Influence of virgin coconut oil-enriched diet on the transcriptional regulation of fatty acid synthesis and oxidation in rats – a comparative study

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Abstract

The present study was carried out to evaluate the effects of virgin coconut oil (VCO) compared with copra oil, olive oil and sunflower-seed oil on the synthesis and oxidation of fatty acids and the molecular regulation of fatty acid metabolism in normal rats. Male Sprague–Dawley rats were fed the test oils at 8% for 45 d along with a synthetic diet. Dietary supplementation of VCO decreased tissue lipid levels and reduced the activity of the enzymes involved in lipogenesis, namely acyl CoA carboxylase and fatty acid synthase (FAS) \( (P<0.05) \). Moreover, VCO significantly \( (P<0.05) \) reduced the \textit{de novo} synthesis of fatty acids by down-regulating the mRNA expression of FAS and its transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c; 68 kDa), compared with the other oils. VCO significantly \( (P<0.05) \) increased the mitochondrial and peroxisomal \( \beta \)-oxidation of fatty acids, which was evident from the increased activities of carnitine palmitoyl transferase I, acyl CoA oxidase and the enzymes involved in mitochondrial \( \beta \)-oxidation; this was accomplished by up-regulating the mRNA expression of PPAR\( \alpha \) and its target genes involved in fatty acid oxidation. In conclusion, the present results confirmed that supplementation of VCO has beneficial effects on lipid parameters by reducing lipogenesis and enhancing the rate of fatty acid catabolism; this effect was mediated at least in part via PPAR\( \alpha \)-dependent pathways. Thus, dietary VCO reduces the risk for CHD by beneficially modulating the synthesis and degradation of fatty acids.

Key words: Virgin coconut oil; Fatty acid synthesis; \( \beta \)-Oxidation; PPAR\( \alpha \)

CVD, which is often associated with hyperlipidaemia, has become the major cause of death in many countries\(^{1,2}\). Elevated peripheral NEFA and \textit{de novo} lipogenesis predominantly contribute to the accumulation of lipids in the liver\(^{3}\), which is mostly controlled by the intake of different dietary fats through changes in hepatic enzyme activities related to the metabolism of fatty acids\(^{4}\). In general, fat accumulation in the body may be considered as the net result of the balance among dietary absorbed fat, endogenous synthesis of fat and the catabolism of fat via \( \beta \)-oxidation.

It has been shown that different fatty acids exert different effects on the transcription of specific genes involved in hepatic lipid metabolism\(^{5,6}\). Transcriptional regulation of fatty acid metabolism is considered to be the major regulatory mechanism controlling lipid homeostasis, which is executed by a variety of transcription factors\(^{7}\). Sterol response element-binding protein-1c (SREBP-1c; 68 kDa) is a transcription factor known to be the major regulator of lipogenic enzymes\(^{8}\) and reduced expression of SREBP-1c is a primary mechanism by which dietary fatty acids reduce hepatic lipogenesis. In contrast with the numerous factors involved in fatty acid synthesis and storage, the transcriptional regulation of fatty acid oxidation has long been dominated by PPAR\( \alpha \), which is a nuclear receptor activated by natural ligands such as fatty acids and stimulates \( \beta \)-oxidation of SFA in mitochondria and PUFA in the peroxisomes\(^{9}\). Some of the enzymes of these two systems are highly inducible by natural and synthetic ligands of PPAR\( \alpha \)\(^{10,11}\).

Nutritional status, particularly the dietary fatty acid pattern of the individual, plays a key role in regulating fatty acid metabolism\(^{12}\). The diversity in fatty acid structure resulting from differences in chain length, degree of unsaturation, position and stereoisomeric configuration of the double bonds may affect the rate of fatty acid oxidation. Although the usual dietary fats composed of mainly long-chain fatty acids are known to be powerful inhibitors of lipogenesis in the liver and adipose tissue\(^{13}\), paradoxically they produce obesity\(^{14}\).

