Cholesterol Distribution in the Bulk Tissues of Man: Variation with Age

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Abstract Lacking reliable data on cholesterol concentrations in muscle, adipose tissue, skin, and connective tissues (i.e., the "bulk tissues") in "normal" man, we have completed these analyses in 21 men and 8 women who died suddenly and unexpectedly; their ages ranged from 23 to 78 yr.

In 11 of these subjects aged 20–40 yr, the mean cholesterol concentrations ranged from 180–440 mg/100 g dry tissue. In contrast, in 13 subjects aged 60–80 yr the values were 23–28% higher in muscle, adipose, and skin, while in dura mater, biceps, and psoas tendons the concentrations were 130, 260, and 460% higher (respectively). Esterified cholesterol in these connective tissues was found to be the major contributor, increasing 5- to 10-fold in the older group as opposed to a less than 5-fold rise in free cholesterol.

In view of the large proportion of total body weight represented by these tissues, it is clear that large amounts of cholesterol can be accumulated there over a lifetime; indeed, the dense connective tissues appear to act uniquely as a trap for cholesterol, especially in the esterified form. Whether analyses of tendinous tissues in man, accessible during life, mirror the pattern of cholesterol deposition in arterial connective tissue remains to be determined.

INTRODUCTION

Although concentrations of cholesterol in human plasma and diseased tissues have been studied extensively in respect to the effects of various hereditary and environmental factors (1), little is known in man about the concentrations and contents of cholesterol in the various "bulk tissues" (muscle, adipose tissue, skin, and connective tissues). In previous investigations of some of these tissues (2–4), materials were sampled largely from individuals dying of wasting diseases. In the present studies, we have analyzed tissues only from victims of sudden death who showed no evidence of chronic illness. Our data indicate striking differences among age groups, among tissues, and in the forms of cholesterol deposited.

METHODS

Tissue samples were obtained in the course of autopsies performed by the Medical Examiner of New York City. In order to avoid terminal ante mortem changes, we assayed tissues only from individuals who had suffered a sudden unexpected death unassociated with chronic illness or with coronary or cerebrovascular occlusion; skin and tendon xanthomata were sought but never found (Table I). No attempt was made to quantitate the degree of atherosclerosis in these 29 individuals, nor did we consider it valid to draw conclusions from plasma cholesterol levels obtained post mortem due to variable hemolysis and proteolysis.

Determination of total cholesterol. Samples of tissues weighing 4–5 g were obtained: within 3–4 hr of sampling, 0.5–1.0-g portions were weighed to the nearest milligram. These were then lyophilized to constant dry weight and cholesterol-4-^{14}C (New England Nuclear Corp., Boston, Mass.) was added as an internal recovery standard. (Concentrations calculated in terms of wet weight were not included in this report in order to avoid confusion due to postmortem shifts in tissue water; the age-related changes to be described below were noted also when concentrations were expressed in terms of wet weights of the various tissues.)

After addition of boiling chips and 20 ml of 1 N NaOH in 90% ethanol, the mixture was refluxed for 1 hr. Water (10 ml) was added, and nonsaponifiable components were extracted three times with 50-ml portions of petroleum ether (PE)[1] (b.p. 66–69°C). The combined PE extracts were evaporated to dryness under reduced pressure, and the residue was dissolved in ethyl acetate; one portion was taken for counting of radioactivity in a Packard Tri-Carb scintillation counter (model 3003, Packard Instrument Co., Downers Grove, Ill.), and another portion for measurement of cholesterol concentration. The cholesterol concentration in adipose tissue, skin, and rectus fascia was quantitated by gas-

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[1] *Abbreviations used in this paper:* EE, ethyl ether; GLC, gas-liquid chromatography; PE, petroleum ether.

