Inhibition of 20-Hydroxyeicosatetraenoic Acid Synthesis Using Specific Plant Lignans
In Vitro and Human Studies

Jason H.Y. Wu, Jonathan M. Hodgson, Michael W. Clarke, Adeline P. Indrawan, Anne E. Barden, Ian B. Puddey, Kevin D. Croft

Abstract—Sesamin, the major lignan found in sesame, has been shown to increase vitamin E levels by inhibiting its metabolism via the cytochrome P450 isozyme CYP4F2. CYP4F2 and CYP4A11 are the predominant human isoforms that synthesize 20-hydroxyeicosatetraenoic acid (20-HETE) from arachidonic acid. Considerable evidence suggests that 20-HETE may play a role in the pathogenesis of hypertension. We hypothesized that sesamin could be an inhibitor of 20-HETE synthesis. This study investigated the effects of sesamin on 20-HETE synthesis in vitro and the effect of sesame supplementation on plasma and urinary 20-HETE concentrations in humans. Human microsomes were used to investigate the potency and selectivity of sesamin inhibition of 20-HETE synthesis. Sesamin inhibited human renal and liver microsome 20-HETE synthesis with IC50 <20 μmol/L. It was selective toward CYP4F2 (IC50: 1.9 μmol/L) and had reduced activity toward CYP4A11 (IC50: >150 μmol/L), as well as cytochrome P epoxygenation of arachidonic acid (IC50: >50 μmol/L). In a randomized, controlled crossover trial, overweight men and women (n=33) consumed 25 g/d of sesame (∼50 mg/d of sesame lignan) or an isocaloric matched control for 5 weeks each. Relative to control, sesame supplementation resulted in a 28% decrease in plasma and a 32% decrease in urinary 20-HETE (P<0.001). Urinary sodium, potassium, and blood pressure were not affected. This study demonstrates for the first time that sesame supplementation in humans reduces the plasma and urinary levels of 20-HETE, likely via inhibition of CYP4F2 by sesame lignans. These results suggest that sesame lignans could be used for the investigation of potential roles of 20-HETE in humans. (Hypertension. 2009;54:1151-1158.)

Key Words: 20-HETE • cytochrome P450 • sesame • vitamin E • cardiovascular disease

The mono-oxygenation of arachidonic acid by cytochrome P450 enzymes is recognized as an important metabolic pathway.1 The major products generated via this enzymatic conversion include 20-hydroxyeicosatetraenoic acid (20-HETE) and regioisomeric forms of epoxyeicosatrienoic acids (EETs).2 These arachidonic acid metabolites possess physiological functions that may influence the development of kidney diseases and hypertension.2 20-HETE, formed via ω-hydroxylation of arachidonic acid, has been a focus of recent investigations that suggest that it plays a key role in the regulation of vascular and renal function. 20-HETE acts as a vasoconstrictor in renal, cerebral, and mesenteric arteries.3 Furthermore, it has been suggested to mediate pressure natriuresis by inhibiting sodium reabsorption in the renal proximal tubule.3 20-HETE, thus, possesses activities that could be prohypertensive or antihypertensive depending on its site of production, and this has been supported by animal models of hypertension.4,5

Sesame seeds are a popular and commonly consumed food.6 The abundance of unique lignans in sesame seeds has been suggested to contribute to health benefits. The predominant lignan in sesame is sesamin (Figure 1). One biological activity of sesamin that has been the target of several recent investigations is its ability to modulate γ-tocopherol metabolism. γ-Tocopherol is a major dietary isof orm of vitamin E, and human intervention studies have demonstrated that a higher dietary intake of sesame (as seeds or oil) leads to elevated serum γ-tocopherol concentrations.6,7 In humans, a major pathway of γ-tocopherol metabolism is via conversion to carboxyethyl hydroxychromans (CEHCs), which are water soluble metabolites excreted in the urine.8 This metabolic pathway is initiated by the ω-hydroxylation of γ-tocopherol by cytochrome P (CYP). It has been shown that the major CYP isoform responsible for γ-tocopherol hydroxylation is CYP4F2. Sesamin has been shown in vitro studies to inhibit CYP4F2 hydroxylation of γ-tocopherol.9 This likely
leads to a decrease in γ-tocopherol metabolism, thus providing a potential mechanistic explanation for the observed increase in γ-tocopherol concentration after sesame supplementation.6,7

