Consumption of Cholesterol Oxides from Fast Foods Fried in Beef Fat in New Zealand

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ABSTRACT: Levels of cholesterol oxides were determined in samples of beef dripping used for deep frying in retail fast-food outlets in Christchurch, New Zealand. The average levels (n = 8) of cholesterol oxides were: β-epoxy cholesterol (15 mg/kg), α-epoxy cholesterol, 7β-hydroxy cholesterol, 7-keto cholesterol (10 mg/kg each), 7α-hydroxy cholesterol (5 mg/kg), and cholestane triol, 25-hydroxy cholesterol (1 mg/kg each). Based on the amount of fat in a typical deep-fried "fish and chips" meal, the amounts of individual cholesterol oxides consumed would then range from 0.05–0.7 mg. This is three to nine times less than reported in a test meal experiment with humans but could still be expected to cause detectable increases in plasma cholesterol oxide levels.

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KEY WORDS: Beef dripping, cholesterol oxides, consumption, deep-frying.

Oxidized derivatives of cholesterol have been shown to have a variety of potentially atherogenic effects, as shown by both *in vivo* and *in vitro* studies (1,2). The elevation of plasma cholesterol oxide levels also has been demonstrated in humans fed a meal rich in these compounds (3).

A significant source of cholesterol oxides in the diet of New Zealanders will be deep-fried food cooked in animal fat. In New Zealand, the most common fat for deep-frying is beef dripping (4,5), and a popular meal is deep-fried "fish and chips." Such meals are likely to represent the largest amount of deep-frying fat consumed at a single time. In this study, the amounts of cholesterol oxides in beef dripping from several fast-food retail outlets in Christchurch have been determined, and these data have been used to estimate the amounts of cholesterol oxides that could thus be consumed in a typical "fish and chips" meal. This information is compared with the amounts ingested in a cholesterol oxide test meal experiment with humans (3).

The full names of the various cholesterol oxides and the common names used in this paper are given in Table 1.

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EXPERIMENTAL PROCEDURES

Materials. Cholesterol oxide standards were obtained from Steraloids (Wilton, NH) (7-keto cholesterol, 7α-hydroxy cholesterol, cholestane triol, β-epoxy cholesterol, α-epoxy cholesterol) or Sigma (St. Louis, MO) (cholesterol, 7-β-hydroxy cholesterol, 25-hydroxy cholesterol, 5α-cholestane, 5α-cholestanol). Stock solutions of standards were prepared as approximately 1 mg/mL solutions in ethyl acetate and stored at −4°C. Dilutions were made in ethyl acetate. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and butylated hydroxytoluene (BHT) were also obtained from Sigma. All solvents (ethyl acetate, diethyl ether, dichloromethane) used were of Analar quality and obtained from BDH Laboratory Supplies (Poole, England).

Samples. Samples of frying fat were obtained from fast-food retail outlets in Christchurch during November 1995. These were taken directly from the frying vat in use, and approximately 100 g was poured into 100-mL Schott bottles (Duran, Mainz, Germany) that contained 100 mg BHT (final antioxidant concentration 0.1%). Samples were stored under nitrogen at 4°C. In addition, unused samples of beef dripping and canola oil were purchased from a local supermarket.

Saponification and extraction. Saponification and extraction were carried out according to the procedures of Sarantinos *et al.* (6). Approximately 300 mg of fat or oil was weighed into a screw-capped test tube. Surrogate standard, 5- α-cholestanol (15 μg), and freshly prepared methanolic KOH (1 M, 20 mL) were then added. The samples were capped and then vortex-mixed for at least 1 min. The samples were left in the dark at room temperature for 18–24 h. Aqueous sodium sulfate (10 mL; 0.47 M) and diethyl ether (25 mL) were added. The mixture was again vortex-mixed and allowed to settle. The diethyl ether was decanted into a separating funnel, and the residue was reextracted with further diethyl ether (25 mL). The combined ether extracts were washed with distilled water (3 × 10 mL). The ether was then dried with sodium sulfate and filtered, and the extract was rotary-evaporated to dryness. The residue was then transferred to a vial (1.5 mL) with diethyl ether and evaporated to dryness under a stream of nitrogen.

