Low Plasma Adiponectin Levels and Risk of Colorectal Cancer in Men: A Prospective Study

Esther K. Wei, Edward Giovannucci, Charles S. Fuchs, Walter C. Willett, Christos S. Mantzoros

Background: Adiponectin is an insulin-sensitizing hormone secreted by adipocytes. Levels of adiponectin are inversely associated with adiposity and insulin resistance. Because both adiposity and insulin resistance have been associated with risk of colorectal cancer, we hypothesized that adiponectin is associated with colorectal carcinogenesis. Methods: We evaluated the association between adiponectin and colorectal cancer among 18,225 men in the Health Professionals Follow-up Study who provided blood samples in 1994. Between blood collection and January 31, 2002, 179 incident colorectal cancer cases occurred. Each case patient was matched to two control subjects on year of birth and date of blood draw. Information on lifestyle factors and diet was collected using biennial questionnaires and food frequency questionnaires. Logistic regression models were used to estimate relative risks (RRs) and confidence intervals (CIs). All statistical tests were two-sided. Results: We observed a statistically significant inverse association between plasma adiponectin levels and risk of colorectal cancer (for the highest quintile [Q5] versus the lowest quintile [Q1], RR = 0.42, 95% CI = 0.23 to 0.78; P_trend = .01). The association was only slightly attenuated after adjustment for body mass index (Q5 versus Q1, RR = 0.48, 95% CI = 0.25 to 0.90; P_trend = .04) or for body mass index and other major risk factors for colorectal cancer (family history, physical activity, multivitamin use, smoking, alcohol, aspirin use, history of endoscopy, dietary calcium, folate, vitamin E, and vitamin D; Q5 versus Q1, multivariable RR = 0.50, 95% CI = 0.26 to 0.97; P_trend = .08). Relative risks were not linear in any of the analyses; the second quintile had a lower relative risk than the lowest quintile, but further decreases in risk were not evident with increasing levels of adiponectin. Conclusions: In this prospective nested case–control study, men with low plasma adiponectin levels had a higher risk of colorectal cancer than men with higher levels. More prospective observational studies, particularly in women, and mechanistic studies are required to fully understand the relationship between adiponectin and carcinogenesis. [J Natl Cancer Inst 2005;97:1688–94]
Adiponectin assay. Each case patient was individually matched to two control subjects on year of birth and on year and month of blood draw. We also had information from each participant on his fasting status at the time of blood collection (hours since last meal before blood draw in categories of 0–2, 3–4, 5–8, >8, or missing, based on the distribution in the population). We excluded one mislabeled blood sample from a control subject and another from a control subject who was identified as a cancer case patient. Therefore, a total of 356 control subjects were included in this analysis. The average time from blood collection to date of diagnosis was 53.0 months (range = 1–103 months). The study was approved by the Human Subjects Committee Review at the Harvard School of Public Health, and written informed consent was obtained from all participants.

**Exposure Assessment**

Participants reported information regarding age, weight, aspirin use, and smoking habits on each of the biennial mailed questionnaires. Family history was asked about on the 1986, 1990, and 1992 questionnaires. In 1986, each participant reported his height and weight on the main questionnaire. In 1987, another optional questionnaire, a tape measure, and specific illustrated instructions on how to measure waist and hip circumference were sent to all participants, of whom approximately 65% returned the questionnaire. In a previous study, this self-reported measurement method was validated among a sample of 123 men in the cohort by comparing the self-reported measurements and trained technician measurements (18). The correlations between the two measures (weight, \( r = .97 \); waist circumference, \( r = .95 \); hip circumference, \( r = .88 \); waist-to-hip ratio, \( r = .69 \)) suggest that particularly among men, the self-measured data are reasonably accurate (18). Although we had direct measurement data for approximately 80% of the study population, we used the reported body mass index (BMI) and age to derive a predicted waist circumference and waist-to-hip ratio for men who were missing data for these measurements (missing waist circumference: \( n = 101 \); missing waist-to-hip ratio: \( n = 103 \)) to increase our statistical power to evaluate the association of adiponectin after adjusting for waist circumference and waist-to-hip ratio. Among the 434 men with self-reported waist measurements, the Spearman correlation between the reported waist circumference and their predicted waist circumference using the method described above was 0.75. Among the 432 men with self-reported waist-to-hip ratio, the correlation was 0.39.

