Serum Oxidized Low-Density Lipoprotein Levels and Risk of Colorectal Cancer: A Case-Control Study Nested in the Japan Collaborative Cohort Study

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Abstract

Oxidative stress plays an important role in carcinogenesis, but few epidemiologic studies have examined associations with risk of colorectal cancer. Relationships between serum levels of oxidized low-density lipoprotein (oxLDL) and oxLDL antibody (oLAB) and colorectal cancer risk were investigated in a case-control study nested in the Japan Collaborative Cohort Study for Evaluation of Cancer Risk. Serum samples and lifestyle information were collected at baseline from 39,242 men and women between 1988 and 1990. Of these, 161 incidents and deaths from colorectal cancer were identified through 1999, and 395 controls were matched for gender, age, and study area. Measurements were taken of serum oxLDL levels in 119 cases and 316 controls and serum oLAB levels in 153 cases and 376 controls. Odds ratios (95% confidence intervals) across quartiles, adjusted for confounding factors, were 1.55 (0.70-3.46), 1.90 (0.84-4.28), and 3.65 (1.50-8.92) for oxLDL (P_trend = 0.004) and 0.98 (0.54-1.80), 0.75 (0.39-1.48), and 1.68 (0.90-3.13) for oLAB (P_trend = 0.140). Further adjustment for serum total cholesterol and α-tocopherol did not materially change these associations. Odds ratio (95% confidence interval) of the highest quartile of serum oxLDL compared with the lowest quartile was 3.40 (1.09-10.58; P_trend = 0.045). Analyses restricted to colon cancer cases and corresponding controls yielded similar relationships between serum oxLDL and oLAB levels and risk. In conclusion, higher levels of serum oxLDL may increase risk of colorectal cancer.

Introduction

Reactive oxygen species (ROS) cause oxidation of lipids, proteins, and DNA in vitro (1, 2), and free radical and lipid peroxides have been considered very important in carcinogenesis (3). Some studies have reported high lipid peroxidation in human colorectal cancer tissue (4, 5). However, few epidemiologic studies have investigated relationships between lipid peroxidation and colorectal cancer.

Oxidized low-density lipoprotein (oxLDL) is generated by the actions of ROS in vitro. The oxLDL is taken up by macrophages, which develop into foam cells, and oLAB is present in both atherosclerotic lesions and plasma (6). Thus, oxLDL is believed to play a critical role in the development and progression of atherosclerosis.
atherosclerosis (7). Serum oxLDL levels may be consid-
ered as a biomarker reflecting the state of oxidative stress
and lipid metabolism in vivo. Experimental studies have
indicated that oxLDL increases intracellular levels of
ROS and lipid peroxidation products (thiobarbituric acid
reactive substances; ref. 8). The oLAB plays a positive
role in maintaining low levels of serum oxLDL.

Various lifestyle factors such as physical activity and
diets reportedly affect oxLDL (9-14). Regular physical
activity has been found to increase LDL resistance to
oxidation and decrease plasma oxLDL concentration (9).
Another study identified correlations between weight
reduction and decreased oxLDL (10). Some epidemio-
logic studies have reported that physical activity (15-17)
displays significant inverse associations with colorectal
cancer and that obesity (18, 19) is associated with
increased risk of colorectal cancer.

Vitamin E and lycopene have been shown to display
powerful antioxidant properties, reducing LDL oxidation
and oxidative damage to plasma proteins (11). Sup-
plementation with antioxidant nutrients (vitamin E,
vitamin C, and carotenoids) has been shown to protect
LDL from oxidation (12-14). High dietary carotenoid
intake possibly decreases the risk of colorectal cancer
(20) and a meta-analysis (21) of five prospective nested
case-control studies indicated that high plasma levels
of α-tocopherol were associated with a modest de-
crease in the subsequent incidence of colorectal cancer.

Given the results of these previous studies, we hy-
pothese that serum oxLDL levels represent a biomarker
reflecting oxidative stress and lifestyle factors such as
physical activity and diet as related to colorectal cancer.

