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Research Article

Elimination and metabolism of sesamol, a bioactive compound in sesame oil, in rats

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Sesamol, generally regarded as a main antioxidative component in sesame oil, is generated from sesamolin upon roasting of sesame seed or during bleaching process of sesame oil. This investigation studied the bioavailability and excretion of sesamol in Sprague-Dawley rats. After oral administration of sesamol (p.o. 100 mg/kg) to SD rats, the changes in concentration of sesamol were determined in various excreta within 24 h period. Our results showed that sesamol conjugated metabolites were rapidly eliminated from urine and feces in 0 – 4 h. The majority of intact sesamol glucuronide was excreted in the urine. It is suggested that sesamol conjugated metabolites are primarily eliminated from the plasma *via* the kidney by active tubular secretion. LC-MS/MS analyses of rat excreta showed that sesamol can be converted to 2-methoxybenzene-1,4-diol and benzene-1,2,4-triol *in vivo* by rat.

Keywords: Benzene-1,2,4-triol / Elimination / 2-Methoxybenzene-1,4-diol / Sesamol / Sesamol glucuronide

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1 Introduction

Sesame, an important oilseed derived from *Sesamum indicum*, is one of the oldest oilseeds known to humans and is considered to have nutritional value as well as medicinal effects. The seed contains two lignans, sesamin, and sesamolin. Upon roasting sesame seeds, sesamolin is converted to sesamol [1, 2]. Sesamol has been found to have antioxidative effects [3] and to induce growth arrest and apoptosis in cancer cells [4]. Recently, the antiphotooxidative activity of sesamol for oil has been reported to be due to the scavenging of singlet oxygen [5].

Sesamol has a phenolic and a benzodioxole group in its molecular structure. The phenolic groups of molecules are generally responsible for the antioxidant activity of many natural products [6 – 9]. On the other hand, benzodioxole derivatives are widely distributed in nature and have been shown to possess antitumor, antioxidant and many other biological activities [10 – 13]. These activities have been attributed to the effect on various enzymes as well as scavenging of reactive oxygen species. Sesamol could also attenuate the production of nitric oxide and hydrogen peroxide and reduce monoamine oxidase activity in glial astrocyte cells [14]. Since a distinct relationship exists between monoamine oxidase activity and the development of neurodegenerative diseases associated with aging such as Alzheimer's disease and stroke, sesamol might play a role in the prevention of these types of diseases. In addition, fibrinolysis is considered as a risk factor for severe cardiovascular diseases such as myocardial infarction and stroke [15 – 17]. Sesamol may enhance overall vascular fibrinolytic capacity through regulating gene expression of plasminogen activator [18]. However, there are no comprehensive and in-depth investigations on the elimination and metabolism of sesamol *in vivo*. The aim of this study was to elucidate the metabolic fate of sesamol after its oral administration in rats.

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Abbreviations: SGF, simulated gastric; **SIF,** intestinal fluid; **SRM,** selected reaction monitoring

2 Materials and methods

2.1 Chemicals

Sesamol, D-saccharic acid-1,4-lactone, hesperetin, 2-methoxybenzene-1,4-diol, benzene-1,2,4-triol, and sulfatase (*Helix pomatia*, S-9626) were obtained from Sigma – Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade. Liquid chromatographic grade solvents and reagents were obtained from Mallinckrodt

Baker (Phillipsburg, NJ, USA). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2 Simulated gastric (SGF) and intestinal fluids (SIFs) digestion stability assay

SGF andSIFs were freshly prepared on the day of experiment. SGF was prepared as described in the United States Pharmacopoeia [19–21] and consists of 3.2 mg/mL pepsin in 0.03 M NaCl at pH 1.2. Aliquots (200 μL) of SGF were placed in 1.5-mL microcentrifuge tubes and incubated in a water bath at 37°C. Ten microliters of the test sample (0.5 mM in 0.03 M NaCl) was added to each of the SGF vials to start the digestion reaction. At intervals of 0, 5, 15, 30, 60, 120, 240, 360, and 540 min, 75 μL of 1 N NaOH, or 0.2 M $NaCO_3$ was added to each vial to stop the reaction. Subsequently, all samples were deproteinized by centrifuging for 10 min at 10 000 × *g* and 4°C. A 250 μL of internal standard (hesperetin, 2.0 μg/mL in ACN) solution was added to each sample before deproteinization. After centrifugation, 200 μL supernatant was injected into the HPLC system.

SIF was prepared as described in the United States Pharmacopoeia [19–21] and consists of 10 mg/mL of pancreatin in 0.05 M KH_2PO_4, pH 7.5. Aliquots (64 μL) of SIF were placed in 1.5 mL microcentrifuge tubes and incubated at 37°C for 10 min in a water bath. The test sample (10 μL) at a concentration of 0.5 mM (in 0.05 M KH_2PO_4, pH 7.5) was added to each of the microcentrifuge tubes to start the reaction. At intervals of 0, 5, 15, 30, 60, 120, 240, 360, and 540 min, the reaction was immediately stopped by placing the tube in a boiling water bath for 10 min. The subsequent deproteinization condition was the same as described for the stability in SGF study.

