Cholesterol Feeding Increases C-Reactive Protein and Serum Amyloid A Levels in Lean Insulin-Sensitive Subjects

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Background—Inflammatory markers associated with elevated cardiovascular risk are increased by cholesterol feeding in animal models. However, whether dietary cholesterol increases inflammatory marker levels in humans is not known.

Methods and Results—C-reactive protein (CRP), serum amyloid A (SAA), and lipoprotein levels were compared in 201 healthy subjects on an American Heart Association–National Cholesterol Education Program step 1 diet at baseline and after addition of 4 eggs per day for 4 weeks. Subjects were classified a priori into 3 groups based on their body mass index (BMI) and insulin sensitivity index (SI): lean insulin sensitive (LIS), mean±SEM BMI, 23.2±0.3 kg/m², and SI, 6.7±0.3×10⁻⁴ min⁻¹/μU/mL, n=66; lean insulin resistant (LIR), BMI, 24.5±0.2 kg/m² and SI, 2.9±0.1×10⁻⁴ min⁻¹/μU/mL, n=76; or obese insulin resistant (OIR), BMI, 31.4±0.5 kg/m² and SI, 2.1±0.1×10⁻⁴ min⁻¹/μU/mL, n=59. Insulin resistance and obesity each were associated with increased baseline levels of both CRP (P for trend, <0.001) and SAA (P for trend=0.015). Egg feeding was associated with significant increases in both CRP and SAA in the LIS group (both P<0.01) but not in the LIR or OIR groups. Egg feeding also was associated with a significant increase in non-HDL cholesterol (P<0.001) in LIS subjects; however, there was no correlation between the change in non-HDL cholesterol or changes in either CRP or SAA in this group.

Conclusions—A high-cholesterol diet leads to significant increases in both inflammatory markers and non-HDL cholesterol levels in insulin-sensitive individuals but not in lean or obese insulin-resistant subjects. (Circulation. 2005;111:3058-3062.)

Key Words: cholesterol ■ C-reactive protein ■ insulin resistance ■ lipoproteins ■ serum amyloid A

Inflammation is a hallmark of atherosclerosis. Associations exist between increased risk of cardiovascular events and elevated levels of inflammatory markers such as C-reactive protein (CRP)1-4 and serum amyloid A (SAA).5-7 In addition, both CRP and SAA levels are increased with obesity and with insulin resistance.5-15

Although a high intake of dietary cholesterol also is associated with increased cardiovascular risk,13,15-16 the effect of dietary cholesterol on plasma lipid levels varies substantially17 and is influenced by the presence of lipid disorders,13,19 as well as by obesity and insulin resistance.20 Dietary cholesterol also increases SAA in hyperlipidemic mice.21 However, it is not known whether dietary fat and cholesterol affect CRP and SAA levels in humans or whether the response is influenced by obesity and/or insulin resistance. Therefore, we studied the effects of egg feeding on CRP and SAA levels in a cohort of healthy, community-dwelling individuals stratified according to their degree of obesity and insulin resistance. We report here the surprising finding that high dietary cholesterol, in the form of 4 eggs per day added to the diet, increased CRP and SAA levels in lean, insulin-sensitive only subjects but not in obese or lean insulin-resistant subjects.

Methods

Subjects

Subjects (n=201) had participated previously in a study examining the effect of insulin resistance and/or obesity on lipid and lipoprotein responses to egg feeding.20 As described previously, all subjects had baseline measurements of height; weight; waist-hip ratio (WHR); fasting plasma levels of glucose, insulin, cholesterol (total, LDL, and HDL), triglycerides, and apolipoprotein B; abdominal and subcutaneous fat (from a computed tomographic image taken at the level of the umbilicus); and insulin sensitivity index (SI) (with use of the minimal model of glucose kinetics from the results of a frequently sampled, tolbutamide-modified, intravenous, glucose-tolerance test).20 The study was approved by the University of Washington Human Subjects Review Committee, and all subjects gave written, informed consent.

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Subjects were divided into 3 a priori–defined groups: lean insulin-sensitive (LIS); lean insulin-resistant (LIR); or obese insulin-resistant (OIR). Body mass index (BMI) was used to classify patients as lean (BMI<27.5 kg/m²) or obese (BMI≥27.5 kg/m²). The BMI cut point was based on criteria used in the National Health and Nutrition Examination Survey II, which did not include the current “overweight” category. Thus, both lean and obese groups of the present study contain some individuals who would be classified as overweight under current criteria. Baseline measurement of S_{I} was used to classify patients as insulin sensitive [S_{I} = 4.2 (10^{-4} min^{-1} (μU/mL))] or insulin resistant [S_{I} = 4.2×10^{-4} min^{-1} (μU/mL)]. S_{I} measurements were not repeated at the end of the study.

