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Comparative study of fatty acid composition in human and monkey aorta

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Abstract. The fatty acid composition of thoracic and abdominal aortic intima and media of normal human subjects and rhesus monkeys has been studied. Significantly higher values of unsaturated fatty acids as compared to saturated fatty acids have been noted in the intima of monkey as compared to man. The fatty acid profile of the aortic wall in these two species has provided a probable biochemical basis for the lesser incidence of atherosclerosis in macaques.

Keywords. Fatty acids; human and monkey aorta.

Introduction

In our previous study significant differences were observed in the lipid constituents of the aortic wall of man and monkeys (Dahiya *et al.,* 1983). The important differences pertained to the content of total lipids, total cholesterol and its fractions and phospholipids which were significantly more in human aorta than in monkeys. Further, it was observed that the content of neutral lipids was much higher in the intima than in media of both species. Although total phospholipids were not significantly different between intima and media, phosphatidylcholine and phosphatidylethanolamine showed significant alterations. As there was much less atherosclerosis in monkeys as compared to man (Chakravarti and Kukreja, 1981) the above findings with respect to lipid constituents of aorta provide a possible biochemical basis, for this difference. It was, therefore, thought worthwhile to study in detail the fatty acid composition of different lipid constituents of the aortic media and intima which could throw further light on this aspect.

Materials and methods

Collection of aorta samples and separation of intima and media

As reported earlier (Dahiya *et al.,* 1983), samples of aorta were collected from 18 normal healthy male rhesus monkeys and from 11 young male human subjects who had died in

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street accidents. The upper 2/3rd of the thoracic and abdominal aorta which were sampled for study of lipid profile and intima-media were separated as reported earlier (Smith *et al.,* 1967).

Extraction and separation of lipids and preparation of methyl ester

The lipids were extracted by the procedure of Folch *et al.* (1957). The different components of neutral lipids like triglycerides, esterified cholesterol and free fatty acids along with total phospholipids were separated by thin layer chromatography using different solvent systems. The different lipid were converted to methyl esters by dissolving them in 1 ml of benzene and 2 ml of 0·5 N sodium methoxide in anhydrous methanol (Christie, 1973); incubated at 50°C for 10 min and 0·1 ml of acetic acid then added. A mixture of 5 ml of water and 5 ml of hexane was added twice, the hexane layer was separated, dried over anhydrous sodium sulphate containing 10% solid sodium bicarbonate and then filtered. The solvent was evaporated in a nitrogen atmosphere. The resulting methyl ester was dissolved in 1 ml of benzene and used in gas liquid chromatography for determination of fatty acid composition.

Gas liquid chromatography

The methyl esters of fatty acids of different lipid constituents were analysed by gas liquid chromatography using a Pye Unichem 104 instrument fitted with a flame ionization detector under isothermal condition (180°C). The column (6'×4 mm) contained 10% diethylene glycol succinate on diatomic C-AW (100-200 mesh). Nitrogen gas (40ml/min) was used as a carrier gas. Fatty acids were identified by comparing their retention times with those of standard fatty acid methyl esters.

Results

Table 1 shows the fatty acid composition of monkey thoracic aorta. The main fatty acids in the phospholipids were stearic, palmitic, oleic, linoleic and arachidonic acid in both intima and media. There was a significant increase in oleic and linoleic acid, and decrease in stearic and palmitic acid of phospholipids in the media as compared to intima. The total saturated fatty acids of phospholipids were significantly decreased while unsaturated fatty acids were higher in media as compared to intima. The main fatty acids of esterified cholesterol were palmitic, palmitoleic, oleic and linoleic acid. There was a significant decrease in palmitic acid in media as compared to intima of monkey thoracic aorta; other fatty acids of esterified cholesterol remained unaltered. The fatty acid content of triglycerides was mainly constituted of palmitic, palmitoleic, oleic and linoleic acid. There was significant decrease in the content of myristic, palmitic and stearic acid and increase in palmitoleic, linoleic and arachidonic acid in media as compared to intima. Total free fatty acid of intima and media of monkey thoracic aorta mainly comprised of palmitic, palmitoleic, stearic, oleic and linoleic acid. There was significant decrease in palmitic and stearic acid content in media as compared to intima. Total saturated fatty acids of esterified cholesterol, triglycerides and free fatty acids

Table 1. Fatty acid composition of monkey thoracic aorta.