2.3 Animals and diets

The experimental protocol was approved by the National Laboratory Animal Center (Taipei, Taiwan.) Inbred male Sprague Dawley rats (body wt 275 ± 25 g, mean ± SD) were housed in pairs in cages in a room with controlled temperature (20–22°C) and relative humidity (50–70%), and a 12-h light: dark cycle (lights on at 7 h). The rat diet was AIN 93 M diet (Purina Mills, St. Louis, MO, USA). Rats consumed their food ad libitum and had unlimited access to water; their weight and food consumption were recorded weekly.

2.4 Excretion experiment

Animals ($n = 6$) were administered *via* gastric gavage, 100 mg/kg body weight sesamol dissolved in normal saline for four days to three daily doses (300 mg/kg/day). After consuming the sesamol diet for 4 days, urine and feces samples were collected at the following time periods: 0–4, 4–8, 8–12, and 12–24 h after administration of sesamol. Urine and feces from rats housed in metabolic cages were collected for the 24 h experiment. The rats were anesthetized at fourth day, without overnight fasting, using CO_2 as a carrier. Ascorbic acid (200 mg/mL) was added to urine samples, which were then stored at −80°C until analysis.

2.5 Preparation of feces samples

The feces were lyophilized and then homogenized individually (Polytron and mill) before further processing. Samples were stored in airtight containers at −80°C.

2.6 Extraction of feces samples

For extraction, feces of rat were weighed in 50 mL tubes. The samples were homogenized (Polytron) in 10 mL of ACN with a vortex. The extract was transferred to a 25 mL tube and centrifuged for 10 min at 10 000 × *g* and 4°C. The supernatant was transferred to a clean tube and the residue was extracted two more times. The organic solvent from the supernatant was evaporated at 50°C. Tubes were weighed before and after evaporation of extraction solvent. Subsequently, the residues were dissolved with 1 mL 0.1 N NaOAc buffer (pH 5.0, with 200 mg/mL ascorbic acid). Each sample (100 μL) was either hydrolyzed with 50 μL enzyme 1000 U sulfatase or 1000 U sulfatase activity with β-glucuronidase inhibitor (D-saccharic acid-1,4-lactone, 7.5 mg/mL) in 0.1 N NaOAc buffer (pH 5.0, with 200 mg/mL ascorbic acid)]/g excreta for incubation at 37°C, or not hydrolyzed but processed immediately with the addition of the same volume of NaOAc buffer without enzyme mix [22–25]. Subsequently, all samples were deproteinized by centrifuging for 10 min at 10 000 × *g* and 4°C. A 250 μL of internal standard (hesperetin, 2.0 μg/mL in ACN) solution was added to each sample before deproteinization. After centrifugation, 1 mL supernatant was injected into the HPLC system.

2.7 Determination of sesamol excluded in urine and feces

The HPLC system consisted of Hitachi L7100 pumps (Hitachi, Tokyo, Japan) and a Hitachi L7420 UV–Vis detector (Hitachi). Separation was achieved by injecting 20 μL sample onto a Luna C18 column (4.6 × 250 mm, 5 μm, Phenomenex), protected by a RP18 guard column (15.0 mm × 3.2 mm, 5 μm, Phenomenex, Torrance, CA, USA). Columns were housed in a column heater set at 30°C. The solvents for elution were 100% methanol (solvent A) and 1% acetic acid, pH 6 (solvent B). The elution program of rat feces at a flow rate of 1 mL/min was as follows: 0–15 min, linear gradient from 40 to 70% A; 15–16 min, linear gradient from 70 to 100% A. Peaks were

detected with a UV–Vis detector at 290 nm. The elution program of rat urine at a flow rate of 1 mL/min was as follows: 0–15 min, linear gradient from 30 to 40% A; 15–19 min, linear gradient from 40 to 100% A. Peaks were detected with a UV–Vis detector at 290 nm.

The concentrations of sesamol sulfate and sesamol glucuronide were calculated from the following equation:

$$\mathrm{Conc}_{\mathrm{sulfate}} = \mathrm{Conc}_{\mathrm{parent\ form\ +\ conjugates\ (sulfatase)}} - \mathrm{Conc}_{\mathrm{parent\ form}}$$

$$\mathrm{Conc}_{\mathrm{glucuronide}} = \mathrm{Conc\ conjugates}_{\mathrm{(sulfatase)}} - \mathrm{Conc}_{\mathrm{sulfate\ form\ (sulfatase\ with\ inhibitor)}}$$

2.8 Sesamol and its metabolites analysis of urine samples by LC-MS/MS

Urinary sesamol and its metabolites analysis was performed using a previously described method [10] with slight modifications. Briefly, 2 mL of 0.1 mol/L sodium acetate buffer (pH 4.5) and 50 μL of 1000 U sulfatase activity with β-glucuronidase inhibitor (D-saccharic acid-1,4-lactone, 7.5 mg/mL) in 0.1 mol/L sodium acetate buffer were added to 1 mL urine sample, and the mixture was incubated in a 37°C water bath overnight to hydrolyze the sesamol conjugates. The samples were then passed through Sep-ed SPE Cartridges (Octadecyl C18:14%, 200 mg: 3 mL) that were preconditioned with 5 mL of chloroform/methanol (1:1 v/v), 5 mL of methanol, and 5 mL of distilled water [26]. They were then washed with 5 mL of distilled water and 4 mL of methanol. The samples were completely evaporated using a vacuum rotary evaporator at 60°C. The residue was dissolved in 1 mL of methanol before LC and LC-MS/MS.