The CYP4F subfamily of enzymes has gained recent recognition for its important role in eicosanoid and drug metabolism.10 In particular, several recent studies have suggested a potential association between CYP4F2 and hypertension through the production of 20-HETE.11,12 It has been shown that CYP4F2 is responsible for the majority of 20-HETE synthesis in human liver and kidney, with CYP4A11 being the other isoform contributing toward 20-HETE synthesis.13,14 Previous studies have demonstrated that sesamin inhibits CYP4F2 catalyzed γ-tocopherol hydroxylation.8 We, therefore, hypothesized that this lignan may also inhibit 20-HETE synthesis in humans. In the current study, we carried out in vitro studies to characterize the potency and specificity of sesamin toward CYP-catalyzed formation of 20-HETE. We also carried out a human intervention study to investigate the effect of sesame supplementation on plasma 20-HETE levels and urinary excretion of 20-HETE. We additionally measured urinary excretion of γ-CEHC, the major metabolite of γ-tocopherol, as an additional surrogate measure of in vivo CYP4F2 activity.

**Subjects and Methods**

### Sesamin Inhibition of 20-HETE Synthesis: In Vitro Experiments

**Microsome Experiments**

The ability of sesamin and structurally related lignans to inhibit eicosanoid synthesis was examined in isolated microsomes. Pooled human liver (n=50; mixed gender) and kidney (n=8; mixed gender) microsomes were prepared by Xenotech LLC (Lenexa). Microsomes prepared from baculovirus-infected insect cells that express recombinant CYP4F2 and 4A11 were obtained from BD Gentest. Inhibitions of the conversion of arachidonic acid to oxygenated metabolites were assayed according to Lasker et al.13 Briefly, microsomes (0.1 to 0.5 mg of protein) were preincubated for 5 minutes at 37°C with arachidonic acid (100 μmol/L) and various concentrations of plant lignans or vehicle (as control) in 200 μL of 100 mmol/L of potassium phosphate buffer (pH 7.4). Reactions were initiated with 1 mmol/L of NADPH. Depending on the microsome system, the reaction mixture was incubated for between 8 and 40 minutes and terminated with 20 μL of 2 N HCl. The protein concentrations and incubation times chosen for each type of microsome were shown in optimization assays to be within the range for which product formation was linear. The mixture was extracted with 1 mL of ethyl acetate. The extract was dried under nitrogen and analyzed by liquid chromatography-mass spectrometry.

**Mass Spectrometry Analysis**

Eicosanoids generated from in vitro microsomal experiments were analyzed by high-performance liquid chromatography. Thermo
Fisher Accela Pump and autosampler interfaced to a triple-quadrupole mass spectrometer (Thermo-Fisher TSQ Quantum Ultra). Analytes were eluted using acetonitrile/water/formic acid (37.00:63.00:0.02; solvent A) and acetonitrile/isopropanol (50:50; solvent B) in a gradient as follows (0 minutes, 0% B; 6.0 minutes, 33% B; 15.0 minutes, 33% B; 15.5 minutes, 100% B; 17.5 minutes, 100% B; 17.6 minutes, 0% B; and 19.6 minutes, 0% B). Negative ions were generated using Heated Electrospray Ionization. The autotune function of the instrument was used to optimize the source conditions and ion optics for maximum sensitivity using eicosanoid standards (Cayman Chemical) before sample analysis. Quantitative analyses were obtained by the stable isotope dilution method using ion transitions, as described. Data from the in vitro microsome inhibition assays are expressed as the percentage of the control activity. Curve fitting and IC50 estimation were carried out by nonlinear regression using Prism (version 4, GraphPad Software).