Physical activity was estimated using information provided on the biennial questionnaires in response to questions about time spent per week on several listed activities. The time spent on each activity was multiplied by the average metabolic equivalents (MET) for that activity, and the resulting values were summed to obtain a total MET-hour score for each man. The validity and reliability of these methods have been evaluated in a subset of this cohort and shown to be adequate (19).

Information on diet was provided by participants on self-administered semiquantitative FFQs collected before blood collection (1986, 1990, and 1994). To calculate specific nutrient intakes, we multiplied the reported frequency of consumption of each specified food item by the nutrient content of the specified portion size; these products were then summed for all food items. Specific nutrient contributions from supplemental sources were derived based on information provided on use of multivitamins and other supplements (including details on which brand and type was used), using an extensive database of supplement formulations. These nutrient contributions were then added to the specific nutrient intake from foods to calculate a total daily intake for each man. This method of dietary assessment has been directly validated in this cohort of men (20) and in a similar cohort of women using the same instrument (21).

**Laboratory Procedures**

Blood samples were placed on ice, stored in Styrofoam containers, and shipped by overnight courier. More than 95% of the samples arrived within 24 hours of collection. Blood samples were processed immediately upon receipt and placed in the vapor phase of liquid nitrogen freezers (−130 °C or below). Plasma adiponectin concentrations were measured in a single run at the Beth Israel Deaconess Medical Center (Boston, MA) using a commercially available radioimmunoassay kit from Linco Research (St. Charles, MO) that has a sensitivity of 2 ng/mL, as previously described (17). Blood samples for the cancer case patients and control subjects were handled together, shipped together, and assayed in the same analytical run. To assess laboratory precision, each batch included masked replicate plasma samples that were labeled identically to the regular sample. All laboratory personnel were blinded with respect to case patient or control subject status. The mean intra-assay coefficient of variation was 9.97%. The stability of adiponectin under the transport conditions we used (i.e., in blood kept on ice and shipped overnight) has been reported by others to be good (22), and the BMI-adjusted intraclass correlation coefficient over a 1-year period was high [\( r = .84, 95\% \) CI = 0.65 to 0.94 (22)], suggesting that plasma adiponectin levels assayed from a single blood sample are reasonably accurate over a long period.

**Statistical Analyses**

We compared case patients and control subjects with respect to various factors and analyzed how the distribution of these factors varied across quintiles of plasma adiponectin. Continuous variables are presented as means and standard deviations; categorical variables are presented as percentages. Statistical significance of the comparisons was calculated using generalized linear models controlling for age. Adiponectin levels were categorized into quintiles based on the distribution in the control subjects and were age standardized using direct standardization to the age distribution among case patients and control subjects. We used the extreme Studentized deviate Many-Outlier procedure (23) to assess for outliers in each set of laboratory results. From results of this procedure, no values were excluded.

Relative risks and 95% confidence intervals for the association between plasma adiponectin and colorectal cancer and colon cancer separately were calculated using conditional logistic regression, adjusting for fasting status. To test for linear trend across the quintiles, we modeled the median of each quintile as a continuous variable. We evaluated the potential confounding effect of BMI, physical activity, waist circumference, waist-to-hip ratio, and total dietary intake of folate, calcium, vitamin D, vitamin E, and alcohol (all modeled as continuous variables). To estimate average long-term dietary intake and to reduce measurement error from a one-time measure, we used the average of the intake reported on the three FFQs between 1986 and 1994. Other potential confounders we included were having a family history of colorectal cancer (yes
RESULTS

