal mucosa does not counteract the activation of oxidative stress-responsive pathways such as NF-κB and Nrf2.

In conclusion, the present study shows that ingestion of an oxidized fat causes a significant up-regulation of several oxidative stress-responsive genes including SOD1, GPX-1, AKR1B8, and vanin-1 in the intestinal mucosa which is likely mediated by the activation of oxidative stress-sensitive transcription factors such as NF-κB and Nrf2. We postulate that up-regulation of these genes that have antioxidant, cytoprotective, and detoxifying functions is an adaptive response of the intestinal mucosa to cope with the luminal diet-derived oxidants thereby preventing ROS-mediated damage to the intestinal mucosa. In this context, we further postulate that oxidized fat, similar to the previously reported effects of resveratrol and physical exercise [60, 61], may induce the generation of low levels of ROS which could comprise a positive "hormetic redox signal" in the enterocyte thereby enhancing stress resistance. This is in contrast to the detrimental "cellular stress signal" induced by high levels of ROS generated from high doses of ascorbic acid resulting in inactivation of Nrf2 [60].

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