### Human Intervention Study

**Study Population**

Overweight (body mass index: &gt;25 kg/m²) men and postmenopausal women were recruited from the general population. Details of the study population and study design have been published previously. Briefly, volunteers were included if they had ≥1 of the risk factors for the metabolic syndrome, as defined by the American Heart Association Adult Treatment Panel III criteria, or if they had for the metabolic syndrome, as defined by the American Heart Association Adult Treatment Panel III criteria. If any of the volunteers provided written informed consent, and the study was approved by the University of Western Australia Human Ethics Committee. Thirty-three volunteers completed the study.

**Study Design**

This was a randomized, placebo-controlled crossover study. After an initial 4-week washout period, half of the volunteers replaced their usual breakfast with a sesame breakfast bar and the other half with a placebo breakfast bar for 5 weeks. At the end of the first treatment period, volunteers had another 4 weeks of washout (during which time the volunteers reverted to their usual breakfast intake). The volunteers then commenced a second 5-week treatment period where they replaced their breakfast with the alternative bar. The order of treatment was randomized via permutated block randomization, using computer-generated random numbers. Volunteers were advised to maintain their lifestyle, including diet and physical activity, throughout the study period. Volunteers visited the study center on the morning before the start of each treatment period, as well as the morning after the end of each treatment period, where fasting blood and 24-hour urine samples were collected. They were also fitted with an ambulatory blood pressure monitor, as described previously, which was worn during awake hours.

### Supplements

The composition of the bars has been published previously (Figure S1, please see the online Data Supplement at http://hyper.ahajournals.org). During the sesame supplementation, the volunteers received 60 g of each bar per day, with the sesame bar providing 26.2 g of sesame seeds per day, and the placebo bars matched for energy, macronutrient, vitamin E, and sodium composition. Sesamin content of the seeds used in this study (151 mg/100 g of seeds) was comparable to the mean value reported previously for 65 natural sesame seed samples (163 mg/100 g).

### Biochemical Measurements

All of the biochemical measurements were carried out in a blinded fashion. Plasma unesterified 20-HETE, urinary 20-HETE, and vitamin E metabolites were measured using gas chromatography-mass spectrometry, as described previously. Internal standards were added to plasma and urine samples before extraction. Plasma arachidonic acid was measured by gas chromatography, and plasma tocopherols were measured by high-performance liquid chromatography with electrochemical detection following published methods.

### Statistical Analysis

Data are shown for participants who completed the study (n=33). Examination of the relationship between the variables. Nonnormally distributed data were log-transformed before analysis and are presented as geometric mean (95% CI). All of the outcome data from the clinical study were analyzed using linear mixed-models (PROC MIXED, version 9.1; SAS Institute Inc). Subjects were included as a random factor nested under treatment sequence within a linear mixed model. The following factors were always included as fixed effects in the model: baseline values, treatment, period, and treatment sequence. Differences were considered significant when P&lt;0.05.

### Results

#### In Vitro Inhibition of Eicosanoid Synthesis by Sesame Lignans

We initially examined the ability of sesame lignans to inhibit 20-HETE synthesis in human renal microsomes. We found that sesame lignans inhibited 20-HETE synthesis in a dose-dependent manner, with IC50 values of 5.31 µmol/L and 3.39 µmol/L for sesamin and sesamolin (another sesame lignan with similar structure to sesamin), respectively (Figure 2A). Because sesame lignans are precursors to the mammalian lignans, enterolactone and enterodiol, we also examined
the inhibitory activities of these compounds. In contrast to their precursors, mammalian lignans had comparatively much weaker inhibitory activity (Figure 2A, at 50 \( \mu \text{mol/L} \), enterolactone and enterodiol inhibited 20-HETE production by 27\(\pm\)4.3\% and 19\(\pm\)1.6\%, respectively). To investigate the structural features of sesame lignans, which may be related to their inhibitory activity, we further examined 2 other structurally related dietary lignans found in flaxseeds, pinoresinol and secoisolariciresinol. As shown in Figure 2A, both compounds exhibited minimal activity for the inhibition of 20-HETE synthesis.