To the best of our knowledge, no studies have
identified relationship between oxLDL and risk of
colorectal cancer. We therefore examined correlations
between serum levels of oxLDL and oLAB and risk of
colorectal cancer in a case-control study nested in a large-
scale Japanese cohort.

Materials and Methods

Study Subjects and Serum Samples. Study subjects
were recruited in the Japan Collaborative Cohort (JACC)
Study for Evaluation of Cancer Risk sponsored by
Monbukagakusyo (Ministry of Education, Culture,
Sports, Science, and Technology of Japan; ref. 22).
This study involves 110,792 residents who were ages 40 to
79 years at baseline from 45 areas all over Japan. An
epidemiologic survey of lifestyle factors was conducted
using a self-administered questionnaire about health
conditions and lifestyles such as medical history,
smoking habits, and alcohol consumption. Details of this
study have been published elsewhere (22).

In addition to the questionnaire survey, participants
in the JACC Study provided peripheral blood samples at
health screening checkups sponsored by municipalities
between 1988 and 1990. A total of 39,242 subjects (35.4%
Of respondents to the questionnaire survey) provided
blood samples. Sera were separated from samples at
laboratories in or near the surveyed municipalities as
soon as possible after sampling. Serum derived from
each subject was divided into three to five tubes (100-500
μL/tube) and stored at −80°C until analyzed.

Written informed consent for participation was
obtained individually from subjects, with the exception
of those in a few study areas in which informed consent
was provided at the group level after the aim of the study
and confidentiality of the data had been explained to
community leaders. This study was approved by the
Ethical Committee of Medical Care and Research at
Fujita Health University.

Case Ascertainment and Control Selection. Subjects
who died or moved away from study areas were
identified using population registries, and causes of
death were confirmed from death certificates. Incident
cases of cancer could be identified by linkage with cancer
registries in 24 of the 45 study areas. Follow-up for death
was conducted from baseline to the end of 1999, and
follow-up for incidence was conducted from baseline to
the end of 1997, excluding three study areas (from
baseline to the end of 1994, 1995, and 1996, respectively).
Only 4% of subjects were lost to follow-up due to moving
during the study period.

Death and incidence of colorectal cancer were defined
by the codes “C18,” “C19,” and “C20” in the Interna-
tional Statistical Classification of Diseases and Related Health
Problem, 10th Revision (23). During follow-up, 76 deaths
from colorectal cancer [colon (C18), n = 50; rectum (C19
and 20), n = 26] and 185 incident cases of colorectal
cancer (colon, n = 123; rectum, n = 62) were identified
from subjects who had provided serum samples at
baseline. Of these, 23 subjects with a history of colorectal
and other cancers at baseline were excluded. For each
case of colorectal cancer, two or three controls were
selected from the remaining population without incident
cancer or previous history of cancer, matching for
gender, age (±3 years), and study area. A total of 49
cases and 56 controls without sufficient samples for
measurement of serum levels of both oxLDL and oLAB
were excluded from analysis. Following these exclusions,
subjects without corresponding cases or controls were
also excluded. Finally, serum levels of either oxLDL or
oLAB could be measured in 161 cases (111 colon cancer
cases and 50 rectum cancer cases) and 395 controls
in this study. Of these, sufficient serum samples for
determination of oxLDL and oLAB were available for
103 cases and 279 controls and 135 cases and 330 controls,
respectively. For analyses using only incident cases and
corresponding controls, the subjects were 82 cases and
216 controls for oxLDL and 111 cases and 266 controls
for oLAB, respectively. Incident and dead cases were
analyzed together to maximize sample size for main analysis.

Biochemical Analyses of Sera. All samples were
analyzed by trained staff blinded to case-control status
in 2001. Serum oxLDL and oLAB were determined by
enzyme-linked immunoassay using commercially avail-
able kits (oxLDL: Oxidized LDL ELISA kit, Mercodia,
Uppsala, Sweden; oLAB: oLAB ELISA kit, Biomedica,
Vienna, Austria) in our laboratory. With regard to
intraassay and interassay reproducibility, coefficients of
variation for oxLDL (24) and oLAB (25) were <10%.
Serum α-tocopherol levels were measured separately
using high-performance liquid chromatography (26)
in our laboratory. Serum total cholesterol was measured
using an autoanalyzer at a single laboratory (SRL,
Hachioji, Japan). Values for oxLDL and oLAB could not be measured in all serum samples, because some initial samples yielded insufficient sera and other various substances were also measured from the same samples.

