Concentration and Distribution of Cholesterol in Muscle and Adipose Tissue.* (22061)

ALFONSO DEL VECCHIO,† ANCEL KEYS, AND JOSEPH T. ANDERSON.
Laboratory of Physiological Hygiene, University of Minnesota.

It is widely assumed that most or all of the cholesterol in muscles, for example in meats, is associated with adipose tissue and interstitial fat and that, in general, with increasing fatness there is an increasing cholesterol content of the whole muscle. This view is reflected in common dietetic practice in which fatty meats are proscribed in low-cholesterol diets, though lean, fat-trimmed meats are allowed.

Actually, however, data have not been available on the distribution of cholesterol between the muscle proper and the associated fat. The present paper summarizes a study on this question with muscle and adipose tissue of 3 species of meat animals, of rabbits, of chickens and of man.

Materials and methods. Beef, veal, pork, suckling pig, mutton, lamb and chickens were purchased fresh in Minneapolis markets. Rabbits were obtained from the University Medical School. Fresh human tissues (abdominal wall muscle and associated adipose tissue and psoas muscle) were provided by the Department of Pathology of the University of Minnesota Medical School. Both commercial and choice grades of round and rib steaks of beef were studied. Leg and loin cuts of veal, mutton and lamb and the longissimus dorsi muscles of rabbit were used. Light (breast) and dark (thigh) meats from roosters, hens and chicks were separately studied. Subcutaneous fat of rabbits, hens and roosters were also analyzed. For each sample the adipose tissue and gross interstitial fat were separated from the lean as completely as possible by careful scalpel dissection. The separated portions were minced in a meat grinder and stored in well-sealed containers at –20°C before analysis. Moisture was determined by drying at low temperature in a vacuum desiccator(1). "Fat" was measured as the weight of the dried ether extract obtained by prolonged extraction in the Soxhlet apparatus. For cholesterol the procedure of Pihl(2) was followed until the chloroform extract was obtained. This was then evaporated, the residue was dissolved in acetone-alcohol, and the cholesterol was precipitated and measured as the digitonide according to the method of Foldes and Wilson(3). Tests of the method gave 98 to 104% recovery of added cholesterol. All measurements were made in duplicate on each sample.

Results. There were no significant differences in the cholesterol results for the different grades and cuts of meat so these are not differentiated in Table I which summarizes 8 independent samples of beef, 4 each of lamb and pork and 2 each for the other

TABLE I. Results from Analyses on Meat. EE = ether extract; cholesterol, mg % EE = mg of cholesterol/100 g of ether extract. Mean values and ± stand. deviations (beef, pork, lamb) or half the range (veal, pig, mutton, rabbit).

	Dissected muscle			Adipose tissue			
						Cholesterol	
Item	EE, %	Water, %	Chol., mg %	EE, %	Water, %	mg %	mg % EE
Beef	5.5 ± 2.3	72.6 ± 3.0	46.8 ± 4.7	75.7 ±10.9	21.6 ± 9.7	81.5 ±18.9	104.6 ±30.0
Veal	1.6 ± .4	74.5 ± 0	71.9 ± 1.0	85.5 ± 3.6	13.6 ± 3.9	76.4 ± 4.3	89.2 ± 1.0
Pork	5.0 ± 1.0	71.0 ± .7	46.4 ±10.1	90.0 ± 3.9	7.9 ± 3.0	72.6 ±11.2	80.2 ±14.8
Pig	3.0 ± .5	76.4 ± .9	83.7 ± .6	68.5 ± 4.3	24.9 ± 3.1	89.3 ±10.0	121.6 ±22.4
Mutton	3.0 ± .5	73.7 ± .1	51.8 ± .8	90.2 ± 1.5	6.6 ± 1.2	76.3 ± 6.0	84.4 ± 5.3
Lamb	5.3 ± 2.4	70.6 ± 1.1	60.9 ± 1.3	92.7 ± 4.9	4.7 ± 3.6	67.4 ±20.0	73.7 ±26.8
Rabbit	.9 ± .3	74.5 ± 2.8	43.5 ± 1.0	89.2 ± 0	8.5 ± .7	133.7 ±47.6	149.8 ±53.3

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† Present address: Institute of Pharmacology, University of Milan, Italy.

TABLE II. Results from Analyses on 5 Patients. Column headings as in Table I.

Case	Age	Sex	Dissected muscle EE, %	Dissected muscle Water, %	Dissected muscle Chol., mg %	Adipose tissue EE, %	Adipose tissue Water, %	Adipose tissue Cholesterol mg %	Adipose tissue Cholesterol mg % EE
BS	43	♀	3.4	77.4	65.8	55.5	28.7	150.2	270.5
M	65	♀	22.0	58.9	112.7	96.9	2.3	189.8	195.9
AJ	67	♂	8.3	71.6	138.4	87.4	11.0	323.1	396.6
LB	57	♂	5.6	74.3	59.2	88.4	10.5	95.4	107.9
CM	7	♀	6.6	71.8	64.3	93.9	—	101.1	107.7

items. Table II gives the data from muscle and adipose tissues from 4 adults and a 7-year-old girl. Subjects M and CM had brain tumors, subject BS had carcinoma of the breast, subject AJ had carcinoma of the pancreas, and subject LB had rheumatic heart disease. Table III summarizes the data from chickens. It was impossible to obtain enough adipose tissue from the chick for separate analysis. Inspection of Tables I, II and III shows that, in general, the concentration of cholesterol in the ether extract of the dissected muscle is much higher than in the ether extract of the adipose tissue. However, some of the ether extract obtained from the dissected muscles represents adipose tissue not removed by dissection so some allowance must be made for this. The concentration of cholesterol in such residual adipose tissue should be the same, per unit of ether extract, as in the adipose tissue actually dissected out and analyzed so it should be possible to calculate the amount of cholesterol in the muscle associated with this adipose tissue.

