Free Rad. Res., Vol. 30, pp. 341–350
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Oxysterols in Cap and Core of Human Advanced Atherosclerotic Lesions

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Accepted by Prof. R. Stocker

(Received 24 June 1998; In revised form 25 September 1998)

Objective: Different parts of the advanced atherosclerotic lesion have characteristic differences in lipid content, but the distribution of lipid oxidation products has not been reported. This study provides novel data on oxysterol and hydroxyoctadecadienoic acids quantification in core versus cap. It compares the lipid composition of core and cap to assess the topographical distribution of evidence of lipid oxidation.

Methods: Lipids and oxidised lipids were analysed by gas chromatography (GC) and GC–mass spectrometry (GC–MS) in samples of human atheromatous lipid core and fibrous cap of individual advanced atherosclerotic plaques (Stary, Type V) in necropsy samples.

Results: The total lipid was of course massively greater in the core than in the cap. The oxidation products, cholest-5-en-3β,26-diol (26-OH–CHOL) and cholest-5-en-3β,7β-diol (7β-OH–CHOL) were detected in all the samples. 26-OH–CHOL was more abundant in the core than in the cap when related both to wet weight and to cholesterol. 7β-OH–CHOL levels were significantly higher in the core than in the cap when related to wet weight but not when related to cholesterol. Because the processing included a sodium borohydride reduction step, the 7β-OH–CHOL detected could partly originate from 7-ketocholesterol or 7-hydroperoxycholesterol. Several isomeric hydroxyoctadecadienoic acids were detected in both core and cap, more in the cap when related to cholesterol content. Most of the components of the cap showed a high degree of cross-correlation on linear regression analysis, but cross-correlations were weaker for the core. The core samples contained a larger proportion of linoleate relative to oleate than the fibrous cap.

Conclusion: The findings suggest that the different lipid and oxidised lipid contents of cap and core may be due to variations in oxidative activity in different parts of the lesion.

Keywords: Atherosclerosis, cap, lipid, lipid core, oxysterol, hydroxyoctadecadienoic acid

1. INTRODUCTION

Advanced atherosclerotic (Stary, Type V)[1] lesions usually contain two easily differentiated parts, the atheromatous core and the fibrous cap. To the naked eye, the core appears soft and glistening yellow, whereas the cap is firm and grey-white.

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Microscopically, the basal core is a pool of acellular debris containing much lipid, including cholesterol monohydrate crystals. Macrophage foam cells are found scattered round the core, often in considerable numbers at the periphery, or shoulders, of the plaque.[2] This type of advanced atherosclerotic lesion is prone to rupture at the shoulders, leading to thrombus formation. The fibrous cap contains smooth muscle cells and collagen fibres.[2,3]

This study is the first attempt to quantify oxysterols and hydroxyoctadecadienoic acids (HODEs) in cap versus core, and the fatty acid data reported here (free plus esterified) are also novel findings in that previous data on cap versus core were restricted to cholesterol ester fatty acids. In previous studies,[4] we have analysed the lipid content of whole advanced lesions, including both fibrous cap and atheromatous core, and compared them to earlier lesions. Lesion formation has been directly correlated with increased lipid oxidation products, especially 7β-OH–CHOL and 26-OH–CHOL.[4–7] The possible consequences of the oxidation of lipids in lesions are many, amongst which is the toxicity of such compounds for the macrophage foam cells.[8] Evidence of oxysterol toxicity towards various cell types has been repeatedly shown *in vitro*.[8–11] In view of the possible relevance of such compounds to the development of atherosclerosis, an attempt has been made to compare the lipids and oxidised lipids of the fibrous cap to those in the atheromatous core.

2. MATERIALS AND METHODS

2.1. Sampling Procedures

Samples of advanced atherosclerotic lesions (Stary Classification, Type V) were obtained from human abdominal aortas post mortem at Addenbrooke's Hospital, Cambridge. A total of 9 advanced lesions were dissected into their respective fibrous caps and cores, resulting in 9 pairs of subsamples. Care was taken not to contaminate the cap subsample with the core subsample or vice versa. The fibrous caps were sampled by shaving off a superficial slice with a scalpel. The plaques were then incised vertically with a different scalpel and the atheromatous core scooped out with the blunt side of the scalpel blade. The samples were stored at −70°C under argon within an hour of dissection.

The 9 lesions were from a total of 8 male patients, whose ages ranged from 67 to 92, the time intervals after death ranging from 28 to 97 h. The length of this time interval has been shown not to affect results significantly.[5] The wet weight of the cap samples ranged from 1.7 to 31.3 mg, that of the cores from 5.6 to 26.8 mg.

2.2. Extraction and Work-up of Lipids

Lipids were extracted from the samples and processed for analysis as described previously.[5] The procedure involved weighing each sample (wet weight), adding internal standards (*n*-heptadecanoic acid 90 µg, 5α-cholestane 81.5 µg, and coprostane 10.5 µg) and BHT, Bligh and Dyer extraction (with sonication), sodium borohydride reduction, saponification and derivatisation to methyl esters and trimethylsilyl ethers. Sodium borohydride reduction was included to convert hydroperoxides, which are unstable to heat and are thus unsuitable for gas chromatography (GC) and GC–mass spectrometry (GC–MS) analysis, to the stable alcohols. Processed samples were stored at −20°C under argon until GC analysis, which was carried out within a week of dissection.