Identification of sesamol and sesamol metabolites were carried out by LC-MS/MS and analyzed. These analyses were performed on a Thermo HPLC system equipped with electrospray–ionization IT mass spectrometer (Thermo-Finnigan LXQ Advantage, San Jose, CA, USA). The separation was achieved using a Luna C18 column (2.0 × 150 mm id; 5 μm, YMC, Tokyo, Japan). For the operation in MS/MS mode, a mass spectrometer with an electrospray interface (ESI) was used. During the analyses, the ESI parameters were set as follows: capillary voltage, –9.52 kV for negative mode; source voltage, 3.94 kV; source current, 6.15 μA; sheath gas flow rate, 34.57 au; capillary temperature, 200°C; tube lens voltage, –30 V. The collision energy of *m*/*z* 139 $[M-H]^-$ was adjusted to maximize the intensity of the deprotonated molecular ion (precursor) as 15% and the collision energy was also adjusted to optimize the product ion signals as 15% for sesamol metabolite (2-methoxybenzene-1,4-diol) analysis. The cone energy of *m*/*z* 125 $[M-H]^-$ was 12% and the collision energy was 12% for sesamol metabolite (benzene-1,2,4-triol) analysis. The cone energy of *m*/*z* 137 $[M-H]^-$ was 13% and the collision energy was 13% for sesamol (free from) analysis. Selected reaction monitoring (SRM) was highly sensitive and accurate for multiple metabolites quantitation from rats extracts on a 2-D linear IT mass spectrometer. The SRM was used to monitor the transition of the molecule to the product ion for sesamol and its metabolites analysis. All LC-MS/MS data were processed by the Xcalibur version 2.0 data acquisition software.

2.9 Statistical analysis

Individual excreta of six rats were collected after varying times of sesamol and its metabolites. Samples were extracted individually before analysis ($n = 6$). Sesamol, sesamol glucuronide, and sesamol sulfate concentrations were expressed in nmol/g feces or nmol/mL urine. Data were analyzed by *t*-test analysis of variance, and differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Stability of sesamol in SGF and SGI

In vitro gastrointestinal digestion models with a sequential use of digestive enzymes in physiological concentrations and the stomach/duodenal environment were used to assess the digestibility of sesamol. In addition to acid, sesamol in the gastrointestinal tract was exposed to a digestive juice containing components such as pepsin in stomach and pancreatin in small intestine. After exposure sesamol to gastrointestinal digestion models for 540 min, sesamol was stable to both acid and enzymes (Fig. 1).

3.2 Elimination study

To investigate the exclusion of sesamol in rats, the concentrations of sesamol were determined in urine and feces within 24 h after administration to rats. Excreta concentrations of sesamol and sesamol-conjugated metabolites were analyzed by HPLC. Sesamol-conjugated metabolites (glucuronide/sulfate) were excluded in rat excreta. Sesamol may be, at first, incorporated into the liver and then transported to the kidney, and then excreted in bile undergoes enterohepatic circulation to be eventually excreted in urine.

A quantitative method for the measurement of the concentrations of sesamol and its conjugated metabolites was established. The levels of sesamol in urine samples with and without the treatment of sulfatase or sulfatase (with β-glucuronidase inhibitor) were determined by HPLC. The sulfatase (from *H. pomatia*), an enzyme has both activity of sulfatase and β-glucuronidase, not only hydrolyzes sesamol sulfate, but also causes the hydrolysis of sesamol glucuronide. It was necessary for the quantitative analysis of sesamol sulfate in excreta by adding the saccharolactone (D-saccharic acid 1,4-lactone) as an inhibitor for β-glucuronidase. On the other hand, β-glucuronidase did not contain the activity of sulfatase.