Let E and e represent the grams of ether extract obtained from 100 g of adipose tissue and dissected muscle, respectively, and let K and k be the grams of cholesterol contained in these. Further, let R be the grams of ether extract in 100 g of dissected muscle that is attributable to adipose tissue residue in that muscle, and let C be the grams of cholesterol per 100 g of dissected muscle that is not attributable to residual adipose tissue. Then: 1), $e = R + C$, and 2), $(k-C)/R = K/E$, so 3), $C = (Ek-Ke)/(E-K)$.

Computations from the data in Tables I, II and III are summarized in Table IV. Very little of the cholesterol in the dissected muscle is attributable to residual adipose tissue like that dissected away and it is indicated that truly "lean" muscle contains a very significant amount of cholesterol in all animals, including man.

Further inspection of Table IV shows that this non-adipose tissue cholesterol tends to be higher in the young than in the mature animals of the same species (beef vs. veal, pork vs. pig, mutton vs. lamb). The data do not allow exact calculations for the chicken but in this case, too, it would appear that the rule must hold (Table III). The data from the human subjects are inadequate for the examination of this question and, in any case, may be unrepresentative of normal persons.

Discussion. In most of the previous studies of cholesterol in tissues the Liebermann-Burchard reagent has been applied directly to the non-saponifiable residue from the lipid extraction without separating the digitonide. In

TABLE III. Results from Analyses on Roosters, Hens, and Chicks. L = light meat, D = dark meat. Column headings as in Table I.

Item		Dissected muscle EE, %	Dissected muscle Water, %	Dissected muscle Chol., mg %	Adipose tissue EE, %	Adipose tissue Water, %	Adipose tissue Cholesterol mg %	Adipose tissue Cholesterol mg % EE
Rooster	L	.6	72.6	31.7	97.5	4.0	76.4	78.4
"	D	.8	73.1	55.3				
Hen	L	1.5	70.3	31.7	97.3	4.6	91.3	93.9
"	D	4.9	71.1	53.7				
Chick	L	.6	74.6	66.1	—	—	—	—
"	D	1.5	77.3	97.6	—	—	—	—

TABLE IV. Computed Mean Values for Concentration of Cholesterol in mg per 100 g of Muscle Free of Adipose Tissue.

	Cholesterol		Cholesterol
Patient BS	58.8	Beef	43.3
" M	89.4	Veal	71.6
" AJ	117.8	Pork	44.6
" LB	56.4	Pig	82.4
" CM	61.3	Mutton	50.7
Rooster L	31.5	Lamb	60.3
" D	55.1	Rabbit	42.6
Hen L	30.8		
" D	51.6		

the present study this "direct" proceeding was applied in parallel with our digitonin method in aliquots from 34 samples. The "direct" result averaged 16.9% higher (s.d. $= \pm$ 10.8%) than the digitonin result so it appears that about a sixth of the non-saponifiable Liebermann-Burchard reacting material in muscle is not cholesterol. This explains, in part, the fact that most of the previously published analyses for cholesterol in meats are higher than those given here(cf, 4-6).

It has been reported that different muscles in the same animal may differ somewhat in cholesterol concentration(7-9), and it has been suggested that these differences are related to differences in the customary activity of the muscles(10-12). It is interesting that red muscles (semi-tendinosus) contain more cholesterol than white (biceps femoralis) muscle in the rabbit(8) and that, as reported here, the same rule holds in the chicken(7).

If to these considerations we add the fact of higher concentrations of cholesterol in the muscle tissue of younger animals it would appear that perhaps there is a general rule for the cholesterol concentration in muscle to be related to the total energy metabolism representing growth of the muscle and muscular work, or both. And it is difficult to avoid the conclusion that, far from being inert, cholesterol has an important role in structure or function of the muscle.

The present calculations for the non-adipose tissue cholesterol in muscle are approximations, of course, because there are certainly other ether-soluble materials in muscle besides cholesterol that are not associated with adipose tissue. Equation 1), above, more properly should be written 4), $e = R + C + X$, where X represents other ether-soluble materials not accounted for by adipose tissue. Presumably, X might include some of the lecithin, for example, found in muscle. But X must be a small quantity compared to R and the error in the estimation of C resulting from its neglect should be trivial.

Summary. 1. Analyses are reported for cholesterol contents of muscle and adipose tissue from beef, veal, pork, pig, mutton, lamb, rabbit, chicken and man. It is shown that a large proportion of the cholesterol in muscle is not attributable to residual adipose tissue or any tissue of similar composition. 2. Concentration of cholesterol in muscle, apart from associated adipose tissue, is of the order of 40 to 50 mg per 100 g in the adult mammals studied and in the dark meat of chickens. Higher values were found in two elderly human beings. The corresponding cholesterol concentration in the white meat of chickens is of the order of 30 mg per 100 g. 3. There is a general tendency for muscle cholesterol concentration to be considerably higher in young, growing animals than in adults of the same species. This reinforces the theory that most of the cholesterol in muscle has a definite metabolic or structural function.

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