Effect of Dietary Fat Selection on Plasma Cholesterol Synthesis in Older, Moderately Hypercholesterolemic Humans

Peter J.H. Jones, Alice H. Lichtenstein, Ernst J. Schaefer, Gayle L. Namchuk

Abstract To study factors controlling plasma cholesterol levels, the effect of dietary fat type on cholesterol synthesis was examined in 15 hypercholesterolemic subjects (low-density lipoprotein [LDL] cholesterol > 130 mg · dL⁻¹) consuming over a period of 32 days (1) a baseline diet (36% kcal as fat: 15% saturated, 15% monounsaturated, and 6% polyunsaturated fat; 180 mg cholesterol · 1000 kcal⁻¹) and diets meeting National Cholesterol Education Program step 2 criteria (30% kcal as fat, ≤7% saturated fat, 80 to 85 mg cholesterol/Mcal), where two thirds of the fat was either (2) olive, (3) corn, or (4) canola oil. Plasma total, LDL, and high-density lipoprotein (HDL) cholesterol and triglyceride levels were determined at the end of each period. Cholesterol fractional synthesis rate (FSR) was also measured as the deuterium (D) incorporation into plasma total cholesterol relative to body D₂O level (1.2 g D₂O · kg⁻¹ estimated body water) over 24 hours. Absolute synthesis rates (ASRs) were determined as the product of FSR and rapid turnover cholesterol pool size. Plasma total and LDL cholesterol levels declined significantly (P < .005) on all plant-oil diets compared with the baseline diet; however, triglyceride levels were not different. FSRs were higher (P < .05) for the corn oil (0.0665 ± 0.0097 pool · d⁻¹) compared with baseline (0.0412 ± 0.0060 pool · d⁻¹) and olive oil (0.0492 ± 0.0072 pool · d⁻¹) but not canola oil (0.0492 ± 0.0072 pool · d⁻¹) diets. Mean ASR for the corn oil diet (1697 ± 271 mg · d⁻¹) was elevated (P < .05) relative to baseline (1081 ± 170 mg · d⁻¹) and olive oil (1054 ± 140 mg · d⁻¹) but not canola oil (1169 ± 137 mg · d⁻¹) diet phases. These data suggest a more rapid rate of cholesterol synthesis with consumption of corn oil versus olive oil diets, indicating differential mechanisms that control circulating cholesterol level control across plant oil types. (Arterioscler Thromb. 1994;14:542-548.)

Key Words • cholesterol • synthesis • deuterium • humans • corn oil • olive oil • canola oil • fatty acids

Compelling evidence links plasma cholesterol levels to risk of human coronary heart disease.¹ Both earlier²⁻⁴ and more recent⁵⁻⁹ studies indicate that cholesterol levels are raised with consumption of fats containing saturated fatty acids (SAFAs) and reduced with fats high in monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA). Therefore, the type of dietary fat selected may influence heart disease risk. Despite this knowledge, the mechanism by which dietary fats influence circulating cholesterol levels remains to be completely understood. In animals, studies comparing the effects of various dietary fats on both the removal of cholesterol from the circulating compartment¹⁰,¹¹ and cholesterogenesis¹²⁻¹⁴ suggest higher rates with PUFA consumption. In humans, although consumption of SAFAs versus PUFA fat does not influence cholesterol synthesis as measured by sterol balance¹⁵ and mononuclear leukocytes,¹⁶ enhanced fecal sterol excretion has been reported with PUFA feeding,¹⁷⁻¹⁹ with resultant negative sterol balance.¹⁵ The only previous systematic study in humans has shown no effect of plant oils on cholesterol synthesis.²⁰ Any effect of dietary fat on cholesterol synthesis could potentially be attributable to factors including fatty acid composition or the presence of plant sterols or other constituents.

The present objectives were to examine (1) the influence of the ratio of MUFA to PUFA on circulating cholesterol levels and plasma cholesterol synthesis rates in moderately hypercholesterolemic subjects and (2) associations between fatty acid and plant sterol contents of the oils consumed and plasma cholesterol synthesis rate. Cholesterol synthesis was determined as the fractional synthesis rate (FSR) of the rapid-turnover body cholesterol pool and as a calculated estimate of absolute synthesis rate (ASR). Hypercholesterolemic subjects were examined because they are the group for whom the current dietary recommendations are targeted and are individuals who would derive the greatest physiological benefit from any changes in plasma lipids.