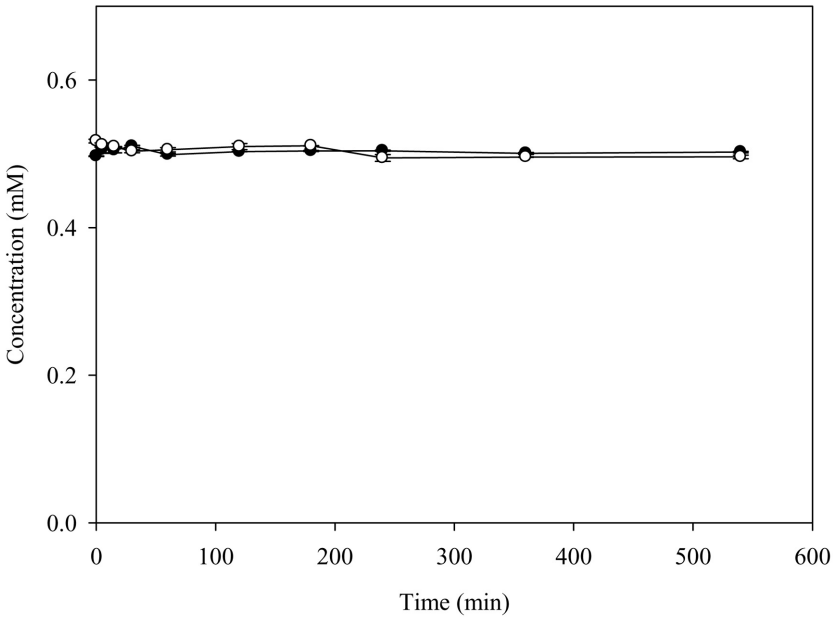

Figure 1. Time-dependent changes of sesamol in SGF (●) and SIF (○).

Table 1. Individual urinary excretion of sesamol and its conjugated metabolites during each time interval after oral administration of sesamol 100 mg/kg

Conc. (nmol/mL) Time (h)	Sesamol	Sesamol glucuronide	Sesamol sulfate
0–4	202.9 ± 76.1	4538.6 ± 446.4[a)]	2614.4 ± 257.2[a,b)]
4–8	109.4 ± 4.8	8426.2 ± 108.5[a)]	4853.8 ± 62.5[a,b)]
8–12	93.48 ± 23.8	3675.7 ± 169.0[a)]	2117.3 ± 97.4[a,b)]
12–16	97.83 ± 16.9	3871.1 ± 270.4[a)]	2229.9 ± 155.7[a,b)]
16–24	301.4 ± 34.0	4941.5 ± 82.2[a)]	2846.5 ± 47.4[a,b)]
% of dose	0.4 ± 0.1	13.4 ± 0.6	7.7 ± 0.3

Data are expressed as mean ± SD ($n = 6$).
a) $p < 0.05$ compared with group sesamol.
b) $p < 0.05$ compared with group sesamol glucuronide.

In urinary excretion (Table 1), sesamol glucuronide concentration was significantly greater than the concentrations of sesamol and sesamol sulfate. The highest concentrations of sesamol conjugated metabolites were found at 4–8 h after administration. The elimination of sesamol glucuronide was rapid at 0–4 h from urine. The majority of intact sesamol glucuronide was excreted in the urine. Thus, sesamol conjugated metabolites are primarily eliminated from the plasma *via* the kidney by active tubular secretion. On the other hand, sesamol sulfate appears to be mediated by an organic anion transport system with a high affinity for sesamol sulfate. The percentage of conjugated metabolites excreted in urine in rats during 0–24 h accounted for 13.4 ± 0.6 and 7.7 ± 0.3% of the total excretion for sesamol glucuronide and sesamol sulfate, respectively. Much smaller quantities of sesamol were excreted in the two subsequent 4–8 and 16–24 h periods. Urinary amounts of sesamol and its conjugated metabolites increased in response to sesamol ingestion, reaching a peak at 4–8 h. The measured amounts of sesamol were 109.4 ± 4.8 nmol/mL for the free form, 8426.2 ± 108.5 nmol/mL for sesamol glucuronide, and 4853.8 ± 62.5 nmol/mL for sesamol sulfate. The unchanged sesamol was present in very low concentrations and represented only 0.4 ± 0.1% up to 24 h after administration. Glucuronidation was the main metabolic pathway for sesamol in urine, and urinary elimination was a major route of elimination.

To investigate the elimination of sesamol in rat, we determined the concentration of sesamol and its conjugated metabolites in rat feces within 24 h after administration of sesamol. In the feces (Table 2) the concentrations of sesamol and conjugated metabolites reached a maximum at 4–8 h after administration. However, the concentration of free sesamol was significantly greater than its conjugated metabolites in the feces. The percentage of sesamol and sesamol glucuronide excreted in feces in rats was highest in

Table 2. Individual fecal excretion of sesamol and its metabolites during each time interval after oral administration of sesamol 100 mg/kg

Conc. (nmol/mL) Time (h)	Sesamol	Sesamol glucuronide	Sesamol sulfate
0–4	240.6 ± 135.8	121.4 ± 25.0	69.9 ± 14.4[a,b)]
4–8	635.5 ± 27.4	458.4 ± 127.7*	264.1 ± 73.5[a,b)]
8–12	312.3 ± 74.0	208.3 ± 114.7	120.0 ± 66.1[a)]
12–16	426.8 ± 136.6	218.4 ± 78.5	125.8 ± 45.2[a)]
16–24	510.9 ± 178.1	458.4 ± 137.6	264.1 ± 79.2[a,b)]
% of dose	1.2 ± 0.3	0.8 ± 0.3	0.5 ± 0.2

Data are expressed as mean ± SD ($n = 6$).
a) $p < 0.05$ Compared with group sesamol.
b) $p < 0.05$ Compared with group sesamol glucuronide.

