Effect of Choline and Cholesterol on Lipoprotein Patterns of Rats

By George F. Wilgram, M.A., M.D., Lena A. Lewis, Ph.D. and Charles H. Best, M.D., F.R.C.P.

Serum lipid values (low-density lipoproteins, alpha lipoproteins and serum cholesterol) are decreased in choline deficiency. It is believed that this is due specifically to a choline lack rather than to a nonspecific effect of a deficiency. The addition of cholesterol to a choline deficient diet does not lead to hyperlipemia and hypercholesterolemia. It appears that dietary choline is one of the many requirements necessary for production of hyperlipemia and hypercholesterolemia. A metabolic interdependence between choline and cholesterol is pointed out in rats.

The addition of cholesterol to diets supplemented with adequate choline may lead to atheroma-like lesions in coronary arteries and in aorta of older rats. Addition of cholesterol to borderline choline-deficient diets greatly aggravates the occurrence of renal damage and of the Moenkeberg-type of medial sclerosis in young rats.

Our approach to one aspect of the problem of the relationship of cholesterol to vascular disease in the presence and absence of dietary choline has utilized determinations of plasma lipids and lipoproteins. The results suggest that hyperlipemia and hypercholesterolemia, induced in rats by feeding cholesterol, can occur only when adequate amounts of choline are being fed.

Methods

Eighty male Wistar rats weighing between 190 and 210 Gm. were separated into groups of 10 animals each. The animals were fed our standard synthetic diet* for a period of three weeks, after which time they were sacrificed by decapitation. Their blood was immediately collected in wide-mouthed jars, and great care was taken to avoid contamination by fur or tissues. To insure uniform food consumption prior to sacrifice, the animals were starved for eighteen hours before their last feeding. During their final meal they were allowed to feed for a period of two hours ad libitum, when the colony was kept dark and undisturbed. Two hours after withdrawal of food, half the animals were killed. The remaining animals were sacrificed at the end of eight hours.

The composition of the basal choline deficient diet is shown in the footnote. It was supplemented at the expense of starch with either 0.85% choline, or 2% cholesterol, or with both. To ascertain the final dietary intake, not only was the last food consumption recorded, but the degree of filling of the stomach was checked by visual inspection at autopsy.

Small pieces of liver and kidney were examined histologically.

The serum lipoproteins were studied ultracentrifugally by Lewis, Green and Page's modification7 of the Gofman technic.8 Cholesterol was determined by the method of Abel, Levy, Brodie and Kendall.*

Results

On histologic examination the livers of all choline deficient animals were fatty, but the kidneys appeared normal. Cholesterol supplementation of a choline deficient diet greatly accentuated the fattiness of the liver and led also to fat accumulation in the tubules of the kidney, with occasional “renal frosting” as described by Hartroft.10 This latter finding suggests again that cholesterol aggravates the symptoms of choline deficiency, although the animals were beyond the weight range in which this effect may be demonstrated most conspicuously. All organs of the choline supplemented rats were normal, while the rats


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TABLE 1—Effect of Choline and Cholesterol on Lipoproteins

<table>
<thead>
<tr>
<th>Group</th>
<th>Time interval after last food intake (hr.)</th>
<th>Avg. Wt./Gm. at sacrifice</th>
<th>Final food consumption Avg./Gm.</th>
<th>Dietary choline per cent</th>
<th>Dietary cholesterol per cent</th>
<th>Lipoproteins*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S20-40</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>318</td>
<td>15.1</td>
<td>—</td>
<td></td>
<td>18.7</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>287</td>
<td>13.7</td>
<td>—</td>
<td>2</td>
<td>33.4</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>282</td>
<td>16.0</td>
<td>.85</td>
<td>—</td>
<td>5.7</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>298</td>
<td>15.5</td>
<td>.85</td>
<td>2</td>
<td>74.5</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>302</td>
<td>13.9</td>
<td>—</td>
<td>—</td>
<td>27.0</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>275</td>
<td>13.5</td>
<td>—</td>
<td>2</td>
<td>37.7</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>266</td>
<td>13.8</td>
<td>.85</td>
<td>—</td>
<td>77.5</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>308</td>
<td>14.5</td>
<td>.85</td>
<td>2</td>
<td>86.5</td>
</tr>
</tbody>
</table>

* Determined at 1.21, using NaCl-KBr.

† $p > 0.1$
‡ $p > 0.02$
§ $p > 0.01$

given choline plus dietary cholesterol had so-called cholesterol fatty livers.

Food consumption and rate of growth were approximately the same in all groups. We feel, therefore, that the different lipoprotein patterns indicated in table 1 are due specifically to a choline lack rather than to a nonspecific effect of a deficiency. The following is discernible from the table:

1. Cholesterol supplementation of a choline deficient diet leads to a slight increase in the -S20-40 class. Choline deficient cholesterol supplemented animals (groups 2 and 6) do not exhibit any other significant lipoprotein or serum cholesterol changes as compared with choline deficient rats without dietary cholesterol (groups 1 and 5).