Chemicals were as described previously,[5,12] as was the cleaning of analytical glassware.[5] Reagent blanks were run to check for contamination. Care was taken to minimise exposure of samples to air, and sodium borohydride reduction, saponification, derivatisation and storage of samples were all under argon.

2.3. GC and GC–MS Analysis

After workup, GC analysis was performed for all the samples as described previously, except that a

cold on-column injector was used and the oven programmed at 50–120°C at 11°C/min, 120–200°C at 4°C/min, 200–280°C at 3°C/min and held at 280°C for 10 min.[4] Quantitation was by peak areas, measured electronically using an integrator, relative to internal standards. GC–MS was performed on selected samples, using a similar capillary column, to verify identities of components. GC instrumentation and standards were as described previously.[5,12] The GC–MS instrumentation comprised a Carlo Erba 8000 GC coupled to a Masslab MD800 mass spectrometer, operated in the electron-impact, positive ionisation mode.

2.4. Statistical Analysis of Data

Student's paired *t*-tests and linear regression analysis were carried out using SigmaPlot 3.0™ software. Significance as judged by Student's paired *t*-test was taken as $P \leq 0.05$. Levels of significance for r^2 values were computed using StatView 4.5® software.

3. RESULTS

3.1. Lipid Components of Lesions

The lipids measured in all samples were cholesterol, linoleic acid (18 : 2), oleic acid (18 : 1), stearic acid (18 : 0), arachidonic acid (20 : 4) and the oxysterols 7β-hydroxycholesterol (7β-OH–CHOL) and 26-hydroxycholesterol (26-OH–CHOL). As before, no information on esterification state was sought.[4]

The results of the GC analysis were expressed in two ways: (i) micrograms of each component per milligram wet weight of tissue; (ii) micrograms of each component per 100 μg of cholesterol. All the concentrations of components were found to be lower in the cap than in the core when expressed as μg per mg wet weight. However, when expressed as μg per 100 μg cholesterol, the fatty acids were relatively higher in the cap than in the core; 7β-OH–CHOL levels were approximately the same in cap and core; 26-OH–CHOL levels were lower in the cap than in the core.

The 9 pairs of fibrous caps and cores each came from the same lesion. For each component, comparisons were made between the cap and core from a given lesion (Student's *t*-test, paired). The main features of the results are described below.

3.2. Cholesterol

Cholesterol levels were significantly lower in the cap ($P = 0.0003$) than in the core (Figure 1 and Table I).

3.3. Fatty Acids (18 : 2, 18 : 1, 18 : 0, 20 : 4)

Each fatty acid was present at lower levels in the caps than in the matching cores when expressed as μg/mg wet weight (Table I). These differences were significant for all the fatty acids measured, 18 : 2 ($P = 0.007$), 18 : 1 ($P = 0.003$), 18 : 0 ($P = 0.043$) and 20 : 4 ($P = 0.006$). When expressed as μg/100 μg cholesterol, however, all the fatty acids had higher levels in the cap than in the core, due to the higher cholesterol content of the core: 18 : 2 ($P = 0.030$), 18 : 1 ($P = 0.006$), 18 : 0 ($P = 0.006$) and 20 : 4 ($P = 0.011$).

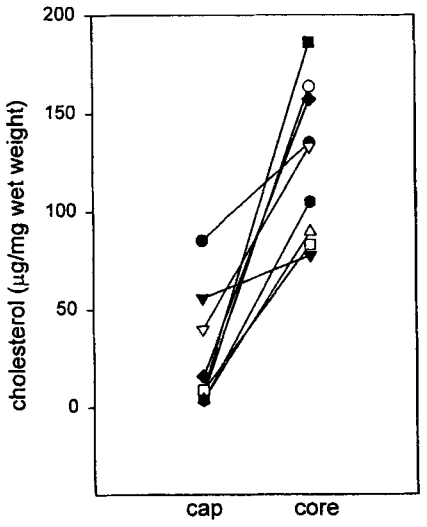

FIGURE 1 Cholesterol contents in the 9 caps compared to the matching cores (μg/mg wet weight) $P = 0.0003$. Each data point represents the mean of extracts analysed in duplicate.

TABLE I Mean levels and standard deviations for selected components of atheromatous core and fibrous cap expressed as μg/mg wet weight and as μg/100 μg cholesterol

	Atheromatous core				Fibrous cap			
	μg/mg wet wt.		μg/100 μg chol.		μg/mg wet wt.		μg/100 μg chol.	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
18:2	12.76	8.22	8.17	4.87	3.03	2.32	13.85	6.09
18:1	11.08	4.48	7.37	3.17	4.10	4.31	19.62	11.59
18:0	1.83	0.72	1.25	0.66	1.18	1.13	7.04	5.43
20:4	2.20	1.24	1.46	0.87	0.72	0.80	2.80	0.93
HODE 1	0.49	0.24	0.40	0.15	0.16	0.11	0.95	0.47
HODE 2	0.36	0.16	0.52	0.09	0.11	0.08	0.60	0.32
HODE 3	0.13	0.13	0.10	0.09	0.06	0.08	0.33	0.28
HODE 4	0.14	0.11	0.11	0.08	0.10	0.12	0.80	0.77
Total HODEs	1.17	0.57	1.02	0.15	0.42	0.34	2.64	0.45
Cholesterol	125.48	38.82	100	—	25.35	28.81	100	—
7β-OH–CHOL	0.09	0.06	0.06	0.05	0.01	0.01	0.04	0.04
26-OH–CHOL	0.33	0.16	0.24	0.18	0.03	0.03	0.08	0.06