TABLE I

Clinical Data in 30 Medical Examiner Cases Dying a Sudden Unexpected Death

Age group	Subject No.	Subject Initials	Age	Sex	Height	Weight	Ideal weight*	Cause of death
			yr		*in.*	*lb.*	%	
I. 20–39 yr								
	1	P. McC.	23	M	—	163	—	Gunshot wound
	2	S. M.	24	M	67	135	96	Stab wound
	3	S. C.	25	F	65	105	85	Drug overdosage
	4	H. B.	25	M	70	155	101	Stab wound
	5	E. S.	28	M	69	205	136	Cause unknown
	6	L. C.	29	M	67	130	93	Gunshot wound
	7	R. R.	31	M	73	175	104	Cause unknown
	8	E. B.	32	F	69	165	118	Drowning
	9	R. J.	32	M	68	175	120	Gunshot wound
	10	C. H.	34	F	64	130	108	Cause unknown
	11	J. H.	34	M	66	210	153	Stab wound
II. 40–59 yr								
	12	A. B.	42	M	69	195	130	Automobile accident
	13	H. S.	45	M	67	137	99	Gunshot wound
	14	R. H.	50	M	65	130	99	Fracture of cervical vertebrae
	15	G. L.	56	F	—	140	—	Suffocation
	16	G. J.	57	M	—	185	—	Subdural hematoma (trauma)
III. 60–79 yr								
	17	N. A.	62	M	66	105	80	Subdural hematoma (trauma)
	18	R. H.	63	F	—	110	—	Drug overdosage
	19	T. F.	65	M	68	130	93	Subdural hematoma (trauma)
	20	M. M.	65	F	64	140	110	Automobile accident
	21	S. H.	66	F	59	125	118	Drug overdosage
	22	F. G.	69	M	67	125	92	Subdural hematoma (trauma)
	23	J. K.	72	M	67	120	91	Subdural hematoma (trauma)
	24	D. M.	73	M	64	174	136	Automobile accident
	25	A. H.	75	M	68	135	95	Subdural hematoma (trauma)
	26	S. W.	75	M	—	120	—	Subdural hematoma (trauma)
	27	M. L.	77	F	60	135	128	Pulmonary embolism
	28	A. N.	77	M	—	138	—	Automobile accident
	29	L. K.	78	M	66	127	94	Automobile accident

* According to Life Insurance Tables (5).

liquid chromatography (GLC) as previously described (6), using 5α-cholestane or β-sitosterol as an internal standard; that in extracts from muscle and dense connective tissue was measured by AutoAnalyzer (7). Recovery of radioactive standards was > 90% for all samples except adipose tissue (75–85%). GLC and AutoAnalyzer data checked within 10% (60 duplicate determinations of tendon and muscle cholesterol).

Determination of esterified cholesterol. Free and esterified cholesterol were determined in 50 of the 85 samples of dura mater, biceps tendon, and psoas tendon. Each sample (0.5–1.0 g) was suspended in 10–15 ml of water, homogenized in a stainless steel micro Waring Blendor, and then dehydrated as described above. 60 ml choloroform : methanol (2 : 1) was added, along with cholesterol-1,2-^{3}H (New England Nuclear Corp.) and cholesterol-1,4-^{14}C palmitate (Nuclear-Chicago Corp., Des Plaines, Ill.) as internal recovery standards. The sample was refluxed for 3 hr; 12 ml 0.04% $CaCl_2$ was added, and the vessel was shaken. Lipids were extracted three times with 20-ml portions of "pure solvents lower phase" (8), and the combined lower phases were evaporated. Free and esterified cholesterol in the residue were separated by thin-layer chromatography on Silica Gel H plates developed with PE : ethyl ether (EE) (80 : 20) and sprayed with a half-saturated aqueous solution of rhodamine G. The bands of interest were detected under ultraviolet light, collected individually, and eluted with EE. After evaporation of the eluate under a stream of nitrogen, the free and esterified cholesterol concentrations were analyzed after saponification as described for total cholesterol.

RESULTS

Variations in cholesterol concentrations in different portions of the same tissue. In order to determine the variation in concentration of cholesterol from site to site

TABLE II

Cholesterol Concentrations in Various Tissues as a Function of Age

Age group	Cholesterol form	Mean cholesterol, *mg/100 g dry tissue**						
					Connective tissues			
		Muscle	Adipose	Skin	Rectus fascia	Dura mater	Biceps tendon	Psoas tendon
I. 20–39 yr	Total	259±29 (10)	180±39 (10)	267±67 (9)	223±27 (10)	428±79 (11)	269±182 (11)	190±51 (11)
	esterified					88 (7)	67 (5)	34 (5)
II. 40–59 yr	Total	278±24 (4)	237±75 (4)	297±55 (3)	231±30 (4)	667±189 (5)	575±393 (4)	506±207 (4)
	esterified							
III. 60–79 yr	Total	319±48 (10)	224±42 (8)	343±59 (7)	283±50 (10)	1002±290 (13)	959±566 (13)	1056±492 (13)
	esterified					460 (11)	540 (9)	345 (10)
III vs. I‡ (% increase and *P* value)	Total	+22% (<0.01)	+24% (<0.05)	+28% (<0.05)	+27% (<0.01)	+130% (<0.01)	+260% (<0.01)	+460% (<0.01)
	esterified					+445% (<0.01)	+710% (<0.01)	+855% (<0.05)

* ±SD for (n) individuals in three age groups. Muscle was sampled in duplicate in 16 individuals and in quintuplicate in 8. Adipose tissue was sampled in duplicate n 14 subjects and in quintuplicate in 8. Skin was sampled singly in 11 subjects and in quadruplicate in 8. Rectus fascia was sampled singly in 24 subjects. Multiple samples were taken from dura mater in all subjects, biceps tendon in 28 subjects, and psoas tendon in 28 subjects.