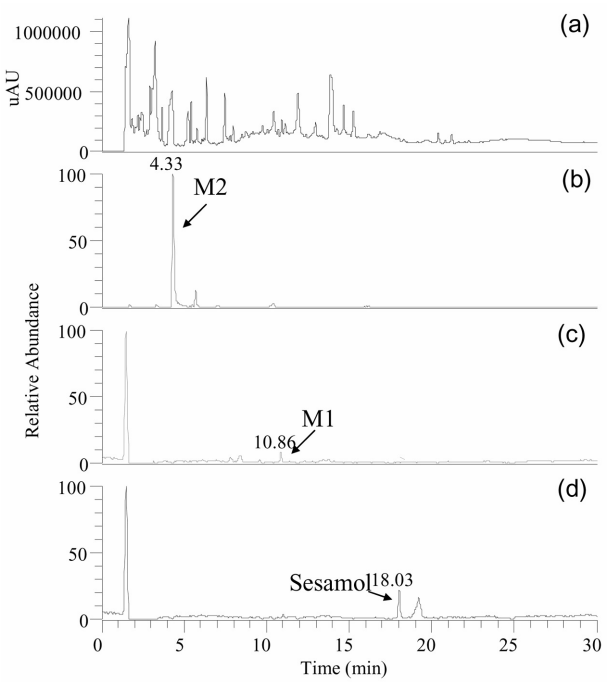

Figure 2. HPLC chromatogram and LC–SRM chromatograms of sesamol and its metabolites in rats. (a) UV at 280 nm, (b) SRM for sesamol metabolites (*m/z* 125, M2), (c) SRM for sesamol metabolites (*m/z* 139, M1), and (d) SRM for sesamol (*m/z* 139).

the first 24 h, accounting for 1.170 ± 0.304 and 0.806 ± 0.266% of the total excretion for sesamol and sesamol glucuronide, respectively.

3.3 Metabolism study

Besides the conjugated metabolites, other sesamol metabolites were observed and identified in rat urine based on their mass spectra and HPLC chromatograms, as shown in Fig. 2. In the analytical condition of LC-MS/MS, the full scan in negative ion modes (scan range from *m/z* 50 to 200) was used to identify the analyte. With SRM mass spectra for the determination of sesamol and its metabolites, as shown in Fig. 2. Panels a–c of Fig. 3 show the mass spectra of collected rat urine after sesamol administration (100 mg/kg, p.o.), with mass transitions of *m/z* 125 $[M-H]^- \rightarrow$ 107 $[M-H-18]^-$ for sesamol metabolite (M2), *m/z* 139 $[M-H]^- \rightarrow 124$ $[M-H-15]^-$ for sesamol metabolite (M1) and *m/z* 137 $[M-H]^- \rightarrow 109$ $[M-H-28]^-$ for sesamol, respectively (Table 3). Panels d–f of Fig. 3 show the mass spectra of authentic sesamol, benzene-1,2,4-triol and 2-methoxybenzene-1,4-diol, respectively. The metabolites were, therefore, identified as benzene-1,2,4-triol and 2-methoxybenzene-1,4-diol (Fig. 3).

For the quantitation of M1 and M2 in urine, the standard curves for these two metabolites were established. The linear regression analysis showed that the correlation coefficients of all standard curves were better than 0.993 over the range of 1–100 nmol. LOQ were calculated from the LODs with the LOQ being determined as five times the LOD. The LOQ ranged from 0.7 nmol for benzene-1,2,4-triol to 1.2 nmol for 2-methoxybenzene-1,4-diol. After sesamol administration, the sesamol metabolites could be detected in the urine. The urinary elimination of benzene-1,2,4-triol was determined to be 6.450 ± 0.121 nmol/16–24 h and of 2-methoxybenzene-1,4-diol to be 2.592 ± 2.201 nmol/16–24 h.

4 Discussion

In this study, we measured the concentration of sesamol and metabolites in rats to investigate: (i) sesamol and conjugated metabolites may be incorporated into the body, after which they are excluded to urine; (ii) the concentration of sesamol was significantly lower than sesamol conjugated metabolites in rat urine; (iii) sesamol may be converted to 2-methoxybenzene-1,4-diol and benzene-1,2,4-triol *in vivo* by rat.

