Total Cholesterol and High Density Lipoprotein Cholesterol Levels in Populations Differing in Fat and Carbohydrate Intake

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This paper reviews epidemiological studies on the relationship between diet and high density lipoproteins (HDL), with emphasis on the authors' studies of boys and men from different countries and with different dietary habits. Sera were collected from boys (ages 7 to 9 years) and men (ages 33 to 48 years) in 20 countries, and these were analyzed in one standardized laboratory. In boys, total and HDL cholesterol changed in parallel, from low values in populations in developing countries with low-fat, high-carbohydrate diets to high values in affluent populations. The correlation between HDL and total cholesterol was 0.90 (n = 16 populations). A similar trend was seen in groups of vegetarians and omnivores boys within one region. Detailed analyses of individual diets of boys in five countries showed a negative relation between carbohydrate intake and HDL cholesterol both for group means (r = -0.99, n = 5) and for individual boys within one country (r = -0.26 to 0.04, n = 109 to 133 boys per country). In these boys, differences in obesity and physical activity were slight, and unrelated to differences in HDL. Total cholesterol rose with saturated fat intake (r = 0.87 for five population means; r = 0.07 to 0.26 within population groups). In adult men, total and HDL cholesterol also tended to rise simultaneously with affluence. However, the relation was much weaker (r = 0.60, n = 13 population groups). In adult men, body mass index was negatively related to the ratio of HDL to total cholesterol both between and within countries and also between and within groups of omnivores and vegetarian men within one region. We suggest that three partly opposing dietary factors determine an adult population's total cholesterol/HDL cholesterol ratio; namely, the proportion of energy from saturated fat, which raises total cholesterol; the proportion of energy from total fat, which raises HDL cholesterol; and the excess in dietary energy, which produces obesity and in that way lowers HDL cholesterol.

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Following Keys' finding that there was little difference in the concentration of alpha lipoprotein (high density lipoprotein, HDL) between Japanese and Finn men, it was thought that HDL cholesterol was relatively stable and independent of diet.1,2 This may be one reason why HDL received little attention for some time. In the 1970s interest in HDL revived, and the concentration of HDL cholesterol was found to be negatively related to the risk of coronary heart disease (CHD) within a number of populations.3-8 However, the results of international comparisons are contradictory and suggest that this inverse association between the concentration of HDL cholesterol and the risk of CHD is absent or even opposite to that within populations. For example, low HDL cholesterol values have been reported for Tarahumara Indians, Masal, vegetarians and those on macrobiotic, low-fat, high-carbohydrate diets.9-12

However, high values have been reported for Nigerians who are relatively free from coronary heart disease and for black South African children.13,14 In addition, the comparison of Japanese and Finnish men mentioned above and comparisons of Koreans and Chinese with Belgians, and of adults from four European countries all brought to light minimal differences in HDL cholesterol in spite of major differences in total and LDL cholesterol.1,15,16

In the present paper we review epidemiological studies on dietary and other determinants of HDL cholesterol. Emphasis is placed on four recent international studies carried out by our department in collaboration with many investigators abroad.17-21 The main aim of these studies was to obtain data on the concentrations of total and HDL cholesterol in groups of boys and men from populations differing in fat and carbohydrate intake and living in countries with different rates of mortality from coronary heart disease. The study of young boys reduced the influence of confounding factors known to affect HDL cholesterol, because factors such as smoking, drinking, obesity, drug use and physical activity are either absent or constant in young boys from different countries.22-23

Because it is sometimes argued that low concentrations of cholesterol in people from African and Asian countries
are a result of poor health, we also studied men and boys living in Holland and Belgium who were on macrobiotic, vegetarian or omnivorous diets. Macrobiotic diets are rich in carbohydrate and poor in total fat and persons on this diet are, in this respect, comparable to Africans and Asians except that they enjoy the health care and other services of a modern welfare state. Studies within one region are less hampered by the confounding factors that affect cross-cultural comparisons.

In this review we attempt to present a unified explanation for the sometimes contradictory results of international studies on diet, HDL, and coronary heart disease.