Coconut oil extracted from dried copra is the characteristic cooking medium in most of the culinary preparations in all coconut-producing countries. Nowadays, virgin coconut oil (VCO) extracted from fresh coconut kernel by wet processing is

\textbf{Abbreviations}: ACC, acetyl CoA carboxylase; ACDH, acyl CoA dehydrogenase; ACO, acyl CoA oxidase; CO, copra oil; CPT I, carnitine palmitoyl transferase I; FAS, fatty acid synthase; MSE, mannitol–sucrose–EDTA; OO, olive oil; SFO, sunflower-seed oil; SIRT1, sirtuin 1; SREBP-1c, sterol regulatory element-binding protein-1c; VCO, virgin coconut oil.

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becoming very valuable because of its biological properties. Individual fatty acid analysis of the two types of coconut oil has revealed the presence of higher amounts of short- and medium-chain TAG, mainly lauric acid\(^{15}\). The major advantage of medium-chain fatty acids in coconut oil is that they are more rapidly oxidised by both mitochondrial and peroxisomal pathways compared with MUFA and PUFA\(^{16}\). Moreover, VCO extraction by wet processing retains higher amounts of unsaponifiable components such as polyphenols, tocopherols and phytosterols\(^{17,18}\). Previous studies have reported that VCO possesses significant hypolipidemic, antioxidant and antithrombotic effects compared with copra oil (CO)\(^{15,17,19}\). In addition, our recent reports have revealed that VCO has beneficial effects on regulating hepatic lipid metabolism in vivo\(^{20}\).

In view of these findings, the present study was carried out to evaluate the effects of VCO compared with CO, oleate-rich olive oil (OO) and linoleate-rich sunflower-seed oil (SFO) on the synthesis and oxidation of fatty acids and its effect on the transcriptional regulation of fatty acid metabolism in rats.

**Materials and methods**

**Chemicals**

Acetyl CoA, malonyl CoA, NAD phosphate, l-carnitine, ATP, TRI reagent and primers used for RT-PCR were obtained from Sigma Chemicals. cDNA synthesis and PCR reactions were performed using a kit purchased from Fermentas, Thermo Fisher Scientific Inc. and all other chemicals used were of the highest analytical grade.

**Extraction of virgin coconut oil and copra oil**

The solid endosperm of mature coconut (West coast tall variety) was crushed, made into viscous slurry and squeezed through cheesecloth to obtain coconut milk, which was refrigerated for 48 h, then subjected to mild heating (50°C) in a thermostat oven. The obtained VCO filtered through cheesecloth was used for the present study\(^{17}\). CO was extracted from coconut meat, which was dried in sunlight continuously for 4 d to remove moisture and the resulting copra was pressed in a mill to obtain CO\(^{17}\).

**Olive oil and sunflower-seed oil**

OO (Pietro Coricelli brand) and SFO (Sun drop brand) were purchased from a local market.

**Animals**

Male Sprague–Dawley rats \((100–120 \text{ g})\) bred in our departmental animal house were used for the study. Animals were individually housed under hygienic conditions in polypropylene cages in a room maintained at an ambient temperature of 25 ± 1°C with a 12h light–12h dark cycle. The entire protocol was approved by the Animal Ethics Committee, University of Kerala (IAEC-KU-8/09-10-BC-TR [17]) and the animals were handled using welfare guidelines for laboratory animals.

**Experimental design**

A total of twenty-four rats were divided into four groups. The experimental design was as follows: group I rats were given 8% CO; group II rats were given 8% VCO; group III rats were given 8% OO; and group IV rats were given 8% SFO. Each rat was given 12 g synthetic diet containing 8% dietary oils daily for 45 d as described previously (Table 1)\(^{17,21}\). The fatty acid compositions of test oils were estimated by GC-MS and reported previously (Table 2)\(^{22}\). Food intakes of rats were noted daily and the body weight was determined weekly. After 45 d, animals were fasted overnight and killed by sodium pentathione injection. Blood and tissues were collected for various estimations.

