Interaction of dietary fat saturation and cholesterol level on cholesterol synthesis measured using deuterium incorporation

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Abstract To examine interactive effects of dietary fat saturation and cholesterol level on serum lipids and de novo cholesterogenesis, moderately hypercholesterolemic subjects were fed solid-foods diets containing 30% fat (80 mg C.1000 $Kcal^{-1}$) in which 2/3 fat was either corn oil or beef tallow, with and without 120 mg C.1000 $Kcal^{-1}$, for 5 wk. At the end of each diet period, subjects were given deuterium (D) oxide orally and de novo cholesterol synthesis was measured over 24 h from D incorporation into cholesterol as fractional synthesis rates (FSR) and absolute synthetic rate (ASR) into the rapid exchangeable cholesterol pool. Plasma total and low density lipoprotein levels were elevated ($P < 0.01$) with both beef tallow and cholesterol feeding. High density lipoprotein (HDL) levels were not influenced by fat saturation or cholesterol level; however, HDL was higher with addition of cholesterol to corn oil versus beef tallow ($P < 0.02$). Plasma triglycerides were higher ($P < 0.02$) with beef tallow feeding but were not influenced by cholesterol level. FSR was increased ($P < 0.02$) by feeding corn oil, versus beef fat, but not by dietary cholesterol level. Calculated cholesterol pools sizes did not differ across groups; however, ASR was also elevated with corn oil versus beef tallow feeding ($P < 0.02$). Results indicate that corn oil feeding lowers circulating cholesterol by mechanisms other than reduced synthesis, and that cholesterol at the level of supplementation used is not associated with feedback inhibition of cholesterogenesis. However, with the exception of HDL levels, dietary fat saturation and cholesterol levels do not interactively influence circulating lipoprotein cholesterol levels and cholesterol synthesis.—**Jones, P. J. H., A. H. Lichtenstein, and E. J. Schaefer.** Interaction of dietary fat saturation and cholesterol level on cholesterol synthesis measured during deuterium incorporation. *J. Lipid Res.* 1994. **35:** 1093–1101.

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Dietary fatty acid composition and cholesterol level both potentially influence circulating cholesterol levels. The cholesterol-raising properties of fats containing saturated fatty acids (SAFA), in comparison to the cholesterol-lowering action of monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) fats, has been repeatedly demonstrated in humans (1–11). In contrast, the impact of dietary cholesterol on circulating cholesterol levels is more controversial. Increases in dietary cholesterol exert no effect (12–16) or a cholesterol-raising effect (8, 10, 17–22) in humans. Concensus opinion is that raising dietary cholesterol results in higher circulating levels, with variable responses attributed to factors including inter-individual variability in response and a plateauing of the dietary–circulating cholesterol level relationship at higher cholesterol intakes (23).

Mechanistic actions of dietary fat and cholesterol on in vivo cholesterol metabolism have been partially elucidated. Human studies showed that consumption of PUFA compared with more saturated fats had no influence on sterol balance or synthesis (24), or resulted in increased sterol excretion in normal subjects (25, 26) and patients with hypertriglyceridemia (27). Elevated triglyceride-rich lipoprotein (28) and low density lipoprotein (LDL) (11) removal from circulation has also been reported in subjects fed PUFA, versus SAFA. In animals, dietary fat type increases both uptake from (29, 30), and synthesis into (31–33), circulating cholesterol pools. For dietary cholesterol, increased dietary cholesterol levels either modify (7, 34) or have no effect on (35–37) cholesterol absorption and synthesis in humans. Animal studies (32, 38–40) have shown that increasing dietary sterol consumption depresses cholesterol synthesis.

Abbreviations: FSR, fractional synthesis rate; ASR, absolute synthetic rate; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; MRU, Metabolic Research Unit; TLC, thin-layer chromatography; D, deuterium.

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Although interactive effects of dietary fat type and cholesterol level on circulating cholesterol levels have been determined (7–10, 16, 32) less is known concerning mechanisms of independent and interactive effects. In humans consuming normal diets, the degree of saturation of dietary fat exerted no effect on cholesterol synthesis or feedback response, whereas addition of dietary cholesterol resulted in a reduction in fractional absorption and feedback inhibition of cholesterogenesis (7). In hamsters, feeding fat containing SAFA versus PUFA fat augmented the influence of added dietary cholesterol in lowering hepatic LDL receptor activity, concurrently decreasing cholesterol synthesis rates (32). Further studies are required to clarify the response of human cholesterol metabolism to changes in diet fatty acid composition and cholesterol content.