	Intima				Media			
	PL	ECH	TG	FFA	PL	ECH	TG	FFA
$C_{12:0}$ Lauric acid	1·8 ± 0·5	1·1 ± 0·05	1·7 ± 0·06	1·2 ± 0·27	1·2 ± 0·07	0·9 ± 0·08	1·5 ± 0·09	0·9 ± 0·29
$C_{14:0}$ Myristic acid	3·2 ± 0·7	1·4 ± 0·07	5·1 ± 1·2	4·1 ± 1·2	2·1 ± 0·3	1·2 ± 0·1	2·5 ± 0·7**	3·8 ± 1·2
$C_{16:0}$ Palmitic acid	22·0 ± 4·6	17·3 ± 4·2	18·6 ± 2·3	16·5 ± 3·2	17·7 ± 4·2*	11·3 ± 2·1*	10·8 ± 2·3***	10·7 ± 2·8*
$C_{16:1}$ Palmitoleic acid	4·8 ± 0·9	14·2 ± 4·3	14·7 ± 2·3	5·7 ± 1·2	6·2 ± 1·2	11·9 ± 3·2	21·7 ± 4·2*	8·7 ± 2·9
$C_{18:0}$ Stearic acid	19·7 ± 4·2	6·7 ± 1·2	10·5 ± 1·8	16·4 ± 2·3	10·4 ± 1·8**	7·2 ± 1·7	5·6 ± 1·2**	9·7 ± 1·8**
$C_{18:1}$ Oleic acid	14·1 ± 2·7	31·5 ± 8·7	36·5 ± 8·2	31·2 ± 5·6	22·4 ± 4·5*	33·5 ± 8·5	34·3 ± 8·5	35·2 ± 9·2
$C_{18:2}$ Linoleic acid	6·2 ± 1·2	24·1 ± 4·6	10·2 ± 2·1	20·5 ± 4·2	12·1 ± 2·3***	29·4 ± 3·8	20·2 ± 6·8*	25·5 ± 6·7
$C_{20:4}$ Arachidonic acid	27·3 ± 3·2	3·1 ± 0·7	1·8 ± 0·07	3·5 ± 0·7	26·8 ± 3·7	3·7 ± 0·4	2·9 ± 0·7*	4·7 ± 1·3
$C_{22:0}$ Behenic acid	0·9 ± 0·1	0·6 ± 0·05	0·9 ± 0·26	0·9 ± 0·07	0·8 ± 0·09	0·9 ± 0·08	0·5 ± 0·28	0·8 ± 0·09
Saturated	47·3 ± 4·5	34·7 ± 4·7	36·6 ± 2·9	38·9 ± 3·2	32·1 ± 3·1***	22·1 ± 2·4**	20·7 ± 2·5***	25·4 ± 2·1***
Unsaturated	52·7 ± 4·8	65·3 ± 7·1	63·4 ± 5·9	61·1 ± 4·7	67·9 ± 4·3***	77·9 ± 6·8*	79·3 ± 6·9*	74·6 ± 6·8*

Values are Mean ± SE of 5 observations. *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.
PL, Phospholipids; ECH, esterified cholesterol; TG, triglycerides; FFA, free fatty acid.

were significantly less and total unsaturated fatty acids were more in media as compared to that of intima of thoracic aorta of monkeys.