**Change in body weight**

The body weight of each animal was noted before starting the feeding experiment and also on the experimental day. Change in body weight was calculated as follows:

\[
\text{Change in body weight (g) = final weight} - \text{initial weight.}
\]

**Lipid analysis**

Total lipids from serum and tissues were extracted using chloroform–methanol \((2:1, \text{v/v})\) as described previously\(^{23}\). Tissue (500 mg) or serum \((0.5 \text{ ml})\) was homogenised with chloroform–methanol \((2:1, \text{v/v})\), filtered and washed with chloroform–methanol at least three times. Calcium chloride \((0.027 \text{ g})\) was added to the mixture \((20 \% \text{ of the total volume of the extract})\), mixed vigorously and allowed to stand for a few minutes. The upper layer was washed three times with 5 ml chloroform–methanol–calcium chloride \((3:48:48, \text{by vol.})\). The washed upper layer was evaporated to dryness and the residue was redissolved in a known volume of chloroform. From this, samples were used for lipid analysis. Total cholesterol was estimated as described by Carr & Drekter\(^{24}\). TAG were estimated by the method of Van Handel & Zilversmit\(^{25}\).

**Table 1. Formulation of synthetic diet used for the study**

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Casein</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Copra oil</td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Virgin coconut oil</td>
<td>–</td>
<td>8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olive oil</td>
<td>–</td>
<td>–</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>Sunflower-seed oil</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>Salt mixture*</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Composition of salt mixture (per kg diet)\(^{27}\): NaCl, 105 mg; KCl, 120 mg; KH\(_2\)PO\(_4\), 310 mg; Ca\(_3\)(PO\(_4\))\(_2\), 149 mg; CaCO\(_3\), 210 mg; MnSO\(_4\), 0.2 mg; KI(SO\(_4\))\(_2\), 0.09 mg; MgSO\(_4\), 90 mg; FeSO\(_4\).7H\(_2\)O, 14.7 mg; CuSO\(_4\), 0.37 mg; NaF, 0.57 mg; KI, 0.05 mg; ZnCl\(_2\), 0.02 mg; CoCl\(_2\), 0.15 mg.
† Composition of vitamin mixture (per 100 g diet): retinyl palmitate, 3 mg ergocalciferol, 3.75 µg; α-tocopherol, 12 mg; menadione, 0.3 mg; thiamine, 1 mg; riboflavin, 1 mg; pyridoxine, 0.6 mg; niacin, 10 mg; calcium pantothenate, 5 mg; inositol, 20 mg; folic acid, 0.4 mg; vitamin B\(_12\), 3 µg; biotin, 20 µg; p-aminobenzoic acid, 5 mg; choline chloride, 300 mg.
Isolation of mitochondria and peroxisomes

Mitochondria were separated from the heart according to Tyler 
& Gonzalez(27). Rat heart tissue was washed in cold MSE medium 
(0·225M-mannitol, 0·075M-sucrose and 0·05 mM-EDTA, pH 7·4) 
and finely chopped. The chopped heart tissue was washed 
continuously with MSE medium to remove adhering blood. 
The heart tissue was then homogenised in 5 ml of MSE medium, 
and the sample digested again until it became colourless. The Kjeldahl flask was cooled, 1 ml water was added and heated in a boiling water-bath for 5 min. Then 1 ml of ammonium molybdate (2·5 %) and 0·1 ml of 1-amino 
2-naphthol 4-sulfonic acid (ANSA) reagent were added and the 
colourless. The Kjeldahl flask was cooled, 1 ml water was 
added and the sample digested until it became light brown. 
After a second centrifugation at 700

g for 10 min, the supernatant fraction was collected and the pellets were discarded. The mitochondrial pellet was then re-suspended in 40 ml of MSE medium.

