Virgin coconut oil maintains redox status and improves glycemic conditions in high fructose fed rats

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Abstract Virgin Coconut Oil (VCO), extracted from fresh coconut kernel possess similar fatty acid composition to that of Copra Oil (CO), a product of dried kernel. Although CO forms the predominant dietary constituent in south India, VCO is being promoted for healthy life due to its constituent antioxidant molecules. High fructose containing CO is an established model for insulin resistance and steatohepatitis in rodents. In this study, replacement of CO with VCO in high fructose diet markedly improved the glucose metabolism and dyslipidemia. The animals fed VCO diet had only 17 % increase in blood glucose level compared to CO fed animals (46 %). Increased level of GSH and antioxidant enzyme activities in VCO fed rats indicate improved hepatic redox status. Reduced lipid peroxidation and carbonyl adducts in VCO fed rats well corroborate with the histopathological findings that hepatic damage and steatosis were comparatively reduced than the CO fed animals. These results suggest that VCO could be an efficient nutraceutical in preventing the development of diet induced insulin resistance and associated complications possibly through its antioxidant efficacy.

Keywords Virgin coconut oil · Copra oil · High fructose diet · Oxidative stress · Insulin resistance · Hepatosteatois

Abbreviations

VCO	Virgin Coconut Oil
CO	Copra Oil
HDLc	High Density Lipoprotein Cholesterol
GSH	Reduced Glutathione
GR	Glutathione Reductase
GPx	Glutathione Peroxidase
SOD	Super Oxide Dismutase
GST	Glutathione S Transferase

Introduction

Copra oil is the commonly used edible oil in south India, extracted from the dried coconut kernel. It is composed mainly of medium chain saturated fatty acids, especially lauric acid. VCO is the unrefined oil extracted from the fresh coconut kernel through natural or mechanical procedures as per codex alimentarius commission (Commission 1999). Even though the fatty acid profiles of both these oils are the same, VCO has a higher amount of polyphenolic antioxidants such as caffeic acid, ferulic acid, syringic acid, catechin and epigallocatechin (Marina et al. 2008). These polyphenolics are well known for their antidiabetic and insulin sensitizing effects (Ramar et al. 2012; Srinivasan et al. 2014; Xie et al. 2013). Supplementation of VCO has been reported to have beneficial effects on lipid parameters by reducing lipogenesis and enhancing the rate of fatty acid catabolism (Arunima and Rajamohan 2012). In addition, VCO is reported to have chemopreventive, anti-inflammatory (Nair et al. 2015), analgesic and anti-pyretic activities (Vysakh et al. 2014). The biological efficacy of the VCO is thought to be due to its high polyphenolic content (Marina et al. 2008).

Diabetes mellitus is a common disease with tremendous impact on human health worldwide, which is characterized

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by insulin resistance, impaired insulin and glucagon secretion. Various studies suggest that insulin resistance has a central role in the development of dyslipidemia and oxidative stress (Kunde et al. 2011). The resulting chronic hyperglycemia often leads to chronic inflammation that forms the basis of secondary complications involving cardiomyopathy, kidney failure, neurodegenerative disorders, cataract and even cancers of various types (Onitilo et al. 2014).

Obesity is closely associated with diabetes and its complications. Essential factor prevailing under obesity is the elevated plasma free fatty acid levels and increased utilization of lipids by tissues, leading to a reduction of glucose metabolism (Boden 2008). This in turn will lead to the development of insulin resistance and also dyslipidemia. Role of fatty acids in the development of insulin resistance and other diabetic complications are also well established (Riccardi et al. 2004). Among these, long chain saturated and free fatty acids are mainly involved in the pathogenesis of diabetes mellitus (Funaki 2009). In addition, long term consumption of medium chain saturated fat such as copra oil along with high fructose diet has reported to alter serum lipid profiles (Prakash et al. 2014).

High fructose (Chandramohan and Pari 2014; Suwannaphet et al. 2010) or high sucrose (Cao et al. 2012) along with high saturated fat diet are known to induce insulin resistance and associated complications in murine models. Previously, several such studies used CO as a fatty acid source in high fructose diet to induce glycemic conditions and insulin resistance (Prakash et al. 2014). In this study influence of dietary modification by replacing CO with VCO in high fructose diet is evaluated. Glycemic conditions, oxidative and carbonyl stress, hepatic antioxidant status and lipid profile changes under these dietary modifications are examined.