**Diets**

All subjects were counseled on the National Cholesterol Education Program step 1 diet, and compliance was evaluated periodically. Subjects ingested 0, 2, or 4 egg/d for 4-week periods, in random order, with each intervention period separated by a 4-week “washout” period during which the subjects consumed only the National Cholesterol Education Program step 1 diet.20 Maintenance of stable weight was emphasized. Subjects were given individual frozen daily portions of egg preparation (homogenized natural eggs). The 4-egg preparation consisted of 68 g egg yolk, 20 g Egg Beaters egg substitute, and 20 g water and provided 253 kcal, 13.4 g protein, 21 g fat, 871 mg cholesterol, and 6.5 g saturated fat. Three-day food records were collected at the beginning and end of each intervention period, and the records were analyzed to demonstrate consistency of dietary intake throughout the study. The present study evaluated measurements from before and after the 4 egg/d visits.

**Inflammatory Marker Assays**

CRP (high-sensitivity assay) and SAA levels were determined on deeply frozen (−80°C), not previously thawed samples by a nephelometric method. Interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α were measured simultaneously with a multiplex bead system on a Luminex analyzer. Subjects with values too low to be detected by the assays were assigned a value of 50% of the assay’s lowest detectable value (for CRP, 0.08 mg/L; for SAA, 0.4 mg/L; for IL-1β, 1.6 pg/mL; and for TNF-α, 1.6 pg/mL; no values for IL-6 or IL-8 were below the lower limit of detection).

**Statistics**

Data are presented as mean±SEM unless otherwise specified. CRP, SAA, and cytokines are presented as medians and interquartile ranges. Tests for significant differences among the 3 groups were performed by 2-way ANOVA evaluating the effect of group and sex, with pairwise multiple comparisons made with the Holm-Sidak method. The effects of egg feeding on various parameters were analyzed by paired t test when the variables were satisfactory for parametric tests or by Wilcoxon signed-rank tests. Lipid values were normally distributed. Because CRP and SAA values were not normally distributed, regression analyses with these variables were performed with logarithmically (natural) transformed values. Four subjects (all in OIR) had baseline CRP values >10 mg/L, and 5 subjects (1 each in LIS and LIR and 3 in OIR) had subsequent CRP values >10 mg/L. CRP values >10 mg/L generally are considered to indicate clinically relevant inflammation. However, because exclusion of these subjects did not affect results, all subjects were included in analyses. All figures depict raw (untransformed) data. The strength of associations of CRP and SAA (dependent variables) with lipoprotein variables (independent variables) was evaluated by linear regression on each variable separately, unadjusted for covariates. Data were considered significant at (2 sided) P<0.05.

**Results**

**Subject Characteristics**

The present study includes 201 subjects who completed the 4 egg/d intervention (Table). The LIS group was slightly younger than the LIR group. By definition, body weight and BMI were greatest in the OIR group. There was an incrementral increase in subcutaneous fat, intra-abdominal fat, and WHR among the 3 groups, with the greatest increment in the OIR group over the LIR group. Insulin sensitivity, by definition, was greatest in the LIS group, although there was a small but significant difference between the LIR and OIR groups. Non-HDL cholesterol and triglycerides were significantly lower in the LIS group compared with either the LIR or OIR group (Table). HDL cholesterol was highest in the LIS group, but HDL also was higher in the LIR group compared with the OIR group (Table).20 Significant effects of sex were found for weight, WHR, subcutaneous and intra-abdominal fat areas, glucose, CRP, SAA, and HDL. However, there were no significant interactions between group and sex for CRP or SAA; thus, the observed differences in CRP and SAA among groups are not accounted for by sex effects. The data for age, BMI, subcutaneous and intra-abdominal fat areas, and non-HDL and HDL cholesterol levels did have significant interactions between group and sex. However, the differences in mean values for these factors (age, BMI, subcutaneous and intra-abdominal fat areas, and non-HDL and HDL cholesterol levels) among the different groups remained significant after allowing for the effects of sex.

Insulin resistance was associated with higher CRP levels (P<0.001 for LIS versus LIR), as was obesity (P<0.001 for LIR versus OIR and for LIS versus OIR; Table), as has been reported previously.8–14 Similarly, insulin resistance and obesity were associated with higher SAA levels (P<0.001 for LIS versus OIR). Although there was a trend toward higher levels of cytokines with increased insulin resistance and obesity, there were no significant differences for any cytokine...
measures between groups (P values [1-way ANOVA]: IL-1β, 0.27; IL-6, 0.10; IL-8, 0.46; and TNF-α, 0.14).

Egg Feeding and Changes in CRP and SAA
CRP and SAA levels from before and after addition of 4 eggs/d to the diet were compared. Although baseline levels of both CRP and SAA were higher in the LIR and OIR groups than in the LIS group, egg feeding was associated with a significant increase in levels of both CRP and SAA in the LIS group alone (Figure 1). Within the LIS group and for all subjects, the change in CRP was highly correlated with the change in SAA (LIS group only, r=0.955, P<0.001; all subjects combined, r=0.754, P<0.001). Body weight did not change with egg feeding (P=NS; data not shown).

Egg Feeding and Changes in Lipoproteins
Egg feeding increased non-HDL cholesterol only in the LIS group (Figure 2). As has been reported previously for cholesterol feeding,20,22–24 egg feeding was associated with significant increases in HDL cholesterol in all 3 groups. Although triglycerides tended to decrease slightly in all 3 groups, none of these changes reached statistical significance.

