Effects of Dietary Proteins, Methionine and Vitamins on Plasma Lipids and Atherogenesis in Cholesterol-Fed Cockerels

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With the assistance of Mrs. D. Century and Mr. P. Johnson

Methionine deficiency in the presence of a high-fat, high-cholesterol diet aggravates atherogenesis in the cockerel. High-protein, high-vitamin supplementation suppresses the atherogenic effect of a cholesterol-fat-containing diet. High-protein supplementation alone had a suppressive effect; high-vitamin supplementation had no effect. However, the combined protein and vitamin supplement was the most effective.

Recent work in this department led to the tentative conclusion that patterns of cholesterolemia and atherogenesis in man and experimental animals may be influenced by the total dietary pattern. An hypothesis was put forward, suggesting that dietary imbalance—excess of some nutrients (e.g., total calories, total fats, saturated fats, refined carbohydrates) and inadequacy (absolute or relative) of others (e.g., proteins, vitamins, minerals, essential fatty acids)—may be particularly pernicious in inducing hypercholesterolemia and atherosclerosis.

The present experiments were undertaken to test this hypothesis in chicks, particularly with respect to inter-related effects of lipid, cholesterol, amino acids, proteins and vitamins.

METHODS

Four series of experiments were accomplished, utilizing a total of 180 cockerels (10 birds per group) (table 1). The department's established techniques for studies on experimental atherosclerosis were used throughout. One-day-old Hy-line chicks were obtained from a commercial hatchery and reared in a battery brooder. The experimental periods were ages 16 to 21, 14 to 21, 8 to 15, and 13 to 23 weeks in series 39, 41, 46, and 47 respectively.

In all series chicks were fed a diet supplemented with 5 per cent cottonseed oil and cholesterol (1, 2, 2, and 1 to 2 per cent in series 39, 41, 46, and 47 respectively). Over-all, two types of diets were utilized, one with commercial chick starter mash as its base, the other with purified ingredients (table 1). In the latter, two types of purified basal proteins were used, casein-gelatin (series 39, 41, and 46) or soy protein (series 46). In the control groups on purified ration, the protein level was 35 per cent (groups 2 and 2D, table 1); in the high-protein groups on purified ration, it was increased to 63 per cent (groups 3 and 3D); in the reduced-protein group, it was decreased to 20 per cent (group 4, series 39). The sulphydryl amino acid d,l-methionine was incorporated in the purified rations of all groups, except the low-methionine groups (table 1). In all control groups, choline and inositol (0.2 to 0.3 and 0.1 to 0.2 per cent, respectively, for the control groups on purified ration) were increased to 1.0 to 1.5 per cent and 0.5 to 1.0 per cent, respectively. In the high-vitamin groups, premixes of multiple fat-soluble and water-soluble vitamins were incorporated in the ration at levels five times as great as for the control groups on purified ration. Complete details concerning the vitamin and salt mixtures utilized, as well as other aspects of the diets, are available from the authors upon request.

In series 39, the effects of high cholesterol-high

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TABLE 1.—General Experimental Design

<table>
<thead>
<tr>
<th>Series no. and cholesterol oil supplement*</th>
<th>Group no.</th>
<th>Over-all dietary characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>1</td>
<td>Control—Mash</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Control—Purified</td>
</tr>
<tr>
<td>-1 C-O</td>
<td>3</td>
<td>High protein—High vitamin—Purified</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Reduced protein—Purified</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Low methionine—Purified</td>
</tr>
<tr>
<td>41</td>
<td>1</td>
<td>Control—Mash</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Control—Purified</td>
</tr>
<tr>
<td>-2 C-O</td>
<td>3</td>
<td>High protein—High vitamin—Purified</td>
</tr>
<tr>
<td>46</td>
<td>1</td>
<td>Control—Mash</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Control—Purified—Casein and gelatin</td>
</tr>
<tr>
<td>-1-2 C-O</td>
<td>3D</td>
<td>High protein—High vitamin—Purified (D)</td>
</tr>
<tr>
<td></td>
<td>3M</td>
<td>High protein—High vitamin—Mash</td>
</tr>
<tr>
<td></td>
<td>5D</td>
<td>Borderline—Low methionine—Purified (D)†</td>
</tr>
<tr>
<td>47†</td>
<td>1</td>
<td>Control—Mash</td>
</tr>
<tr>
<td>-0.5 C-O</td>
<td>3M</td>
<td>High protein—High vitamin—Mash</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>High protein—Mash</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>High vitamin—Mash</td>
</tr>
</tbody>
</table>

* 0.5 C-O, 1 C-O and 2 C-O are mash or purified feed supplemented with 0.5, 1 or 2 per cent cholesterol plus 5 per cent cottonseed oil. In series 46, mash birds ate 1 C-O, purified diet chicks 2 C-O.
† In this series, each group was made up of 7 or 8 birds at the start of the experimental feeding regimen. Two birds from each group were sacrificed after 5 weeks.
‡ The purified ration of this group contained the usual soy protein (not α-protein) at the 35 per cent level, but methionine supplementation was omitted.