Serum samples of subjects had been stored for ~10 years until assay. Distribution of mean ± SD values for serum oxLDL levels in study controls [males: 36.1 ± 11.1 units/L (n = 144); females: 39.0 ± 11.4 units/L (n = 172)] was similar to that in our previous study (24) using fresh sera [males: 41.6 ± 12.2 units/L (n = 158); females: 42.7 ± 13.9 units/L (n = 158)]. Distributions of serum oxLAB were also similar, with median values (25th-75th percentiles) at 191.0 (128.0-241.0) units/L in males (n = 179) and 192.0 (142.0-304.0) units/L in females (n = 197) for the present study compared with 170.7 (130.9-301.2) units/L in males (n = 158) and 209.0 (152.6-312.5) units/L in females (n = 158) for the previous study (25). Subjects in this and our previous study were Japanese ages 40 to 79 years, and the same ELISA kits were used. Serum levels of oxLDL and oLAB had thus not changed substantially during long-term storage.

Statistical Analyses. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared. Baseline characteristics were compared between cases and controls using \( \chi^2 \) tests. Mean differences for serum total cholesterol levels and BMI between cases and controls were examined using \( t \) tests. Because serum oxLDL, oLAB, and \( \alpha \)-tocopherol levels are log normally distributed (25, 26), mean differences between cases and controls were examined using \( t \) tests after converting serum levels of oxLDL, oLAB, and \( \alpha \)-tocopherol to logarithmic values. Relationships among serum levels of oxLDL, oLAB, total cholesterol, and \( \alpha \)-tocopherol were examined using Spearman correlation coefficients. \( \alpha \)-Tocopherol was included in this analysis because it binds to LDL and may be associated with decreased risk of colorectal cancer (21).

Conditional logistic regression models with gender, age, and study area strata were applied to calculate odds ratios (OR) and 95% confidence intervals (95% CI) for colorectal cancer. ORs were computed according to quartile levels of serum oxLDL and oLAB. Cases were categorized into four groups according to the quartile in controls for serum oxLDL and oLAB. To test for linear trends in ORs over quartiles, each quartile was coded as 0, 1, 2, or 3 and then incorporated into logistic models as a single variable.

Potential confounding was considered by smoking habits (never, former, or current smokers and unknown), drinking habits (never, former, or current drinkers and unknown), intake frequency of green leafy vegetables (1-2 times/mo or less, 1-2 times/wk or more, and unknown), time spent in sports or physical exercise (little, 1 h/wk or more, and unknown), intake frequency of green leafy vegetables, or time spent in sports or physical exercise.