Intimal and medial fatty acid composition of monkey abdominal aorta is shown in table 2. The major phospholipid fatty acids were palmitic, stearic, oleic, linoleic and arachidonic acid. There was a decrease in phospholipid palmitic and stearic acid, while an increase in linoleic acid in media as compared to that of intima in monkey abdominal aorta was observed. Esterified cholesterol fatty acid profile showed the palmitic, palmitoleic, stearic, oleic and linoleic acids. Palmitic and stearic acids were significantly less while palmitoleic, oleic and linoleic acids were more in media as compared to intima. The triglycerides mainly contained palmitic, palmitoleic, stearic, oleic and linoleic acid. There were significantly lower values of palmitic and stearic acids while palmitoleic and linoleic acids in media were more as compared to intima. The main constituents of total free fatty acids were palmitic, palmitoleic, stearic, oleic and linoleic acid. There was a significant increase in palmitoleic acid and decrease in stearic acid in media as compared to intima. Total saturated fatty acids of phospholipids, esterified cholesterol, triglycerides and free fatty acids were significantly lower than total unsaturated fatty acids in media as compared to intima of monkey abdominal aorta. A comparison of the fatty acid composition of thoracic and abdominal segments of monkey aorta, revealed that there was more saturated fatty acids in abdominal aorta as compared to thoracic aorta, except for phospholipid fatty acids which were not changed. This shows that increased level of saturated fatty acids and decreased level of unsaturated fatty acids in abdominal aorta might be one of the factors responsible for more atherosclerotic lesions in abdominal segment as compared to thoracic aorta of the same species.

Table 3 shows fatty acid composition of human thoracic aorta. The main phospholipid fatty acids in human thoracic aorta were palmitic, stearic, oleic, linoleic and arachidonic acids. In thoracic aorta, a significant increase in linoleic acid in media was obtained when compared to that in intima. The esterified cholesterol fatty acids, mainly comprised of palmitic, palmitoleic, oleic and linoleie acids. There was a significant increase in palmitoleic acid and decrease in palmitic acid in media as compared to that of intima of human thoracic aorta. The main fatty acid components of triglycerides were palmitic, stearic, oleic and linoleic acids. Stearic and behenic acids were significantly less, while linoleic acid was higher in media as compared to intima. The fatty acid consituents of free fatty acid component were mainly palmitic, stearic, oleic, and linoleic acids. There was a significant decrease in stearic and behenic acid while increase in palmitoleic and oleic acid in media as compared to intima of human thoracic aorta. Total saturated fatty acids of phospholipids, esterified cholesterol, triglycerides and free fatty acids were significantly higher and total unsaturated fatty acids were lower in intima as compared to media of human thoracic aorta.

If fatty acid constituents of human and monkey thoracic aorta were compared, then it was observed that the saturated fatty acid content in human thoracic aorta was higher than that of monkey. The unsaturated fatty acids were more in monkey thoracic aorta.

The fatty acid composition of human abdominal aorta is given in table 4. The main phospholipid fatty acids in intima and media of abdominal aorta were palmitic, stearic, oleic and linoleic acids. The stearic acid was significantly less while oleic, linoleic and arachidonic acids were significantly higher in media as compared to intima. The