3.4. Hydroxyoctadecadienoic acids and Hydroxyeicosatetraenoic acids

Four GC peaks were identified as HODEs. On GC–MS, HODEs 1, 2, 3 and 4 all showed the diagnostic ions for 9-hydroxyoctadeca-10,12-dienoic acid and 13-hydroxyoctadeca-9,11-dienoic acid (as methyl esters, TMSi-ethers), i.e. molecular ion m/z 382, and key fragment ions m/z 225 and m/z 311. A similar pattern of HODEs has been found previously for *in vitro* macrophage-mediated oxidation of cholesteryl linoleate[12] and of LDL.[13] HODE 1 was previously shown to coelute on GC with the main product of soybean lipoxygenase-mediated oxidation of linoleic acid, which is 13-hydroxyoctadeca-9,11-dienoic acid,[14] suggesting that HODE 1 might be the same compound.[12] On GC–MS HODEs 1 and 2 eluted together as a single slightly broadened peak, with a shoulder being discernible for HODE 2 in some of the samples. Further work would be needed for more detailed structural characterisations of the HODEs, to distinguish the permutations of the above structures arising from *cis* and *trans* double bond isomers.

As found for the fatty acids, the HODEs were present at low levels in the cap and at significantly higher (HODE 4 is an exception) levels in the core when related to wet weight (Table I): HODE 1 ($P=0.015$), HODE 2 ($P=0.005$), HODE 3 ($P=0.012$), Total HODEs (Figure 2(a); $P=0.012$). HODE 4 appeared to be slightly higher in core than cap, but not significantly ($P=0.593$). When expressed as μg/100 μg cholesterol, HODEs were significantly higher in cap than in core (HODE 2 is an exception): HODE 1 ($P=0.004$), HODE 3 ($P=0.019$), HODE 4 ($P=0.036$), Total HODEs (Figure 2(b); $P=0.019$). HODE 2 appeared to be slightly higher in cap than core, but not significantly ($P=0.188$).

On GC–MS the diagnostic ions of the hydroxyeicosatetraenoic acids (HETEs)[15] were not present in significant amounts.

3.5. 7β-Hydroxycholesterol

7β-OH–CHOL levels were significantly lower ($P=0.005$) in fibrous cap than in core when expressed as μg/mg wet weight (Figure 3(a)). However, when related to cholesterol, the 7β-OH–CHOL level in cap was not significantly different from that in core ($P=0.220$; Figure 3(b)). The means and standard deviations for 7β-OH–CHOL are shown in Table I.

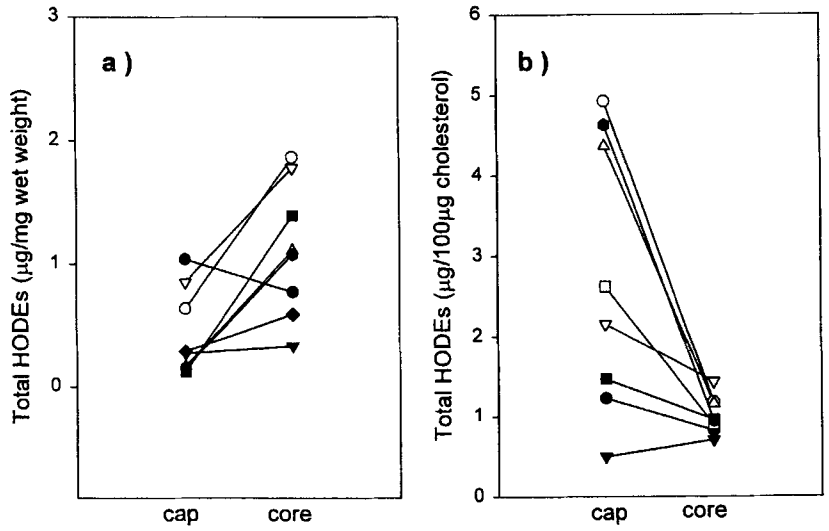

FIGURE 2 (a) Total HODEs contents in the 9 caps and matching cores (µg/mg wet weight) $P = 0.012$. (b) Total HODEs contents in the 9 caps and matching cores (µg/100 µg cholesterol) $P = 0.019$. Each data point represents the mean of extracts analysed in duplicate.