Table 1. Baseline characteristics of colorectal cancer cases and controls

<table>
<thead>
<tr>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>( P (\chi^2 \text{ test}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>161 (100.0)</td>
<td>395 (100.0)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>75 (46.6)</td>
<td>187 (47.3)</td>
</tr>
<tr>
<td>Female</td>
<td>86 (53.4)</td>
<td>208 (52.7)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>14 (8.7)</td>
<td>36 (9.1)</td>
</tr>
<tr>
<td>50-59</td>
<td>48 (29.8)</td>
<td>131 (33.2)</td>
</tr>
<tr>
<td>60-69</td>
<td>65 (40.4)</td>
<td>159 (40.3)</td>
</tr>
<tr>
<td>70-79</td>
<td>34 (21.1)</td>
<td>69 (17.5)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>37 (23.0)</td>
<td>96 (24.3)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>23 (14.3)</td>
<td>51 (12.9)</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>93 (57.8)</td>
<td>218 (55.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>8 (5.0)</td>
<td>30 (7.6)</td>
</tr>
<tr>
<td>Drinking habit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current drinker</td>
<td>75 (46.6)</td>
<td>168 (42.5)</td>
</tr>
<tr>
<td>Ex-drinker</td>
<td>2 (1.2)</td>
<td>12 (3.0)</td>
</tr>
<tr>
<td>Nondrinker</td>
<td>78 (48.4)</td>
<td>195 (49.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (3.7)</td>
<td>20 (5.1)</td>
</tr>
<tr>
<td>Family history of colorectal cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (6.2)</td>
<td>14 (3.5)</td>
</tr>
<tr>
<td>No</td>
<td>151 (93.8)</td>
<td>381 (96.5)</td>
</tr>
<tr>
<td>Intake frequency of green leafy vegetables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 times/mo or less</td>
<td>23 (14.3)</td>
<td>57 (14.4)</td>
</tr>
<tr>
<td>1-2 times/wk or more</td>
<td>126 (78.3)</td>
<td>325 (82.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (7.5)</td>
<td>13 (3.3)</td>
</tr>
<tr>
<td>Time spent in sport or physical exercise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little</td>
<td>97 (60.2)</td>
<td>247 (62.5)</td>
</tr>
<tr>
<td>1 h/wk or more</td>
<td>57 (35.4)</td>
<td>125 (31.6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (4.3)</td>
<td>23 (5.8)</td>
</tr>
</tbody>
</table>

We therefore calculated these ORs to know the risk in relation to serum oxLDL and oLAB independent of \( \alpha \)-tocopherol and total cholesterol.

Two-sided \( P \)s < 0.05 were considered statistically significant. All statistical analyses were done using the Statistical Analysis System.

Results

Table 1 summarizes baseline characteristics of study subjects. No significant differences between cases and controls were observed for age distribution, smoking and drinking habits, family history of colorectal cancer, intake frequency of green leafy vegetables, or time spent in sports or physical exercise.

Table 2 compares serum levels of oxLDL, oLAB, total cholesterol, and \( \alpha \)-tocopherol and BMI between cases and controls. Serum oxLDL levels were significantly higher in cases than in controls. BMI and serum levels of oLAB, \( \alpha \)-tocopherol, and total cholesterol did not differ significantly between cases and controls.

Table 3 shows relationships among serum levels of oxLDL, oLAB, total cholesterol, and \( \alpha \)-tocopherol in control subjects. Serum oxLDL levels were significantly and positively correlated with serum levels of total cholesterol and \( \alpha \)-tocopherol in both genders. Serum
oLAB levels displayed no correlation with serum levels of oxLDL, total cholesterol, or \( \alpha \)-tocopherol in either gender. Table 4 shows ORs and 95% CIs for colorectal cancer by serum levels of oxLDL and oLAB after adjusting for confounding factors. ORs (95% CIs) across quartiles for serum oxLDL adjusted for gender, age, and study area (OR1) were 1.21 (0.57-2.55), 1.49 (0.71-3.14), and 2.34 (1.03-5.30; \( P \) trend = 0.030). OR (95% CI) for serum oxLDL adjusted for gender, age, study area, smoking and drinking habits, intake frequency of green leafy vegetables, time spent in sports or physical exercise, family history of colorectal cancer, and BMI (OR2) was significantly higher in the highest quartile compared with the lowest quartile [3.65 (1.50-8.92); \( P \) trend = 0.004]. OR1 and OR2 for oLAB tended to be higher in the highest quartile of serum oLAB but not significantly (OR1, 1.66; 95% CI, 0.91-3.01; \( P \) trend = 0.148; OR2, 1.68; 95% CI, 0.90-3.13; \( P \) trend = 0.140).

When the analysis was limited to incident cases and corresponding controls, the higher risk was still found in relation to higher serum levels of oxLDL. OR2s (95% CIs) for colorectal cancer across quartiles of serum oxLDL were 3.11 (1.09-8.87), 2.25 (0.79-6.39), and 4.77 (1.50-15.10; \( P \) trend = 0.027). OR2s (95% CIs) for colorectal cancer across quartiles of serum oLAB were 0.67 (0.32-1.41), 0.89 (0.41-1.92), and 1.22 (0.51-2.62; \( P \) trend = 0.412).