We also investigated the inhibitory activity of sesame lignans in human liver microsomes, because both major 20-HETE producing CYPs (4F2 and 4A11) are also expressed in the human liver.\(^{14}\) Furthermore, human liver microsomes generate EET, the other major class of eicosanoids generated by CYP enzymes.\(^{22}\) This allowed for the investigation of whether sesame lignans demonstrate differential inhibitory activity toward the \( \omega \)-hydroxylation and epoxidation pathways. Both sesamin and sesamolin dose-dependently inhibited 20-HETE synthesis in human liver microsomes (IC\(_{50}\) values of 17.3 \( \mu \text{mol/L} \) and 9.6 \( \mu \text{mol/L} \), respectively; Figure 2B). Mixed human liver microsomes produced regioisomeric 14,15-, 11,12-, and 8,9-EET, as well as their respective dihydroxyeicosatrienoic acid. Total epoxygenase activity was calculated as the sum of all EETs and dihydroxyeicosatrienoic acid. We found that sesame lignans showed weaker inhibitory activity toward EET synthesis (at 50 \( \mu \text{mol/L} \), sesamin and sesamolin inhibited epoxygenase activity by 47\(\pm\)2.1\% and 35\(\pm\)4.5\%, i.e., IC\(_{50}\) =50 \( \mu \text{mol/L} \)).

Finally, we determined the specificity of sesamin toward the 2 major human 20-HETE CYP synthases, CYP4F2 and CYP4A11. We focused on sesamin, because it is the major lignan found in sesame. As shown in Figure 3, sesamin strongly inhibited 20-HETE synthesis in microsomes expressing human CYP4F2 (IC\(_{50}\) \( = 1.87 \mu \text{mol/L} \)). Conversely, it only weakly inhibited CYP4A11-catalyzed 20-HETE production with IC\(_{50}\) >150 \( \mu \text{mol/L} \) (Figure 3).

**Human Supplementation Study**

**Participant Characteristics**

Subject baseline profiles are shown in Table 1. Five subjects were taking antihypertensive medication, and 4 had elevated fasting glucose levels. None of the volunteers were taking hypoglycemic medication. We were careful to ensure that each subject's medication status remained unchanged throughout the study. None of the subjects reported any adverse effects from the supplement bars. Subject compliance was 99.8\(\pm\)0.7\% and 98.3\(\pm\)3.3\% for sesame and placebo bars, respectively, on the basis of counting the number of bars returned at the end of each treatment period. As reported previously, sesame treatment did not lead to any changes to blood lipids, blood pressure, glucose, or circulating inflammatory and oxidative stress markers.\(^{16}\)

**Tocopherol Metabolism**

Compared with placebo, sesame supplementation led to an \( \approx \)20\% increase in serum \( \gamma \)-tocopherol (\( P=0.009; \) Table 2). This result was unaltered by correction for serum lipid concentrations. To examine whether sesame supplementation inhibited the metabolism of \( \gamma \)-tocopherol via the CYP pathway, we measured the excretion of water-soluble metabolites in the urine. Sesame supplementation led to a significant