Derivatization. The dried extracts were derivatized by dissolving in pyridine (350 μL) and adding BSTFA (350 μL). After approximately 30 min at room temperature, the extracts were blown to dryness under a stream of nitrogen. Dichloromethane (0.5 mL) was added to redissolve the extract, the vial

TABLE 1
List of Cholesterol Oxides and Common Names

Order of chromatographic elution[a]	Chemical name	Common name
1	5α-Cholestane	5α-Cholestane
2	5-Cholesten-3β, 7α-diol	7α-Hydroxy cholesterol
3	5-Cholestene-3β-ol	Cholesterol
4	5α-Cholestan-3β-ol	5α-Cholestanol
5	5-Cholesten-3β, 7β-diol	7β-Hydroxy cholesterol
6	Cholestan-5β, 6β-epoxy-3β-ol	β-Epoxy cholesterol
7	Cholestan-5α, 6α-epoxy-3β-ol	α-Epoxy cholesterol
8	5-Cholestene-3β, 20α-diol	20-Hydroxy cholesterol[b]
9	Cholestan-3β, 5α, 6β-triol	Cholestane triol
10	5-Cholestene-3β, 25-diol	25-Hydroxy cholesterol
11	5-Cholesten-3β-ol-7-one	7-Ketocholesterol

[a]As shown in Figures 1–3.
[b]Not quantitated in analyses.

was crimp-capped, and then vortex-mixed. Internal standard 5-α-cholestane (5 µl; 1 mg/mL) was then added.

Gas chromatography–mass spectrometry (GC–MS). Analysis of the derivatized extracts was performed on a Hewlett-Packard HP 5970B gas chromatograph, equipped with an HP 8590 mass selective detector (Hewlett-Packard Associates, Palo Alto, CA). Separation was on a 5% phenyl, 95% methyl silicone column (DB-5ms, 30 m × 0.25 µm i.d., 0.25 µm film; J & W Scientific, Folsom, CA). Temperature settings were: injector, 280°C; detector, 300°C; transfer line, 300°C. The temperature program used was: initial, 175°C (1 min); 175–290°C at 30°C/min; 290°C (15 min). The carrier gas used was helium (head pressure, 17.5 psi). Splitless injections were performed. The software for analysis and control was Hewlett-Packard ChemStation B.02.02. Selective ion monitoring (SIM) was used for detection and quantitation. Standards were run individually in the total ion full-scan mode to determine order of elution and to provide a representative mass spectrum. A series of mixed standards (100, 50, 10, 2, and 0.4 ppm) were then run to provide a calibration curve (fitted with a quadratic equation). Each compound was quantitated from the level of a selected major ion; a second major ion was also monitored for each compound to provide an ion ratio that was characteristic for each compound. The response to each target ion was divided by the response of the internal standard (5α-cholestane) to normalize for any small volume differences. The selected ions used are shown in Table 2. Although 20-hydroxy cholesterol was originally included in the standards mixture, the GC–MS response was too low to permit quantitation.

Three samples were repeated on the GC–MS in full-scan mode to provide full mass spectra of the identified cholesterol oxides present in the samples. The full mass spectra were fully consistent with those obtained from the derivatized standards.

The surrogate standard 5α-cholestanol eluted immediately after cholesterol in the GC–MS procedure. The large quantity of cholesterol in these samples had the effect of "quenching" the mass spectrometer detector briefly, so the GC–MS values for the surrogate standard in the beef dripping samples were unreliable. Consequently, it was necessary to conduct gas-chromatographic analyses with a flame ionization detector (GC–FID) to quantitate this one peak. The quantitation values reported are all from the GC–MS results except for the surrogate standard levels in the animal fat samples. The recoveries of the surrogate standard ranged from 45–97% with a mean of 71%. The detection limit for the cholesterol oxides by GC–MS analysis was 0.4 mg/kg in the final extract, and this converts to 1 mg/kg in the sample. All results given of 1 mg/kg for cholesterol oxides were positively identified by GC–MS.