Associations of serum oxLDL and oLAB with risk of colorectal cancer were also evaluated after further adjustment for quintiles of total cholesterol and \( \alpha \)-tocopherol (OR3). However, no substantial change in results was observed. When evaluated by gender, no apparent difference between males and females was noted. The same analyses were attempted using only colon cancer cases (\( n = 80 \) for oxLDL and \( n = 106 \) for oLAB) and corresponding controls (\( n = 215 \) for oxLDL and \( n = 261 \) for oLAB). OR3s (95% CIs) for colon cancer across quartiles of serum oxLDL were 2.97 (0.97-9.06), 1.90 (0.55-6.59), and 4.68 (1.19-18.38; \( P \) trend = 0.062). A similar trend was observed for serum oLAB levels: OR3s (95% CIs) across quartiles were 1.75 (0.73-4.20), 1.69 (0.68-4.15), and 2.20 (0.90-5.37; \( P \) trend = 0.119).

 Furthermore, modified data sets excluding cases diagnosed within 2 years from baseline were also analyzed. Results of these analyses were consistent with those of analyses without exclusion (data not shown).

### Discussion

The present investigation represents the first prospective study to examine associations between serum oxLDL and risk of colorectal cancer. Significant positive associations were observed between serum oxLDL levels and risk of colorectal cancer. There was no association between serum oLAB levels and risk of colorectal cancer. Risk of colorectal cancer was higher in the presence of higher levels of serum oxLDL, independent of confounders. The mechanisms involved in this association between oxLDL and colorectal cancer remain unclear.

The adjustment for lifestyle factors, family history, and BMI somewhat strengthened the positive association between oxLDL and risk of colorectal cancer. This may not be in line with our initial hypothesis that serum oxLDL levels represent a marker reflecting lifestyles related to the cancer. Serum oxLDL may be a predictor of the risk independently of other risk factors.

There are some reports that studied the association between serum or plasma oxLDL levels and coronary heart disease (7, 28). It is well known that oxLDL is found in monocyte-derived macrophages in atherosclerosis lesions and that plasma oxLDL levels were significantly higher in patients with coronary artery disease (28). Several studies have been carried out on the modified forms of oxLDL, which are prepared by oxidizing LDL under various conditions in vitro (28). However, there is little information about oxLDL present in vivo (28).

We have also studied associations between serum carotenoids levels and risk of colorectal cancer in this prospective epidemiologic study. We found inverse associations of some carotenoids with colorectal cancer risk in men.\(^6\) Crohn disease is a chronic inflammatory disorder and is associated with increased risk of colon cancer (29). Although the etiology of Crohn disease is unknown, patients with Crohn disease have increased

### Table 2. Serum levels of oxLDL, oLAB, total cholesterol, and \( \alpha \)-tocopherol and BMI for colorectal cancer cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxLDL (units/L), median (25th-75th percentiles)</td>
<td>119</td>
<td>316</td>
<td>0.045</td>
</tr>
<tr>
<td>oLAB (units/L), median (25th-75th percentiles)</td>
<td>153</td>
<td>376</td>
<td>0.120</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L), mean ± SD</td>
<td>159</td>
<td>382</td>
<td>0.225</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol (µmol/L), median (25th-75th percentiles)</td>
<td>155</td>
<td>377</td>
<td>0.834</td>
</tr>
<tr>
<td>BMI (kg/m²), mean ± SD</td>
<td>158</td>
<td>380</td>
<td>0.779</td>
</tr>
</tbody>
</table>

### Table 3. Spearman correlation coefficients (no. subjects) among serum levels of oxLDL, oLAB, total cholesterol, and \( \alpha \)-tocopherol among control subjects

<table>
<thead>
<tr>
<th></th>
<th>oxLDL</th>
<th>oLAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-LAB</td>
<td>0.066 (136)</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.525* (138)</td>
<td>-0.023 (172)</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>0.397* (140)</td>
<td>0.011 (169)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \alpha )-LAB</td>
<td>0.021 (161)</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.429* (166)</td>
<td>-0.093 (191)</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>0.227* (168)</td>
<td>0.006 (192)</td>
</tr>
</tbody>
</table>

\( *P < 0.001.\)

\( ^6 \) Unpublished data.
production of ROS (29). It was reported that lipid peroxidation and F2 isoprostane was significantly higher in patients with Crohn disease than in healthy control subjects (30).