The present objective was to examine interactive effects of dietary fat composition and cholesterol level on plasma cholesterol concentrations and synthesis in moderately hypercholesterolemic individuals, with a view towards describing the mechanism through which these dietary factors modulate circulating cholesterol levels. It was hypothesized that changes in cholesterol synthesis account for the response of circulating total and LDL levels to dietary fat composition and cholesterol level. Cholesterogenesis was measured using deuterium incorporation in subjects consuming diets containing predominantly corn oil and beef tallow as the source of fat, at two levels of dietary cholesterol, within the typical range of intakes of the population. Hypercholesterolemic individuals were examined as they are the group for whom dietary recommendations are targeted, and are individuals who would derive the greatest physiological benefit from any changes in plasma lipids.

METHODS

Subjects

Healthy volunteers with LDL cholesterol levels over 130 mg • dl^{-1} were screened for chronic illness including cardiac, hepatic, renal, and thyroid dysfunction before admission to the study. Subjects were non-smokers and not taking lipid-lowering drugs, beta-blockers, diuretics or hormones. All females were post-menopausal. The protocol was approved by the Human Investigation Review Committee of New England Medical Center and Tufts University.

Protocol

Subjects each underwent four 5-wk dietary phases as described (41). During each dietary phase subjects consumed experimental diets containing 30% of kcal as fat and 80 mg • 1000 $kcal^{-1}$ cholesterol, with two-thirds of the diet fat derived from corn oil or beef tallow. Non-oil constituents of each diet were identical. Subjects consumed each diet fat phase twice, once with and once without about 120 mg cholesterol • 1000 $kcal^{-1}$ added as egg yolks. Triplicate preparations of each complete meal cycle (3 days) for each diet phase were analyzed for macronutrient and fatty acid content by Hazelton Laboratories America, Inc. (Madison, WI). Food for each phase was provided by the Metabolic Research Unit (MRU) of the USDA Human Nutrition Center on Aging, Tufts University for consumption on site or packaged for take-out. Caloric intakes of subjects were tailored to individual requirements, as verified by ability to maintain body weight. During the final week of each dietary phase, subjects resided within the MRU while consuming their 3 meals per day and snack at scheduled intervals. Breakfast, lunch, and supper meals were provided at 08:00, 12:00, and 17:00 h, with a snack given at 20:00 h.

Plasma lipid levels

Fasting blood samples from wk 5 were collected in tubes containing EDTA (0.1%). Plasma was separated at 3,000 rpm at 4°C and assayed for total, LDL, and high density lipoprotein (HDL) cholesterol and total triglyceride levels as described (41). After removal of very low density lipoprotein, the infranatant fraction was assayed for total cholesterol level using an enzymatic technique (42). HDL was determined following precipitation of other lipoproteins using dextran sulphate magnesium as described (43). LDL-cholesterol levels were calculated by subtracting HDL cholesterol from the 1.006 g • ml^{-1} infranatant fraction cholesterol level.

Cholesterol synthesis determinations

On wk 5 of each study phase, deuterium incorporation was measured after administration of 1.2 g deuterium oxide per kg body water, estimated as 60% of body weight. Blood samples were collected just prior to, and 24 h after, dosing for plasma total cholesterol and water deuterium enrichment measurement as reported (38, 44, 45). Lipids were extracted from 2–3 ml plasma in duplicate, saponified, then dissolved in chloroform and separated on thin-layer silica (TLC) gel plates (20 × 20 cm, 250 mm, Whatman Inc, Clifton, NJ). Plates were developed in petroleum ether–diethyl ether–acetic acid 135:15:1.5 (v/v/v) for 60 min and air dried. Lipid fractions were visualized in iodine vapor; the free cholesterol band was scraped from the plate and eluted from the silica scrapings using hexane–chloroform–diethyl ether 5:2:1 (v/v/v). Eluted cholesterol was transferred to Pyrex (Corning Glass Works, Corning, NY) combustion tubes (12 cm × 6 mm) containing 0.5 g ground cupric oxide and a 2.5-cm length of 1 mm silver wire. Chloroform was removed under vacuum and tubes were sealed. Tubes were placed in an oven at 520°C for 4 h to combust the cholesterol. Combustion product water was transferred by vacuum distilla-

tion into a second Pyrex tube containing 60 mg of zinc reagent (Biogeochemical Laboratories, Indiana University, Bloomington, IN) (44, 45).