Materials and methods

Physicochemical parameters of the oils

Copra oil was purchased from local market. VCO was prepared based on the method by Nevin and Rajamohan (2004).

a. *Para anisidine and aldehyde content assay*

Extent of lipid peroxidation in the edible oils were estimated by thiobarbituric acid reactive substances assay according to the method of Ohkawa et al. (1979). Amount of conjugated dienes in the samples was determined spectrophotometrically by reading the absorption at 234 nm (Corongiu and Banni 1994). Para-anisidine value of the oils was determined according to the standard protocols by codex alimentarius commission.

b. *Polyphenol estimation*

Polyphenols in the oils were extracted according to the method described by Nevin and Rajamohan (2004). Approximately 10 g of oil was mixed with 50 mL of n-hexane, and extracted using 20 mL methanol (60 %). It was repeated three times to ensure complete extraction of polyphenols. The samples are dried in a vacuum concentrator (Eppendorf, India) and the residue was dissolved in 2 mL HPLC grade methanol. The total polyphenol content of this solution was estimated using Folin-Ciocalteu reagent.

Animals

Male Wistar rats, weighing about 150–160 g were purchased from Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, were maintained under standard conditions as per OECD guidelines and fed with standard rat chow (Sai Duraga feeds, Bangalore) and water ad libitum. The compositions of the diets are shown in Table 1. All experiments were conducted with prior permission from Institutional Animal Ethical Committee (No.149/1999/CPCSEA), followed the CPCSEA, Govt. of India guidelines.

Experimental design

The animals were divided into three groups of six animals each. Group 1 fed with reference diet was kept as control. The other two groups were fed with semi synthetic diet, composed of 60 % fructose, 20 % protein, 10 % nutrients, vitamins and minerals with 10 % of either CO (Group 2) or VCO (Group 3) as fatty acid source (Table 1) for a period of 4 weeks. At the end of experimental period, oral glucose tolerance test was carried out (Islam et al. 2009) and on the next day animals were sacrificed following an overnight fasting.

Biochemical measurements for blood glucose, serum total cholesterol, high density lipoprotein cholesterol (HDLc), triglycerides, serum glutamine pyruvate transaminase (GPT), glutamine oxaloacetate transaminase (GOT) and alkaline phosphatase (ALP) were performed using commercially available kits (Agappe, India). Hydroxymethyl glutaryl CoA (HMG CoA) - Mevalonate ratio was estimated according to the method of Navarro-Gonzalez et al. (2014).

Liver tissue was excised and washed in ice cold saline. TBARs (Ohkawa et al. 1979), conjugated dienes (Corongiu and Banni 1994) and protein carbonyls (Levine et al. 1990) were estimated in liver homogenate (10 % w/v in 0.1 M Tris HCl, pH 7.4). The clarified supernatant (5000 rpm for 60 min at 4 °C) was collected and used for the assays of GSH (Moron et al. 1979), SOD (McCord and Fridovich 1969), GPx (Hafeman et al. 1974), catalase (Abel et al. 2001), GST and GR (Carlberg and Mannervik 1975).

Table 1 Composition of the reference diet, CO and VCO containing diets

Nutrient (g/100 g feed)	Type of diet		
	Reference	CO	VCO
Protein	20.0	20.0	20.0
Corn starch	60.0	0.0	0.0
Fructose	0.0	60.0	60.0
Ground nut oil	10.0	–	–
Coconut oil	–	10.0	–
Virgin coconut oil	–	–	10.0
Vitamin mix	1.5	1.5	1.5
Mineral mix	3.5	3.5	3.5
Fiber	5.0	5.0	5.0
Saturated fat (%)	16.9	91	91
Monounsaturated fat (%)	46.1	6	6
Polyunsaturated fat (%)	32.0	3	3
Total (g)	100	100	100
Energy (Mean Kcal/Kg)	3600	3600	3600

Histopathological analysis

A portion of the liver was sliced, washed in PBS, fixed in 10 % buffered formalin and embedded in wax. Serial sections of the tissues were taken in a microtome at a thickness of 4 μ m and stained with hematoxylin and eosin. Histopathological examination was carried out by a pathologist who was blind to the design of this study.