Various potentially toxic oxidized lipids are contained in oxLDL such as lipid peroxides, oxysterol, and aldehydes (31). These oxidized lipids elicit oxidative stress and lipid peroxidation (31). As oxLDL reduces antioxidant enzymes such as Cu/Zn superoxide dismutase (32) and glutathione peroxidase (33) and ROS degradation is decreased following increases in oxLDL (31), ROS levels are elevated. Lipid peroxidation is initiated by ROS attacks, generating large amounts of reactive products that have been implicated in tumor initiation and promotion (34). Increased levels of malondialdehyde, a major genotoxic carbonyl compound generated by lipid peroxidation (34), have been reported in tumor tissue from colorectal cancer patients compared with normal mucosa from the same individuals (35).

In another experimental study (8), oxLDL-induced oxidative stress enhanced p53 DNA binding activity and p53 protein synthesis. As a tumor suppressor, p53 is induced by various kinds of cell stress (36) to protect the cell. Genetic information is protected by the functions of p53, including induction of cell cycle arrest or apoptosis after DNA damage and maintenance of genomic stability (37). Given the above, high levels of oxLDL might induce excess stress against the cell. This stress may induce DNA damage and mutation, because oxidative stress is known to cause such damage (38). Mutation of p53 gene is found in >50% of all human cancers and >75% of colorectal adenocarcinomas (39). Mutation of the p53 gene is known to play crucial roles in tumor development and progression (37).

Cyclooxygenase-2 (COX-2) expression is reportedly induced by oxLDL in a murine macrophage-like cell line (40) and human monocytes (41). COX is an enzyme that initiates the conversion of arachidonic acid into all of the prostaglandins and thromboxanes (42). Lipid peroxidation is necessary for initiation of COX activity (43), and reactive oxygen intermediates (ROI) induce COX-2 (44). Levels of arachidonic acid (45) and prostaglandin E2 (46) are higher in colon tumor than in normal colon mucosa. Prostaglandin E2, a major product of COX, stimulates proliferation and growth of human colorectal cancer cells (47).

Analysis of COX-2 expression (induced by cytokines, growth factors, and mitogens) has revealed elevated levels in up to 90% of sporadic colon carcinomas and 40% of colonic adenomas but no elevation in normal colonic epithelium (48). Recent clinical epidemiologic studies have shown that COX inhibitors such as aspirin and other nonsteroidal anti-inflammatory agents exert preventive effects on colorectal cancer (49, 50). Such inhibition of COX-2 is considered to lead to decreased incidence of colorectal cancer, although the mechanisms are not fully understood.

Functions of oxLDL such as increasing oxidative stress and inducing COX-2 expression might play an important role in colorectal carcinogenesis. At the very least, oxidative stress is increased in subjects with high levels of serum oxLDL, and oxidative stress should be related to colorectal carcinogenesis.

Epidemiologic studies showed the close association between insulin resistance and colon cancer risk (51). The consumption of excess dietary energy results in the development of insulin resistance with increased circulating levels of insulin, triglycerides, and nonesterified fatty acids. These circulating factors subject colonic epithelial cells to a proliferative stimulus and also expose them to reactive oxygen intermediates. Other study reported that colonic epithelial cells to a proliferative stimulus and also expose them to reactive oxygen intermediates. Other study reported that LDL oxidizability is increased in insulin resistance subjects compared with healthy subjects (52). These long-term exposures are expected to result in the promotion of colon cancer.