![Figure 3. Effect of sesamin on the formation of 20-HETE in microsomes prepared from baculovirus-infected insect cells that express recombinant CYP4F2 and 4A11. Results are expressed as the percentage of inhibition vs control, and each point represents a mean\(\pm\)SEM from 3 independent experiments.](http://hyper.ahajournals.org/doi/10.1161/JAHA.109.002215)
Table 2. Serum γ-Tocopherol and Urine γ-CEHC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sesame Before</th>
<th>Sesame After</th>
<th>Placebo Before</th>
<th>Placebo After</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-Tocopherol, μmol/L</td>
<td>2.40 ± 0.69</td>
<td>2.80 ± 0.93</td>
<td>2.28 ± 0.72</td>
<td>2.25 ± 0.81</td>
<td>0.009</td>
</tr>
<tr>
<td>γ-Tocopherol/lipids, μmol/mmol†</td>
<td>0.33 ± 0.09</td>
<td>0.38 ± 0.10</td>
<td>0.30 ± 0.09</td>
<td>0.31 ± 0.10</td>
<td>0.009</td>
</tr>
<tr>
<td>γ-CEHC/creatinine, μmol/mmol§</td>
<td>0.52 (0.42 to 0.63)</td>
<td>0.36 (0.31 to 0.42)</td>
<td>0.50 (0.41 to 0.60)</td>
<td>0.67 (0.53 to 0.84)</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine, mmol/L§</td>
<td>5.59 (4.62 to 6.75)</td>
<td>5.95 (4.97 to 7.13)</td>
<td>5.68 (4.66 to 6.92)</td>
<td>6.18 (5.00 to 7.64)</td>
<td>0.578</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD unless otherwise described. *Data show the comparison of after-treatment means by mixed-model analysis. †Serum γ-tocopherol is expressed normalized to lipids, which are calculated as total cholesterol plus triglycerides. §Urinary γ-CEHCs are expressed normalized to creatinine excretion. ¶Data were log-transformed before statistical analysis and are presented as geometric mean (95% CI).

Table 3. Plasma, Urinary 20-HETE, and Urinary Electrolytes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sesame Before</th>
<th>Sesame After</th>
<th>Placebo Before</th>
<th>Placebo After</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma arachidonic acid, mmol/L</td>
<td>0.27 ± 0.06</td>
<td>0.27 ± 0.05</td>
<td>0.28 ± 0.06</td>
<td>0.27 ± 0.06</td>
<td>0.783</td>
</tr>
<tr>
<td>Plasma 20-HETE, pmol/μmol†</td>
<td>832 (714 to 969)</td>
<td>596 (525 to 677)</td>
<td>830 (720 to 958)</td>
<td>768 (663 to 890)</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma 20-HETE/AA, pmol/μmol†‡</td>
<td>3.13 (2.66 to 3.68)</td>
<td>2.25 (1.98 to 2.55)</td>
<td>3.10 (2.68 to 3.58)</td>
<td>2.96 (2.52 to 3.48)</td>
<td>0.002</td>
</tr>
<tr>
<td>Urine 20-HETE/creatinine, pmol/mmol§</td>
<td>148 (125 to 175)</td>
<td>101 (84 to 120)</td>
<td>149 (125 to 176)</td>
<td>153 (129 to 182)</td>
<td>0.001</td>
</tr>
<tr>
<td>Urine sodium/creatinine, mmol/mmol§</td>
<td>12.2 ± 3.9</td>
<td>12.4 ± 4.4</td>
<td>12.3 ± 4.1</td>
<td>11.9 ± 4.3</td>
<td>0.556</td>
</tr>
<tr>
<td>Urine potassium/creatinine, mmol/mmol§</td>
<td>6.8 ± 2.4</td>
<td>6.3 ± 2.2</td>
<td>7.2 ± 2.7</td>
<td>6.2 ± 2.3</td>
<td>0.227</td>
</tr>
<tr>
<td>SBP†</td>
<td>132.8 ± 12.2</td>
<td>132.1 ± 11.9</td>
<td>134.2 ± 11.9</td>
<td>133.4 ± 13.7</td>
<td>0.835</td>
</tr>
<tr>
<td>DBP†</td>
<td>80.7 ± 8.1</td>
<td>81.0 ± 7.2</td>
<td>82.0 ± 7.5</td>
<td>80.8 ± 8.4</td>
<td>0.223</td>
</tr>
</tbody>
</table>

AA indicates arachidonic acid; SBP, systolic blood pressure; DBP, diastolic blood pressure. Data are presented as mean ± SD except where indicated. *Data show a comparison of after-treatment means by mixed-model analysis. †Data were log-transformed before statistical analysis and are presented as geometric mean (95% CI). ‡Data were log-transformed before statistical analysis and are presented as geometric mean (95% CI). §Urinary 20-HETE, sodium, and potassium are expressed normalized to creatinine excretion. ¶Mean awake blood pressure was measured by ambulatory blood pressure monitor.