Gas chromatographic–flame ionization detector (GC–FID) analysis. This analysis was performed on a Hewlett-Packard HP 5890A instrument, fitted with the same column used for the GC–MS analysis. The temperature settings were: injector and detector, 310°C; column, isothermal at 280°C. The FID was run with a hydrogen/air flame with helium as makeup gas. The carrier gas was hydrogen (head pressure 22 psi). Chromatograms were recorded on a Hewlett-Packard 3393A integrator. As before, each standard was injected singly to confirm the elution series, a dilution series of mixed standards was injected (100, 50, 10, 2 ppm) to provide a calibration line (fitted by least-square linear regression), and standard and

TABLE 2
Ions Used for Quantitation and Identification of Cholesterol Oxides

Compound as TMS[a] derivative (in order of elution)	Target ion (*m/z*) (quantitation)	Qualifying ion (*m/z*)
5α-Cholestane	217	372 (M^+)
7α-Hydroxy cholesterol	456 (M^+)	129
Cholesterol	329	368
5α-Cholestanol	215	355
7β-Hydroxy cholesterol	456 (M^+)	129
β-Epoxy cholesterol	197	384
α-Epoxy cholesterol	197	384
Cholestane triol	403	321
25-Hydroxy cholesterol	131	271
7-Ketocholesterol	472 (M^+)	367

[a]TMS, trimethylsilyl. M^+ indicates a parent ion.

sample responses were normalized by dividing by the area of the internal standard (5-α-cholestane).

Recovery estimation. To improve accuracy of the recovery information, quadruplicate analyses of unused canola oil, spiked with cholesterol oxide standards (approximately 50 mg/kg each), were undertaken. The canola oil was also analyzed unspiked to confirm the absence of cholesterol oxides. These analyses provided recovery factors to account for differences in recovery between the surrogate standard and the actual cholesterol oxides. We found that most of the cholesterol oxides were recovered better than 5α-cholestanol. Relative to 5α-cholestanol (set at 100%), the mean recovery of the other standards was: 7-keto cholesterol (130%), 7α-hydroxy cholesterol (126%), cholestane triol (98%), β-epoxy cholesterol (150%), α-epoxy cholesterol (121%), cholesterol (86%), 7β-hydroxy cholesterol (130%), and 25-hydroxy cholesterol (185%). Thus, the sample results were corrected to 100% for each extraction by means of the surrogate standard recovery and then corrected for the differences in recovery performance by using the average recovery factor.

RESULTS AND DISCUSSION

Examples of GC–FID chromatograms from a derivatized sample and from the cholesterol oxide standard mixture are shown in Figure 1. Total ion chromatograms from the GC–MS runs of the cholesterol oxides standard mixture and of the same sample are shown in Figures 2 and 3, respectively. Finally, example mass spectra of three cholesterol oxide trimethylsilyl derivatives from standards and from the same sample are shown in Figure 4.

The cholesterol oxide levels detected by these analyses are summarized in Table 3. The results for the unused sample of beef dripping show little or no cholesterol oxides present. The overall levels of oxides in the used fat samples were remarkably consistent with moderate amounts of α- and β-epoxy cholesterol, 7β- and 7α-hydroxy cholesterol, and 7-keto cholesterol. Cholestane triol and 25-hydroxy cholesterol were either undetected or at low levels.

The level of cholesterol in these samples was far higher than any of the standards used and was approximately calculated by extrapolation. The mean value obtained of 618 mg/kg is comparable with that given for cholesterol in beef dripping of 600 mg/kg (7).

Previous literature reports on the levels of cholesterol oxides in frying fats or deep-fried foods were examined. Cholesterol oxides in lard, heated for up to 200 h at 180°C, have been determined by liquid chromatography (8). The oxides detected were 7-keto cholesterol, cholesta-4,6-diene-3-one, α- and β-epoxy cholesterol, 7β-hydroxy cholesterol and cholestane triol. All of these increased to approximately 15–30 mg/kg after 20–40 h of heating, except for cholestane triol, which did not appear in any great quantities until after 40 h of heating. 25-Hydroxy cholesterol was not detected.