Methods

Subjects

Fifteen healthy volunteers with low-density lipoprotein (LDL) cholesterol levels > 130 mg · dL⁻¹ were screened for chronic illness including hepatic, renal, and cardiac dysfunction before admission to the study. Subjects were nonsmokers and were not taking lipid-lowering drugs, β-blockers, diuretics, or hormones. All women were postmenopausal. Protocols were approved by the Human Investigation Review Committee of New England Medical Center and Tufts University.

Protocol

Subjects consumed four experimental diets composed of solid foods typical of North American intakes. Each diet was consumed for a 32-day period, separated by a washout period of 7 days. Each diet was designed to meet National Cholesterol Education Program step 2 criteria (30% kcal as fat, ≤7% saturated fat, 80 to 85 mg cholesterol/Mcal) and was examined because they are the group for whom the current dietary recommendations are targeted and are individuals who would derive the greatest physiological benefit from any changes in plasma lipids. (Arterioscler Thromb. 1994;14:542-548.)

Key Words • cholesterol • synthesis • deuterium • humans • corn oil • olive oil • canola oil • fatty acids

Received September 3, 1993; revision accepted January 12, 1994.

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interval of 7 to 14 days. Subjects first consumed, over a period of 32 days, a baseline diet that contained 16.5% and 35.4% of calories from protein and fat, respectively, and a cholesterol content of 128 mg · 1000 kcal⁻¹. The fat contained 15% SAFA, 15% MUFA, and 6% PUFA. Subjects then consumed experimental diets consistent with National Cholesterol Education Program guidelines containing 30% of kcal as fat, with two thirds of dietary fat derived from olive, corn, or canola oil and 80 to 85 mg · 1000 kcal⁻¹ cholesterol. These three diets were provided in randomized order according to a double-blinded study design as part of a larger study involving other dietary treatments not included in this report. Foods and beverages containing the targeted energy, cholesterol, and fatty acid contents were provided by the Metabolic Research Unit of the US Department of Agriculture Human Nutrition Center on Aging for consumption on site or packaged for take-out. Subjects reported to the unit on at least three occasions per week. Energy intakes of subjects were tailored to individual requirements, as verified by constant body weight. Adjustments to energy intakes were made only during the initial 10-day period. PUFA to SAFA ratios for baseline and olive, corn, and canola oil and 80 to 85 mg · 1000 kcal⁻¹ energy, cholesterol, and fatty acid contents were provided by the Metabolic Research Unit of the US Department of Agriculture Human Nutrition Center on Aging for consumption on site or packaged for take-out. Subjects reported to the unit on at least three occasions per week. Energy intakes of subjects were tailored to individual requirements, as verified by constant body weight. Adjustments to energy intakes were made only during the initial 10-day period. PUFA to SAFA ratios for baseline and olive, corn, and canola oil and 80 to 85 mg · 1000 kcal⁻¹

Plasma Lipid Levels

Fasting blood samples from week 5 were collected in tubes containing EDTA (0.1%). Plasma was separated and assayed for total, LDL, and high-density lipoprotein (HDL) cholesterol and triglyceride levels by enzymatic procedures previously described.