Samples of plasma were diluted sevenfold to reduce the deuterium 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 generated from cholesterol and plasma samples was reduced at 520°C for 30 min before analysis of product hydrogen gas-deuterium enrichment by isotope ratio mass spectrometry (VG Isomass 903D, Cheshire, UK). Mean internal and external precision (SD) levels of the mass spectrometer were 0.16 and 2.3 del (parts per thousand relative to Standard Mean Ocean Water, °/oo), respectively. The sample H_3^+ contribution was checked daily and appropriate corrections were applied. The instrument was calibrated using water standards of known isotopic composition. Samples for each subject were analyzed concurrently against a single set of standards.

Cholesterol fractional synthesis rates (FSR) were determined from the initial incorporation rate of deuterium-labeled cholesterol into the rapid exchangeable cholesterol pool, relative to the initial precursor enrichment as determined using the body water deuterium level, as defined by Foster et al. (46). Theoretical and practical considerations of the deuterium incorporation approach have been described previously (38, 44, 45) and synthesis results have been shown to accord well with levels of circulating cholesterol precursors (47). The central, rapid turnover pool requires months to attain plateau deuterium enrichment in humans and the initial deuterium incorporation rate, excepting circadian periodicity, is highly linear. This initial linear rate of tracer appearance is unaffected by flux rates of unlabeled material through the system, thus it represents a direct index of synthesis independent of the total production rate. Maximum attainable enrichment was calculated as the body water pool enrichment corrected for the fraction of protons in de novo synthesized cholesterol that derive from water, relative to non-water sources using the equation:

$$\text{FSR } (d^{-1}) = \frac{\text{del } (^{o}/oo)_{init} \text{ cholesterol}}{\text{del } (^{o}/oo)_{max\text{-}init} \text{ cholesterol}} \qquad Eq.\ 1)$$

where del_{init} refers to the difference in plasma cholesterol deuterium enrichment the initial 24 h, and $del_{max\text{-}init}$ the maximum initial cholesterol enrichment predicted as del (°/oo) plasma water × 0.478 (45).

Absolute synthesis rate (ASR) g • d^{-1}) was derived by multiplying FSR by total rapid exchangeable cholesterol pool volume, as calculated from the equation of Goodman et al. (48). Data were analyzed using a two-factor analysis of variance design, with tests for interactions. The level of significance was set at $P = 0.05$.

RESULTS

Demographics of study participants are shown in **Table 1.** Subjects were 63 ± 3 years (mean ± SEM) of age, weighed 74 ± 4 kg and were 165 ± 3 cm in height. Body mass index 27.2 ± 1.1 kg • m^{2-1}. Screening plasma cholesterol levels were 238 ± 6 mg • dl^{-1}; LDL cholesterol levels were 164 ± 6 mg • dl^{-1}; HDL cholesterol levels were 48 ± 3 mg • dl^{-1}; plasma TG levels were 135 ± 12 mg • dl^{-1}.

Composition of diets consumed is shown in **Table 2.** Protein, carbohydrate, and fat contents of the four diets were within 3–4%. Fatty acid composition differences between corn oil and beef tallow diets reflected a direct substitution of PUFA for SAFA. Fatty acid composition of diets containing each diet fat, with and without added cholesterol, were similar. Due to the higher natural cholesterol content of the beef tallow, each day's diet with tallow contained approximately 25 mg more cholesterol

TABLE 1. Demographics of study subjects

Variable	Females (n = 8)	Males (n = 6)	Mean (n = 14)
Age (year)	70 ± 5	57 ± 5	63 ± 3
Body weight (kg)	67 ± 4	80 ± 6	74 ± 4
Height (cm)	159 ± 1	173 ± 4	165 ± 3
Blood pressure (mm Hg)	132/70	128/79	130/74
Body mass index [Weight (kg)/height²(m)]	27.8 ± 1.9	26.3 ± 1.0	27.2 ± 1.1
		mg• dl⁻¹	
Total cholesterol	236 ± 11	240 ± 6	238 ± 6
LDL-C	159 ± 10	170 ± 7	164 ± 6
HDL-C	52 ± 3	43 ± 4	48 ± 3
Triglyceride	124 ± 13	149 ± 23	135 ± 12