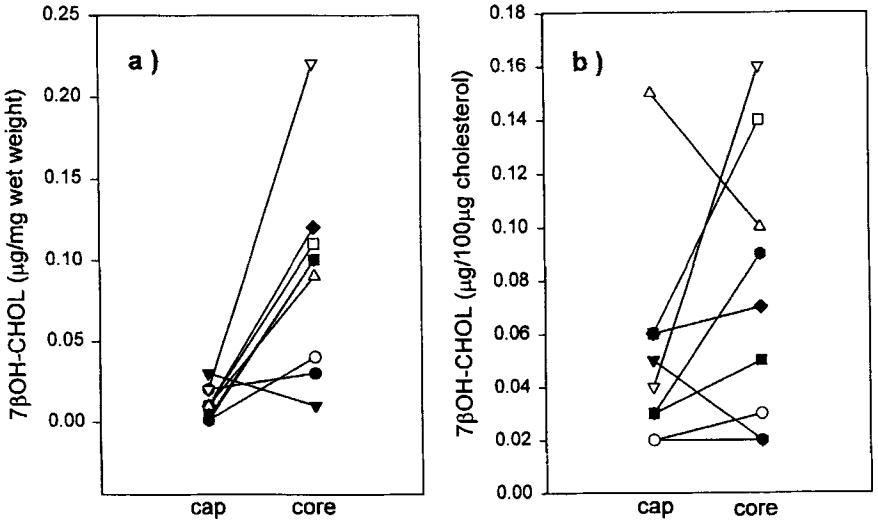

FIGURE 3 (a) 7β-OH–CHOL contents in the 9 caps and matching cores (µg/mg wet weight) $P = 0.005$. (b) 7β-OH–CHOL contents in the 9 caps and matching cores (µg/100 µg cholesterol). The difference was not significant ($P = 0.220$). Each data point represents the mean of extracts analysed in duplicate.

3.6. 26-Hydroxycholesterol

Table I shows that 26-OH–CHOL levels were significantly lower in the fibrous cap than in the core, whether expressed as µg/mg wet weight ($P = 0.0007$) or as µg/100 µg cholesterol ($P = 0.016$). The upward trends for 26-OH–CHOL in the individual cores compared to the caps can be seen in Figure 4(a) and (b).

3.7. Fatty Acid Ratio 18 : 2/18 : 1

The ratio of the two fatty acids 18 : 2 and 18 : 1 was lower in the cap than in the core (mean

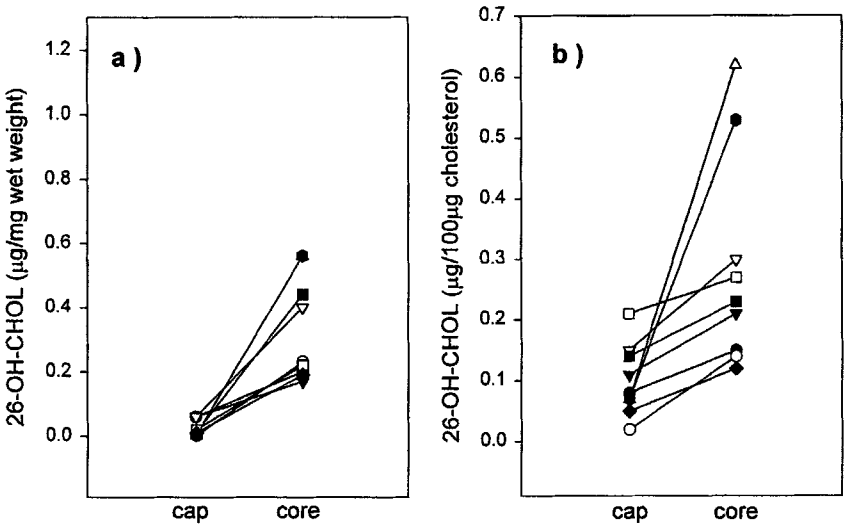

FIGURE 4 (a) 26-OH–CHOL contents in the 9 caps and matching cores (µg/mg wet weight) $P = 0.0007$. (b) 26-OH–CHOL contents in the 9 caps and matching cores (µg/100 µg cholesterol) $P = 0.016$. Each data point represents the mean of extracts analysed in duplicate.

TABLE II Cross-correlations of components of atheromatous core and of fibrous cap using least squares linear regression analysis

Components correlated	Atheromatous core		Fibrous cap	
	r^2	p	r^2	p
18 : 2 vs. 18 : 1	0.65	0.006	0.57	0.015
Cholesterol vs. 18 : 2, 18 : 1	0.31–0.34	> 0.1	0.83–0.85	< 0.0002
18 : 0 vs. cholesterol	0.19	0.25	0.70	0.003
20 : 4 vs. cholesterol	0.22	0.22	0.92	< 0.0001
20 : 4 vs. 18 : 0	0.30	0.13	0.87	< 0.0001
20 : 4 vs. 18 : 2, 18 : 1	0.66–0.85	≤ 0.006	0.77–0.94	≤ 0.0008
7β-OH–CHOL vs. cholesterol	0.02	0.75	0.93	< 0.0001
26-OH–CHOL vs. cholesterol	0.0003	0.89	0.86	< 0.0001
26-OH–CHOL vs. 7β-OH–CHOL	0.15	0.32	0.81	0.001
26-OH–CHOL vs. 18 : 2	0.20	0.25	0.85	0.0001
7β-OH–CHOL vs. 18 : 2	0.05	0.57	0.72	0.005
HODE 1 vs. 2	0.94	< 0.0001	0.83	0.0002
HODE 1 vs. 3	0.20	0.29	0.77	0.0008
HODE 2 vs. 3	0.35	0.13	0.57	0.016
HODE 1 vs. 4	0.11	0.43	0.23	0.20
HODE 2 vs. 4	0.25	0.22	0.59	0.013
HODE 3 vs. 4	0.94	< 0.0001	0.05	0.56

For linear regression analysis, all components were expressed as µg/mg wet weight.