Serum oLAB levels were not significantly associated with risk of colorectal cancer. Serum oLAB is generated from immunoresponses against oxLDL. Serum oLAB levels, in addition to serum oxLDL levels, may therefore also depend on various lifestyle factors such as dietary intake of antioxidants and smoking habits. Plasma oLAB levels are reported to show a negative correlation with plasma oxLDL levels in healthy subjects (53), and oLAB may play a role in maintaining low levels of blood oxLDL. Wide ranges of serum oLAB levels might reflect interindividual differences in immune responses rather than in oxLDL generation. The immune system is also affected by various lifestyle factors such as smoking habits. We considered that almost no relationship between serum oLAB and oxLDL in controls was derived from interindividual differences in immune responses. Interindividual difference in immune responses may

| Table 4. ORs and 95% CIs for colorectal cancer risk by serum levels of oxLDL and oLAB |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Range           | Cases | Controls | OR1  | 95% CI | P_trend | OR2  | 95% CI | P_trend | OR3  | 95% CI | P_trend |
| oxLDL (units/L) |       |          |      |        |         |      |        |         |      |        |         |
| Q1 ≤ 29.1       | 22    | 79       | 1.00 | —      | 0.030   | 1.00 | —      | 0.004   | 1.00 | —      | 0.038   |
| Q2 29.2-36.1    | 26    | 79       | 1.21 | 0.57-2.55 | 1.55   | 0.70-3.46 | 1.15 | 0.49-2.72 |
| Q3 36.2-44.6    | 31    | 79       | 1.49 | 0.71-3.14 | 1.90 | 0.84-4.28 | 1.38 | 0.54-3.51 |
| Q4 ≥ 44.7       | 40    | 79       | 2.34 | 1.03-5.30 | 3.65 | 1.50-8.92 | 3.10 | 1.04-9.23 |
| oLAB (units/L)  |       |          |      |        |         |      |        |         |      |        |         |
| Q1 ≤ 135.4      | 34    | 94       | 1.00 | —      | 0.148   | 1.00 | —      | 0.140   | 1.00 | —      | 0.212   |
| Q2 135.5-191.9  | 41    | 96       | 1.14 | 0.64-2.01 | 0.98 | 0.54-1.80 | 1.11 | 0.58-2.11 |
| Q3 192.0-272.4  | 28    | 92       | 0.87 | 0.46-1.64 | 0.75 | 0.39-1.48 | 0.74 | 0.36-1.52 |
| Q4 ≥ 272.5      | 50    | 94       | 1.66 | 0.91-3.01 | 1.68 | 0.90-3.13 | 1.69 | 0.85-3.35 |

NOTE: OR1: OR adjusted for gender, age, and study area; OR2: OR adjusted for gender, age, study area, smoking and drinking habits, intake frequency of green leafy vegetables, time spent in sport or physical exercise, family history of colorectal cancer, and BMI; OR3: OR adjusted for gender, age, study area, smoking and drinking habits, intake frequency of green leafy vegetables, time spent in sport or physical exercise, family history of colorectal cancer, BMI, and serum levels of total cholesterol and a-tocopherol.
have also attenuated the association between serum oxLDL and risk of colorectal cancer.

Although oxLDL is an oxidant and α-tocopherol is an antioxidant, our results show positive association between serum oxLDL and α-tocopherol levels. We suggest that this association was observed because serum LDL binds to α-tocopherol (54). Similarly, serum oxLDL is positively associated with serum cholesterol levels.

Cases included both colon and rectal cancers. Risk for colon cancer only was increased with high serum oxLDL levels after adjusting for gender, age, study area, and potential confounders. The sample population for rectum cancer cases was too small to analyze associations between serum levels of oxLDL and oLAB and risk of rectum cancer. These associations warrant further study.

In conclusion, the present study showed that increased levels of serum oxLDL represent a risk factor for colorectal cancer among Japanese. Although further investigations are needed to clarify the role of oxLDL in tumorgenesis for colorectal cancer, serum oxLDL levels may be one biomarker for predicting risk of colorectal cancer.

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