Table 2. Fatty acid composition of monkey abdominal aorta

	Intima				Media			
	PL†	ECH†	TG†	FFA†	PL†	ECH†	TG†	FFA†
$C_{12:0}$ Lauric acid	1·6 ± 0·07	1·2 ± 0·09	1·9 ± 0·07	1·4 ± 0·07	1·4 ± 0·08	0·08 ± 0·06	1·6 ± 0·07	0·8 ± 0·09
$C_{14:0}$ Myristic acid	4·7 ± 1·3	4·5 ± 0·8	6·7 ± 1·3	5·1 ± 1·1	3·3 ± 0·6	4·7 ± 0·12	3·2 ± 0·5	4·2 ± 0·8
$C_{16:0}$ Palmitic acid	24·1 ± 3·8	26·6 ± 4·8	27·2 ± 5·6	16·5 ± 5·1	16·5 ± 2·7*	11·9 ± 2·1***	11·8 ± 2·3***	12·7 ± 3·2
$C_{16:1}$ Palmitoleic acid	4·6 ± 0·8	8·8 ± 1·2	7·8 ± 1·5	6·3 ± 1·2	6·1 ± 1·2	12·2 ± 2·8*	11·6 ± 3·6*	9·5 ± 1·7*
$C_{18:0}$ Stearic acid	16·4 ± 5·2	12·9 ± 3·2	18·8 ± 3·4	21·5 ± 4·2	10·5 ± 2·1*	7·5 ± 1·3*	7·2 ± 1·2***	14·3 ± 2·7*
$C_{18:1}$ Oleic acid	13·1 ± 3·4	22·5 ± 3·8	26·5 ± 5·7	27·2 ± 6·8	8·2 ± 4·1	31·5 ± 4·2*	33·2 ± 8·9	29·7 ± 8·7
$C_{18:2}$ Linoleic acid	7·5 ± 1·7	18·9 ± 2·8	9·6 ± 2·3	18·5 ± 4·7	12·4 ± 2·7*	27·3 ± 3·9*	28·1 ± 6·2***	25·3 ± 6·4
$C_{20:4}$ Arachidonic acid	27·4 ± 2·8	3·8 ± 0·9	1·2 ± 0·07	2·7 ± 0·5	30·9 ± 2·2	3·7 ± 0·7	2·8 ± 0·7	2·9 ± 0·8
$C_{22:0}$ Behenic acid	0·6 ± 0·08	0·8 ± 0·06	0·3 ± 0·03	0·8 ± 0·05	0·7 ± 0·06	0·4 ± 0·05	0·5 ± 0·06	0·6 ± 0·03
Saturated	46·8 ± 5·3	48·2 ± 6·3	53·6 ± 5·2	44·9 ± 3·2	33·4 ± 4·6**	26·4 ± 3·1***	23·3 ± 2·5***	32·4 ± 2·2***
Unsaturated	53·2 ± 4·9	51·8 ± 5·7	46·4 ± 3·9	55·1 ± 5·4	66·6 ± 4·9**	73·6 ± 4·9***	76·7 ± 6·5***	67·6 ± 6·1*

Values are Mean ± SE of 5 observations. *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.
† For expansion please see foot note in table 1.

Table 3. Fatty acid composition of human thoracic aorta

	Intima				Media			
	PL†	ECH†	TG†	FFA†	PL†	ECH†	TG†	FFA†
$C_{12:0}$	2·1 ± 0·3	0·9 ± 0·1	1·2 ± 0·2	1·1 ± 0·2	1·8 ± 0·2	0·6 ± 0·09	1·3 ± 0·2	1·2 ± 0·1
$C_{14:0}$	4·2 ± 1·2	2·4 ± 1·2	4·2 ± 1·2	3·9 ± 0·9	2·9 ± 0·7	1·8 ± 0·3	2·4 ± 0·6*	3·7 ± 1·1
$C_{16:0}$	26·8 ± 4·7	21·5 ± 5·7	26·7 ± 7·3	20·5 ± 4·3	22·8 ± 3·7	13·8 ± 2·8*	26·8 ± 6·5	20·4 ± 5·2
$C_{16:1}$	3·3 ± 0·8	9·8 ± 2·3	6·4 ± 1·8	4·4 ± 1·2	2·6 ± 0·7	15·8 ± 2·2*	6·7 ± 1·7	6·9 ± 0·12**
$C_{18:0}$	22·5 ± 5·3	6·5 ± 2·1	14·7 ± 3·2	19·9 ± 6·3	20·6 ± 6·2	4·6 ± 1·3	7·6 ± 2·1*	11·6 ± 2·7*
$C_{18:1}$	16·8 ± 4·2	30·5 + 7·2	33·9 ± 8·7	24·1 ± 6.5	20·4 ± 3·5	32·9 ± 6·7	39·4 ± 6·9	33·7 ± 5·6*
$C_{18:2}$	12·9 ± 3·7	25·1 ± 4·5	9·8 ± 3·2	19·3 ± 4·7	18·7 ± 3·1*	24·9 ± 4·8	16·3 ± 2·3*	18·2 ± 2·1
$C_{20:4}$	10·2 ± 2·8	2·9 ± 0·8	1·9 ± 0·8	6·2 ± 2·1	9·5 ± 4·20	4·8 ± 1·20	0·8 ± 0·04	3·8 ± 1·2
$C_{22:0}$	1·2 ± 0·3	0·4 ± 0·1	1·2 ± 0·3	0·6 ± 0·1	0·7 ± 0·1	0·8 ± 0·1	0·3 ± 0·05***	0·3 ± 0·06***
Saturated	57·8 ± 6·5	34·7 ± 4·7	47·5 ± 3·4	45·4 ± 3·2	45·8 ± 4·9*	29·6 ± 4·8	37·9 ± 2·5**	36·9 ± 2·9*
Unsaturated	42·2 ± 4·9	65·3 ± 7·1	52·5 ± 5·4	54·6 ± 4·9	54·2 ± 4·7*	71·4 ± 8·5	62·1 ± 6·1*	63·1 ± 6·1