**Methods**

The particulars of our studies that examined the concentrations of total and HDL cholesterol in populations in different countries have been described in detail, 17-21 but we will reiterate the main features.

**Laboratory Methods**

To ensure uniformity of the methods for blood sampling and transport, detailed standardized protocols were used. All the necessary materials including needles and tubes for blood drawing and serum preparation, as well as insulated shipping containers and cooling elements for transport of serum to the Netherlands, were supplied from the laboratory in Wageningen. Blood was drawn into evacuated tubes and was allowed to clot. The serum was separated from the blood cells and stored at -20°C for a maximum of 4 weeks and was then shipped frozen to the Netherlands by air express with cooling elements or dry ice. All samples arrived frozen at the laboratory and were stored at -20°C for no more than 6 weeks until analysis. The concentrations of total and HDL cholesterol were measured as described previously. 17-21, 29, 30 The combined reproducibility within and between runs was 0.9% (one coefficient of variation) for total and 2.2% for HDL cholesterol. Throughout the whole period of the studies, accuracy (agreement between target and measured values) of total cholesterol determinations was within 2.5% and that of HDL cholesterol was within 1.8% of the target values of the external control sera provided by the Centers for Disease Control, Atlanta, Georgia.

**Study I. Relation Between Total and HDL Cholesterol in Boys from 16 Countries**

This study was aimed at measuring the concentrations of total and HDL cholesterol in young boys having different dietary habits 22 and living in countries with different rates of mortality from coronary heart disease. The following countries were involved in this study: Ghana, Ivory Coast, Nigeria, U.S.A., Pakistan, the Philippines, Austria, Denmark, Finland, Greece, Hungary, Ireland, the Netherlands, Poland, Portugal, and Sweden. In each country, between 35 and 80 boys were selected through several elementary schools. In total, 908 boys participated. Originally, separate urban and rural samples of children were selected and studied, but as urban-rural differences within countries were minimal, all boys from one country were pooled for the present study. 17 Ten ml of blood was sampled per boy.

**Study II. Total and HDL Cholesterol Concentrations in Vegetarian and Nonvegetarian Men and Boys in the Netherlands and Belgium**

This study was carried out in boys (ages 6 to 11 years) and men (ages 30 to 39 years) who were eating macrobiotic, lactovegetarian, or omnivorous diets and who were living in Belgium and the Netherlands. 18 Persons on macrobiotic diets consume high-carbohydrate, low-fat food based on whole grains, beans, and vegetables supplemented with seaweed and fermented soy products. Lactovegetarians eat milk and egg products, but rarely or never eat meat or fish products. Semilactovegetarians eat meat or fish products less than once a week. In total, 52 omnivorous men, 54 omnivorous boys, 132 vegetarian men, and 36 vegetarian boys were studied.

**Study III. Determinants of Total and HDL Cholesterol in Boys, with Special Reference to Diet**

This study was carried out in 8- and 9-year-old boys from Finland, the Netherlands, Italy, the Philippines, and Ghana. 19 About 120 boys per country were selected through at least four schools to participate in this study. In addition to measurements of serum lipids, food intake was measured on 7 nonconsecutive days for each individual boy. Analysis of foods from the different countries was carried out to check the data in food composition tables and to supplement or update these tables. 30 Before the study, a training period of 2 weeks was held in the Netherlands to discuss all aspects of the working plan and to train the research teams in the different aspects of food intake assessment. The boys' hemoglobin and albumin levels were measured to evaluate their general health. Their usual physical activity was assessed by use of a questionnaire.

**Study IV. Total and HDL Cholesterol in Adult Men from 13 Countries**

This study was similar to Study I, and was carried out in about 80 adult men in each country, half of whom aged 33 to 38 years and the other half, 43 to 48 years. 30 As trends between countries were similar for both age groups, age groups were pooled for this study. The following countries were involved: Ghana, Ivory Coast, Nigeria, Surinam, Pakistan, the Philippines, Finland, Hungary, Italy, the Netherlands, Poland, Portugal, and Spain. The men were generally farmers or laborers such as factory workers and drivers. Their heights and weights were estimated according to a standardized protocol.