The levels of four cholesterol oxides in fat extracted from french fries have been determined in samples taken from fast-food restaurants (9), although the type of fat was not clearly identified. Hot saponification was used, to which was attributed the destruction of any 7-keto cholesterol that was not observed. The cholesterol oxides present in french fries, fried in an animal/vegetable shortening used in two U.S. restaurants, were studied by GC–MS (10). The results were expressed as

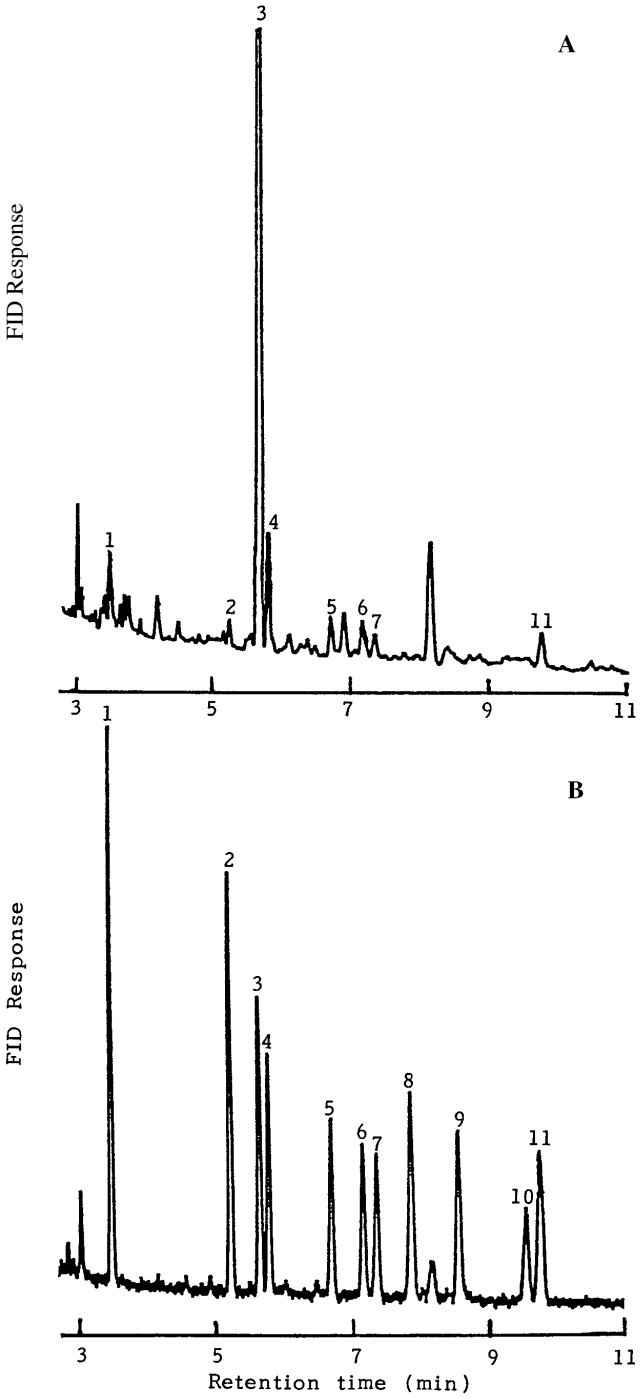

FIG. 1. Gas chromatograph–flame ionization detection (FID) chromatogram of the trimethylsilyl ether derivatives of cholesterol oxides from (A) a sample of beef dripping and (B) standard mixture. Cholesterol oxides numbered as in Table 1.