‡ Difference between groups I and III expressed as per cent of group I value, and significance of difference as a *P* value by Student's *t* test.

within tissues we selected four individuals 25–34 yr old and four 62–73 yr old. We chose samples from each individual as follows: muscle from five sites, adipose tissue from five sites (three subcutaneous and two intra-abdominal sites), and skin from four sites. Replicate samples of muscle, adipose tissue, and skin showed av-

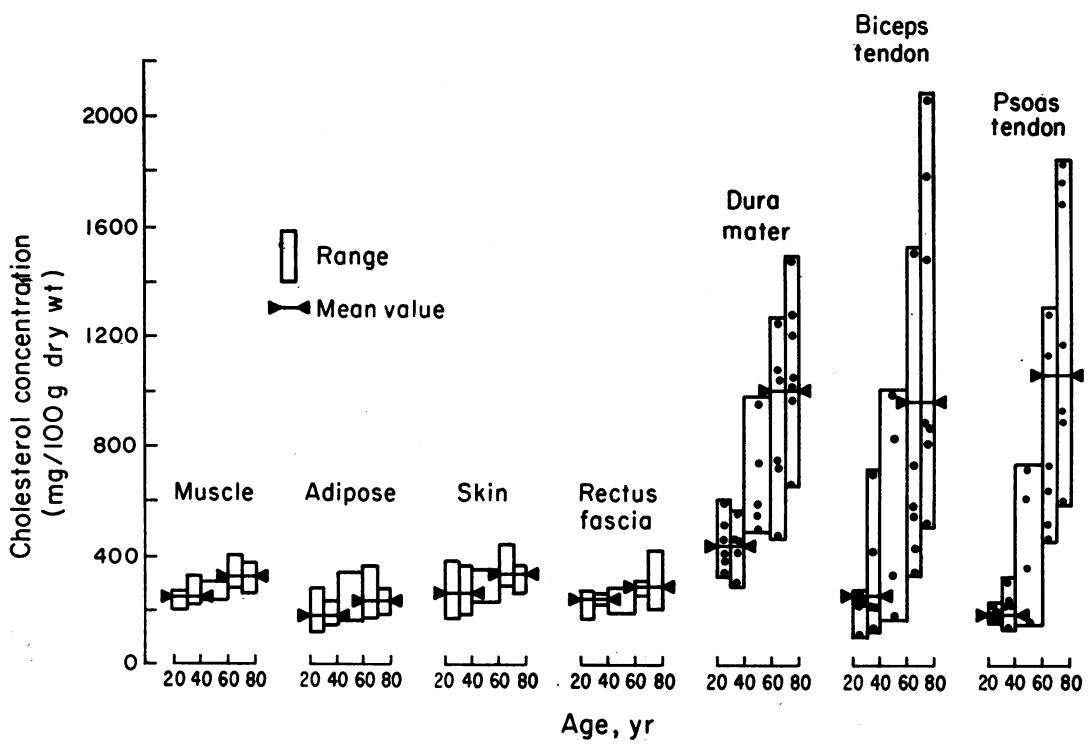

FIGURE 1 Range of cholesterol concentrations in four tissues (including four sites of connective tissue) in 29 individuals separated into 5 subgroups according to age. Note that the indicated line (▶——◀) represents the mean for the entire age group 20–40 and 60–80, and corresponds to the mean values represented in Table II. Individual values are shown (●) for the cholesterol concentrations in dura mater, biceps, and psoas tendons: only one overlap is seen between the 20–30 yr and 60–80 yr age groups.

erage coefficients of variation among the several sites of 4, 17, and 18%, respectively.

Replicate samples of connective tissue from biceps and psoas tendon in the entire study group of 29 individuals showed more variability. 18 of 47 pairs of replicates differed by more than 25%. However, when we related these data to the subjects' ages, it became clear that these variations occurred more frequently in the older age groups: only 5 of 21 replicates in the 20–40 yr group varied by more than 25%, whereas 13 of 26 analyses in the 60–80 yr group varied that much. Since our analyses consumed one-half to three-quarters of the entire biceps tendon, and 10% of the psoas tendon, we consider that the means give a reasonably accurate picture of these tissue concentrations of cholesterol. For dura mater the replication was far less variable: only two replicates from 29 pairs varied by more than 25%, and the mean variation between pairs in a given subject was 10% or less, regardless of age. The mean cholesterol concentration in the dura of six elderly individuals dying of subdural hematoma (1026 mg/100 g dry weight) was not significantly different from that of seven others in the same age group (III) who died of other causes (945 mg/100 g dry weight).