**Statistical Methods**

The relations to be tested were determined in the design phase of the studies. Simple Pearson correlation coefficients were calculated between the mean concentrations of total and HDL cholesterol and for the concentrations of total and HDL cholesterol with dietary variables (Study III) both within and between groups.

The relationships of the concentrations of total and HDL cholesterol with dietary variables (Study III) and body mass index were also examined by linear regression equa-
tions using the Statistical Package for the Social Sciences. The regression coefficients for the groups from the different countries were estimated from multiple regression analyses on the pooled data and by using a model allowing slopes and intercepts specific for age and country or group. Indicators of country-specific (Studies III and IV) or group-specific (Study II) levels were introduced into the regression model followed by dietary variables (Study III only), body mass index, and physical activity (Study III only). Additional variables were not included when this did not result in a significant improvement of the accuracy of estimation of the dependent variable.

Results

Parallelism of HDL and Total Cholesterol in Boys

Study I showed that there were low concentrations of total cholesterol in the Asian and African groups (2.6 to 4.2 mmol/l; 101 to 163 mg/dl) and intermediate to high concentrations in the European and U.S. groups (4.2 to 5.2 mmol/l; 163 to 201 mg/dl) (Figure 1). Thus serum total cholesterol values were highest in boys from countries with the highest incidence of ischemic heart disease. Remarkably, a similar gradient was found for HDL cholesterol (Figure 1): low concentrations in the African and Asian groups (0.8 to 1.2 mmol/l; 31 to 46 mg/dl) and high concentrations in the European and U.S. groups (1.2 to 1.7 mmol/l; 46 to 66 mg/dl). There was a good agreement between the mean of the U.S. sample studied here (Figure 1, filled circle) and that of the much larger, nationwide sample studied in the Lipid Research Clinics Prevalence Study (Figure 1, filled square). The boys from Finland, a country noted for its high incidence of ischemic heart disease, had the highest mean HDL cholesterol levels (1.7 mmol/l; 66 mg/dl). The correlation coefficient between the mean levels of total and HDL cholesterol was 0.90 (n = 16 countries). For the European groups alone the correlation coefficient was also 0.90 (n = 10 countries). The concomitant increase of total and HDL cholesterol was reflected in the constancy of the HDL cholesterol/total cholesterol ratio. It ranged between 0.30 and 0.36 in the African groups, between 0.32 and 0.37 in the European and U.S. groups, and between 0.24 and 0.27 in the Asian groups.

Low Levels of Total and HDL Cholesterol in Boys on Macrobiotic Diets in the Netherlands and Belgium

Study II was carried out with boys differing widely in dietary habits but living in the same affluent region of Northwestern Europe. In Figure 1 the results of this study (open squares) are combined with those of the international study (closed symbols). The concentration of total cholesterol was low in the boys on macrobiotic diets (MAC in Figure 1) and higher in the lacto-vegetarians, semilacto-vegetarians, and the omnivores (LV, SLV, and NE in Figure 1). The concentration of HDL cholesterol was also lowest in those on macrobiotic diets (1.2 mmol/l; 46 mg/dl) and equal in the other three groups (1.4 mmol/l; 54 mg/dl). The ratio of HDL to total cholesterol was almost identical for the four groups (0.33 to 0.35). The low levels of both total and HDL cholesterol seen in the boys on macrobiotic diets were similar to those in boys from less privileged countries (Figure 1). This supports the concept that diet may be an important determinant of HDL cholesterol independent of race, climate, or medical care.