Statistical analysis

The values represented are Mean $\pm$ SD of six animals per group. Statistical analysis of the data was done by one way ANOVA followed by Tukey-Kramer multiple comparison test using Graph pad Instat software.

Results

Physico-chemical parameters of the oils

Physicochemical parameters assessed for the oils are given in Table 2. Total polyphenol content in VCO was found to be significantly higher than those of CO and ground nut oil ($P < 0.001$). The ground nut oil contained in the reference diet had the least polyphenol content (10.9 ± 1.7 mg/100 g of oil). The amount of polyphenols in VCO was 32.24 ± 1.2 mg/100 g of oil, whereas in CO, it was 18.1 ± 2.01 mg. The *p*-anisidine value of the oils depicts the aldehyde content which was less in VCO than other oils ($P < 0.05$). Thiobarbituric acid reactive substances (TBARs), an indicator of the extent of lipid peroxidation in the oils were

Table 2 Physicochemical properties of groundnut oil, copra oil and virgin coconut oils

	Ground nut oil	Copra oil	Virgin coconut oil
Total Phenols (mg/100 g oil)	10.9 ± 1.7	18.1 ± 2.01	$32.24 \pm 1.2^{***}$
TBARs (mmol/kg)	0.365 ± 0.044	0.351 ± 0.061	$0.251 \pm 0.035^{***}$
Conjugated Dienes (mmol/kg)	0.458 ± 0.041	0.165 ± 0.012	$0.185 \pm 0.027^{**}$
<i>p</i> -anisidine	2.819 ± 0.114	2.685 ± 0.084	$2.565 \pm 0.065^*$

* indicates significant difference at $P < 0.05$, ** indicates significance at $P < 0.01$ and *** indicates $P < 0.001$

also low in the VCO. Conjugated dienes were found to be similar in CO and VCO, whereas in ground nut oil, it was found to be significantly higher ($P < 0.01$).

Blood glucose levels and oral glucose tolerance

Blood glucose levels in the VCO containing diet and reference diet fed animals were similar and found to be in the normal ranges of blood glucose of rats (50–135 mg/dL) at the end of the experimental period. The initial blood glucose in the VCO containing diet fed animals was 97.2 ± 6.95 mg/dL which reached 118.1 ± 12.94 mg/dL in 4 weeks time. The values of CO containing diet fed animals were found to be significantly varied from the reference and VCO containing diet fed rats ($P < 0.001$). In CO containing diet fed group, there was a change in blood glucose level from an initial level of 84.40 ± 6.48 mg/dL to a final level of 156.03 ± 12.04 mg/dL. The variation in blood glucose level among the reference diet fed group was from an initial value of 80.73 ± 10.11 to 111.73 ± 4.21 mg/dL during the same experimental period. The percentage increase in blood glucose level in reference group was 26 %, however, in CO group it was 46 %. In VCO groups, the increase in glucose level was only 17 %.

In this study, oral glucose tolerance was measured as an indicative of insulin resistance at the end of the experimental period. In the reference diet fed group of rats, blood glucose level was found to increase from a basal level (111.73 ± 4.21 mg/dL) to 158 ± 4.2 mg/dL within 30 mins after the oral administration of 5 g/kg bw glucose. Within 90 min, this level was reduced to 80 ± 6.1 mg/dL and at the end of 120 mins reached to 76.0 mg/dL (Fig. 1i). Compared to reference group, glucose level of CO group rats initially raised to 187.0 ± 5.3 mg/dL at 30 mins from the basal glucose level of 156.03 ± 12.04 mg/dL. Later, the blood glucose levels declined to 160.0 ± 6.4 and 141.0 ± 3.6 mg/dL at 60 and 90 min, which were further reduced to 130.5 ± 5.97 mg/dL at the end of 120 min. On the other hand, VCO fed animals had a basal blood glucose level of 118.08 ± 12.94 mg/dL, which was raised to 170 ± 4.6 mg/dL in 30 mins following oral administration of glucose. Glucose level at 60 mins was