0.84 ± 0.47 for cap, 1.12 ± 0.35 for the core; $P = 0.011$).

3.8. Correlations between Components

Each component was assessed for correlations with the other components in the samples, using linear regression analysis. Each of the two subsamples (cap and core) was tested separately. Most components listed in Table II, expressed as µg/mg wet weight, showed significant cross-correlation in the fibrous cap, which suggests that subsamples of the cap are quite similar in the abundance of the various components. There

were some correlations between components in the cores but not as significant as those in the caps.

Using the same linear regression analysis, each component was assessed for the correlation of its abundance in the core versus the cap. Most components correlated very weakly or not at all, giving values of $r^2 \leq 0.18$; $P > 0.26$, except 18 : 0 ($r^2 = 0.49$; $P = 0.03$) and HODE 3 ($r^2 = 0.86$; $P = 0.0002$, all the components expressed as μg/mg wet weight).

4. DISCUSSION

Not all advanced lesions (Stary, Type V) have a basal core big enough to be recognisable with the naked eye. Some are predominantly fibrous. The lesions chosen for the present study were restricted to those with a recognisable core and cap bulky enough to yield adequate samples and therefore cannot be regarded as representative of all advanced lesions.

4.1. Cholesterol and Fatty Acids

The lipid core was, unsurprisingly, richer in all of the lipid species analysed than the fibrous cap, when expressed relative to wet weight. In the core, cholesterol appeared at much higher concentration than the fatty acids and was also the most abundant lipid in the cap.

The ratio 18 : 2/18 : 1 was found to be lower (0.84) in cap than in core (1.12). Several explanations are possible, which are not mutually exclusive. First, linoleate is more susceptible to peroxidation than is oleate,[12,13] implying that peroxidation might be more pronounced in the cap than the core. Secondly, oleate might be biosynthesised in the cap. Linoleate originates only from diet. A third possible explanation is differential hydrolysis. One reported analysis of lipid composition of cap and core measured the fatty acid distributions within cholesterol esters and suggested that a lower 18 : 2/18 : 1 ratio in the cap was due to differential hydrolysis.[16] A second relevant study on fatty acid distributions within cholesterol esters showed a lower 18 : 1/(18 : 1 + 18 : 2) ratio in the core,[17] which is in agreement with the present study. Our analytical procedure does not distinguish esterification state or free state because it includes a saponification step, but it is known that fatty acids in lesions are predominantly in the esterified state (cholesterol esters, triglycerides, phospholipids). Differential hydrolysis might cause preferential loss of linoleic acid into the plasma, since free fatty acids are less hydrophobic than cholesterol esters and *cis*-polyunsaturated chains are less hydrophobic than *cis*-monounsaturates. A fourth possible explanation for a lower 18 : 2/18 : 1 ratio in the cap may be a different distribution of lipid classes between cap and core, each favouring different fatty acids. A fifth possible explanation worth considering is that 18 : 2 may be metabolised by cells in the cap into longer chain, more highly polyunsaturated fatty acids. In support of this, the ratio 18 : 2/20 : 4 was found to be lower in the cap (5.00 ± 1.35) than in the core (5.99 ± 1.49). This difference was significant ($P = 0.047$) when a paired Student's *t*-test was carried out.

4.2. HODEs

The 9- and 13-HODEs detected may partly derive from 9- and 13-hydroperoxides and 9- and 13-keto analogues of the HODEs, as a result of our borohydride reduction. Reduction within the lesion, e.g. by a glutathione peroxidase, could also convert the hydroperoxides to HODEs.[18] Hydroperoxy- and hydroxy-octadecadienoic acids are products of lipoxygenase- or free radical-mediated peroxidation of linoleate. Relative to linoleate, the HODE contents of the cap and core were similar. This implies that peroxidation of linoleate is at least as great in the cap as in the core, but the hydroperoxide precursors of HODEs can fragment into shorter chain molecules such as aldehydes, which were not analysed and which moreover can bind to protein. Thus amounts of hydroperoxides or HODEs forming in the early stages of peroxidation can fall as oxidation progresses, as for *in vitro* oxidations

of LDL.[13,19] Hence, the HODEs detected by us probably only represent a small proportion of hydroperoxides originally generated within the lesion *in vivo*.

4.3. Oxysterols

Two products of cholesterol oxidation, 7β-OH–CHOL and 26-OH–CHOL were determined. The levels of these oxysterols reported in the present study were on average lower than, but overlapped the range reported previously,[5] when a comparable sampling technique was used. However, the previous study of atheroma had also included much larger and ulcerated lesions, which were not included in the present study.