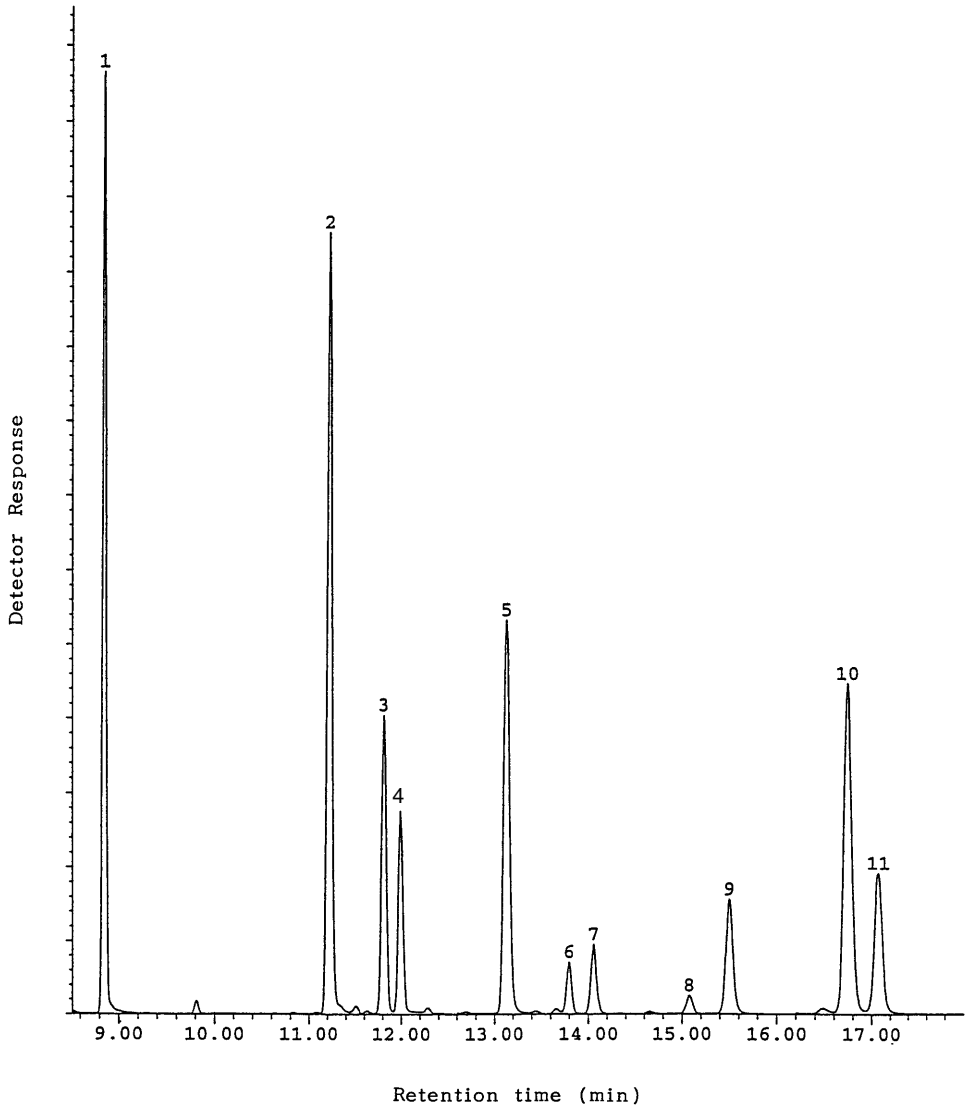

FIG. 2. Total ion chromatogram (from gas chromatography–mass spectrometry) of the trimethylsilyl ether derivatives of cholesterol oxide standard mixture. Cholesterol oxides numbered as in Table 1.

mg/kg of french fries, and fat content was not reported. The cholesterol oxides in tallows, taken from deep-fat fryers, have been determined by gas chromatography and thin-layer chromatography–FID (11). 25-Hydroxy cholesterol was not detectable by this method. Finally, the cholesterol oxides in a tallow/cottonseed mixture (90:10) after simulated french-fry cooking were reported (12). The data from these reports have been summarized in Table 4, along with the ranges of results from this study for comparison.