Table 2. Fatty acid composition of test oils in the diet

<table>
<thead>
<tr>
<th>Fatty acid (%)</th>
<th>Copra oil</th>
<th>Virgin coconut oil</th>
<th>Olive oil</th>
<th>Sunflower-seed oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 : 0</td>
<td>0·68 ± 0·02</td>
<td>0·80 ± 0·03</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8 : 0</td>
<td>6·7 ± 0·25</td>
<td>7·5 ± 0·28</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10 : 0</td>
<td>7·0 ± 0·27</td>
<td>7·7 ± 0·34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>12 : 0</td>
<td>45·4 ± 1·72</td>
<td>46·4 ± 1·77</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14 : 0</td>
<td>17·5 ± 0·67</td>
<td>16·9 ± 0·64</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>16 : 0</td>
<td>9·2 ± 0·50</td>
<td>8·5 ± 0·41</td>
<td>22·5 ± 0·86</td>
<td>17·0 ± 0·65</td>
</tr>
<tr>
<td>16 : 1</td>
<td>–</td>
<td>–</td>
<td>2·9 ± 0·11</td>
<td>–</td>
</tr>
<tr>
<td>18 : 0</td>
<td>3·9 ± 0·15</td>
<td>4·2 ± 0·16</td>
<td>10·1 ± 0·39</td>
<td>11·6 ± 0·44</td>
</tr>
<tr>
<td>18 : 1</td>
<td>7·6 ± 0·28</td>
<td>6·6 ± 0·26</td>
<td>62·3 ± 2·37</td>
<td>20·9 ± 0·65</td>
</tr>
<tr>
<td>18 : 2</td>
<td>2·1 ± 0·08</td>
<td>1·4 ± 0·05</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>18 : 3</td>
<td>0·15 ± 0·01</td>
<td>0·21 ± 0·01</td>
<td>0·67 ± 0·03</td>
<td>0·45 ± 0·02</td>
</tr>
<tr>
<td>20 : 0</td>
<td>–</td>
<td>–</td>
<td>2·2 ± 0·11</td>
<td>1·4 ± 0·05</td>
</tr>
<tr>
<td>22 : 0</td>
<td>–</td>
<td>–</td>
<td>2·1 ± 0·08</td>
<td>–</td>
</tr>
</tbody>
</table>

Mitochondrial and peroxisomal β-oxidation

Mitochondrial β-oxidation(30) and the activities of carnitine 
palmitoyl transferase I (CPT I)(31), acyl CoA dehydrogenase 
(ACDH)(32), enoyl CoA hydratase(33), β-hydroxy acyl CoA 
dehydrogenase(34) and 3-ketoacyl CoA thiolase(35) were deter-
mined in isolated mitochondria. The peroxisomal fraction was used for determining the activity of acyl CoA oxidase (ACO)(36).

De novo fatty acid synthesis

The enzymes involved in de novo fatty acid synthesis, namely 
acyl CoA carboxylase (ACC)(37) and fatty acid synthase 
(FAS)(38), were assayed in liver using a Shimadzu 1240 
spectrophotometer.

Isolation of total genomic RNA

Total genomic RNA was isolated from the heart using TRI 
reagent (Sigma Aldrich) by the method described by 
Chomczynski & Mackey(39).

cDNA synthesis and RT-PCR

cDNA synthesis and PCR reactions were performed in a thermo-
cycler (Eppendorf AG) according to the manufacturer’s instruc-
tions using a kit purchased from Fermentas. The primers used 
were: FAS (forward) 5′-AGG TGC TAG AGG CCC TGC TA-3′ 
and (reverse) 5′-GTG CAC AGA CAC CTT CCC AT-3′; SREBP- 
1c (forward) 5′-GGAGCCATGGATTTGACATT-3′ and (reverse) 
5′-AGGAAGGCTTCCAGAGAGGA-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward) 5′-TCATGTGTCGCTCTTCC 
and (reverse) 5′-GCACTTCTGCTTCTGCTCCTTTTA-3′; PPARα (forward) 5′-CTACGACTAATGCAACGTCTT-3′ and (reverse) 5′-GGCCCTGGCTCATCTTGTTCTGATG-3′; glycerol-3-phosphate dehydrogenase 
(GAPDH) (forward) 5′-CTTCTGCTGCCACTCACC-3′ and (reverse) 5′-GGGAAGGCTTCCAGAGAGGA-3′. 

The PCR products were electrophoresed on 1·5 % agarose gel and visualised by staining with ethidium bromide. The gels were subjected to densitometric scanning (Bio-Rad Gel Doc) to determine the optical density of each and then normalised against an internal control (GAPDH) using Quantity One imaging software (Bio-Rad Laboratories).