Case patients had statistically significantly lower adiponectin levels and higher BMIs, waist circumferences, and waist-to-hip ratios than the control subjects (Table 1). Also, case patients were more likely than control subjects to have a family history of colorectal cancer. Men in the highest quintile of adiponectin level were older than those in the lowest quintile; they also had lower BMI, waist circumference, and waist-to-hip ratio; were more physically active; had higher intakes of total folate, total vitamin E, vitamin E from food only, total vitamin D, and total calcium; and were more likely to be multivitamin users in 1994. Fasting status was not related to adiponectin levels (data not shown).

Plasma adiponectin levels were inversely associated with risk of colorectal cancer (Table 2). In a simple conditional logistic model that adjusted for age, other matching factors, and fasting status, men in the highest quintile of adiponectin had a 58% lower risk of colorectal cancer than those in the lowest quintile, with a statistically significant linear trend across the categories (relative risk [RR] = 0.42, 95% confidence interval [CI] = 0.23 to 0.78; \( P_{\text{trend}} = .01 \)). Adding various measures of body fatness, such as BMI, to the model attenuated the associations only slightly (Q5 versus Q1, RR = 0.48, 95% CI = 0.25 to 0.90; \( P_{\text{trend}} = .04 \)). In a model adjusting for BMI and other potential confounders, including family history, physical activity, multivitamin use, folate, calcium, vitamin D, current smoking, vitamin E, aspirin use, and endoscopy before 1994, the inverse association between colorectal cancer risk and adiponectin levels remained statistically significant for the highest quintile compared with the lowest (multivariable RR = 0.50, 95% CI = 0.26 to 0.97), although the linear trend was no longer statistically significant (\( P_{\text{trend}} = .08 \)). Similar results were obtained in models including the other measures of body fatness, such as waist circumference and waist-to-hip ratio. The relative

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients</th>
<th>Control subjects</th>
<th>( P^{\dagger} )</th>
<th>Adiponectin quintile 1</th>
<th>Adiponectin quintile 5</th>
<th>( P^{\ddagger} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin ± SD, μg/mL</td>
<td>7.4 ± 2.1</td>
<td>7.8 ± 1.9</td>
<td>.02</td>
<td>4.8</td>
<td>10.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>66.6</td>
<td>66.5</td>
<td>.09</td>
<td>63.7</td>
<td>68.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current smoker (1994) (176 case patients, 352 control subjects)</td>
<td>14.9</td>
<td>15.2</td>
<td>.89</td>
<td>14.6</td>
<td>14.4</td>
<td>.74</td>
</tr>
<tr>
<td>Current smoker (1994) (176 case patients, 352 control subjects)</td>
<td>5.1</td>
<td>5.4</td>
<td>.91</td>
<td>6.9</td>
<td>3.6</td>
<td>.11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8</td>
<td>25.4</td>
<td>.05</td>
<td>26.5</td>
<td>24.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist circumference (1987), in</td>
<td>38.2</td>
<td>37.4</td>
<td>.01</td>
<td>38.5</td>
<td>36.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio (1987)</td>
<td>0.95</td>
<td>0.94</td>
<td>.04</td>
<td>0.95</td>
<td>0.92</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Height, in</td>
<td>70.4</td>
<td>70.2</td>
<td>.51</td>
<td>70.4</td>
<td>70.6</td>
<td>.50</td>
</tr>
<tr>
<td>Physical activity, MET-hours/wk</td>
<td>28.6</td>
<td>28.9</td>
<td>.87</td>
<td>26.9</td>
<td>33.3</td>
<td>.04</td>
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<tr>
<td>Aspirin use for 8 y, %</td>
<td>11.7</td>
<td>14.9</td>
<td>.32</td>
<td>13.9</td>
<td>17.7</td>
<td>.34</td>
</tr>
<tr>
<td>Family history of colon or rectal cancer, %</td>
<td>20.7</td>
<td>12.6</td>
<td>.02</td>
<td>17.8</td>
<td>13.5</td>
<td>.37</td>
</tr>
<tr>
<td>Endoscopy before 1994, %</td>
<td>59.8</td>
<td>67.1</td>
<td>.08</td>
<td>61.7</td>
<td>72.0</td>
<td>.10</td>
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<tr>
<td>Alcohol, g/day</td>
<td>12.1</td>
<td>12.1</td>
<td>.99</td>
<td>11.8</td>
<td>13.0</td>
<td>.59</td>
</tr>
<tr>
<td>Multivitamin use in 1994, %</td>
<td>48.0</td>
<td>50.3</td>
<td>.63</td>
<td>13.0</td>
<td>64.6</td>
<td>.002</td>
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<tr>
<td>Total folate intake, μg/day</td>
<td>503</td>
<td>526</td>
<td>.24</td>
<td>506</td>
<td>582</td>
<td>.006</td>
</tr>
<tr>
<td>Folate from food only, μg/day</td>
<td>360</td>
<td>367</td>
<td>.28</td>
<td>369</td>
<td>378</td>
<td>.26</td>
</tr>
<tr>
<td>Total Vitamin D, IU/day</td>
<td>128</td>
<td>131</td>
<td>.88</td>
<td>102</td>
<td>173</td>
<td>.004</td>
</tr>
<tr>
<td>Total Vitamin D, IU/day</td>
<td>11.6</td>
<td>11.7</td>
<td>.80</td>
<td>11.6</td>
<td>12.6</td>
<td>.03</td>
</tr>
<tr>
<td>Calcium from food only, mg/day</td>
<td>252</td>
<td>256</td>
<td>.66</td>
<td>247</td>
<td>263</td>
<td>.10</td>
</tr>
<tr>
<td>Calcium from food only, mg/day</td>
<td>895</td>
<td>922</td>
<td>.39</td>
<td>871</td>
<td>987</td>
<td>.01</td>
</tr>
</tbody>
</table>