Cholesterol Synthesis Determinations

Lipids were extracted from 2 to 3 mL plasma in duplicate. Extracts were saponified, dissolved in chloroform, and separated on thin-layer chromatography (TLC) silica gel plates (20x20 cm, 250 mm, Whatman Inc) as described. Plates were developed in petroleum ether/diethyl ether/acetic acid (135:15:1.5, vol/vol/vol) for 60 minutes and air-dried. Lipid fractions were visualized in iodine vapor; free cholesterol was removed from the plate and eluted from the silica by use of hexane/chloroform/diethyl ether (5:2:1, vol/vol/vol). Extracts containing approximately 2 mg cholesterol were transferred to Pyrex (Corning Glass Works) combustion tubes (12 cm x 6 mm) containing 0.5 g ground cupric oxide and a 2.5-cm length of 1-mm silver wire. Chloroform was removed, and tubes were sealed under vacuum. Tubes were placed in an oven at 520°C for 4 hours to combust the cholesterol. Combustion product water was transferred by vacuum distillation into a second Pyrex tube containing 60 mg of zinc reagent (Biogeochemical Laboratories). To measure plasma water D enrichment, plasma samples were diluted sevenfold to reduce the D enrichment to within the range of working standards. Baseline plasma samples were not diluted. Plasma samples were distilled into Pyrex tubes containing zinc and sealed under vacuum. Water from cholesterol and plasma samples was reduced at 520°C for 30 minutes before analysis of product hydrogen gas D enrichment by isotope ratio mass spectrometry (VG Isomass 903D). Mean internal and external precision (SD) levels of the mass spectrometer were 0.17 and 2.1 per mil (%o), respectively, for mean enrichment changes of about 150%o. The sample H2O contribution was checked daily, and appropriate corrections were applied. The instrument was calibrated against water standards of known isotopic composition.

Calculations of Cholesterol Synthesis Rates

Cholesterol FSRs were determined as incorporation of precursor D into plasma total cholesterol relative to the maximum
Theoretical enrichment with the linear-regression model. Considerations of the technique, which is based largely on the titrated water uptake method developed in animals, have been described previously.\(^{23,24}\) With humans, the present technique is restrictive, in that other than plasma, tissue cholesterol cannot be sampled over time. However, the 24-hour measurement period enables equilibration of D-labeled cholesterol across plasma and tissue pools. The peak and nadir of the diurnal rhythm in D incorporation have been shown to accord well with theoretical enrichment with the linear-regression model. Considerations of the technique, which is based largely on the titrated water uptake method developed in animals, have been described previously.\(^{23,24}\)

### Table 2. Plasma Cholesterol Levels and Fractional Synthesis Rates of Study Subjects

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<th>Subject</th>
<th>Sex</th>
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<th>Weight, kg</th>
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<th>Corn</th>
<th>Canola</th>
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</table>

**Results**

The mean±SEM age of the subjects was 62±3 years. Mean weight and height were 75.2±3.6 kg and 167±3 cm, respectively (Table 2). Mean caloric intake of the subjects was 2700±153 kcal·d\(^{-1}\). Cholesterol intakes were 346±20 and 224±12.7 mg·d\(^{-1}\) during baseline and corn and canola (194±5 mg·d\(^{-1}\)) oil diets were significantly (\(P<0.005\)) lower than those on the baseline diet (221±8 mg·d\(^{-1}\)) (Table 2). Total cholesterol levels when subjects consumed the corn oil diet were higher (\(P<0.01\)) than corn and canola phases. LDL levels during consumption...
Incorporation rates expressed as percent of plasma water deuterium enrichment (Enr) (solid bars) and as fractional synthetic rate (FSR) (hatched bars) in subjects consuming baseline and olive, corn, and canola oil-enriched diets. Values are mean±SEM. *Significant difference relative to corn oil diet (P<.05).

D incorporation rates are shown in Fig 2 for subjects consuming each diet. Rates are expressed both as percent precursor incorporation relative to plasma water enrichment and as calculated FSR. Percent incorporation for baseline and olive, corn, and canola oil diets was 1.97±0.29%, 1.96±0.24%, 3.18±0.44%, and 2.35±0.33%, respectively. FSR values for baseline and olive, corn, and canola oil diets were 0.0412±0.0060, 0.0409±0.0052, 0.0665±0.0097, and 0.0492±0.0072 mol d⁻¹, respectively (Table 2). In each case, rates were significantly (P<.05) higher for the corn oil phase compared with olive oil and baseline diet phases. The FSR for the canola oil phase tended to be similar to those of olive oil and baseline phases; however, the difference from the corn oil phase did not reach statistical significance.