7β-OH–CHOL levels, relative to wet weight, were higher in the core than the cap, but relative to cholesterol were not significantly different. 7β-OH–CHOL could originate in part from 7β-hydroperoxycholesterol, which might be a relatively minor contributor since it has been reported as only a trace oxysterol in human atherosclerotic plaque.[20] A major contributor is likely to be 7-ketocholesterol, which is formed by free radical oxidation.[21] The 7-position of cholesterol is most susceptible to abstraction of a hydrogen, because the radical so produced is resonance-stabilised by the double bond, i.e. an allylic radical, which then reacts with oxygen to result in 7-oxysterols. The proportion of standard 7-ketocholesterol that was converted to 7β-OH–CHOL by our borohydride reduction procedure was 85.2%, while the rest was converted to 7α-OH–CHOL. This result was similar to the 78% reported by Brown *et al.*[20]

The abstraction of a hydrogen at the 7-position appears to be effected by a polyunsaturated fatty acid-derived radical (e.g. a peroxyl or pentadienyl radical).[12,22,23] Cholesterol 7-oxidation appears linked with linoleate oxidation, during both copper- and macrophage-mediated oxidations of LDL and model lipoproteins.[12,13,24] In a study of lesions of various stages, fatty streaks, composed of macrophage foam cells, possessed the highest ratio of 7β-OH–CHOL to cholesterol, and the lowest 18 : 2/18 : 1 ratio.[4] In the present study, the similar cap and core levels of 7β-OH–CHOL, relative to cholesterol, are accompanied by a lower 18 : 2/18 : 1 ratio in the cap than in the core. The relationship between linoleate depletion and 7β-OH–CHOL therefore does not appear as straightforward here, and differential escape of cap components into the plasma, and/or biosynthesis of 18 : 1 within the cap, may have influenced the observed levels.

The greater abundance of 26-OH–CHOL in the core than in the cap indicates a greater contribution of cytochrome P-450 sterol 26-hydroxylase-mediated oxidation of cholesterol in the core. This enzyme is present in various mammalian tissues[25] and in a number of cell types, including macrophages and endothelial cells.[26,27] 26-OH–CHOL in the core might derive from the activity of macrophage foam cells at the shoulders of the plaque which are thought to die and spill their contents into the core.[2] Its lower abundance in the cap may be because numbers of smooth muscle and endothelial cells, or their activity of sterol 26-hydroxylase, are lower than those of macrophages in the shoulders.

26-OH–CHOL levels in the cap might be diminished by removal into the plasma, where it is carried in the lipoproteins.[28,29] 26-OH–CHOL may be metabolised further to 3β-hydroxycholestenoic acid, which is more water-soluble and also might escape into the blood. This pathway appears to be a cellular cholesterol removal mechanism and these metabolites are ultimately converted to bile acids by the liver.[27,30,31] The 26-hydroxylase activity in foam cells might be up-regulated by the high level of cholesterol accumulated.[4] Oxysterols including 26-OH–CHOL can down-regulate HMGCoA reductase and LDL receptors, and may have a regulatory role in cholesterol biosynthesis and uptake.[32,33]

Individuals with the genetic disease cerebrotendinous xanthomatosis, who lack a functional sterol 26-hydroxylase, show elevated cholesterol levels in most tissues and suffer from premature atherosclerosis, despite normal plasma

cholesterol levels.[34] On the other hand oxysterols, especially 26-OH–CHOL, are cytotoxic, including for macrophages, and may contribute to the cell death which leads to core formation.[4,8] Escape of oxysterols from the core into the blood would presumably be much slower than from the fibrous cap.

4.4. Overall Considerations

The accumulation of lipids and lipid oxidation products within the core of the advanced lesion is consistent with influx of blood monocytes, which mature into macrophages, accumulate and oxidise lipid, and die, spilling their contents into the core. The fibrous cap has a different composition which implies a different history and suggests that free radical-mediated oxidation might be at least as great in the cap as in the core, whilst the reverse appears true for cytochrome P-450 enzymatically mediated oxidation.

The fibrous cap, consisting of smooth muscle cells, collagen and elastin fibres and proteoglycan, first appears beneath the endothelium of intermediate lesions and, as the lesion advances, it becomes more fibrous and less cellular. Advanced lesions (Stary, Type V) can rupture at the macrophage-rich shoulders, exposing their thrombogenic contents.[35] The presence of lipids and oxidised lipids in the fibrous cap might theoretically diminish the elasticity of elastin,[36,37] and render it more susceptible to elastases,[36,38] thus destabilising the lesion.

The present study suggests that the cap and the core of (Stary, Type V) advanced atherosclerotic lesions have not only different cellular patterns but also different chemical composition, presumably resulting from complex interactions between the blood, shoulders, core and cap. Dynamic exchanges between these four compartments might follow free radical and enzymatic oxidation, or in situ biosynthesis. In our opinion, these findings justify attempts at more detailed chemical study of the different parts of both advanced and earlier lesions.

Acknowledgements

We gratefully acknowledge the British Heart Foundation and Universitat de Barcelona-Beques de Formació en la Recerca i la Docència 1997–1998 (Spain) for financial support. We thank Drs. D.E. Bowyer and C. Fitzsimmons (Department of Pathology, University of Cambridge) for helpful advice. We also thank Dr. J.A. Ballantine, Mrs. B. Stein and Mr. C. Llewelyn (EPSRC National Mass Spectrometry Service Centre, Chemistry Department, University of Wales, Swansea) for GC–MS.