Values are Mean ± SE of 5 observations. *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.
† For expansion please see foot note in table 1.

Table 4. Fatty acid composition of human abdominal aorta

	Intima				Media			
	PL†	ECH†	TG†	FFA†	PL†	ECH†	TG†	FFA†
$C_{12:0}$	1·3 ± 0·2	1·3 ± 0·3	1·4 ± 0·2	1·2 ± 0·2	0·7 ± 0·05	1·1 ± 0·06	1·3 ± 0·03	1·3 ± 0·05
$C_{14:0}$	4·4 ± 1·3	3·2 ± 0·9	5·8 ± 1·5	3·8 ± 0·9	2·8 ± 1·2	1·8 ± 0·05	2·6 ± 0·3**	3·5 ± 0·7
$C_{16:0}$	28·7 ± 4·2	22·7 ± 5·2	30·8 ± 4·8	31·4 ± 7·2	23·5 ± 3·5	14·3 ± 3·8*	22·4 ± 7·2	20·3 ± 4·7*
$C_{16:1}$	3·4 ± 0·3	8·9 ± 1·3	4·7 ± 1·2	4·2 ± 0·9	2·0 ± 0·7	12·5 ± 2·8	7·3 ± 2·1*	6·3 ± 0·2
$C_{18:0}$	33·2 ± 4·8	16·3 ± 2·3	24·3 ± 5·8	27·1 ± 4·2	24·7 ± 3·2*	10·2 ± 1·7**	8·2 ± 2·3***	12·5 ± 1·7***
$C_{18:1}$	12·5 ± 2·1	21·4 ± 3·8	22·7 ± 4·1	14·4 ± 2·3	18·7 ± 2·3*	27·4 ± 4·2*	38·4 ± 7·8*	35·8 ± 6·3***
$C_{18:2}$	10·2 ± 1·2	22·7 ± 4·2	8·1 ± 1·2	12·7 ± 1·9	15·7 ± 3·1*	27·8 ± 6·3*	17·5 ± 3·1***	18·2 ± 2·5*
$C_{20:4}$	5·1 ± 1·7	2·8 ± 0·3	1·3 ± 0·5	4·3 ± 0·8	10·2 ± 2·3*	4·3 ± 1·2*	1·5 ± 0·3	2·1 ± 0·5**
$C_{22:0}$	1·2 ± 0·2	0·7 ± 0·05	0·9 ± 0·06	0·9 ± 0·07	1·7 ± 0·07	0·6 ± 0·05	0·8 ± 0·05	0·5 ± 0·06
Saturated	63·8 ± 6·2	45·2 ± 4·3	62·9 ± 6·2	63·3 ± 6·4	52·8 ± 3·9*	32·7 ± 3·9**	34·3 ± 2·7***	37·7 ± 2·9***
Unsaturated	36·2 ± 3·1	54·8 ± 5·4	37·1 ± 3·2	36·7 ± 2·9	45·2 ± 3·4**	67·3 ± 6·4**	65·7 ± 6·6***	62·3 ± 6·2***

Values are Mean ± SE of 5 observations. *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

† For expansion please see foot note in table 1.