Lack of Correlation Between Changes in CRP or SAA and Changes in Lipoproteins
Because egg feeding led to significant increases in CRP and SAA as well as lipid variables in the LIS group, regression analysis was performed to evaluate whether individual changes in non-HDL cholesterol predicted changes in CRP or
SAA levels. However, within the LIS group, the change in non-HDL cholesterol was not correlated with changes in either CRP ($r = -0.04$, $P = 0.77$) or SAA ($r = -0.03$, $P = 0.82$). Also, although egg feeding was associated with significant increases in HDL cholesterol in all 3 groups, there were no correlations between changes in CRP and changes in HDL cholesterol in any group (LIS, $r = -0.001$, $P = 0.99$; LIR, $r = 0.002$, $P = 0.99$; and OIR, $r = -0.12$, $P = 0.35$). Similarly, there were no significant correlations between changes in SAA and changes in HDL cholesterol in any group (LIS, $r = 0.05$, $P = 0.71$; LIR, $r = 0.13$, $P = 0.26$; and OIR: $r = -0.09$, $P = 0.51$).

**Discussion**

In this study, we found that among healthy, community-dwelling individuals, egg feeding was associated with significant increases in CRP and SAA levels in LIS subjects. In addition, egg feeding was associated with a significant increase in non-HDL cholesterol in the same subjects. Moreover, because the change in non-HDL cholesterol was not correlated with a change in either CRP or SAA, a lack of change in non-HDL cholesterol could not be taken to indicate a lack of increase in either of these 2 inflammatory markers.

Previous studies have shown relations between CRP and obesity and, taken together, they generally suggest that CRP is largely determined by BMI and/or total fat mass.8,25–27 Insulin sensitivity also has been shown to be independently associated with CRP.28 Our data confirm that a higher BMI and reduced insulin sensitivity are associated with higher CRP levels and also demonstrate that a higher BMI and reduced insulin sensitivity are associated with higher levels of SAA.

The present study found that in subjects who were non-obese and insulin sensitive, egg feeding had effects on inflammatory markers similar to those seen in mice with genetic dyslipidemias29 and dietary cholesterol feeding.21 In contrast, egg feeding did not have a significant effect on CRP or SAA levels in human subjects who were obese and/or insulin resistant, ie, groups whose inflammatory marker levels were already elevated at baseline. In addition, the effect on lipoprotein levels of adding dietary cholesterol was much less pronounced in obese and/or insulin-resistant subjects. Although this finding may seem surprising, previous studies have demonstrated that cholesterol absorption may be inhibited in patients who are either obese or insulin resistant.30,31 In addition, a recent study of weight loss diets found that among obese women randomized to a high-fat, cholesterol-rich, Atkins-type diet, CRP and SAA did not rise but rather decreased in proportion to the amount of weight lost.32 Obesity is associated with hypersecretion of biliary cholesterol,1,33,34 which may inhibit dietary cholesterol absorption through competition for micellar solubilization and membrane uptake.35 Thus, we hypothesize that decreased cholesterol absorption associated with obesity may account, in part, for the relative lack of an adverse effect of dietary cholesterol on inflammatory markers and lipoprotein levels in the LIR and OIR groups.

Similar to what has been found in previous studies, dietary cholesterol was associated with increased HDL cholesterol, which has been associated with a lower cardiovascular disease risk in epidemiological studies. However, it is not clear that increases in HDL cholesterol induced by dietary cholesterol feeding are necessarily beneficial. First, many studies have demonstrated that high dietary cholesterol intake is associated with increased cardiovascular risk, despite the ability of dietary cholesterol to increase HDL cholesterol. Second, dietary cholesterol increases SAA levels, at least in LIS subjects, and SAA is carried on HDL cholesterol particles.36,37 Several recent studies have demonstrated that increased SAA levels are associated with increased cardiovascular risk.1,5–6 Moreover, in vitro and animal studies have demonstrated that SAA-containing HDL binds more avidly to arterial wall proteoglycans21,29 and that SAA levels are correlated with atherosclerotic lesion size in LDL receptor–deficient mice.21 Thus, at least some of the HDL cholesterol increase seen with dietary cholesterol feeding is caused by an increase in particles that contain SAA, a protein associated with increased atherosclerosis.

It is interesting to note that, as reported previously for LDL cholesterol levels, the LIS group had the most significant changes in non-HDL cholesterol levels with egg feeding.20 However, we were unable to demonstrate a correlation between individual changes in non-HDL cholesterol and changes in either CRP or SAA in LIS subjects. LIS subjects showed greater adverse effects of egg feeding with respect to a number of markers of cardiovascular risk compared with LIR or OIR subjects, although the levels attained were not as high as the baseline levels in LIR or OIR subjects. These findings are consistent with the hypothesis that in LIS subjects, a diet high in cholesterol might induce inflammatory stress similar to that observed with abdominal obesity and insulin resistance.

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