2. There is a shift towards the low-density lipoproteins in the choline supplemented animals given dietary cholesterol (groups 4 and 8) as compared with the choline supplemented rats without added cholesterol (groups 3 and 7).

3. The alpha lipoproteins and low-density lipoproteins are decreased in the choline deficient animals (groups 1 and 5) as compared with their choline supplemented controls (groups 3 and 7).11

4. The plasma cholesterol levels of the choline deficient rats with and without dietary cholesterol are similar (groups 1, 2, 5 and 6). Choline supplementation elevates the serum cholesterol significantly (groups 3 and 7). It is only when choline is present in the food that the serum cholesterol may be further elevated by dietary cholesterol supplements (groups 4 and 8).

DISCUSSION

It has been shown previously that rats may develop atheroma-like lesions accompanied by hypercholesterolemia and hyperlipemia when fed diets high in fat, choline and cholesterol.1-3 The present study was undertaken to determine the requirements for the production of hyperlipemia and hypercholesterolemia and to investigate the significance of choline and cholesterol in the induction of these two phenomena which are believed to be important in the etiology of atherosclerosis.

The data show clearly that marked elevation of the plasma lipids (low density lipoproteins, alpha lipoproteins and serum cholesterol) in rats is possible only in the presence of dietary choline. The addition of cholesterol to a choline deficient diet does not lead to any significant plasma lipid changes. It would appear from our results that adequate dietary choline is one of the many requirements to make hyperlipemia and hypercholesterolemia possible.

The same choline deficient diets used in these experiments, when given to younger animals (150 Gm.), may lead to a Moenckeberg-type
of arterial sclerosis, and addition of cholesterol to these diets increases the severity of renal damage and the Moenckeberg-type of arterial disease. Our results suggest that this aggravating effect of cholesterol in choline-deficient rats is not accompanied by marked changes in plasma lipids. We think, therefore, that present evidence favors the view that the Moenckeberg-type of vascular disease in choline-deficient rats does not depend on hyperlipemia or hypercholesterolemia, but is related to renal damage (bilateral renal hemorrhagic cortical necrosis) which is induced by choline deficiency.4-6.12 Supporting this view is the fact that others have been able to produce essentially the same Moenckeberg-type of arteriosclerosis in rats by disturbing renal function sufficiently to cause uremia and electrolyte changes, with or without hypertension.13-15 We do not know why cholesterol increases the effects of choline deficiency, but recent work by Pilgeram and Greenberg16 points to a metabolic interdependence between choline and cholesterol in rats.

That a metabolic relationship exists between choline and cholesterol is also suggested by our finding that dietary cholesterol may lead to increases of serum lipids only when adequate choline is fed and by the aggravating effect of dietary cholesterol on hepatic, renal and vascular lesions in young choline deficient rats.

SUMMARY

The low-density lipoproteins, the alpha lipoproteins and the serum cholesterol values are decreased in choline deficient rats as compared with their choline supplemented controls.

Dietary cholesterol leads to marked hyperlipemia and hypercholesterolemia in rats only in the presence of dietary choline. The addition of cholesterol to choline-deficient diets does not lead to elevations in serum lipids or cholesterol in rats, indicating a close metabolic interdependence between choline and cholesterol.

ACKNOWLEDGMENTS

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SUMMARIO IN INTERLINGUA

Le valores de lipoproteinas a bassa densitate, de lipoproteinas alpha, e de cholesterol seral es reducite in rattos a carentia de cholina in comparation con animales de controlo recipiente supplementos de cholina.

Cholesterol dietari resulta in rattos in marcate grados de hyperlipemia e hypercholesterolemia solmente in le presentia de cholina dietari. Le addition de cholesterol a dietas a carentia de cholina non resulta in rattos in augmentos del cholesterol o del lipidos seral. Isto indica un strict relation metabolic inter cholina e cholesterol.

REFERENCES

Calculation of Blood Volumes in the Left Heart and Pulmonary Bed in Clinical Conditions

The use of dye dilution curves for estimating blood volume introduced by Hamilton and associates (Am. J. Physiol. 99: 534, 1932) has been extended by a number of workers to throw light on the distribution of blood volumes under different circulatory conditions. Some of these reports have appeared in this journal.

Recently, Swedish investigators have presented formulations purporting to show that the blood volumes of the left heart and lungs can be estimated directly from dye dilution curves when the dye is injected directly into the pulmonary artery through a catheter, and blood from the radial arterial is sampled.

According to their formulations the diastolic volume of the left ventricle and the systolic capacity of the left atrium can be obtained from the last part of the dye-dilution curves. Since mean pulmonary circulation time can also be deduced, pulmonary blood volume can be calculated. It is claimed that the calculations for pulmonary blood volume hold for the combined—but not the separate—volumes of the left atrium and ventricle when mitral or aortic regurgitation, or both, are present.

This report will undoubtedly stimulate further experimental studies to test the validity of assuming several empirical constants.

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