Cholesterol pool size and ASRs are shown for subjects on each dietary phase in Fig 3. There were no differences in calculated rapid turnover M, pool sizes among the baseline (25.4±0.9 g) and olive (24.7±0.9 g), corn (24.4±0.9 g), and canola (24.4±0.9 g) oil groups. ASRs for baseline and olive, corn, and canola oil diets were 1080±170, 1034±140, 1697±271, and 1169±137 mg d⁻¹, respectively. For ASR, corn oil feeding was associated with an elevation (P<.05) in synthesis relative to the baseline and olive oil phases. As with FSR, rates during the canola phase were similar to those observed during baseline and olive oil phases. Expressed per unit body weight, synthesis rates were, for baseline, olive, corn, and canola diet phases, 13.9±1.9, 13.4±1.6, 21.3±2.8, and 16.1±1.3 mg kg⁻¹ d⁻¹, respectively.

Fatty acid composition and nonsaponifiable lipid contents of dietary oils are compared in Table 1. Olive and canola oils were relatively rich in oleic acid, whereas corn oil contained higher levels of linoleic acid. Canola oil contained greater concentrations of linoleic acid and less palmitic acid compared with olive oil. Combined nonsaponifiable lipid levels were highest in corn oil, with lowest levels in olive oil. Campesterol levels were virtually undetectable in the olive oil sampled. No lanosterol was found in any of the oils studied.

Both FSR and ASR were in part related to the 18:2 n6 and plant sterol fractions of the oils. Correlation analyses comparing FSR against total plant sterol level, sitosterol content, and percent dietary 18:2 n6 yielded coefficients of R²=.119 (P=.025), R²=.129 (P=.019), and R²=.129 (P=.020), respectively. Comparison of ASR against total plant sterol level, sitosterol content, and percent dietary 18:2 n6 yielded coefficients of R²=.108 (P=.059), R²=.121 (P=.024), and R²=.125 (P=.022), respectively. No other significant associations were detected between cholesterol synthesis and fatty acid content.

**Discussion**

Present findings, indicating enhanced cholesterol synthesis in humans consuming corn relative to olive and

**Fig 1.** Bar graph showing plasma total cholesterol (Chol), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels in subjects consuming baseline and olive, corn, and canola oil-enriched diets. Values are mean±SEM. *Difference relative to baseline diet (P<.005 for total, P<.001 for LDL, and P<.01 for HDL, respectively). **Difference relative to olive oil diet (P<.01).

**Fig 2.** Bar graph showing plasma cholesterol deuterium (D) incorporation rates expressed as percent of plasma water deuterium enrichment (Enr) (solid bars) and as fractional synthetic rate (FSR) (hatched bars) in subjects consuming baseline and olive, corn, and canola oil-enriched diets. Values are mean±SEM. *Significant difference relative to corn oil diet (P<.05).

**Fig 3.** Bar graph showing rapid exchange M, cholesterol pool size (solid bars) and absolute cholesterol synthesis rates (ASR) (hatched bars) in subjects consuming baseline and olive, corn, and canola oil-enriched diets. Values are mean±SEM. *Significant difference relative to corn oil diet (P<.05).
perhaps canola oil, suggest a fundamental difference in the mechanism by which these different plant oils elicit their cholesterol-lowering effect. Positive correlations were observed between both the 18:2 n6 and plant sterol of the three plant oils tested but not with other oil constituents. These associations suggest that fatty acid or plant sterol levels of dietary oils consumed may play a role in the regulation of cholesterol biosynthesis.

Differential regulation of circulating cholesterol levels among various plant oils has been suggested previously by two lines of evidence. First, whereas plant oils containing MUFAs and PUFAs lower human total circulating cholesterol concentrations in comparison with SAFAs, lipoprotein distribution may differ with the type of plant oil consumed. Although not without exception, some studies report that oils high in MUFA lower LDL but not HDL cholesterol concentrations, whereas those high in PUFAs result in a reduction of both lipoprotein subspecies. Differences in the mechanism of action may underlie the distinct effects observed in lipoprotein profile with feeding of PUFAs versus SAFAs containing oils.