Values are mean ± SEM; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

TABLE 2. Diet composition as determined by chemical analysis

Composition	Corn Oil	Corn Oil Plus Cholesterol	Beef Tallow	Beef Plus Cholesterol
		percent of calories		
Protein	17.4 ± 1.50	15.7 ± 0.20	16.1 ± 0.10	16.7 ± 0.30
Carbohydrate	53.0 ± 1.10	52.9 ± 0.50	51.2 ± 0.90	52.4 ± 1.20
Fat	29.6 ± 0.60	31.5 ± 0.60	32.7 ± 0.60	30.8 ± 1.50
SFA	6.41 ± 0.80	7.37 ± 0.20	13.69 ± 0.33	12.07 ± 0.60
12:0	0.09 ± 0.01	0.13 ± 0.02	0.06 ± 0.03	0.89 ± 0.01
14:0	0.39 ± 0.03	0.39 ± 0.02	0.87 ± 0.01	0.73 ± 0.04
16:0	3.86 ± 0.64	4.72 ± 0.04	8.39 ± 0.18	7.63 ± 0.35
18:0	1.57 ± 0.14	1.75 ± 0.14	3.75 ± 0.16	3.14 ± 0.20
MUFA	10.71 ± 1.36	9.20 ± 0.16	12.07 ± 0.23	11.28 ± 0.39
16:1n7	0.30 ± 0.04	0.36 ± 0.02	0.85 ± 0.04	0.75 ± 0.02
18:1n9	10.06 ± 1.35	8.58 ± 0.18	10.61 ± 0.21	10.08 ± 0.34
PUFA	9.45 ± 1.47	11.91 ± 0.09	2.62 ± 0.20	3.40 ± 0.12
18:2n6	8.48 ± 1.90	11.73 ± 0.09	2.44 ± 0.16	2.97 ± 0.11
18:3n3	0.83 ± 0.43	0.40 ± 0.02	0.23 ± 0.02	0.24 ± 0.08
20:4n6	0.05 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.12 ± 0.01
Cholesterol (mg • 1000 kcal^{-1})	83 ± 4	197 ± 8	109 ± 7	226 ± 12

Values are mean ± SEM; n = 3. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

than with corn oil. For a 2000 kcal • day^{-1} diet, the average cholesterol intakes for corn oil and beef tallow diets were 166 and 218 mg • day^{-1} without and 394 and 452 mg • day^{-1} cholesterol with added sterol.

Plasma lipid level responses to diet treatment are shown in **Fig. 1.** Plasma total cholesterol levels on corn (193 ± 6 mg • dl^{-1}), corn plus cholesterol (205 ± 8 mg • dl^{-1}), beef (217 ± 9 mg • dl^{-1}), and beef plus cholesterol (229 ± 10 mg • dl^{-1}) were influenced by both dietary fat type (P = 0.002) and cholesterol content (P = 0.008), although there was no interactive effect (P = 0.961). Similarly, plasma LDL levels for corn (125 ± 6 mg • dl^{-1}), corn plus cholesterol (135 ± 7 mg • dl^{-1}), beef (139 ± 8 mg • dl^{-1}), and beef plus cholesterol (156 ± 9 mg • dl^{-1}) were decreased by substitution of corn oil in place of beef tallow dietary fat (P = 0.003), as well as by decreasing the dietary cholesterol content (P = 0.001), however, there was no interaction (P = 0.274). HDL levels for corn (44 ± 3 mg • dl^{-1}), corn plus cholesterol (47 ± 3 mg • dl^{-1}), beef (45 ± 3 mg • dl^{-1}), and beef plus cholesterol (45 ± 3 mg • dl^{-1}) were not influenced by either dietary fat type (P = 0.230) or cholesterol level (P = 0.169), however, an interaction was observed (P = 0.014) where HDL cholesterol was higher with addition of cholesterol to corn oil, compared to beef tallow. Triglyceride levels for corn (109 ± 10 mg • dl^{-1}), corn plus cholesterol (101 ± 9 mg • dl^{-1}), beef (114 ± 11 mg • dl^{-1}), and beef plus cholesterol (116 ± 11 mg • dl^{-1}) were influenced by dietary fat type (P = 0.034), but not diet cholesterol level (P = 0.394).