References

[1] H.C. Stary (1996) The histological classification of atherosclerotic lesion in human coronary arteries. In *Atherosclerosis and Coronary Artery Disease* (eds., V. Fuster, R. Ross and E.J. Topol), Lippincott-Raven, London, pp. 463–474.
[2] N.M. Aqel, R.Y. Ball, H. Waldmann and M.J. Mitchinson (1985) Identification of macrophages and smooth muscle cells in human atherosclerosis using monoclonal antibodies. *Journal of Pathology*, **146**, 197–204.
[3] K.L.H. Carpenter, C. van der Veen, S.E. Taylor, S.J. Hardwick, K. Clare, L. Hegyi and M.J. Mitchinson (1995) Macrophages, lipid oxidation, ceroid accumulation and alpha-tocopherol depletion in human atherosclerotic lesions. *Gerontology*, **41** (suppl. 2), 53–67.
[4] K.L.H. Carpenter, S.E. Taylor, C. van der Veen, B.K. Williamson, J.A. Ballantine and M.J. Mitchinson (1995) Lipids and oxidised lipids in human atherosclerotic lesions at different stages of development. *Biochimica et Biophysica Acta*, **1256**, 141–150.
[5] K.L.H. Carpenter, S.E. Taylor, J.A. Ballantine, B. Fussell, B. Halliwell and M.J. Mitchinson (1993) Lipids and oxidised lipids in human atheroma and normal aorta. *Biochimica et Biophysica Acta*, **1167**, 121–130.
[6] L.L. Smith and J.E. van Lier (1970) Sterol metabolism. Part 9. 26-hydroxycholesterol levels in the human aorta. *Atherosclerosis*, **12**, 1–14.
[7] R. Fumagalli, G. Galli and G. Urna (1971) Cholestanol and 26-hydroxycholesterol in normal and atherosclerotic human aorta. *Life Sciences*, **10**, 25–33.
[8] K. Clare, S.J. Hardwick, K.L.H. Carpenter, N. Weerantunge and M.J. Mitchinson (1995) Toxicity of oxysterols to human monocyte-macrophages. *Atherosclerosis*, **118**, 67–75.
[9] J.T. Bakos, B.H. Johnson and E.B. Thompson (1993) Oxysterol-induced cell death in human leukemic T-cells correlates with oxysterol binding protein occupancy and is independent of glucocorticoid-induced apoptosis. *Journal of Steroid Biochemistry and Molecular Biology*, **46**, 415–426.
[10] H. Hughes, B. Matthews, M.L. Lenz and J.R. Guyton (1994) Cytotoxicity of oxidized LDL to porcine aortic smooth muscle cells is associated with the oxysterols 7-ketocholesterol and 7-hydroxycholesterol. *Atherosclerosis and Thrombosis*, **14**, 1177–1185.