Carbohydrate and Fat Intake: Determinants of HDL Cholesterol in Boys

Study III provided an in-depth assessment of possible determinants of HDL cholesterol in boys with widely different HDL levels from five countries. Measurements of albumin and hemoglobin suggested that boys in Ghana and the Philippines were lighter in weight, but were not malnourished. The country mean concentration of HDL cholesterol was strongly and significantly correlated with the percentage of energy from carbohydrate (r = -0.99, n = 5 populations), and positively correlated with the proportion of energy from total fat (r = 0.97) and monounsaturated fat (r = 0.79) and the intake of cholesterol (r = 0.88). The proportion of energy from carbohydrate was also negatively associated with HDL cholesterol between individual children within one population. The association was particularly strong for Filipino boys (r = -0.40; Figure 2). In the Filipino group, the proportion of energy from protein, total fat, saturated, monounsaturated, and polyunsaturated fat, and cholesterol were all positively associated with the concentration of HDL cholesterol (range of r, 0.28 to 0.39). That so many associations were found in the Filipino group is probably due partly to the lack of day-to-day variation in the diets of these boys; the ratios of intra- to interindividual variance in
nutrient intake were generally lower in the Filipino group than in the other groups.

Upon multiple regression analysis of the pooled data which allowed for differences in level between the groups from the different countries, out of the nutrients examined, only the percentage of energy from carbohydrates was significantly related to HDL cholesterol (regression coefficient, $-0.0074 \pm 0.0023$ mmol/l/%) of energy). On the average, differences in carbohydrate intake explained 23% of the intercountry differences in HDL cholesterol. In addition, body mass index was negatively related to HDL cholesterol (regression coefficient, $-0.015 \pm 0.007$ mmol/l/kg/m$^2$). Physical activity was not significantly related to HDL.

For the mean concentration of total cholesterol per country there was a significant positive correlation with the mean proportion of energy from protein ($r = 0.91$), total fat ($r = 0.84$) and saturated fat ($r = 0.87$) and with the intake of cholesterol ($r = 0.88$), and there was a significant negative correlation with the proportion of energy from carbohydrate ($r = -0.87$). Within countries, a significant positive correlation was found between the concentration of total cholesterol and the intake of saturated fat in four of the five groups (range of $r$, 0.16 to 0.26) (Figure 5). Upon multiple

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**Figure 2.** Relationship between proportion of energy from carbohydrates and serum HDL cholesterol concentration in Study III in boys ages 8 and 9 years old. • = mean values for about 120 boys from each of five countries ($r = -0.99$). ○ = individual values for 114 boys from the Philippines ($r = -0.40$). FI = Finland, GH = Ghana, IT = Italy, NE = the Netherlands, PH = the Philippines.

**Figure 3.** Relationship between proportion of energy from saturated fatty acids and serum total cholesterol concentration in Study III in boys ages 8 and 9 years old. • = mean values for about 120 boys from each of five countries ($r = 0.87$). ○ = individual values for 117 boys from the Netherlands ($r = 0.26$). FI = Finland, GH = Ghana, IT = Italy, NE = the Netherlands, PH = the Philippines.
regression analysis of the pooled data, only the proportion of energy from saturated fat was significantly related to total cholesterol, with a regression coefficient of 0.045 ± 0.011 mmol/l per percentage point of energy from saturated fatty acids.

**HDL Cholesterol in Adults and the Role of Obesity**

As shown in Figure 4, the trend seen in boys was also found in adult men, with total and HDL cholesterol increasing together as one goes from populations with low risk to those with high risk of ischemic heart disease. The results from the men from the Netherlands and Belgium with divergent dietary habits (open squares in Figure 4) again fitted into the international picture. Their concentration of total cholesterol ranged from 3.8 mmol/l (147 mg/dl) for those on macrobiotic diets to 5.5 mmol/l (213 mg/dl) for those on omnivorous diets. The concentration of HDL cholesterol (1.2 mmol/l; 46 mg/dl) in those on macrobiotic diets was lower than that in the lactovegetarians and semivegetarians (1.4 mmol/l; 54 mg/dl) but equal to that in men on omnivorous diets.