Second, dietary fat-dependent variations in both lipoprotein clearance and cholesterol synthesis rates have been reported in animal and human studies. In guinea pigs, high PUFAs corn oil-containing diets were shown to increase specific binding of LDL by elevating LDL receptor numbers compared with diets containing olive oil or lard. In hamsters, an increase in hepatic LDL clearance has been observed in animals consuming diets containing safflower oil compared with coconut oil and olive oil. Elevated LDL removal, associated with enhanced hepatic elimination, may reduce circulating cholesterol levels and result in a compensatory increase in cholesterol synthesis. Support of this mechanism, Fernandez et al demonstrated that guinea pigs incorporate tritium into hepatic digu-ton-precipitable sterols more slowly in oil-fed animals than in those fed corn oil. Similarly, cholesterol synthesis, measured by use of tritiated water incorporation, was shown to be higher in hamsters fed diets with PUFAs versus MUFA and in rats fed PUFAs versus SAFAs. Also, enhanced apolipoprotein B secretion after addition of linoleic versus oleic acid has been reported in CaCo2 cells in vitro. These results are not unequivocal; other studies report no differences in hepatic cholesterol synthesis as a result of feeding oils high in PUFA versus MUFA in rats or hamsters. Lack of agreement in synthesis may be a result of interstudy variation in dietary composition of fatty acids, cholesterol content, or other nonnonsaponifiable components of the oils.

Data specifically comparing the effect of diets enriched with PUFA versus MUFA on cholesterol synthesis in humans are limited. In a single subject, there was no influence of diets with PUFA on synthesis measured by sterol balance compared with diets containing MUFA. Other studies using sterol balance and mononuclear leukocytes similarly have shown no difference in cholesterol synthesis rates between diets rich in PUFA versus MUFA. Nonetheless, these results might imply a difference in synthesis of body cholesterol with negative sterol balance. In the latter study, it was speculated that cholesterogenesis may increase to compensate for the enhanced removal of sterol relative to intake. Similarly, and consistent with findings of animal studies in which LDL clearance was increased with PUFAs feeding, increased body elimination of sterol would be expected to result in more rapid clearance of lipoprotein cholesterol, as has been observed in humans consuming PUFAs. The present results are consistent with the concept that in humans, feeding corn oil causes enhanced cholesterogenesis, either in response to increased whole-body excretion and plasma removal of cholesterol or as a consequence of oil fatty acid profile or content of some nonnonsaponifiable component.

The second objective of the present study was to examine whether levels of fatty acids or some other component of dietary oils may be responsible for the effects observed. Fatty acids may directly alter cholesterol synthesis rates in a structure-specific manner. Increasing evidence suggests that PUFAs are preferentially used for oxidation versus retention in vivo. It is conceivable that upregulated β-oxidation of PUFAs results in greater availability of acetyl coenzyme A as a substrate for cholesterol synthesis. These mechanisms also possibly account for the differential effects of the dietary fatty acids contained in the fats studied here. Alternatively, plant sterols or other compounds such as squalene found in plant oils may upregulate cholesterogenesis indirectly by depressing the absorption of cholesterol or directly by serving as a cholesteryl precursor. Squalene levels were not measured in the present study. However, sitosterol and total plant sterol concentrations in the three oils tested varied directly with cholesterol synthesis, supporting the possibility of their interference with cholesterol absorption. Given that subjects were consuming a diet reduced in fat compared with average North American intakes, it would be predicted that any such effects of dietary fat would be even greater at levels typically consumed.

The canola oil tested can be considered intermediate between corn and olive oil in composition, both in PUFA-to-MUFA ratio and in sitosterol and total plant sterol content (Table 1). The cholesterol-lowering effects of canola oil were more typical of corn oil than of olive oil, as has been reported previously. The intermediate effect of addition of this oil on cholesterol synthesis rate may therefore be explained through its plant sterol or fatty acid composition.

Methodological considerations are important in valid interpretation of the data from this study. Valid results require subject compliance with diet and procedural aspects of the study. Subject testing was conducted on an outpatient basis, with subjects taking out a variable proportion of meals for consumption outside the Metabolic Research Unit. Since consumption of each meal was not verified by unit staff, the compliance level of individual study volunteers could not be confirmed. However, plasma fatty acid patterns reflected those of the diet, as has been previously reported, suggesting good compliance.