Effects of diet on cholesterol FSR and ASR values a shown in **Fig. 2.** Individual values for FSR for subjec consuming each diet are provided in **Fig. 3.** FSRs f

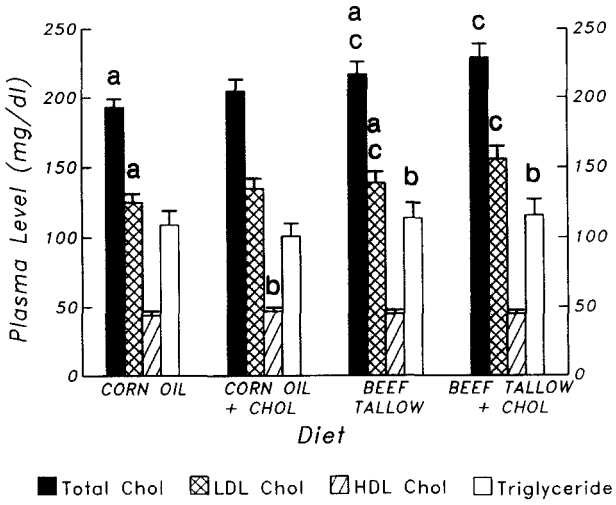

Fig. 1. Plasma levels for total cholesterol, low density lipoprotein (LDL) cholesterol, high density cholesterol (HDL), and triglycerides in subjects consuming diets enriched with corn oil (n=14), corn oil plus added cholesterol (n=14), beef tallow (n=14), and beef tallow with added cholesterol (n=14). Diet fat effect was significant for total (P = 0.002) and LDL (P = 0.003) cholesterol and TG (P = 0.034). Dietary cholesterol effect was significant for total (P = 0.008) and LDL (P = 0.003) cholesterol levels. a: Mean different (P = 0.01) from groups with added cholesterol; b: mean different (P < 0.05) from corn oil groups; c: mean different (P < 0.01) from corn oil groups. Values are means ± SEM.

corn (0.0665 ± 0.007 pools • day^{-1}), corn plus cholesterol (0.0531 ± 0.010 pools • day^{-1}), beef (0.0437 ± 0.007 pools • day^{-1}), and beef plus cholesterol (0.037 ± 0.008 pools • day^{-1}) diets were influenced by diet fat (P = 0.018), but not dietary cholesterol (P = 0.208) level. Calculated rapidly exchangeable cholesterol pool volumes for corn (24.4 ± 1.2 g), corn plus cholesterol (23.3 ± 1.2 g), beef (24.7 ± 1.2 g), and beef plus cholesterol (24.5 ± 1.2 g) diets were not different across groups. Absolute synthesis rates for corn (1.69 ± 0.21 g • day^{-1}), corn plus cholesterol (1.43 ± 0.27 g • day^{-1}, beef (1.08 ± 0.20 g • day^{-1}, and beef plus cholesterol (0.95 ± 0.21 g • day^{-1} diets also showed an effect of diet fat (P = 0.017), but not of cholesterol level (P = 0.375). Similar findings were observed for ASR data described per kg body weight.

DISCUSSION

Despite characterization of the effects of dietary fatty acid composition and cholesterol level on circulating lipids, independent and interactive mechanisms of action of these dietary constituents have not been fully defined. The influence of diet fatty acid composition on circulating cholesterol levels observed presently parallel those seen previously (2, 3, 7–11). For both total and LDL cholesterol levels, we observed higher levels with beef tallow feeding. In this comparison, as observed previously (9), there was no unfavorable lowering of HDL levels by diets containing more PUFA compared with SAFA and MUFA in these moderately hypercholesterolemic subjects fed these diets containing 30% fat. Corn oil substitution for beef tallow would therefore be an advisable strategy in both lowering LDL and optimizing total/HDL cholesterol ratios.