Fig. 1 **i** Variation in the glucose tolerance of animals under the dietary medications: the animals were given 5 g/kg glucose orally and the blood glucose levels were checked at 30, 60, 90 and 120 mins, respectively. **ii** Antioxidant enzyme activities such as GSH, GST, SOD, GR, GPx and Catalase in the liver tissue of reference diet, CO and VCO containing diet fed groups of animals. **iii** Hematoxylin-Eosin stained sections of liver tissues of male Wistar rats; reference diet fed animals (**a**), CO containing diet fed animals (**b**) and VCO containing diet fed animals (**c**) are shown in the Figure. CO fed groups showed signs of hepatic steatosis, observed as vesicles (black arrows)

163.2 ± 6.7 mg/dL, and further reduced to 109 ± 8.9 mg/dL within 90 min. Finally, the blood glucose at 120 mins after the administration of glucose (5 g/Kg) was 90.0 ± 4.89 mg/dL in these group of animals.

Serum and hepatic lipid profile

Total cholesterol in the serum was found to be unchanged (normal ranges 70–120 mg/dL) in VCO, CO and reference diet group of rats (Table 3) at the end of 4 weeks. However, VCO group had higher HDLc level (37.7 ± 1.18 mg/dL), compared to reference group (32.8 ± 2.81 mg/dL; $P < 0.01$) and CO group (35.0 ± 2.02 mg/dL). Serum phospholipid level had a marked decline ($P < 0.01$) in the VCO fed animals with respect to CO fed animals. Compared to reference groups, the levels were higher in CO and VCO. In contrary, a significantly higher level of triglycerides in VCO containing diet fed animals ($P < 0.01$) was observed. The level was 117.51 ± 9.51 mg/dL in this group while in the reference and CO groups, the TG levels were 67.09 ± 15.78 and 87.76 ± 10.15 mg/dL).

In the liver tissue, no variation in total cholesterol was observed between experimental groups (Table 3). However, significant variations in triglyceride level was observed in VCO and CO fed groups with that of reference diet fed group ($P < 0.01$). The difference in triglyceride levels between CO and VCO containing diet fed animals was not significant

(Table 3). HMG CoA/Mevalonate ratio, which indirectly measures the cholesterol synthesis, was found to be significantly increased in the animals fed with VCO ($P < 0.001$). The ratio in VCO fed group was 6.65 ± 0.29, which was 4.31 ± 0.60 and 4.59 ± 0.44 in reference diet and CO containing diet fed groups.

Liver function parameters

The liver marker enzyme analysis showed significant change with respect to SGPT and SGOT activity in VCO and CO containing diet groups. The SGPT activity of VCO was marginally lower than reference diet fed groups. The SGPT and SGOT values in VCO group were 36.47 ± 5.30 and 14.73 ± 2.24 U/L while, in reference group it was 37.60 ± 5.11 and 11.30 ± 1.62 U/L. Compared to VCO and reference diet fed groups SGOT and SGPT values were found to be significantly higher ($P < 0.05$) in animals fed with CO (Table 4). On the other hand, with respect to ALP levels, there was an appreciable change observed among the experimental groups (Table 4).

Hepatic antioxidant status

The antioxidant status measured for the experimental animals were given in the Fig. 1ii. Higher levels of GSH in the liver

Table 3 Serum and liver lipid profile of male Wistar rats fed on reference diet, CO or VCO containing diet, respectively

		Cholesterol (mg/dL)	Triglycerides (mg/dL)	Phospholipid (mg/dL)	HDLc (mg/dL)
Serum	Reference	49.45 ± 7.21	67.09 ± 15.78	108.48 ± 8.64	32.8 ± 2.81
	CO	61.68 ± 11.58	87.76 ± 10.15	121.02 ± 13.04	35.0 ± 2.02
	VCO	62.86 ± 14.66	117.51 ± 9.51 ^a	95.35 ± 9.08 ^a	37.7 ± 1.18 ^c
Liver	Reference	187.90 ± 20.67	1228.71 ± 162.10	4.21 ± 0.60	28.32 ± 2.49
	CO	183.03 ± 25.14	1354.24 ± 123.68	4.94 ± 0.97	23.17 ± 2.97 ^b
	VCO	207.58 ± 28.68	1533.40 ± 196.25	5.03 ± 0.83	25.25 ± 2.30

a- indicates significant difference between CO and VCO; b- indicates significant difference between reference group and CO, c- indicates significant difference between reference diet and VCO