Several studies have shown that phenolic compounds are able to induce phase II enzymes. At low doses the bulk of the phenols appear to be conjugated with sulfate or glucuro-

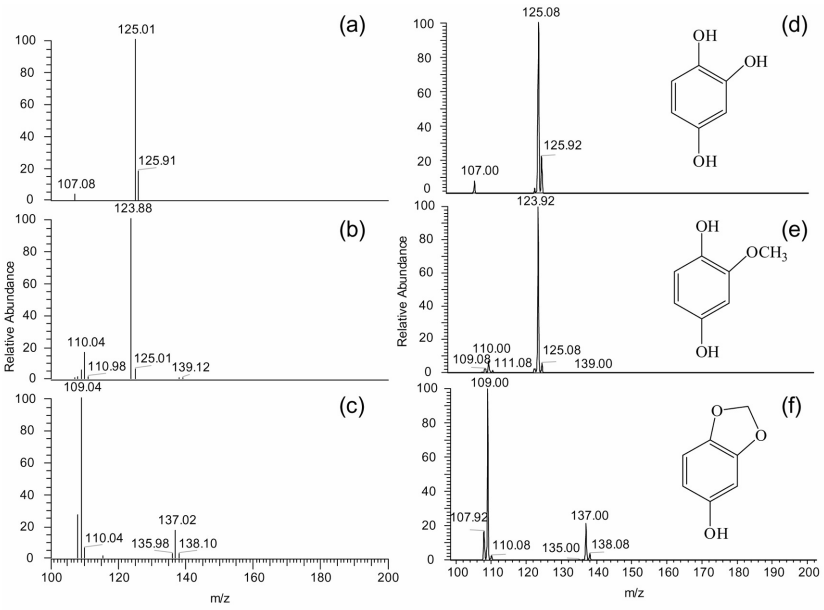

Figure 3. Mass spectra of sesamol metabolites in rats comparing with authentic compounds by LC-MS/MS. (a) Mass fragmentation spectrum of MS/MS for sesamol metabolites (*m*/*z* 125, M2), (b) Mass fragmentation spectrum of MS/MS for sesamol metabolites (*m*/*z* 139, M1), (c) Mass fragmentation spectrum of MS/MS for sesamol (*m*/*z* 137), (d) Mass fragmentation spectrum of MS/MS for benzene-1,2,4-triol (*m*/*z* 125), (e) Mass fragmentation spectrum of MS/MS for 2-methoxybenzene-1,4-diol (*m*/*z* 139), and (f) Mass fragmentation spectrum of MS/MS for sesamol (*m*/*z* 137).

Table 3. LC-MS/MS data of identified sesamol and its metabolites in rat urine

Compound	Metabolite	RT (min)	LC-MS/MS $[M-H]^-$ parent ion selection	Fragment ions, negatively charged (relative abundance)
Sesamol	–	17.9	137	137(18), 109(100)
	M1	10.9	139	139(2), 124(100), 110(19)
	M2	4.25	125	125(100), 107(14)

nide at the portal of entry. As the dose increases, the sulfation pathway becomes saturated, and the relative contribution of glucuronidation and oxidation reactions increases [27]. Urinary concentrations of phenols or their metabolites have been used as biomarkers to assess the prevalence of exposure to these compounds in the general population. Usually, total urinary concentrations of phenolic compounds including both free and conjugated (glucuronide and sulfate) forms are reported as biomarkers [28]. Numerous laboratory animal studies indicate that urinary elimination of sulfate and glucuronide conjugates of phenols accounts for most of the excretion, ranging from 70 to 90% of the administered dose within 24 h, whereas excretion in feces represents only a small fraction, approximately 1 – 3% of the administered dose [29 – 31]. Lesaffer [32] reported that an intravenous administration of *p*-cresol resulted in immediate and extensive metabolism of the compound into *p*-cresylglucuronide. The elimination of *p*-cresol from the body depends largely on the urinary excretion of this metabolite. Our results demonstrated that sesamol eliminated in feces and urine in conjugated forms, mainly glucurono-conjugates, suggesting extensive first-pass intestinal/hepatic metabolism of the ingested sesamol. Sesamol conjugated metabolites could be detected in the feces. This may be in light of enterohepatic recirculation, in which after re-absorption from the gut the newly formed conjugate was different from the one excreted through bile. Sesamol appears rapidly in urine, reaching maximum concentrations 4 – 8 h after sesamol ingestion. A second peak (16 – 24 h) in the time-course of its concentration in urine and feces suggested enterohepatic recirculation of sesamol conjugated metabolites. It was believed that deconjugation in the gastrointestinal tract by β-glucuronidase occurred (in the gut microflora) [33]. This is supported by the metabolite pattern in the feces where sesamol conjugated metabolites were found less than free form, comparatively. A proportion of sesamol undergo reabsorption from the intestine and were mainly transported to the liver for re-conjugation with glucuronic acid to form the corresponding glucuronides, which are then excreted *via* bile or urine.