Table 2.—Feed Intake, Body Weight and Plasma Lipids in Cockerels on Purified Diets

<table>
<thead>
<tr>
<th>Series no. and group no.</th>
<th>Type diet</th>
<th>Feed intake (Gm./chick/day)*</th>
<th>Terminal weight (Gm.)</th>
<th>Terminal total cholesterol (mg. %)</th>
<th>C/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>39-2</td>
<td>Control</td>
<td>70</td>
<td>1910 ±75</td>
<td>201 ±190.84</td>
<td></td>
</tr>
<tr>
<td>39-3</td>
<td>High protein—High vitamin</td>
<td>65</td>
<td>1761 ±113</td>
<td>152 ±90.83</td>
<td></td>
</tr>
<tr>
<td>39-4</td>
<td>Reduced protein</td>
<td>58</td>
<td>1279 ±43</td>
<td>215 ±180.91</td>
<td></td>
</tr>
<tr>
<td>39-5</td>
<td>Low methionine</td>
<td>74</td>
<td>1906 ±68</td>
<td>361 ±59.97</td>
<td></td>
</tr>
<tr>
<td>41-2</td>
<td>Control</td>
<td>78</td>
<td>1912 ±65</td>
<td>228 ±111.00</td>
<td></td>
</tr>
<tr>
<td>41-3</td>
<td>High protein—High vitamin</td>
<td>46</td>
<td>1557 ±77</td>
<td>263 ±28.15</td>
<td></td>
</tr>
<tr>
<td>46-2</td>
<td>Control</td>
<td>71</td>
<td>1296 ±38</td>
<td>321 ±20.16</td>
<td></td>
</tr>
<tr>
<td>46-2D</td>
<td>Control—Soy protein</td>
<td>78</td>
<td>1465 ±68</td>
<td>195 ±11.00</td>
<td></td>
</tr>
<tr>
<td>46-3D</td>
<td>High protein—Soy (Soy)—High vitamin</td>
<td>68</td>
<td>1270 ±28</td>
<td>146 ±5.00</td>
<td></td>
</tr>
<tr>
<td>40-5D</td>
<td>Borderline methionine</td>
<td>81</td>
<td>1355 ±42</td>
<td>258 ±14.00</td>
<td></td>
</tr>
</tbody>
</table>

* Data collected on group as a whole.
† Phospholipids were determined on pooled specimens of plasma. The C/P ratio is the ratio total cholesterol phospholipids.
‡ Standard error of mean.

Effects of Reduced Protein or Low Methionine Content in a Purified Ration Containing Cholesterol Oil. These dietary combinations were studied in series 39 and 46, groups 4, 5 and 5D (tables 2 and 3). The reduced protein diet (series 39, group 4) was associated with a lower feed intake and weight gain. Plasma lipid levels were essentially similar in the reduced protein and control groups. A higher incidence of thoracic aorta lesions was observed in the reduced protein analysis of the separate effects of high proteins and high vitamins individually (table 1). In these experiments with commercial mash, the protein supplement was casein, soy protein, defatted liver, defatted fish meal, and Brewer's yeast, 7 per cent of each; the vitamin supplement was identical with that used in the purified ration.

RESULTS

Effects of Reduced Protein or Low Methionine Content in a Purified Ration Containing Cholesterol Oil. These dietary combinations were studied in series 39 and 46, groups 4, 5 and 5D (tables 2 and 3). The reduced protein diet (series 39, group 4) was associated with a lower feed intake and weight gain. Plasma lipid levels were essentially similar in the reduced protein and control groups. A higher incidence of thoracic aorta lesions was observed in the reduced protein
group; atherosclerosis was otherwise essentially similar in the two groups.

The low methionine group exhibited a level of feed intake and weight gain comparable to that of the control group. Cholesterolemia, cholesterol/phospholipid (C/P) ratio, incidence of thoracic and coronary lesions, and severity of coronary lesions were all increased in the low methionine group. The borderline-adequate methionine group (series 46, 5D) was not significantly different from its matched control.

Effects of High Protein-High Vitamin

Supplementation of a Purified Ration Containing Cholesterol Oil. This experiment was accomplished in series 39, 41, 46 (tables 2 and 3). Feed intake and weight gain tended to be less in high protein-high vitamin groups, compared with controls. In 2 of the 3 series of experiments, hypercholesterolemia was less marked in the high protein-high vitamin groups. Except for one bird in series 46 with a minimal lesion, thoracic aorta atherosclerosis was absent in high protein-high vitamin chicks of all series. No coronary lesions were noted in these birds in series 39 and 46, in contrast to the controls. Coronary atherosclerosis was also less marked in the experimental groups in series 41 (table 3).

Effects of Soy Protein vs. Casein-Gelatin in a Purified Ration Containing Cholesterol Oil. Feed intake and weight gain were greater with 35 per cent soy protein than with the same level of casein-gelatin (series 46, groups 2 and 2D). Hypercholesterolemia, aorta and coronary atherosclerosis tended to be less marked with the soy than with the casein-gelatin protein.