*Dietary intake computed as average of responses to food frequency questionnaires collected between 1986 and 1994. SD = standard deviation; BMI = body mass index.

\( ^{\dagger} \) \( P \) values (two-sided) for case patients versus control subjects calculated using generalized linear models.

\( ^{\ddagger} \) \( P \) values (two-sided) for quintile 5 versus quintile 1 calculated using generalized linear models.

§Matching factor.
risks were not linear across quintiles in any model. Men in the second quintile had a lower relative risk than those in the lowest quintile, but a further decrease in risk was not evident with increasing levels of adiponectin, except in the highest quintile (Table 2).

When we restricted the analysis to cancer arising in the colon only (n = 139 after excluding rectal cancer), the results were similar to those among colorectal cancer patients (Table 3). A statistically significant inverse linear association between adiponectin levels and colon cancer risk was observed using simple matched models adjusting for fasting status. More adjustment for BMI and the other potential confounders attenuated the results only slightly; the relative risk for the top to bottom quintile comparison remained statistically significant and slightly stronger than the association with colorectal cancer. However, the test for trend remained borderline statistically significant (P = .07). The small number of rectal cancer case patients precluded a careful evaluation of the association between plasma adiponectin and rectal cancer.

To evaluate whether preclinical disease may have affected the observed associations, we excluded case patients (n = 34) with cancers that were diagnosed in the first 2 years of follow-up after blood collection. The results were similar to those without this exclusion although slightly stronger (multivariable RR with BMI, Q5 versus Q1, RR = 0.44, 95% CI = 0.20 to 0.98; P_trend = .05). We also evaluated the association among case patients with medium- or high-grade cancer (n = 109) and by Dukes’ stage (24). Overall, the associations remained inverse and were of similar magnitude as the results in Table 2. However, because of the limited number of case patients, our ability to draw conclusions from these results was reduced.