In our study, two sesamol metabolites, 2-methoxybenzene-1,4-diol and benzene-1,2,4-triol were identified. Sesamol (benzo[d][1,3]dioxol-5-ol) may first undergo reductive cleavage of methylenedioxy ring into 2-methoxybenzene-1,4-diol (M1) or methylated in the catechol moiety of benzene-1,2,4-triol (M2) to M1, possibly by liver catechol-*O*-methyl transferase (Fig. 4). We observed very limited conversion of sesamol to M1 and M2 in the urine of rats fed sesamol. In addition, we did not detect any sesamol or its metabolite by *in vitro* fermentation with rat fecal microbiota (data not shown). Therefore, we hypothesized that sesamol is only partially metabolized in the body to M1 and M2; it is

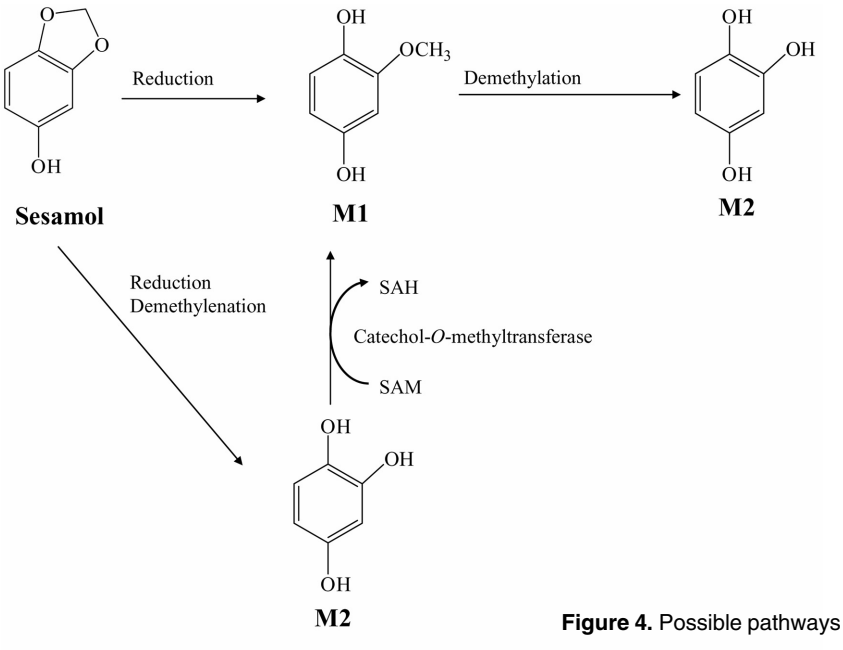

Figure 4. Possible pathways for the transformation of sesamol in rats.

absorbed and metabolized in the liver, which are then excreted in the bile or urine. These metabolites that are excreted in bile undergo enterohepatic circulation and may be metabolized by the CYP 450 enzyme. Furthermore, whilst the glucuronide is the predominate form in the urine, sesamol may represents the main form present in the excreta.

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The authors have declared no conflict of interest.

5 References

[1] Fukuda, Y., Nagata, M., Osawa, T., Namiki, M., Chemical aspects of the antioxidative activity of roasted sesame seed oil and the effect of using the oil for frying, *Agric. Biol. Chem.* 1986, *50*, 857 – 862.

[2] Fukuda, Y., Nagata, M., Osawa, T., Namiki, M., Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil, *J. Am. Oil Chem. Soc.* 1986, *63*, 1027 – 1031.

[3] Uchida, M., Nakajin, S., Toyoshima, S., Shinoda, M., Antioxidative effect of sesamol and related compounds on lipid peroxidation, *Biol. Pharm. Bull.* 1996, *19*, 623 – 626.

[4] Jacklin, A., Ratledge, C., Welham, K., Bilko, D., Newton, C. J., The sesame seed oil constituent, sesamol, induces growth arrest and apoptosis of cancer and cardiovascular cells, *Ann. N. Y. Acad. Sci.* 2003, *1010*, 374 – 380.

[5] Kim, J. Y., Choi, D. S., Jung, M. Y., Antiphotooxidative activity of sesamol in methylene blue- and chlorophyll-sensitized photooxidation of oil, *J. Agric. Food Chem.* 2003, *51*, 3460 – 3465.

[6] Wright, J. S., Johnson, E. R., DiLabio, G. A., Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants, *J. Am. Chem. Soc.* 2001, *123*, 1173 – 1183.

[7] Joshi, R., Adhikari, S., Patro, B. S., Chattopadhyay, S., Mukherjee, T., Free radical scavenging behavior of folic acid: Evidence for possible antioxidant activity, *Free Radical Biol. Med.* 2001, *30*, 1390 – 1399.

[8] Joshi, R., Kapoor, S., Mukherjee, T., Free radical reactions of pyridoxal (Vitamin B6): A pulse radiolysis study, *Res. Chem. Intermed.* 2002, *28*, 505 – 515.

[9] McPhail, D. B., Hartley, R. C., Gardner, P. T., Duthie, G. G., Kinetic and stoichiometric assessment of the antioxidant activity of flavanoids by electron spin resonance spectroscopy, *J. Agric. Food Chem.* 2003, *51*, 1684 – 1690.

[10] Mazziao, E. A., Harris, N., Soliman, K. F., Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells, *Planta Med.* 1998, *64*, 603 – 606.