The correlation of HDL with total cholesterol between countries was much weaker in the men ($r = 0.60$, $n = 13$ countries) than in the boys, and the ratio of HDL to total cholesterol in the adult men was not constant across countries and dietary groups. It varied from 0.15 to 0.22 in the Asian and Surinam men, from 0.20 to 0.28 in the European men, and from 0.26 to 0.32 in the African men and in those on vegetarian and macrobiotic diets. Multiple regression analysis showed that the body mass index was an important determinant of this ratio both in the international study (Study IV; Figure 5, closed symbols) and in the comparison within one region (Study II; open symbols in Figure 5). The ratio of HDL to total cholesterol was lowest in the omnivorous men (NE in Figure 5), and highest in those on macrobiotic diets (MAC in Figure 5) who were quite lean. For the pooled data of these men from the Netherlands and Belgium, the regression coefficients of HDL cholesterol and HDL cholesterol/total cholesterol on body mass index were $-0.023 ± 0.008$ mmol/l per kg/m² and $0.0119 ± 0.0025$ per kg/m², and there were no significant differences in the respective coefficients between the four dietary groups. For the men in the 13 countries in Study IV, multiple regression analysis showed that body mass index was negatively related to the concentration of HDL cholesterol (regression coefficient $-0.0220 ± 0.0034$ mmol/l per kg/m²) and to the HDL/total cholesterol ratio (regression coefficient $-0.0071 ± 0.0008$ per kg/m²), and positively to the concentration of total cholesterol (regression coefficient $0.0719 ± 0.0100$ mmol/l per kg/m²).

![Figure 4. Relationship between serum total and HDL cholesterol concentration in men.](image-url)
Figure 5. Relationship between body mass index and the ratio of serum HDL cholesterol to total cholesterol in men. • = mean values from Study IV in groups of about 80 men ages 33 to 48 years old in each of 13 countries (r = -0.5). ○ = mean values from Study II in various groups of vegetarian men ages 30 to 39 years old in the Benelux countries. LV = 56 lactovegetarians, MAC = 33 men on macrobiotic diets, SLV = 43 semilactovegetarians, EFI = eastern Finland, GH = Ghana, HU = Hungary, IC = Ivory Coast, IT = Italy, NE = the Netherlands, NI = Nigeria, PA = Pakistan, PH = the Philippines, POL = Poland, POR = Portugal, SP = Spain, SU = Surinam, WFI = western Finland.

Discussion

Effects of Dietary Fat and Carbohydrates on HDL Cholesterol

Our studies have shown that in men and boys from countries with a low-fat, high-carbohydrate diet, both total and HDL cholesterol levels tend to be low. Results of controlled experiments support these findings; it has been found quite consistently that replacement of fat by carbohydrate lowers HDL cholesterol. Some of these studies are summarized in Table 1. The fall in HDL cholesterol may be due largely to the HDL₂ fraction, and is accompanied by a fall in apolipoprotein A-I.²⁷

Effect of Obesity

In contrast to the experimental data referred to above, which generally show that carbohydrates lower HDL, results from previous epidemiological studies on HDL and diet have been contradictory. In studies of Tarahumara Indians, in Masai, in vegetarians, and in those on macrobiotic diets, high-carbohydrate, low-fat diets were associated with lower concentrations of both HDL cholesterol and total cholesterol.¹⁹⁻²₂ Studies within affluent countries have also repeatedly shown negative relations between carbohydrate intake and HDL cholesterol at the individual level.²³⁻²⁴ On the other hand, comparisons of Japanese with Finnish men,¹ of Koreans and Chinese with Belgians,¹⁶ and of adults from four European countries with each other¹⁸ all brought to light minimal differences in HDL concentrations in spite of major differences in total and LDL cholesterol.

We suggest that these discrepancies can be largely explained by the effect of two opposing dietary factors on HDL. As one moves from developing to more affluent countries, there is an increase in the proportion of fat in the diet at the expense of carbohydrates, which raises HDL concentrations. This is borne out both by controlled experiments (Table 1), and by our cross-cultural observations on young schoolboys, who differ in little else but diet. At the same time, however, as populations become more affluent, energy expenditure in adult men decreases and no longer matches their energy intake. This excess caloric intake produces obesity which, together with the lack of physical activity, depresses HDL concentrations. In men, we found an average decrease in the concentration of HDL cholesterol of about 0.020 mmol/l (0.8 mg/dl) per unit increase in body mass index, which corresponds well with the published data of Glueck et al.²⁵ We suggest that basically the outcome in terms of HDL levels is determined by a balance between the dietary fuel mix on the one hand (with fat and alcohol raising, and carbohydrates depressing HDL), and by the extent of caloric excess and the accompanying degree of obesity and lack of activity on the other hand. Which of these two factors prevails may differ from