tissue was observed in rats administered with VCO (7.92 ± 0.34 nmols/mg protein) compared to that of the reference diet (6.50 ± 0.48 nmols/mg protein), and CO containing diet fed animals (5.67 ± 0.26 nmols/mg protein). Glutathione reductase activity was found to be increased ($P < 0.01$) in the CO containing diet fed group, in comparison with reference and VCO groups. Catalase activity was 81.70 ± 5.25 U/mg protein in reference animals, which was 87.51 ± 5.74 in the VCO containing diet fed animals and found to be reduced ($P < 0.05$) to 76.8 ± 6.59 U/mg protein in the CO group of animals (Fig. 1ii). Superoxide dismutase activity was 86.84 ± 7.11 U/mg protein in the reference group, which was 79.28 ± 4.29 U/mg protein in CO containing diet fed group, and increased ($P < 0.05$) to 89.50 ± 3.76 U/mg protein in the VCO containing diet fed animals ($P < 0.05$).

Analysis of oxidative stress

In line with these observations, there was also a significant reduction noticed in oxidative and carbonyl stress in VCO fed rats compared to CO fed groups. Significant reduction in serum thiobarbituric acid reactive substances ($P < 0.01$), protein carbonyl adducts and conjugated diene (CD) levels ($P < 0.001$) were observed in VCO containing diet fed animals (Table 5). Significant reduction in the hepatic thiobarbituric acid reactive substances ($P < 0.001$) and conjugated diene ($P < 0.05$) levels were also noticed in these animals. Reference diet fed animals had more or less similar values as that of VCO containing diet fed groups.

Histopathology analysis

The liver tissue architecture was normal with central venous system, the portal triads, sinusoidal spaces and Kupffer cells in reference diet fed (Fig. 1.iii.a) and VCO containing diet fed animals (Fig. 1.iii.c). Copra oil fed groups showed signs of hepatic steatosis, observed as microvesicles (black arrows) (Fig. 1.iii.b).

Discussion

Copra oil and virgin coconut oil used in the experimental diets are two most common edible oils obtained from coconut. They are similar in fatty acid profiles with more than 90 % of medium chain saturated fats. Major fatty acids in these oils are lauric acid (52 %) and myristic acid (15 %). Additionally, VCO is rich in antioxidant polyphenols than the copra oil (Marina et al. 2008). On the other hand, groundnut oil used in the reference diet is rich sources of essential fatty acids which are unsaturated, especially oleic acid (52 %) and linoleic acid (27 %). Both CO and VCO have these unsaturated fatty acid content but with very less amount. However, antioxidant polyphenol levels in ground nut oil are reported to be very less. In the present observation however, the total polyphenols in the CO, VCO is about two fold and 1.5 fold higher amounts than ground nut oil. Hence, animals in the VCO group are likely to consume higher amount of antioxidants along with rich medium chain saturated fats.

Table 4 Variation of serum biochemical parameters in reference, CO and VCO groups

	Reference	CO	VCO
Hemoglobin (mg/dl)	13.95 ± 0.93	15.68 ± 0.90	15.56 ± 0.84
SGPT (U/L)	37.60 ± 5.11	54.11 ± 6.22	36.47 ± 5.30 ***
SGOT (U/L)	11.30 ± 1.62	18.66 ± 2.56	14.73 ± 2.24*
ALP (U/L)	245.20 ± 19.98	281.02 ± 39.40	256.80 ± 21.06

(*** indicates $P < 0.001$, * indicates $P < 0.05$)

Table 5 Oxidative stress status of serum and liver of animals in the reference, CO and VCO fed groups

Sample	Parameters	Reference	CO	VCO
Serum	TBARS ^a	2.05 ± 0.14	3.37 ± 0.15	2.27 ± 0.77**
	Protein Carbonyl ^b	2.99 ± 0.18	1.98 ± 0.17	1.14 ± 0.08***
	CD ^b	0.09 ± 0.00	0.19 ± 0.02	0.13 ± 0.02***
Liver	TBARS ^a	4.35 ± 0.21	6.44 ± 0.14	5.44 ± 0.12***
	Protein Carbonyl ^b	0.15 ± 0.06	0.54 ± 0.19	0.49 ± 0.08
	CD ^b	0.46 ± 0.09	0.71 ± 0.10	0.54 ± 0.11*

a) nmoles of MDA/mg protein, b) nmoles/ml (*** indicates $P < 0.001$, ** indicates $P < 0.01$ and * indicates $P < 0.05$)