The association between plasma adiponectin and colorectal cancer did not vary by BMI (P_interaction = .24). Among men with BMI less than 25 kg/m^2 (75 case patients and 171 control subjects), after adjusting for BMI, family history of colorectal cancer, and physical activity, their relative risk was 0.40 (95% CI = 0.15 to 1.05, P_trend = .06), and among those with a BMI greater than or equal to 25 kg/m^2 (104 case patients and 185 control subjects), the relative risk was 0.65, (95% CI = 0.28 to 1.53, P_trend = .28). To further evaluate the nature of the relationship between BMI...
and adiponectin, we included BMI with and without plasma adiponectin levels in the model. The results were slightly attenuated when adiponectin was added to the model, but overall BMI was associated with colorectal cancer risk in both models (BMI modeled as continuous variable with adiponectin, RR = 1.06 per kg/m², 95% CI = 0.98 to 1.14; without adiponectin, RR = 1.07 per kg/m², 95% CI = 1.00 to 1.15). Similar results were obtained for waist circumference and waist-to-hip ratio (data not shown).

We also evaluated whether the association between plasma adiponectin level and colorectal cancer risk varied by age at blood collection. The association appeared to be slightly stronger among younger men; however, the interaction was not statistically significant (for men <66.5 years, 73 case patients, multivariable RR = 0.25, 95% CI = 0.06 to 1.06, and for men ≥66.5 years, 106 case patients, RR = 0.70, 95% CI = 0.30 to 1.65; P_interaction = .15).

Finally, we evaluated whether the association between adiponectin level and colorectal cancer risk varied by levels of physical activity. We found some evidence of an inverse association between colorectal cancer risk and adiponectin level among those who reported less than 24.4 MET-hours/week of physical activity (adjusting for BMI, physical activity, and family history, n = 89 case patients; RR = 0.41, 95% CI = 0.16 to 1.02, P_trend = .06). The association between adiponectin and colorectal cancer was weaker among those who reported 24.4 or more MET hours/week of leisure time physical activity (n = 90 case patients; RR = 0.59, 95% CI = 0.25 to 1.37, P_trend = .38), but the interaction was not statistically significant.

**DISCUSSION**

In this prospective nested case–control study, plasma adiponectin levels showed a strongly inverse association with risk of colon cancer. Men in the second quintile had a statistically significantly lower risk than the men in the lowest quintile. However, men with adiponectin levels in the third through fifth quintiles had little additional risk reduction compared with those in the second quintile. The association between adiponectin level and risk persisted after adjusting for measures of body fatness, including BMI, waist circumference, and waist-to-hip ratio, and physical activity. Further adjustment for other lifestyle characteristics, including diet, suggested little confounding from environmental factors. Over the approximately 8 years of follow-up, we observed 25 cases of colorectal cancer in the 20% of men in the highest category of adiponectin compared with 54 cases of colorectal cancer in the 20% of men in the lowest quintile of adiponectin (20% of men who gave a blood sample corresponds to 3645 men).

Because BMI and other measures of adiposity are partial determinants of adiponectin levels, the multivariable models must be interpreted cautiously. However, the risk estimates varied only slightly between the simple univariate matched model and the multivariable models, suggesting a minimal effect of the additional covariates. Our results did not suggest a linear dose–response relationship. However, the range of adiponectin levels in the lowest quintile was much wider than for the other quintiles, and we cannot rule out a linear relationship at the lower end of the range.

Several studies have recently reported statistically significant associations between obesity and insulin resistance, hyperinsulinemia, and risk of colorectal cancer (8,10). One mechanism by which hyperinsulinemia may increase the risk of colorectal cancer is by reducing circulating levels of IGFBP-1, which may later lead to higher levels of unbound IGF-I. High levels of circulating IGF-I, which increase cellular proliferation and inhibit apoptosis (25,26), have been associated with increased risk of several common cancers, including CRC (9,27,28). We also recently reported that high levels of insulin (as reflected by increased C-peptide) or high levels of bioavailable IGF-I (assessed by the ratio of IGF-I to IGFBP-3) independently predicted increased risk for colorectal cancer; high levels of both were not associated with more risk (10).