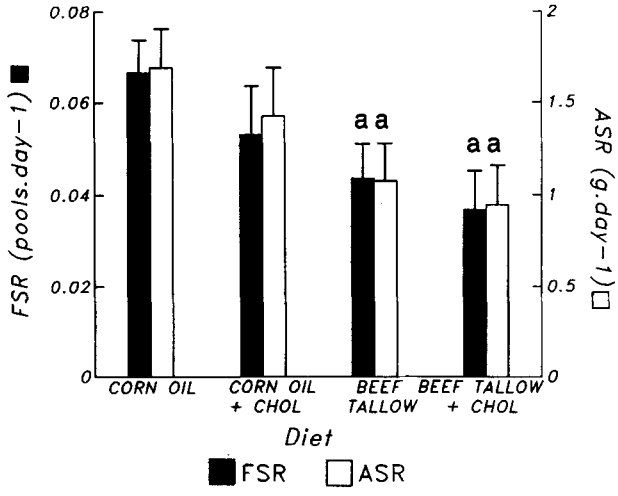

Fig. 2. Fractional synthesis rate (FSR) and absolute synthesis rate (ASR) for total plasma cholesterol in subjects consuming diets enriched with corn oil (n=14), corn oil plus added cholesterol (n=8), beef tallow (n=14), and beef tallow with added cholesterol (n=13). Diet fat effect was significant for FSR (P = 0.017) and ASR (P = 0.018). a: Mean different (P < 0.05) from corn oil groups. Values are means ± SEM.

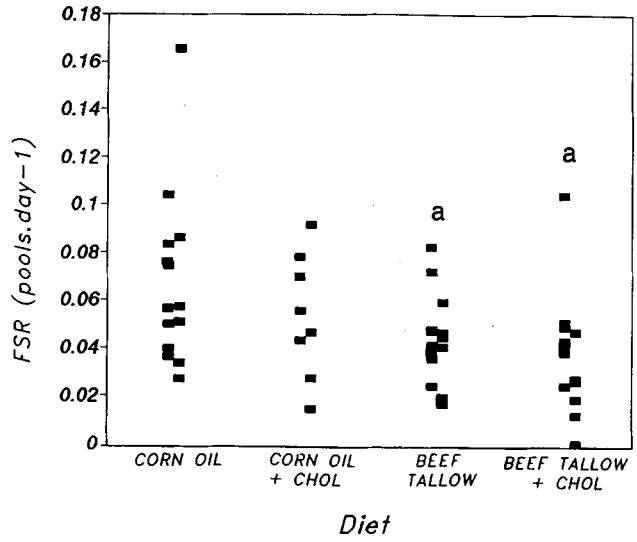

Fig. 3. Individual fractional synthesis rates of total plasma cholesterol in subjects consuming diets enriched with corn oil, corn oil plus added cholesterol, beef tallow, and beef tallow with added cholesterol. a: Mean different (P < 0.05) from corn oil groups.

For cholesterol, our results support findings of several reports (8, 10, 17–22) that indicate a cholesterol-raising effect of addition of varying amounts of dietary cholesterol. Previous findings of absence of effect (12–16) may be attributable to quantity and form of the added cholesterol, as well as to within- and between-subject variability. Our results indicate an interactive influence of dietary fat composition and cholesterol level, where HDL cholesterol levels increased with cholesterol augmentation to the diet containing corn oil, but not the beef tallow. No such interactive influence was observed in a previous similar study design where cholesterol was added to the diet as six eggs per day (8).

Although effects of dietary fats and cholesterol on circulating cholesterol levels have been well defined, it is less clear as to what changes in cholesterol metabolism occur to account for these dietary effects. Several studies in animals and humans have previously addressed this question; however, results are not unequivocal. Physical and biological properties of LDL do not appear influenced by dietary PUFA/SAFA ratio in humans (10), although other findings have shown a fall in the relative percent of cholesterol within LDL (11).

Kinetically, effects of diet fat composition have been noted on both cholesterol clearance from and synthesis into body cholesterol pools. In guinea pigs, consumption

of oils richer in PUFA was associated with enhanced specific receptor binding of LDL compared with consumption of saturated fats (30, 31). Also, greater LDL clearance has been reported in hamsters consuming safflower oil PUFA-containing diets compared with saturated fats (29, 32). Cholesterol synthesis also appears to be elevated in animals consuming PUFA fat diets (32, 33). Incorporation of tritium from tritiated water into tissue cholesterol was greater in hamsters fed PUFA-containing safflower oil, versus those fed coconut and olive oil (32), as well as in rats fed PUFA- versus SAFA-containing diets (33). Our data are not inconsistent with these results. It is suggested that the driving factor in reducing circulating cholesterol levels with PUFA feeding is cholesterol removal, with a concommitant increase in synthesis. However, given that either LDL or total circulating cholesterol pool represent a small fraction of the total exchangeable cholesterol pool size, such a mechanism would require that cholesterogenesis be acutely tuned to respond to this quantitatively minor pool component.