<table>
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<tr>
<th>Study and reference</th>
<th>Number of subjects</th>
<th>Fat content of the diets (% of energy)</th>
<th>Duration (weeks)</th>
<th>ΔHDL cholesterol, high-fat minus low-fat diet (mmol/l)</th>
<th>ΔHDL cholesterol per 10% of energy exchanged (mmol/l)/10 energy %</th>
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<tr>
<td>Antonis and Bersohn ³⁷</td>
<td>32</td>
<td>High-fat diet: 40, Low-fat diet: 15</td>
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<td>Blum et al. ³⁶</td>
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<td>Brunner et al. ³⁰</td>
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<td>0.24</td>
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<tr>
<td>Brussaard et al. ³⁵</td>
<td>29</td>
<td>High-fat diet: 40, Low-fat diet: 30</td>
<td>5</td>
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<td>Kashyap et al. ³⁰</td>
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<td>3</td>
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<td>ΔHDL cholesterol</td>
<td>0.05 to 0.15</td>
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*HDL cholesterol values were calculated from Table III of Antonis and Bersohn³⁷ as total minus beta-cholesterol.

The "beta"-cholesterol had been determined as the manganese-heparin precipitate which probably included the pre-beta cholesterol.

†The low-fat diet also contained less cholesterol.

‡Corrected for the change in the concurrent control group.
one study to another, and often they may balance each other out, so that no cross-cultural differences in HDL are seen in adults. However, most of the studies cited above still fit quite well into the picture resulting from our multicountry comparison of HDL and total cholesterol in adult men; some typical data points from other workers are shown as triangles in Figure 4.

**Effects on Total Cholesterol**

For the total cholesterol concentration, the picture has always been much clearer, because a high saturated fat intake and obesity both work in the same direction. As a result there have been few discrepancies between cross-cultural studies. Within-population studies, however, have yielded conflicting results. Here again, the effects of diet may be clarified by studying children (Figure 3), because many confounding factors present in adults are absent when one confines observations to children of the same age and sex. We are not alone in finding significant correlations between diet and cholesterol in children; similar findings have been reported by other workers.

**Effects of Fatty Acid Composition on the HDL to Total Cholesterol Ratio**

A puzzling, but reproducible, finding in our study was the low level of HDL cholesterol and the low HDL/total cholesterol ratio in boys and men from Asian countries. Miller et al. also reported low HDL values in Indians as opposed to Africans. Although racial features could be involved here, the low HDL/total cholesterol in the Filipino boys (PH in Figure 1) may also be due to their dietary habits. They eat little fat, but what fat they do eat is coconut fat, and is thus highly saturated. As a result, their HDL should be relatively low and their total cholesterol relatively high. The reverse effect was seen when fat intake was high but unsaturated; in the multiple regression analysis of the pooled data of our five-country study of boys (Study III), the percentage of energy from polyunsaturated fat was positively and significantly correlated with the HDL to total cholesterol ratio (regression coefficient 0.0055 ± 0.0023 ratio units per percentage of energy). The favorable effect of monounsaturated fatty acid and of moderate amounts of polyunsaturated fatty acids on the HDL/total cholesterol ratio is also borne out by the results of controlled trials.

**HDL Cholesterol and Coronary Heart Disease**

Evidently the concentration of HDL cholesterol in men and boys from countries with low rates of mortality from coronary heart disease is lower than, or at most equal to, that in countries with high rates. Thus it appears that the average concentration of total cholesterol is the best predictor of between-country differences in rates of mortality from coronary heart disease. The concentration of LDL cholesterol which makes up the bulk of total cholesterol increases when the proportions of saturated fat and cholesterol in the diet increase. This increase in the concentration of total or LDL cholesterol is associated with an increase in HDL cholesterol. Although this increase in HDL cholesterol may diminish the risk of coronary heart disease, it appears to be insufficient to stem the potent atherogenic effects of an increased LDL cholesterol concentration.

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