Discussion

20-HETE influences cardiovascular and renal function and has been suggested to play a major role in the development of hypertension. The recent development of selective inhibitors of 20-HETE formation has enabled improved elucidation of 20-HETE bioactivities in animal models of hypertension. However, to the best of our knowledge, there are currently no such analogous specific inhibitors suitable for administration to humans. This motivated our investigation into the potential of sesame-derived lignans for the inhibition of the 20-HETE synthesis.

Sesamin and sesamolin inhibited human renal and liver microsome 20-HETE synthesis. Although the estimated inhibitory potency of these lignans (IC50 in the low micromolar range) is relatively weak compared with synthetic inhibitors, such as N-Hydroxy-N-(4-butyl-2-methylphenyl)-formamidine (HET0016; IC50 in the nanomolar range), our human study suggests that in vivo concentrations of sesame lignans are sufficient to reduce levels of circulating and excreted 20-HETE after supplementation. Our data also demonstrate that conversion of sesame lignans to the mammalian lignans resulted in a substantial loss in inhibitory activity, highlighting that metabolic transformation of micronutrients can have profound effects on their biological activities.
20-HETE inhibition by structurally related lignans, such as secoisolariciresinol, is consistent with literature that the methylenedioxyphenyl moiety of sesamin is the key structural feature enabling the inhibition of CYP enzymes (Figure 1). Although recent intervention studies have suggested that flaxseeds (rich in secoisolariciresinol) and sesame seeds (rich in sesamin) are similar in their potential to increase mammalian lignans after supplementation, our data suggest important differences in bioactivities between these precursor compounds in the lignan family.

We also examined the effect of sesame lignans on the production of EET. These metabolites have been shown to possess renal vasodilatory and natriuretic activities and are suggested to have antihypertensive effects. We found that sesame lignans exhibited low inhibitory activity toward EET synthesis with IC_{50} values that are ≥3-fold higher compared with the 20-HETE pathway, suggesting relative selectivity toward 20-HETE inhibition. With regard to the major 20-HETE-producing CYP, we showed that in vitro sesamin was a highly selective inhibitor for CYP4F2 and had relatively minimal inhibitory effect on CYP4A11. This is in contrast to recently developed synthetic 20-HETE–selective inhibitors, such as TS-011 and HET0016, which have similar inhibitory activity toward CYP4F2 and 4A11. This suggests that sesamin may be a useful agent for the delineation of respective contributions made by the CYP4F2 and CYP4A11-20-HETE pathways to various physiological states, by allowing a relatively selective inhibition of CYP4F2-20-HETE synthesis.

Previous studies have related a decrease in alcohol and an increase in salt intake (major changes to salt balance achieved) to increased blood pressure in this study involving overweight subjects. However, these results were not unexpected, given the study subjects were ambulatory and no attempts were made to control their salt balance, and urinary electrolytes measurements were made in 24-hour urine collections. Future studies with specific designs are needed to assess whether 20-HETE inhibition after sesame supplementation is associated with alterations in natriuresis in humans.

As reported previously, blood pressure was also unchanged after sesame supplementation in our study. Supplementation with purified sesamin has been shown to reduce blood pressure in animal models of hypertension, possibly related to the ability of sesamin to reduce vascular superoxide production. In Japanese subjects with moderate hypertension, supplementation with 60 mg/d of sesamin for 4 weeks also reduced blood pressure. In these studies, the effect of sesamin supplementation on the 20-HETE pathway was not examined, but our study describes a biological activity of sesame lignans that could provide a potential explanation for the observations. In animal models of hypertension, administration of specific 20-HETE inhibitors, such as HET0016, led to ≥90% reduction in urine levels of 20-HETE, with concomitant changes in blood pressure. It is possible that sesame supplementation in our human study did not result in a sufficient reduction in levels of 20-HETE to affect blood pressure. Furthermore, because 20-HETE possesses prohypertensive or antihypertensive effects depending on its site of synthesis, systemic inhibition of 20-HETE levels after sesame supplementation could have resulted in counteracting effects on BP regulation. We also cannot exclude the possibility that sesame supplementation reduces levels of EET, which may again oppose the physiological effects of decreasing 20-HETE. Additional studies need to investigate whether greater inhibition of 20-HETE synthesis may be achieved using higher doses of sesame seeds or purified sesamin and the possible effect that this has on blood pressure, as well as additional in vivo markers of vascular function, such as flow-mediated vasodilation.