[11] Tseng, T. H., Tsheng, Y. M., Lee, Y. J., Cytotoxicity effects of di- and tri-hydroxybenzaldehydes as a chemopreventive potential agent on tumor cells, *Toxicology* 2001, *161*, 179 – 187.

[12] Tagashira, M., Ohtake, Y., A new antioxidant 1,3-benzodioxole from *Melissa officinalis*, *Planta Med.* 1998, *64*, 555 – 558.

[13] Jurd, L., Narayana, V. L., Pauli, K. D., *In vivo* antitumor activity of 6-benzyl-1,3-benzodioxolederivatives against the P388, L1210, B16, and M5076 murine models, *J. Med. Chem.* 1987, *30*, 1752 – 1756.

[14] Mazzio, E. A., Harris, N., Soliman, K. F. A., Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells, *Planta Med.* 1998, *64*, 603 – 606.

[15] Kohler, H. P., Grant, P. J., Plasminogen activator inhibitor type-1 and coronary artery disease, *N. Engl. J. Med.* 2000, *342*, 1792 – 1801.

[16] Koenig, W., Haemostatic risk factors for cardiovascular diseases, *Eur. Heart J.* 1998, *19*, C39 – C43.

[17] Catto, A. J., Carter, A. M., Stickland, M., Bamford, J. M., *et al*., Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease, *Thromb. Haemost*. 1997, *77*, 730 – 734.

[18] Chen, P. R., Lee, C. C., Chang, H., Tsai, C. E., Sesamol regulates plasminogen activator gene expression in cultured endothelial cells: A potential effect on the fibrinolytic system, *J. Nutr. Biochem.* 2005, *16*, 59 – 64.

[19] United States Pharmacopeia (USP) Convention, *The Pharmacopoeia of the United States of America, The National Formulary*, 29th Revision, USP, Rockville, MD 2006, pp. 3171.

[20] Bhattarai, S., Tran, V. H., Duke, C. C., Stability of [6]-gingerol and [6]-shogaol in simulated gastric and intestinal fluids, *J. Pharm. Biomed. Anal.* 2007, *45*, 648 – 653.

[21] Fu, T. J., Abbott, U. R., Hatzos, C., Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid – a comparative study, *J. Agric. Food Chem.* 2002, *50*, 7154 – 7160.

[22] Hsiu, S. L., Tsao, C. W., Tsai, Y. C., Ho, H. J., Chao, P. D., Determinations of morin, quercetin and their conjugate metabolites in serum, *Biol. Pharm. Bull.* 2001, *24*, 967 – 969.

[23] de Boer, V. C., Dihal, A. A., van der Woude, H., Arts, I. C., *et al*., Tissue distribution of quercetin in rats and pigs, *J. Nutr.* 2005, *135*, 1718 – 1725.

[24] Talavera, S., Felgines, C., Texier, O., Besson, C., *et al*., Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain, *J. Agric. Food Chem.* 2005, *18*, 3902 – 3908.

[25] Graf, B. A., Ameho, C., Dolnikowski, G. G., Milbury, P. E., *et al*., Rat gastrointestinal tissues metabolize quercetin, *J. Nutr.* 2006, *136*, 39 – 44.

[26] Liu, Z., Saarinen, N. M., Thompson, L. U., Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed *in vitro* and in rats, *J. Nutr.* 2006, *136*, 906 – 912.

[27] Pang, K. S., Schwab, A. J., Goresky, C. A., Chiba, M., Transport, binding, and metabolism of sulfate conjugates in the liver, *Chem. Biol. Interact.* 1994, *92*, 179 – 207.

[28] Ye, X., Kuklenyik, Z., Needham, L. L., Calafat, A. M., Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.* 2005, *383*, 638 – 644.

[29] Edwards, V. T., Jones, B. C., Hutson, D. H., A comparison of the metabolic fate of phenol, phenyl glucoside and phenyl 6-*O*-malonyl-glucoside in the rat, *Xenobiotica* 1986, *16*, 801 – 807.

[30] Meerman, J. H. N., Nijland, C., Mulder, G. J., Sex differences in sulfation and glucuronidation of phenol, 4-nitrophenol and N-hydroxy-2-acetylaminoflourene in the rat *in vivo*, *Biochem. Phamacol.* 1987, *36*, 2605 – 2608.

[31] Hughes, M. F., Hall, L. L., Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica* 1995, *25*, 873 – 883.

[32] Lesaffer, G., De Smet, R., Belpaire, F. M., Van Vlem, B., *et al*., Urinary excretion of the uraemic toxin *p*-cresol in the rat: Contribution of glucuronidation to its metabolization, *Nephrol. Dial. Transplant.* 2003, *18*, 1299 – 1306.

[33] Gibson, G., Skett, P., Pathways of drug metabolism, *Introduction to Drug Metabolism*, 3rd Edn., Nelson Thornes, Cheltenham 2001, pp. 1 – 36.