**Statistical analysis**

Data are presented as means with their standard errors. Statistical analysis was performed by one-way ANOVA followed by Duncan’s multiple-range tests using the SPSS/PC (version 17·0; SPSS) software package program. P values < 0·05 were considered significant.

**Results**

**Dietary intake and change in body weight**

There was no significant difference in diet consumption and change in body weight among the experimental groups (Table 3).

**Effect on serum and liver lipids**

Table 4 summarises the levels of lipids in the serum and liver of rats fed the different test oils. The concentration of total cholesterol (1·87 mmol/l serum; 128·57 mg/100 g liver), TAG (0·11 mmol/l serum; 154·78 mg/100 g liver) and phospholipids (1·01 mmol/l serum; 1788 mg/100 g liver) were decreased (0·11 mmol/l serum; 154·78 mg/100 g liver) and phospholipids (40·93 % decrease in serum and 18·34 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO.

A significant increase in TAG and phospholipids levels was observed in OO- (26·66 % increase in serum TAG and 49·50 % increase in serum phospholipids) and SFO- (21·42 % increase in serum TAG and 41·28 % increase in serum phospholipids) compared with VCO-fed rats.

**Effect on synthesis and oxidation of fatty acids**

Activities of enzymes involved in de novo fatty acid synthesis were assayed in the liver cytosolic fraction from rats fed the different test oils. Reduced rates of fatty acid synthesis were evident from the decreased activities of enzymes involved in fatty acid synthesis, namely ACC (9·38 U/mg protein) and FAS (18·78 U/mg protein), in VCO-fed rats compared with rats fed CO, OO and SFO. Supplementation of VCO decreased the cholesterol levels (22·72 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO. Similarly there was a 28·62 % decrease in serum cholesterol levels and a 54·05 % decrease in hepatic cholesterol levels in VCO-fed rats compared with rats fed CO. Decreased levels of TAG (15·38 % decrease in serum and 51·25 % decrease in liver) compared with rats fed CO.

**Table 3. Food intake and change in body weight**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (g/d)</th>
<th>SEM</th>
<th>Mean (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>11·61</td>
<td>0·41</td>
<td>65·55</td>
</tr>
<tr>
<td>Group II</td>
<td>12·12</td>
<td>0·49</td>
<td>61·50</td>
</tr>
<tr>
<td>Group III</td>
<td>12·46</td>
<td>0·37</td>
<td>66·25</td>
</tr>
<tr>
<td>Group IV</td>
<td>12·47</td>
<td>0·44</td>
<td>66·75</td>
</tr>
</tbody>
</table>

* Mean values were not significantly different.

**Table 4. Cholesterol, TAG and phospholipid concentrations in serum and liver**

<table>
<thead>
<tr>
<th></th>
<th>Serum (mmol/l)</th>
<th>Liver (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>2·42</td>
<td>0·11</td>
</tr>
<tr>
<td>Group II</td>
<td>1·87</td>
<td>0·08</td>
</tr>
<tr>
<td>Group III</td>
<td>2·62†</td>
<td>0·10</td>
</tr>
<tr>
<td>Group IV</td>
<td>2·18†</td>
<td>0·08</td>
</tr>
<tr>
<td>TAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0·13</td>
<td>0·01</td>
</tr>
<tr>
<td>Group II</td>
<td>0·11*</td>
<td>0·003</td>
</tr>
<tr>
<td>Group III</td>
<td>0·15†</td>
<td>0·01</td>
</tr>
<tr>
<td>Group IV</td>
<td>0·14†</td>
<td>0·01</td>
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<tr>
<td>Phospholipids</td>
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<tr>
<td>Group I</td>
<td>1·71</td>
<td>0·06</td>
</tr>
<tr>
<td>Group II</td>
<td>1·01*</td>
<td>0·04</td>
</tr>
<tr>
<td>Group III</td>
<td>2·00†</td>
<td>0·07</td>
</tr>
<tr>
<td>Group IV</td>
<td>1·72†</td>
<td>0·07</td>
</tr>
</tbody>
</table>

* Mean value was significantly different from that of group II (P < 0·05).
† Mean value was significantly different from that of group I (P < 0·05).
Effect on transcriptional regulation of fatty acid metabolism

There was a down-regulation in the mRNA expression of FAS (the multi-enzyme complex involved in de novo fatty acid synthesis) and its transcription factor SREBP-1c in VCO-fed rats compared with the other oil-fed groups (Fig. 3). On the other hand, the mRNA expression of PPARα and its target genes involved in fatty acid oxidation, namely CPT I and ACO, were up-regulated in VCO-fed rats compared with CO-, OO- and SFO-fed rats (Fig. 4).