esterified cholesterol fatty acids were mainly palmitic, palmitoleic, stearic, oleic and linoleic acids. There was a significant decrease in palmitic and stearic acids while an increase in oleic, palmitoleic and arachidonic acids in media as compared to intima of human abdominal aorta. The triglyceride fatty acids mainly constitute palmitic, palmitoleic, stearic, oleic and linoleic acids. There was lower value of myristic and stearic acids with increase in palmitoleic, oleic and linoleic acids in media as compared to intima. Similarly, fatty acid constituents of free fatty acid compounds were mainly palmitic, stearic, oleic and linoleic acids. There was significantly higher value of palmitic, stearic acid in intima as compared to media of human abdominal aorta. Total saturated fatty acids of phospholipids, triglycerides, esterified cholesterol and free fatty acids were significantly lesser than that of unsaturated fatty acids in media as compared to intima of human abdominal aorta. If we compare human abdominal aorta with monkey abdominal aorta, then there is more saturated fatty acids and less unsaturated fatty acids in human abdominal aorta as compared to that of monkey.

Discussion

It is evident from this study that total saturated fatty acid (mainly stearic and palmitic) of phospholipids, esterified cholesterol, triglycerides and free fatty acids are less while unsaturated fatty acids (mainly linoleic, oleic and palmitoleic) are more in the media as compared to intima of monkey thoracic aorta. Similar type of results were obtained in monkey abdominal aorta also. It is well known that saturated cholesterol esters are preferentially deposited in the aortic wall (Sinclair, 1956). Hence the presence of higher content of saturated fatty acids in intima than in media may be one of the factors causing intimal lipidosis. Some workers have emphasised the relationship between polyunsaturated fatty acids and the genesis of either spontaneous or experimental atherosclerotic lesions (Kritchevsky *et al.,* 1954,1956). It was reported that in humans (Swell *et al.,* 1960a,b) as well as in cholesterol fed rabbits (Evrard *et al.,* 1961; Zilversmit *et al.,* 1961) and cockerels (Blomstrand and Christensen, 1961) that cholesterol ester fraction of the aortic fatty plaque contained a higher percentage of oleic acid and lower percentage of linoleic acid than the serum fraction. Similar studies were not carried out using monkey aorta. In the present study, we have observed that stearic and palmitic acid were more in intima than in media while linoleic and oleic acids were more in media than in intima in both human and monkey aorta. The high oleate/linoleate ratio is a typical feature of early atheromatous lesions (Swell *et al.,* 1960; Blomstrand and Christensen, 1961). It was observed in this study that this ratio was comparatively more in intima than in media. If we compare the fatty acid composition of different lipid components of monkey thoracic with abdominal aorta, then it was seen that the abdominal segment had more saturated and less unsaturated fatty acids as compared to thoracic aorta. This might explain the greater extent of atherosclerotic lesion in abdominal aorta as compared to thoracic aorta in this species.

As regards human aortic fatty acid composition, a similar trend was observed as in the case of monkey. If we compare fatty acid constituents of human thoracic aorta with monkey thoracic aorta, we find that saturated fatty acids are more in human aorta than in that of monkey, while a reverse relationship holds good with respect to unsaturated fatty acids. Similar findings were noted when human abdominal aorta was compared with monkey abdominal aorta. The most striking difference observed in these two species was in the content of arachidonic acid. The phospholipid arachidonic acid was significantly higher in monkey thoracic and abdominal aorta as compared with corresponding regions of human aorta. The above findings may have important relevance to atherosclerosis since prostacyclin (PGI_2) is the major product of arachidonic acid in walls of arteries and veins in several species including man (Nelson *et al.,* 1961). The most important properties of PGI2 so far elucidated are its vasodilator (Moncada *et al.,* 1976) and inhibitor action on platelet aggregation (Moncada ånd Vane, 1978). PGI_2 applied locally in low concentration inhibits thrombus formation *in vivo* which is caused by ADP in the micro-circulation of hamsters cheek pouch (Higgs *et al.,* 1977). The greater quantity of arachidonic acid in monkey aorta may explain why this species develops less atherosclerosis and thrombosis as compared to that of human. One of the reports from this laboratory has already shown that incidence of naturally occurring atherosclerosis in monkeys is much less (27%), (Chakravarti and Kukrej 1981) as against 80-90% in human aorta. Therefore, the present study has provided some evidence, on the basis of lipid constituents and fatty acid composition of the aorta from man and monkey, why incidences of spontaneously occurring atherosclerosis is much more in man than in the monkey.

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