TABLE 3
Cholesterol Oxide Content of Samples (expressed as mg/kg) of Used and Unused Beef Dripping Analyzed in this Study

	Unused beef drippings	Used beef drippings (n = 8)	
Cholesterol oxide	Level	Mean ± SD	Range
7α-Hydroxy cholesterol	n.d.[a]	5 ± 3	n.d.–7
7β-Hydroxy cholesterol	1	10 ± 5	2–15
β-Epoxy cholesterol	1	15 ± 5	4–18
α-Epoxy cholesterol	1	10 ± 4	2–13
Cholestane triol	n.d.	1 ± 1	n.d.–2
25-Hydroxy cholesterol	n.d.	1 ± 0.5	n.d.–1
7-Keto cholesterol	1	10 ± 6	3–18

[a]n.d., not detected, i.e. <1mg/kg.

The results from the samples in this study are similar and in similar proportions to those in two of the previous studies (9,12). The major differences from the other two reports (10,11) are that cholestane triol and 25-hydroxy cholesterol are present in the Christchurch samples at much lower levels. It is possible that the cleaning and replacement procedures to maintain the quality of the fat prevent buildup of these two cholesterol oxides. However, because cholestane triol is formed from hydrolysis of either α- or β-epoxy cholesterol, the potential for formation of this cholesterol oxide remains. In lard, cholestane triol was not observed until after 40