Discussion

In the present study we compared the effects of VCO with CO, OO and SFO on the synthesis and oxidation of fatty acids and its effect on the molecular regulation of fatty acid metabolism in rats. There was no significant difference in food intake or change in body weight among the experimental groups. Supplementation of VCO decreased the concentration of lipids (cholesterol, TAG and phospholipids) in serum and liver when compared with CO-, OO- and SFO-fed rats. However, OO-fed rats showed an increased level of hepatic lipid accumulation compared with rats fed the other oils, which may be due to an increased lipogenesis and decreased catalysis of fats in OO-fed rats. There are reports that dietary fat influences the occurrences and prognosis of CHD, which is partially mediated by its effect on plasma lipids.

Fatty acid biosynthesis in mammalian cells can be regulated in different ways. On a long-term basis, it is controlled by changes in the rate of synthesis of lipogenic enzymes. As shown in Fig. 1, activities of enzymes involved in the de novo synthesis of fatty acids, namely ACC and FAS, were found to be decreased in VCO-fed rats compared with the other oil-fed rats. ACC catalyses the carboxylation of acetyl-CoA to produce malonyl-CoA for the biosynthesis of fatty acids. There are reports of increased activities of lipogenic enzymes and hepatic lipid accumulation in OO-fed animals. On the other hand, medium-chain TAG have a reductive effect on fat stores and a depressive effect on lipogenesis. Fatty acid analysis of VCO extracted from fresh coconut kernel and CO extracted from dried copra revealed almost similar fatty acid content. Since there was no major difference in the fatty acid profiles among the two varieties of coconut oil, it is evident that the beneficial effect of VCO over CO is mainly due to the presence of unsaponifiable components, namely polyphenols and vitamin E. Studies revealed that apart from fatty acids, the unsaponifiable components present in dietary oils have a role in regulating hepatic lipid metabolism. Chemical analysis of the test oils has revealed that VCO by wet processing has increased polyphenolic contents (84 mg/100 g oil), significantly higher than CO (64.4 mg/100 g oil), OO (75.63 mg/100 g oil) and SFO (55.26 mg/100 g oil). HPLC analysis of the phenolic fraction of VCO has revealed the presence of increased amounts of caffeic acid (0.829 μg/100 g oil), p-coumaric acid (5.43 μg/100 g oil), ferulic acid (0.91 μg/100 g oil), (+)-catechin

Table 5. Effect of test oils on mitochondrial and peroxisomal β-oxidation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mitochondrial β-oxidation (nmol ferricyanide reduced/mg protein per min)</th>
<th>Peroxisomal β-oxidation (nmol NAD reduced/mg protein per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Group I</td>
<td>0.274 ± 0.011</td>
<td>3.76 ± 0.154</td>
</tr>
<tr>
<td>Group II</td>
<td>0.305* ± 0.012</td>
<td>4.53* ± 0.161</td>
</tr>
<tr>
<td>Group III</td>
<td>0.180† ± 0.006</td>
<td>3.62† ± 0.125</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.209† ± 0.007</td>
<td>2.69† ± 0.098</td>
</tr>
</tbody>
</table>

* Mean value was significantly different from that of group I (P<0.05).
† Mean value was significantly different from that of group II (P<0.05).

Fig. 1. Activity of acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) in copra oil-, virgin coconut oil-, olive oil- and sunflower-seed oil- fed rats. Values are means of six rats per group, with their standard errors represented by vertical bars. * Mean value was significantly different from that of group I (P<0.05).