Variations in cholesterol concentration with age. The relationship between cholesterol concentration and age in muscle, adipose tissue, rectus fascia, skin, and other connective tissues in 29 individuals is shown in Table II and Fig. 1. Several points are evident. First, the mean concentrations of total cholesterol increased with age by at least 20% in all tissues studied (group I vs. group III). Second, the increase of total cholesterol in connective tissues (with the exception of rectus fascia) was more extensive than that in other tissues: dura mater, 130%; biceps, 260%; and psoas tendon, 460%. Third, there was no significant increase in total cholesterol concentration between 20 and 40 yr in any tissue; increases occurred after age 40. Fourth, the variability between analyses in any one group of individuals, while relatively constant with age for muscle, adipose tissue, and skin, became larger with increasing age in connective tissue (again, rectus fascia was the exception).

Other correlations. We found no correlation of tissue cholesterol concentration with sex, or with per cent of ideal body weight, nor did the various cholesterol concentrations in the dense connective tissues in a given individual fall in the same rank order.

Variations in fraction of esterified cholesterol in connective tissues with age. The per cent of esterified cholesterol doubled with age (Table II and Fig. 2). In dura mater the percentage increased (group I vs. group III) from 21±8 to 46±10% (expressed as per cent of the total ±SD); in biceps tendon, from 25±10 to 56±12%; and in psoas tendon from 18±5 to 31±12%.

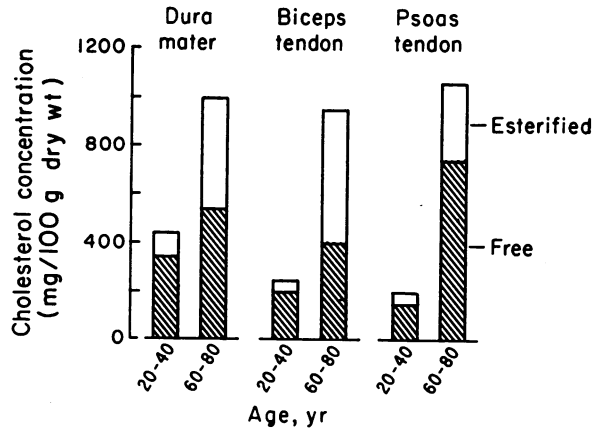

FIGURE 2 Total cholesterol (with relative amounts of free and esterified forms) in three connective tissues as a function of age (groups I vs. III).

(These increases were significant at $P < 0.01$, $P < 0.01$, and $P < 0.05$ levels, respectively.) In all three tissues the absolute rise in esterified cholesterol (5- to 10-fold) was more prominent than that of free cholesterol (< 5-fold).

DISCUSSION

Differences in choice of subjects and in handling of tissues may explain the lack of agreement between our data and those of Khan, Cox, and Asdel (2) and Nieminen (3), who did not note any systematic increase in tissue cholesterol concentration with age. Insull, Hsi, and Yoshimura (9) reported increases in cholesterol concentration in adipose tissue and muscle with age, and Maurizi, Alvarez, Fischer, and Taylor (4) noted similar age related increases in muscle cholesterol concentration; our preliminary report (10) of striking increases in dura mater has recently been confirmed by DiMarco, White, and Adelsen (11), although the latter authors failed to find increases in cholesterol concentration of muscle, adipose tissue, or liver with age.

We defined the site-to-site variation in cholesterol concentration within any given tissue as a base line against which to assess age changes: variations were small in muscle, larger in adipose tissue and skin, and very much larger in the dense connective tissues of elderly individuals. It seems clear from these studies that normal connective tissue is particularly susceptible to focal deposition of cholesterol in elderly individuals (since methodologic difficulties could be eliminated as a factor in site-to-site variations, and since the contribution of plasma cholesterol to postmortem tissue cholesterol appears to be negligible even in vascularized tissues [12]).

Having defined this site-to-site variability, we then examined the variation in total cholesterol concentration

in the bulk tissues with age. In older individuals we found increased total cholesterol concentrations in all tissues studied. In three dense connective tissues these increases were very large; both free and esterified cholesterol concentrations rose, but the increases in cholesterol esters predominated.

It is possible that, in man, the total amount of carcass cholesterol may increase gradually with age. Whether the mass of stored cholesterol can expand or contract acutely under dietary or drug manipulation cannot yet be determined directly; however, we have presented evidence to support the conclusion that it can increase when unsaturated fat diets cause plasma cholesterol concentrations to fall (13) and that it can contract when clofibrate is administered.[2]

Of the tissues studied, connective tissue was most susceptible to cholesterol deposition. This bulk tissue exhibited an affinity for cholesterol (mainly esterified cholesterol) similar to that found in connective tissue in pathologic states such as in xanthomatosis (14) and atherosclerosis (15) and in aging normal aorta (15), and in acute experiments with sponge implants in animals and man (16). In explanation of this tissue's unique sequestration of cholesterol we are particularly attracted by the hypothesis of Walton and Williamson (17) that circulating β-lipoproteins may be aggregated by the acid mucopolysaccharides elaborated in connective tissues.

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