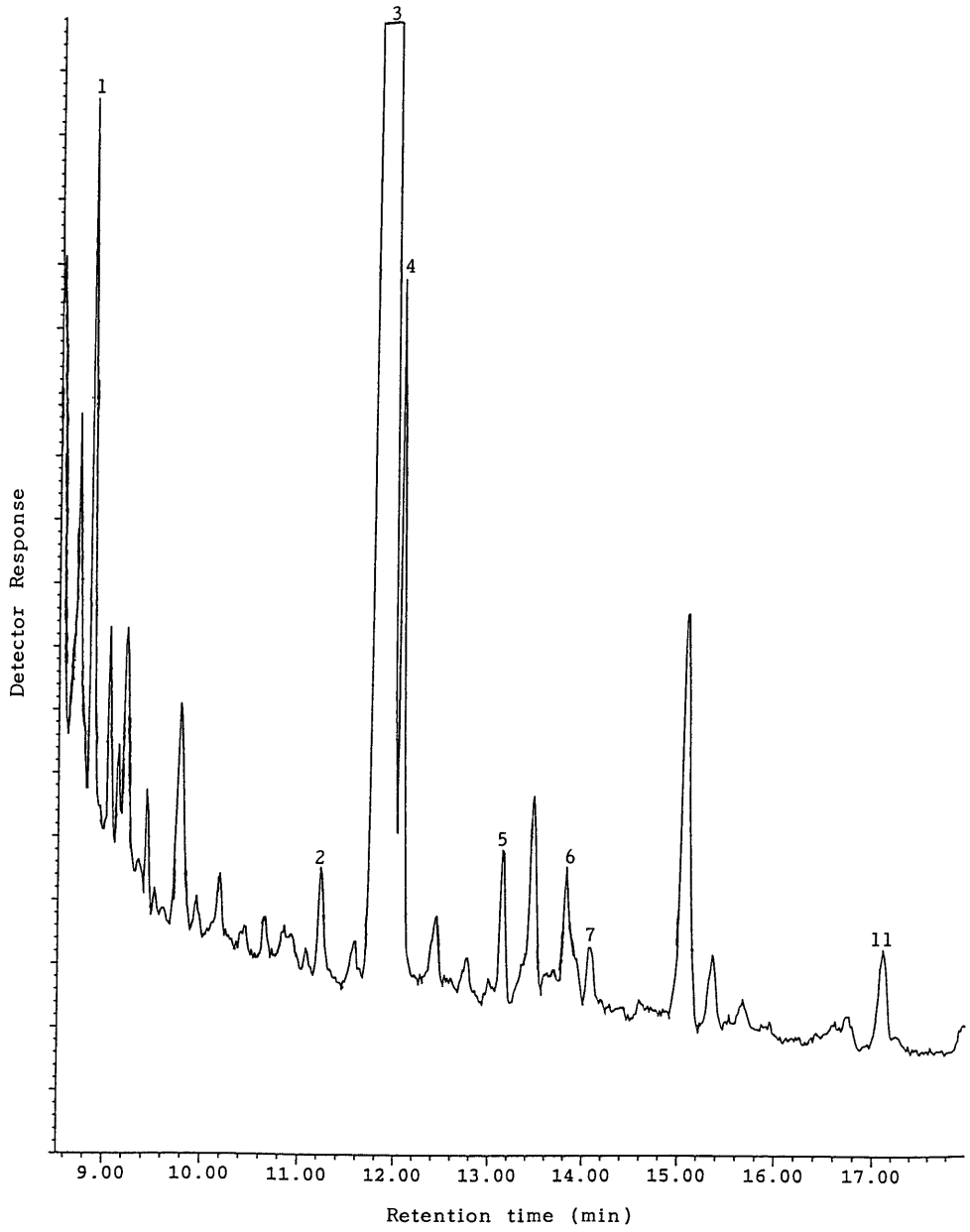

FIG. 3. Total ion chromatogram (from gas chromatography–mass spectrometry) of the trimethylsilyl ether derivatives of cholesterol oxides from a sample of beef dripping. Sample is the same as that for chromatogram shown in Figure 1. Cholesterol oxides numbered as in Table 1.

h of continuous heating, and 25-hydroxy cholesterol was not observed at all (8). It is generally considered that cholestane triol and 25-hydroxy cholesterol are the most active in assays that test the potential atherogenicity of cholesterol oxides (1,13). It is reassuring that the levels of these two oxides are relatively low in the Christchurch samples examined.

Intake of cholesterol oxides. In a previous paper, the estimated consumption of fat in a typical New Zealand "fish and chips" meal was 48.2 g (14). If it is assumed that the contribution of fat from the fish and potato ingredients is minor, then the consumption of cholesterol oxides from a typical "fish and chips" meal, fried in beef dripping, can be calculated. These are shown in Table 5. Also shown in Table 5 are the calculated amounts of cholesterol oxides consumed in a meal of egg powder, used in the test meal experiment with humans (3). That study reported a consumption of 0.7 g egg powder/kg body weight, and the calculations are based on the reported levels of cholesterol oxides and the average weight of New Zealand males and females of 71 kg (15).

The estimated consumption of cholesterol oxides from the "fish and chips" meal would thus be between three and nine times lower than the levels in the test meal. These amounts could be expected to cause smaller but still detectable increases in plasma levels of cholesterol oxides. The fasting level of six