Perspectives
This study demonstrates for the first time that sesame supplementation in humans can lead to decreased plasma levels and urinary excretion of 20-HETE. We further provide evidence that this bioactivity is likely attributed to direct inhibition of CYP4F2, one of the major 20-HETE–producing enzymes in humans, by sesamine lignans. Although urine and plasma 20-HETE levels reduced by ≥30%, there were no changes in sodium excretion or blood pressure in this study involving overweight subjects.
A major hindrance in the investigation of 20-HETE functionality in humans has been the lack of suitable inhibitors, and previous investigations have relied heavily on assessing correlations between urinary 20-HETE excretion and clinical parameters. Sesame is a natural product that is safe for human consumption. We propose that supplementation with sesame (or purified sesame lignan) could be incorporated into future human studies designed specifically to investigate the role of 20-HETE in renal function and its vascular properties. In particular, using sesame to selectively inhibit CYP4F2-20-HETE synthesis in targeted populations (eg, those with CYP4F2 functional polymorphisms and/or untreated hypertension) may yield further insights into the biology of this important pathway.

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Disclosures

None.

References

4. Hoagland KM, Flasch AK, Roman RJ. Inhibitors of 20-HETE formation selectively inhibit CYP4F2-20-HETE synthesis in targeted populations (eg, those with CYP4F2 functional polymorphisms and/or untreated hypertension) may yield further insights into the biology of this important pathway.
5. Zhang Y, Wu JH, Vickers JJ, Ong SL, Temple SE, Mori TA, Croft KD, Mori T. Sesame ingestion with sesame (or purified sesame lignan) could be incorporated into future human studies designed specifically to investigate the role of 20-HETE in renal function and its vascular properties. In particular, using sesame to selectively inhibit CYP4F2-20-HETE synthesis in targeted populations (eg, those with CYP4F2 functional polymorphisms and/or untreated hypertension) may yield further insights into the biology of this important pathway.


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Inhibition of 20-hydroxyeicosatetraenoic acid synthesis using specific plant lignans: *in vitro* and human studies$^{1,2}$

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### S1. Contents of nutrients in sesame and placebo bars

<table>
<thead>
<tr>
<th></th>
<th>Placebo Bar</th>
<th>Sesame Bar</th>
</tr>
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<tbody>
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<td><strong>Unit/60g</strong></td>
<td></td>
<td></td>
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<tr>
<td>Total energy (kJ)</td>
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<td>1171</td>
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<tr>
<td>Protein (g)</td>
<td>7.2</td>
<td>6.9</td>
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<tr>
<td>Total Fat (g)</td>
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<td>14.9</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>30.3</td>
<td>29.3</td>
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<tr>
<td>Dietary Fibre (g)</td>
<td>3.2</td>
<td>3.0</td>
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<tr>
<td>Sodium (g)</td>
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<td>0.44</td>
</tr>
<tr>
<td>α-tocopherol (mg)</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>γ-tocopherol (mg)</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Sesamin (mg)</td>
<td>-</td>
<td>39.5</td>
</tr>
<tr>
<td>Sesamolin (mg)</td>
<td>-</td>
<td>12.2</td>
</tr>
</tbody>
</table>
Scatter-plot between changes in urine 20-HETE excretion and changes in γ-CEHC excretion following sesame and placebo supplementation. Both 20-HETE and γ-CEHC concentrations are adjusted by creatinine excretion and expressed as pmol/mmol. Pearson correlation coefficients were used to quantify relations between the variables. * Estimated r = 0.507 in the sesame group (P = 0.006) whereas in the placebo group r = -0.136, (P = 0.499).