Findings from some human studies are in agreement with a mechanism where synthesis up-regulates in response to PUFA feeding, subsequent to a separate primary influence on body cholesterol removal. In normal men fed formula diets containing either SAFA-containing cocoa butter or PUFA-containing corn oil for 3 wks each, sterol balance was used to determine sterol excretion rates (25). Subjects lived in a metabolic ward during the entire study. Enhanced total fecal steroid excretion was observed during consumption of the PUFA diet, creating a negative cholesterol balance, compared with the SAFA diet. In a similar study, fecal sterol excretion was higher when subjects were fed low and high cholesterol PUFA diets compared with two corresponding SAFA fat periods (9, 26). Increased sterol excretion has also been observed in hypertriglyceridemic individuals consuming PUFA-rich diets (27). PUFA feeding may thus enhance hepatic cholesterol elimination, up-regulating removal of circulating sterol and invoking higher synthesis. A previous report showed an enhanced fractional catabolic rate of LDL in normal males fed PUFA- versus SAFA-enriched diets, without significant alteration of synthetic rate (11).

Other investigations have observed no influence of PUFA feeding on sterol elimination or synthesis. Cholesterol balance studies carried out in hyperlipoproteinemic patients fed liquid diets containing butter, safflower oil, and corn oil showed no fat-related changes in fecal bile acid excretion or synthesis (24). Similarly, when diet fats including corn oil, coconut oil, synthetic trilinolein, and palmitic/oleic glycerides were fed to a single subject (49), no difference in excretion of total fecal sterols occurred, despite changes in plasma cholesterol concentrations. Other studies have failed to observe sterol excretion (50) and synthesis (7) change in response to a dietary shift to PUFA. Some of these studies were carried out on an outpatient basis (7, 24), or used indirect means of measuring synthesis (7). Thus, there exists controversy over the influence of fats containing PUFA on cholesterol metabolism. Although it cannot be excluded that the changes in cholesterol synthesis observed were completely unrelated to LDL metabolism, present findings are consistent with the direction of the results from considerable animal and some human data, which suggest a passive role of synthesis in control of circulating sterol concentrations. Whether PUFA or some other constituent causes increased cholesterol removal from the circulation is uncertain. In humans, ingestion of plant sterols, which are abundant (about 800 mg/dl) in the corn oil used presently (41), has been shown to reduce cholesterol absorption and result in a compensatory increase in synthesis (35).

Similar equivocal findings have been reported in humans concerning the ability of cholesterol feeding to alter sterol absorption and synthesis. Subjects switched from low to high cholesterol diets attained a steady state where the extra sterol absorbed was matched by an elevation in sterol excretion, resulting in no change in synthesis (35). Lack of effect has similarly been observed by others using sterol balance methodology (36) and deuterium incorporation (37). These findings contrast with those of other studies where elevated cholesterol intakes caused reduced synthesis measured in mononuclear leucocytes (7) or using the sterol balance approach (34). Lack of concensus of findings may be due to the large degree of interindividual variation in response to dietary cholesterol (51). Feeding cholesterol to humans produces an expansion of neutral sterol excretion accompanied by reduced sterol formation in some subjects; however, bile acid output increases in others, indicating failure of down-regulation. In general, individuals who respond to a dietary cholesterol challenge with increased circulating levels do not suppress endogenous synthesis; however, those in whom circulating levels remain unchanged compensated by reducing synthesis (7, 34). Our data suggest that synthesis remained unchanged in the groups studied, which may have accounted for the raised plasma total and LDL levels observed with added dietary cholesterol with feeding of both corn oil and beef tallow. However, it cannot be excluded that variability between or within subjects, as well as analytical limitations, prevented identification of true change in synthesis due to dietary cholesterol separate from that of fat, given the P value of 0.2.

The deuterium incorporation method shares several fundamental principles with the tritiated water technique for measuring cholesterogenesis which has been validated (52, 53) and applied (29, 31, 32, 53) in animal studies. Calculation of FSR from initial conditions where tracer is present at natural abundance levels in the system, as used presently, avoids generation of negative FSR values, which have been difficult to interpret previously (46). A potential advantage of the deuterium incorporation