Here in this study, the blood glucose levels and glucose tolerance rate in VCO fed group is found similar to that of reference diet fed animals. However, it is significantly reduced in CO fed group. This indicates that VCO could prevent the development of insulin resistance and hyperglycemia in animals. Possible reason behind these effects may be attributed to their increased polyphenol contents such as caffeic acid, ferulic acid and catechins (Marina et al. 2008), which are known to possess high antidiabetic and insulin sensitizing effects (Ramar et al. 2012; Srinivasan et al. 2014). Wein et al. (2009) has reported that medium chain fatty acids has beneficial effect on glycemic conditions. In view of these it is likely that along with polyphenols, medium chain fatty acids in VCO might have a cumulative effect in glycemic conditions.

Dyslipidemia which is often seen in association with insulin resistance and diabetes (Garg 1996). In this study, it is observed that VCO consumption improved hepatic and serum lipid profile, in comparison with CO fed animals. However, a hike in triglycerides noticed in the VCO containing diet fed rats is obscure. In dyslipidemic individuals, hypertriglyceridemia is observed due to the activation of polyolyl pathway (Yang et al. 2004). If this is the case, CO fed animals also might have increased triglycerides. Therefore it is thought that in this short duration such polyolyl pathway associated changes are unlikely. Differential expression of regulatory molecules of VLDL synthesis and degradation under the influence of CO and VCO may explain the fact.

Studies have shown that hepatic antioxidant system is challenged under prolonged hyperglycemia (Ling et al. 2003). However, in the present study VCO consumption has increased hepatic GSH levels as well as catalase and SOD activities in comparison with reference diet fed and CO fed rats. In CO containing diet fed group animals, GSH level has reduced while there was increase in GR activity. Glutathione reductase activity is a protective mechanism to reduce the peroxide toxicity where in GS-

SG formed is actively reduced to GSH (White et al. 1999). Increased activity of GR thus is necessary under conditions of overwhelming production of peroxides which might be prevailing in the CO containing diet fed groups. Chaiswing et al. (2014) suggest that increase in the catalase and SOD activities is an indication of better oxidative homeostasis in tissues. GSH is an important antioxidant and detoxifying molecule that helps to maintains normal redox status in the body. VCO thus thought to restore antioxidant redox balance. Supporting this, oxidative stress status in terms of TBARS and carbonyl adducts have been found decreased in VCO fed rats. Studies reported the improvement of paraoxonase-1 activity associated prevention of hyperlipidemia and oxidative stress in rats (Arunima and Rajamohan 2013).

Analysis of Liver function markers confirm the oxidative injury to hepatic tissue in CO fed animals. Elevated levels of SGPT, SGOT and ALP are indications of hepatic necrosis or steatosis in this group. Increased lipid peroxidation products, carbonyl adducts and lower levels of antioxidants in CO fed animals may have enhanced the oxidative insults. Histopathological results indicate formation of macrovesicular hepatosteatosis in this group. However the hepatic marker enzymes are found lower in VCO fed animals. There appears a normal hepatic architecture without any changes associated to hepatic steatosis. This suggests that dietary modification with VCO have significantly protected animals from oxidative insults and associated hepatic steatosis.

Aldehyde and peroxide content of VCO is comparatively lower than CO and ground nut oil. More over two fold increase in antioxidant polyphenols are found VCO. This indicates that VCO is highly advantage over CO. It is possible that as a dietary fatty acid source VCO helps to improve the antioxidant capacities and reduces the development of diabetes. Thus VCO may be a useful nutraceutical as well as food additives in alleviating diabetes and associated nonalcoholic hepatic steatosis.

At present, high consumption of fructose containing beverages and intake of high fat is common in societies. Together with sedentary life style, fructose consumption account for most of the degenerative human ailments prevailing today. According to a recent estimation, approximately 1.8 lakhs of people die of sugar sweeten beverages (Singh et al. 2015). Under such conditions, present study suggests the use of VCO as an alternate dietary fatty acid source.

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