[11] S. Ramasamy, G.A. Boissonneault and B. Hennig (1992) Oxysterol-induced endothelial cell dysfunction in culture. *Journal of the American College of Nutrition*, **11**, 532–538.
[12] K.L.H. Carpenter, J.A. Ballantine, B. Fussell, J.H. Enright and M.J. Mitchinson (1990) Oxidation of cholesteryl linoleate by human monocyte-macrophages *in vitro*. *Atherosclerosis*, **83**, 217–229.
[13] K.L.H. Carpenter, G.M. Wilkins, B. Fussell, J.A. Ballantine, S.E. Taylor, M.J. Mitchinson, and D.S. Leake (1994) Production of lipids and oxidised lipids during modification of low-density lipoprotein by macrophages or copper. *Biochemical Journal*, **304**, 625–633.
[14] M. Hamberg and B. Samuelsson (1967) On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. *Journal of Biological Chemistry*, **242**, 5329–5335.
[15] A.R. Boeynaems, A.R. Brash, J.A. Oates and W.C. Hubbard (1980) Preparation and assay of monohydroxy-eicosatetraenoic acids. *Analytical Biochemistry*, **104**, 259–267.
[16] E.B. Smith, R.S. Slater and P.K. Chu (1968) The lipids in raised fatty and fibrous lesions in human aorta. A comparison at different stages of development. *Journal of Atherosclerosis Research*, **8**, 399–419.
[17] J.R. Guyton and K.F. Klemp (1994) Development of the atherosclerotic core region. Chemical and ultrastructural analysis of microdissected atherosclerotic lesions from human aorta. *Atherosclerosis and Thrombosis*, **14**, 1305–1314.
[18] C.J.W. Brooks, G. Steel, J.D. Gilbert and W.A. Harland (1971) Lipids of human atheroma. Part 4. Characterisation of a new group of polar sterol esters from human atherosclerotic plaques. *Atherosclerosis*, **13**, 223–237.
[19] H. Esterbauer, J. Gebicki, H. Puhl and G. Jurgens (1992) The role of lipid peroxidation and antioxidants in the oxidative modification of LDL. *Free Radical Biology and Medicine*, **13**, 341–390.
[20] A.J. Brown, S. Leong, R.T. Dean and W. Jessup (1997) 7-Hydroperoxycholesterol and its products in oxidized low density lipoprotein and human atherosclerotic plaque. *Journal of Lipid Research*, **38**, 1730–1745.
[21] M. Crisby, J. Nilsson, V. Kostulas, I. Bjorkhem and U. Diczfalusy (1997) Localization of sterol 27-hydroxylase immuno-reactivity in human atherosclerotic plaques. *Biochimica et Biophysica Acta*, **1344**, 278–285.
[22] J.I. Teng and L.L. Smith (1973) Sterol metabolism. Part 24. On the unlikely participation of singlet molecular oxygen in several enzyme oxygenations. *Journal of the American Chemical Society*, **95**, 4060–4061.
[23] E. Lund, U. Diczfalusy and I. Bjorkhem (1992) On the mechanism of oxidation of cholesterol at C-7 in a lipoxygenase system. *Journal of Biological Chemistry*, **267**, 12462–12467.
[24] C. van der Veen, K.L.H. Carpenter, S.E. Taylor, J.A. McDonald and M.J. Mitchinson (1997) Factors affecting events during oxidation of low density lipoprotein: Correlation of multiple parameters of oxidation. *Free Radical Research*, **27**, 459–476.
[25] S. Andersson, D.L. Davis, H. Dahlback, H. Jornvall, and D.W. Russell (1989) Cloning, structure, and expression of the mitochondrial cytochrome P-450 sterol 26-hydroxylase, a bile-acid biosynthetic enzyme. *Journal of Biological Chemistry*, **264**, 8222–8229.
[26] A.B. Reiss, K.O. Martin, N.B. Javitt, D.W. Martin, E.A. Grossi and A.C. Galloway (1994) Sterol 27-hydroxylase. High levels of activity in vascular endothelium. *Journal of Lipid Research*, **35**, 1026–1030.
[27] I. Björkhem, O. Andersson, U. Diczfalusy, B. Sevastik, R.J. Xiu, C. Duan and E. Lund (1994) Atherosclerosis and sterol 27-hydroxylase: Evidence for a role of this enzyme in elimination of cholesterol from human macrophages. *Proceedings of the National Academy of Sciences of the USA*, **91**, 8592–8596.
[28] C.J.W. Brooks, R.M. McKenna, W.J. Cole, J. MacLachlan and T.D.V. Lawrie (1983) 'Profile' analysis of oxygenated sterols in plasma and serum. *Biochemical Society Transactions*, **11**, 700–701.
[29] N.B. Javitt, E. Kok, S. Burstein, B. Cohen and J. Kutscher (1981) 26-hydroxycholesterol. Identification and quantitation in human serum. *Journal of Biological Chemistry*, **256**, 12644–12646.
[30] E. Lund, O. Andersson, J. Zhang, A. Babiker, G. Ahlborg, U. Diczfalusy, K. Einarsson, J. Sjövall and I. Björkhem (1996) Importance of a novel oxidative mechanism for elimination of intracellular cholesterol in humans. *Atherosclerosis, Thrombosis and Vascular Biology*, **16**, 208–212.
[31] I. Björkhem (1992) Mechanism of degradation of the steroid side chain in the formation of bile acids. *Journal of Lipid Research*, **33**, 455–471.
[32] N.B. Javitt (1990) 26-hydroxycholesterol: Synthesis, metabolism, and biologic activities. *Journal of Lipid Research*, **31**, 1527–1533.
[33] E. Miao, S.R. Wilson and N.B. Javitt (1988) Cholesterol metabolism. Effect of 26-thiacholesterol and 26-aminocholesterol analogs of 26-hydroxycholesterol on cholesterol synthesis and low density lipoprotein receptor binding. *Biochemical Journal*, **255**, 1049–1052.
[34] I. Björkhem and S. Skrede (1989) Familial diseases with storage of sterols other than cholesterol: Cerebrotendinous xanthomatosis and phytosterolemia. In *The Metabolic Basis of Inherited Disease* (eds., C.R. Scriver, A.L. Beaudet, W.S. Sly and D. Valle), McGraw-Hill, 6th Ed., New York, pp. 1283–1302.
[35] C.V. Felton, D. Crook, M.J. Davies and M.F. Oliver (1997) Relation of plaque lipid composition and morphology to the stability of human aortic plaques. *Atherosclerosis, Thrombosis and Vascular Biology*, **17**, 1337–1345.
[36] W. Hornebeck and L. Robert (1987) Elastin–lipid interaction in atherogenesis. In *Atherosclerosis. Biology and Clinical Science* (ed. A.G. Olsson), Churchill Livingstone, Edinburgh, pp. 147–149.
[37] R. Tarnawski, R. Tarnawski and J. Grobelny (1995) Changes in elastin in human atherosclerotic aorta: Carbon-13 magic angle sample-spinning NMR studies. *Atherosclerosis*, **115**, 27–33.
[38] W. Hornebeck and L. Robert (1987) Elastin and elastases. In *Atherosclerosis. Biology and Clinical Science* (ed. A.G. Olsson), Churchill Livingstone, Edinburgh, pp. 125–134.