Fig. 2. Activity of carnitine palmitoyl transferase I (CPT I) and acyl CoA oxidase (ACO) in copra oil-, virgin coconut oil-, olive oil- and sunflower-seed oil- fed rats. Values are means of six rats per group, with their standard errors represented by vertical bars. * Mean value was significantly different from that of group I (P<0.05).
Coconut oil intake and fatty acid metabolism

Table 6. Effect of test oils on mitochondrial β-oxidation enzymes
(Means values of six rats per group with their standard errors)

<table>
<thead>
<tr>
<th>Groups</th>
<th>ACDH (U/mg protein)</th>
<th>ECHD (U/mg protein)</th>
<th>HOAD (U/mg protein)</th>
<th>KAT (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SEM</td>
<td>Mean SEM</td>
<td>Mean SEM</td>
<td>Mean SEM</td>
</tr>
<tr>
<td>Group I</td>
<td>0.626 0.021</td>
<td>0.204 0.006</td>
<td>0.384 0.045</td>
<td>0.180 0.006</td>
</tr>
<tr>
<td>Group II</td>
<td>0.768* 0.027</td>
<td>0.304* 0.010</td>
<td>0.620* 0.021</td>
<td>0.224* 0.006</td>
</tr>
<tr>
<td>Group III</td>
<td>0.247† 0.009</td>
<td>0.161† 0.005</td>
<td>0.213† 0.007</td>
<td>0.130† 0.004</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.612† 0.022</td>
<td>0.198† 0.007</td>
<td>0.222† 0.042</td>
<td>0.159† 0.005</td>
</tr>
</tbody>
</table>

ACDH, acyl CoA dehydrogenase; ECHD, enoyl CoA hydratase; HOAD, β-hydroxy acyl CoA dehydrogenase; KAT, 3-ketoacyl CoA thiolase; group I, 8% copra oil-fed rats; group II, 8% virgin coconut oil-fed rats; group III, 8% olive oil-fed rats; group IV, 8% sunflower-seed oil-fed rats.

* Mean value was significantly different from that of group I (P<0.05).
† Mean value was significantly different from that of group II (P<0.05).

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The non-saponifiable fraction of VCO contains appreciably higher amounts of antioxidants, namely vitamin E (33·12 μg/100 g oil) and β-carotene (196 μg/100 g oil)(20,22). Increased amounts of these bioactive non-saponifiable components present in VCO may partly be responsible for the decreased lipogenesis and lipid accumulation compared with other oils. Increased amounts of these bioactive non-saponifiable components may partly be responsible for the decreased lipogenesis and lipid accumulation compared with other oils.

There are reports that polyphenols can inhibit hepatic lipogenesis, promote hepatic lipid clearance(47) and decrease serum and hepatic lipid accumulation(48,49). Increased amounts of polyphenols present in VCO may decrease the lipid levels (cholesterol, TAG and phospholipids) compared with other oils. Some of the beneficial metabolic actions of polyphenols are mediated by their ability to activate sirtuin 1 (SIRT1)(50,51) or AMP-activated protein kinase (AMPK)(52). SIRT1 is a NAD+-dependent class III histone deacetylase(53). Polynens stimulate hepatic SIRT1 activity and reduce lipid accumulation(52), however, the underlying mechanisms remain unclear. There are reports that the polyphenols substantially prevent the impairment in phosphorylation of AMPK and its downstream target, ACC, elevation in the expression of FAS and lipid accumulation in human HepG2 hepatocytes(54).

Furthermore, increased rates of mitochondrial and peroxisomal β-oxidation were observed in VCO-fed rats, which was evident from the increased activity of CPT I and ACO in VCO-fed rats compared with the other oil-fed rats. The OO-enriched diet showed a maximum decrease in the activity of CPT I, resulting in increased hepatic lipid accumulation in OO-fed rats. The present results are in agreement with previous reports(55). Moreover, increased activities of mitochondrial β-oxidation enzymes, namely ACDH, enoyl CoA hydratase, β-hydroxy acyl CoA dehydrogenase and 3-ketoacyl CoA thiolase, were observed in VCO-fed rats compared with the rats fed the other oils. There are reports that laurate present in coconut oil has a higher oxidation rate than oleate(56) and the metabolites derived from long-chain fatty acids decrease activity of CPT I and the efficiency of ATP production(57). In addition, medium-chain TAG in coconut oil are transported predominantly in portal venous blood, extensively oxidised in the liver and less effectively incorporated into tissue lipids than other long-chain fatty acids(58). Thus lauric acid present in VCO may be partly responsible for the increased catabolism of fats and decreased accumulation of lipids.