The deuterated water incorporation technique we used provides a novel tool for simple, direct measurement of human cholesterol synthesis. The model shares many assumptions of the tritium uptake technique although developed in animals, enabling cholesterogenesis measurement over short durations with minimal subject involvement. Circadian synthesis rate variations seen with D incorporation...
ration agree well with periodicity in plasma mevalonate levels. Limitations, assumptions, and procedural issues have been previously reviewed. Two issues emerge concerning the capacity of the D-incorporation method to provide an accurate indication of synthesis in the present application. First, label incorporation is influenced by both the synthesis and flux rate of unlabeled cholesterol through the rapidly miscible pool. With higher dietary intakes, more cholesterol would be absorbed and transported to the liver, competing with labeled sterol for packaging into very-low-density lipoprotein and thereby diluting the measurable plasma D-containing cholesterol. In this way, measurable D incorporation would be reduced at a level proportional to the extent of influx of unlabeled sterol. In the present study, dietary cholesterol contents were constant across the three plant oil diets; therefore, the method should yield accurate relative indices of cholesterol formation, unless cholesterol absorption rates varied across oils. If the greater nonsaponifiable lipid levels in corn oil caused a decline in cholesterol absorption, D-incorporation rates might be somewhat increased, although given the low level of cholesterol in these plant oil diets overall, it seems unlikely that the observed differences in cholesterol synthesis could be entirely explained through differences in absorption. Some of the potential interoil effects on absorption are accounted for through the calculation of ASR, which factors in variation in the rapidly miscible pool size.

The second possible limitation of the methodology concerns the potential impact of dietary fatty acid composition on the D-incorporation ratio, a critical ratio in the derivation of FSR values. It has been suggested that the observed disparity between results of tritium incorporation and sterol balance techniques for cholesterogenesis measurement in guinea pigs fed fats differing in fatty acid composition was due to dietary fat-mediated effects on the metabolic source of NADPH. Variation in the tracer enrichment of NADPH caused by the fat type consumed could produce an apparent difference in synthesis, whereas actual rates remained unchanged. Our calculations have shown that any such error would be quantitatively minor and thus unlikely to result in changes in synthesis of the magnitude presently observed.

Present results suggest that differences in cholesterol synthesis in response to dietary fat selection occur in middle-aged, moderately hypercholesterolemic subjects consuming diets relatively low in fat content. Whether these differences occur in normolipidemic or younger subjects cannot be established from this study. The intention was to examine the mechanisms of action of lowering of circulating cholesterol levels in subjects with a greater risk of heart disease, who would derive the largest benefit from cholesterol-lowering diets.

In summary, present findings support work done in animals suggesting that there is a more rapid flux of central-pool cholesterol associated with enhanced synthesis subsequent to corn versus olive oil feeding. Although other factors not presently identified may be responsible for this association, the higher content of oils used are possible contributing factors. Further investigation will be required to delineate the precise underlying mechanism responsible for this effect.

Acknowledgments

This study was supported by grants from the British Columbia and Yukon Heart and Stroke Foundation, the US Department of Agriculture (contract 53-3K06-5-10), the National Institutes of Health (HL-39326), Uncle Ben’s Inc, Dallas, Texas, and the US Egg Marketing Board. The authors thank the staff of the Metabolic Research Unit, Human Nutrition Research Center on Aging at Tufts University, for expert services provided; gratefully appreciate the cooperation of study subjects; and acknowledge the excellent technical assistance of Clement Fung, Isabeau IQbal, Yi Lin, Joost Schulte, and Brian Toy. Thanks are also extended to Drs Lynne Ausman, Tufts University, and Mark Bieber, Best Foods Inc, for assistance with nonsaponifiable lipid analysis, and to Dr Barry Goldin, Tufts University, for help with the fatty acid analysis.

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Arterioscler Thromb Vasc Biol. 1994;14:542-548
doi: 10.1161/01.ATV.14.4.542

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/14/4/542

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