Effects of High Protein-High Vitamin Supplementation of Commercial Mash Containing Cholesterol Oil. Feed intake tended to be slightly less in the high protein-high vitamin groups, as was to be expected in view of this ration's greater caloric content (table 4). Gain in weight was greater in
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the experimental groups. Hypercholesterolemia, aorta and coronary atherogenesis were less marked in the high protein-high vitamin supplemented group.

Effects of Separate Supplementation with High Protein or High Vitamin in Commercial Mash Containing Cholesterol Oil. This single supplementation resulted in lower feed intakes and weight gains (series 47, groups 6 and 7) (table 4). In contrast to the high protein-high vitamin group (3M), both the high protein-alone and the high vitamin-alone groups had levels of hypercholesterolemia as high as or higher than the controls. Correspondingly, the high vitamin-alone group had coronary atherogenesis as marked as the control (table 5). In the group supplemented with high protein-alone, incidence of coronary atherogenesis was markedly lower than in the controls, and was similar to that of the high protein-high vitamin group.

Effects of Cholesterol Oil Supplementation of Purified Ration vs. Commercial Chick Starter Mash. In all series, cholesterol supplementation at the same (series 39 and 41) or twofold greater (series 46) levels in the purified, compared with the commercial mash, group yielded less marked hypercholesterolemia and atherogenesis (group 1, series 46, tables 4 and 5 and group 2, series 46, tables 2 and 3*).

DISCUSSION

The findings of these experiments indicate that high protein-high vitamin supplementation suppressed hypercholesterolemia and atherogenesis in cholesterol-oil-fed cockerels. This was true with both purified and commercial mash diets. Vitamin supplementation alone had no such effect. Protein supplementation alone apparently suppressed coronary atherogenesis in a manner similar to high protein-high vitamin. However, protein alone apparently had less definitive and clear-cut effects than the combination. It would seem, therefore, that the high protein diet is the decisive factor responsible for these results, but that high vitamin intake has an adjuvant, synergistic influence. These results are consistent with the concurrent independent observations in chicks by other workers. Antiatherogenic effects of protein have apparently not been hitherto reported.

These experiments further demonstrate that inadequacy of methionine intake aggravated the hypercholesterolemic and atherogenic effects of cholesterol-oil ingestion. They also confirm in cockerels observations previously reported in monkeys and rats. The present results further indicate that addition of cholesterol to the chick diet induced less hypercholesterolemia and atherogenesis in a purified than in a regular mash ration. The mechanism of this effect is not apparent from the data of this experiment. It may be a resultant of the greater protein intake with the purified ration (35 vs. approximately 20 per cent). However, the results with 20 per cent protein in a purified ration (series 39, group 4) do not support this suggestion, since enhancement of hypercholesterolemia was not observed. These data are difficult to interpret, however, in view of the differences in feed intake and growth pattern. This problem was explored further.

Finally, the data of these experiments further suggest that different results may be obtained with qualitatively different proteins in a cholesterol-oil-supplemented purified ration. Thus, hypercholesterolemia and atherogenesis were less marked with soy protein than with casein-gelatin (series 46, groups 2 and 2D). Similar findings were obtained independently by other workers.

*Data on the other groups involved in this comparison are available from the authors upon request.
The relevance (if any) of these findings for the problem of possible interrelationships among diet, cholesterolemia and atherogenesis in man is dealt with elsewhere.5

Summary

Inadequate methionine intake led to increased hypercholesterolemia, coronary and aorta atherosclerosis in chicks on a cholesterol-oil-supplemented purified ration. High protein-high vitamin supplementation partially suppressed hypercholesterolemia and atherogenesis in birds on cholesterol oil rations, of both the purified and commercial mash varieties. High protein supplementation alone tended to produce some of these effects, whereas high vitamin supplementation alone did not.

Chicks fed a purified ration developed less marked hypercholesterolemia and atherogenesis than birds on a commercial feed, even when the former ingested greater quantities of cholesterol. In purified rations containing cholesterol oil, cholesterolemia and atherogenesis tended to be less marked with soy protein than with casein-gelatin (both at the 35 percent level).

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Summary in Interlingua

Inadequate nivellos dietari de methionina resultava in augmentos de hypercholesterolemia e de atherosclerosis coronari e aortic in gallettes recipiente un dieta purificate con supplementos de cholesterol e oleo.

Alte supplementos de proteina e de vitamina resultava in un suppression partial del hypercholesterolemia e del atherogenesis in aves recipiente dietas a cholesterol e oleo, tanto del typo purificate como etiam de proveniencia commercial. Alte supplementos de protein sol (i.e. sin supplementation de vitaminas) tendeva a producer certes de iste effectos, durante que alte supplementos de vitamina (sin supplementation proteinic) non mostrava un tal tendentia.

Gallettes recipiente un dieta purificate disveloppava minus marcate grades de hypercholesterolemia e de atherogenesis que gallettes recipiente un dieta comercial, mesmo in casos in que le dieta purificate contineva plus alte quantitates de cholesterol. In dietas purificate e supplementate per cholesterol e oleo, le cholesterolemia e le atherogenesis tendeva a esser minus marcate con proteina de soja que con caseina e gelatina (in ambe casos a nivellos de 35 pro cento).

References

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