In addition, an increased rate of peroxisomal β-oxidation was observed in VCO-fed rats, which was evident from the increased activity of ACO in peroxisomes compared with other oil-fed rats. There are reports that long-chain fatty acids (>C20) require peroxisomal β-oxidation to shorten the chain length for further completion of oxidation in mitochondria. Thus both the substrate specificities and alterations in the activities of the β-oxidation enzymes may account for the lipid-lowering effects of dietary VCO. It has been reported that polyphenols can also increase peroxisomal β-oxidation and can suppress body fat accumulation(40); thus, higher
amounts of polyphenols in VCO may beneficially regulate fatty acid oxidation. The present study for the first time has evidence that dietary VCO increases the activities of both mitochondrial and peroxisomal enzymes involved in the fatty acid oxidation pathway.

The role of transcriptional regulation of fatty acid metabolism has been studied extensively. Reports indicate that dietary fatty acids suppress hepatic lipogenesis by reducing the transcription of genes coding for the participating enzymes\(^{59,60}\). Several lines of evidence have shown that down-regulation of SREBP-1c is a primary mechanism by which dietary fatty acids reduce hepatic lipogenesis. SREBP-1c stimulates the transcription of the enzymes that participate in the synthesis of fatty acids and TAG\(^{58}\); there was a down-regulation in the hepatic mRNA expression of FAS and its transcription factor SREBP-1c in VCO-fed rats compared with other oils. SREBP-1c is known to be regulated by the nutritional status of the individual and there are reports that polyphenols can reduce SREBP-1c expression\(^{61,62}\). Since VCO contains higher amounts of polyphenols\(^{17,20}\), this may down-regulate the hepatic expression of SREBP-1c and hence reduce lipogenesis. A potential link between SIRT1 and SREBP-1c has also been suggested\(^{63}\). Considerable evidence suggests that SIRT1 functions as a master metabolic regulator by either directly modifying histones or by indirectly regulating the activities of several transcriptional regulators\(^{63,64}\). There are reports that polyphenols increase SIRT1 deacetylase activity\(^{54}\), deacetylation of SREBP-1c by SIRT1 inhibits SREBP-1c activity by decreasing its stability and its association with its lipogenic target gene promoters\(^{65,66}\).

Moreover, both mitochondrial and peroxisomal β-oxidation systems are under the transcriptional regulation of PPARα, which is the nuclear receptor activated by natural ligands such as fatty acids\(^{67}\). There are reports that medium-chain fatty acids are the natural PPAR ligands\(^{68}\) and supplementation of coconut oil up-regulates the mRNA expression of PPARα\(^{67}\). Moreover, polyphenols are reported to be the natural PPARα activators\(^{49,69}\). PPARα activation up-regulates fatty acid oxidation-related genes such as ACO, CPT I, etc.\(^{60}\) and suppresses postprandial lipidaemia and lipid accumulation through the enhancement of fatty acid oxidation\(^{70}\). An up-regulation in the mRNA expression of PPARα and its target genes involved in mitochondrial and peroxisomal β-oxidation in VCO-fed rats. Thus, from the present results it is clear that supplementation of VCO regulates fatty acid oxidation partly via PPARα-dependent pathways.

In conclusion, dietary VCO beneficially modulates lipid levels and fatty acid metabolism by transcriptional regulation of the enzymes involved in the synthesis and oxidation of fatty acids.

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S. A. designed and performed the experiment, analysed the data and prepared the manuscript. T. R. was responsible for the very concept and design of the study, interpretation of the data and formulation of research questions. All authors read and approved the final manuscript.

The authors declare that they have no conflicts of interest.

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