Adiponectin may also be associated with other obesity and insulin resistance–related cancers. Previously, two case–control studies reported that risks of two cancers that are associated with body size and adiposity, breast cancer and endometrial cancer, are inversely associated with high plasma adiponectin levels (29–31). Importantly, these associations were independent of measures of adiposity. Moreover, these associations were independent of waist circumference, a measure of central adiposity that has been even more closely related with insulin resistance than BMI, particularly in men (3). Plasma adiponectin levels were also lower in patients with gastric cancer than in control subjects (32). However, none of these previous case–control studies could differentiate between the possibility that adiponectin is involved in the progression of cancer, or, alternatively, that advanced-stage disease leads to lower adiponectin levels (32). The latter possibility can be effectively excluded given the prospective nature and results of stratified analysis of our study, which support an important role of adiponectin in the obesity and insulin-related pathways of carcinogenesis. Some evidence suggests that the two forms of adiponectin, low molecular weight and high molecular weight, have different physiologic effects, particularly with respect to insulin sensitivity (33,34). Assessment of high-molecular-weight adiponectin may prove to be more closely associated with cancer risk; this supposition requires further investigation as methods appropriate for epidemiologic research develop.

Studies of adiponectin knockout mice have shown that such mice exhibit moderate to severe diet-induced insulin resistance (35,36). Adiponectin may affect insulin sensitivity through its ability to activate 5′-adenosine monophosphate kinase, which inhibits the synthesis of IGF-1, increases IGFBP-1 production in the liver, and reduces circulating insulin levels (37). In humans, plasma adiponectin levels are positively correlated with insulin sensitivity after adjusting for sex and measures of adiposity (r = .42, P < .001 for men) (11). Furthermore, low adiponectin levels result in increased insulin resistance, even among nonobese individuals (38), and have thus been implicated in the development of type 2 diabetes (15) and cardiovascular disease (39).

Adiponectin may contribute to carcinogenesis by promoting apoptosis. Adiponectin levels have been associated with the activation of apoptotic enzymes in the caspase cascade, which leads to cell death (40), modulation of the expression of several apoptosis-related genes in myelomonocytic cells (41), and reduction of tumor neovascularization (40).

Our study has both strengths and limitations. The strengths of this study include blood collected prospectively with respect to disease outcome; that we had detailed information on potential confounders, including validated anthropometric measures; and that multiple questionnaires were administered before blood draw to better estimate long-term average dietary intake and average body size. One limitation of this analysis was the availability of only a one-time blood measurement. However, any random error in the assays would tend to lead to underestimates of the true
Our laboratory assays had relatively low intra-assay variation, and a previous study showed that the stability and reliability of a one-time measure (as in this study) to be high (22). Furthermore, the correlation between adiponectin levels and time between blood collection and diagnosis was low ($r = -0.06$), suggesting that adiponectin levels did not vary with duration of blood storage. After excluding case patients who were diagnosed in the first 2 years of after blood collection, the associations remained statistically significant, suggesting that our results were not affected by underlying disease.

Despite these limitations, this is the first prospective study, to our knowledge, on adiponectin in relation to colorectal malignancy and supports the hypothesis that low adiponectin levels are not simply a consequence of cancer. In conclusion, in this prospective nested case–control study, plasma adiponectin levels were inversely associated with risk for colorectal cancer in men. Individuals in the lowest quintile had about a twofold increased risk for colorectal cancer compared with all other quintiles. This association was independent of BMI, waist circumference, waist-to-hip ratio, and physical activity. More studies to confirm and expand on these findings, particularly among women, should be undertaken, and mechanistic studies to fully elucidate the mechanisms underlying adiponectin’s effects are warranted.

### References


NOTES

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