method, compared with tritium studies, is that, with the latter, incorporation is often measured over approximately 60 min; a duration so short as to preclude equilibration of newly synthesized cholesterol across the components of the rapidly exchangeable pool of cholesterol that includes plasma. Considering that over 60% of hepatic pool free cholesterol exchanges with total plasma cholesterol per h (54), the 24-h measurement interval used presently enables cholesterol synthesized by any organ to distribute across the subcompartments of the free component of this rapid turnover pool. Similarly, whereas red cell deuterium enrichment lags behind that of plasma over the initial few hours post-deuteration, incorporation rates are parallel from about 6 to 24 h (45). Also, although distinct kinetics exist for HDL versus VLDL and LDL, reported turnover times between these pools are very short (i.e., 2.2 pools per h for VLDL/LDL free cholesterol into HDL) (54), suggesting that, with the present methodology, incoming deuterium-labeled free cholesterol would almost completely equilibrate across lipoprotein pools over the 24-h measurement period used. Use of the 24-h period permits transmission of incoming deuterium-labeled cholesterol across subcomponents of the rapid turnover compartment, thus, enrichment changes in plasma reflect those of this pool as a whole.

In the present experiment, it was important that certain assumptions of the method not be violated by the dietary perturbations involved. Four assumptions were considered. The first concerns the ratio of deuterium to hydrogen atoms incorporated into cholesterol during synthesis. A possible method limitation is that fatty acid composition may shift the deuterium to hydrogen incorporation ratio. Diet fat-related effects on this ratio have been suggested as explaining the previously reported disparity between results of ^{3}H incorporation with sterol balance in guinea pigs fed diets containing different fats (55). A change in the enrichment of deuterium or ^{3}H in NADPH, one of the two sources of tracer in de novo synthesized sterol, could produce artefactual changes in synthesis. Although a possible source of error, we have previously calculated that any such shift in ratio cannot account for the size of the difference in synthesis between corn oil versus beef tallow treatments (45).

Second, the possibility was considered that changes in LDL removal rate may cause errors in FSR determinations. Such a problem was unlikely to result in substantial errors for the following reasons. The pool of measurement, the rapid turnover compartment, includes LDL cholesterol as only a small component (48, 54). The free cholesterol component transfluxes rapidly between subcompartments of this pool (54) such that LDL leaving the pool would be expected to equilibrate with other components. Also, available data indicate that LDL removal is enhanced with PUFA feeding (11, 19, 32). If PUFA feeding resulted in increased loss of deuterated LDL cholesterol out of the rapid turnover pool, apparent FSRs should be lower in this group in contrast to what is presently observed.

Third, the influence of adding dietary cholesterol on the rate of deuterium uptake and thus the interpretation of FSR values was considered. In essence, assumptions applying to our approach were identical to those used in earlier ^{3}H studies where substantial changes in dietary cholesterol level were made (32). As the deuterium enrichment plateau requires a period of months to achieve, initial uptake rates, exclusive of circadian rhythmicity (56), are highly linear, indicating very little exit of label from the rapid turnover pool over the measurement period. Because exit of label from the pool is small, the appearance rate is perturbed minimally by differences in the net flux of recycled or dietary sterol moving through the pool. Thus, although this assumption remains to be fully tested, changes in dietary cholesterol utilized in the present experiment should produce little impact on early deuterium incorporation rates.

Fourth, the present approach considers the free and esterified sub-pools as a single compartment whereby the observed enrichment is taken to reflect an average of individual sub-pool enrichments. It has been shown that most label enters free cholesterol during a 24-h sampling period. After 24 h of deuteration, cholesterol ester deuterium enrichment reached about one-third that of free cholesterol in humans consuming self-selected Western diets (56). The slower deuterium uptake rate of esterified cholesterol reflects the greater proportion of circulating and tissue cholesterol that is esterified. As long as esterification rates do not differ significantly between dietary treatments, consideration of free and esterified pools as the rapid turnover pool described by Goodman et al. (48) should not perturb the model presently used.

In summary, present findings support previous work in animals and humans suggesting that corn oil versus beef tallow feeding reduces circulating levels of cholesterol by increasing the flux of central pool cholesterol with a compensatory enhancement of cholesterol synthesis. Augmenting the diet with cholesterol at the present level elevates circulating cholesterol levels, but does not cause a measureable feedback inhibition of cholesterol synthesis, independent of dietary oil. Although it cannot be ruled out that changes in cholesterol synthesis may be unrelated to those occurring in the circulation secondary to dietary manipulation, our findings suggest that cholesterogenesis in humans responds in a reciprocal manner to diet fat-induced shifts in circulatory cholesterol but is resistant to shift produced by alterations in dietary cholesterol.

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