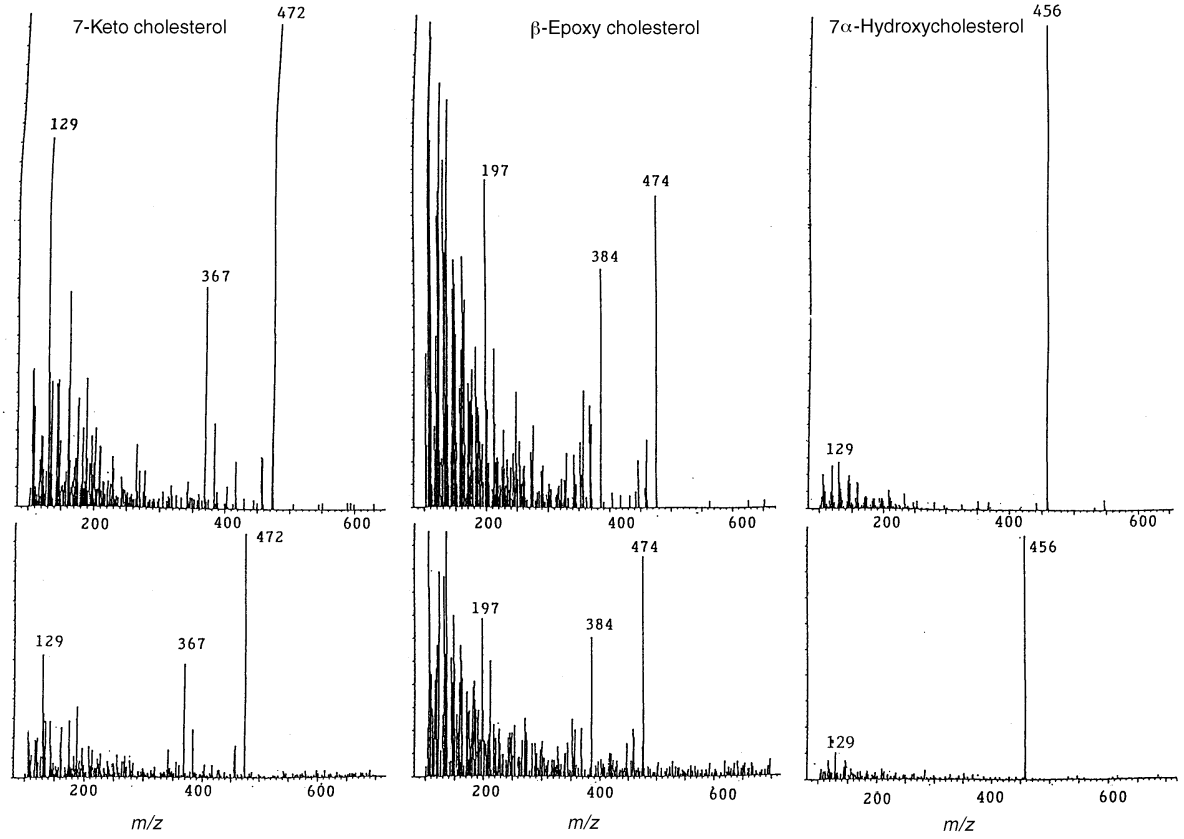

FIG. 4. Mass spectra of trimethylsilyl ethers of cholesterol oxides. Top: standards. Bottom: sample of beef drippings (chromatograms from this sample are shown in Figs. 1 and 3).

TABLE 4
Literature Reports of Cholesterol Oxides in Deep-Frying Fat and Comparison with This Study

Samples (reference)	Cholesterol oxide range (mg/kg)						
	7α-Hydroxy cholesterol	7β-Hydroxy cholesterol	β-Epoxy cholesterol	α-Epoxy cholesterol	Cholestane triol	25-Hydroxy cholesterol	7-Keto cholesterol
French fries (9)	trace–21	n.d.[b]–81	trace–27	n.d.–19	n.a.[c]	n.a.	n.a.
French fries[a] (10)	n.d.	1–3	n.d.–2	n.d.–3	3–16	2–7	4–18
Tallow (11)	40	40	15	1	90	n.a.	10
Tallow/cottonseed oil (12)	2–14	n.d.–42	n.d.–12	n.d.–17	n.d.–4	n.d.–5	n.d.–53
Tallow (this study)	n.d.–7	2–15	4–18	2–13	n.d.–2	n.d.–1	3–18

[a]Reported as mg/kg of french fries.
[b]n.d. = not detected.
[c]n.a. = not analyzed.

TABLE 5
Estimated Consumption of Cholesterol Oxides from a Typical "Fish and Chips" Meal and Comparison with Amounts Used in a Cholesterol Oxide Test Meal (Ref. 3)

Cholesterol oxide	Amount (mg)	
	"Fish and chips" meal	Test meal
7α-Hydroxy cholesterol	0.2	
7β-Hydroxy cholesterol	0.5	3.0
β-Epoxy cholesterol	0.7	2.5
α-Epoxy cholesterol	0.5	4.5
Cholestane triol	0.05	
25-Hydroxy cholesterol	0.05	
7-Keto cholesterol	0.5	1.5

cholesterol oxides in human plasma lipoproteins has been reported (16), and the summed mean levels were approximately 680 μg/dL. In the test meal experiment (3), increases in total plasma cholesterol oxides varied individually and ranged from approximately 200–1500 μg/dL (approximately 30–220% based on an assumed baseline level of approximately 680 μg/dL). Whether such increases are significant with respect to atherosclerosis has yet to be demonstrated, especially given the relatively brief period of elevation observed (2–4 h). However, it is apparent that consumption of cholesterol oxides from such high-fat meals as "fish and chips" has the capacity to cause temporary increases in plasma cholesterol oxide levels, when animal fat is the deep-frying medium.

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