Background and Purpose—The favorable cardiovascular effects attributed to adiponectin may lower risk of stroke. We investigated this in a prospective study and meta-analysis.

Methods—A case–cohort study nested within the Potsdam cohort of the European Prospective Investigation into Cancer was performed, with 170 incident cases of ischemic stroke and a randomly selected subcohort of 2155 participants without major cardiovascular disease at baseline. A random-effects dose–response meta-analysis was performed on prospective studies reporting on adiponectin and incident stroke in healthy populations up to April 2013, identified through MEDLINE and EMBASE.

Results—In European Prospective Investigation into Cancer-Potsdam, after adjustment for cardiovascular risk factors, the hazard ratio of ischemic stroke per 5-µg/mL higher total-adiponectin was 1.10 (95% confidence interval, 0.89–1.37). Participants with higher total-adiponectin had higher high-density lipoprotein-cholesterol and lower high-sensitivity C-reactive protein and triglyceride levels, and had less often diabetes mellitus. Additional adjustment for these putative mediators yielded a hazard ratio of 1.31 (95% confidence interval, 1.04–1.64). Nine studies (19259 participants, 2960 cases), including European Prospective Investigation into Cancer-Potsdam, were meta-analyzed. Pooling relative risks adjusted for cardiovascular risk factors not including putative mediators indicated moderate between-study heterogeneity (I²=52.2%). This was explained by the smallest study, and the pooled relative risk (95% confidence interval) before and after its exclusion was 1.03 (0.98–1.08) and 0.99 (0.96–1.01) per 5 µg/mL, respectively. The pooled relative risk (95% confidence interval) additionally adjusted for potential mediators was 1.08 (1.01–1.15) and 1.05 (1.00–1.11) before and after excluding the same study, respectively.

Conclusions—Adiponectin is not associated with risk of stroke. If anything, adiponectin relates directly to stroke risk after controlling for risk factors that favorably correlate with adiponectin. (Stroke. 2014;45:10-17.)

Key Words: adiponectin ■ meta-analysis ■ myocardial infarction ■ stroke

Adiponectin is a hormone derived from adipose tissue. Its levels are usually higher in women than in men and are downregulated with increasing central adiposity.1 Adiponectin has been suggested to exert anti-inflammatory, antiatherogenic, and insulin-sensitizing effects;2 to promote high-density lipoprotein-cholesterol formation;3 to reduce plasma triglyceride levels;4 and it has been inversely associated with carotid intima-media thickness.5,6 Total-adiponectin circulates in the blood stream as globular-adiponectin, and as full-length fragments of low-molecular weight, medium-molecular weight, and high-molecular weight (HMW). HMW adiponectin has been proposed as the most active fraction for glucose homeostasis,2 whereas the lower weight fractions have been associated with anti-inflammatory effects.7

The favorable cardiovascular effects attributed to adiponectin may lower risk of stroke. We evaluated this in the Potsdam cohort of the European Prospective Investigation into Cancer (EPIC). Because of the antiatherogenic properties of adiponectin, we focused on ischemic stroke (IS). To assess the consistency of the association between adiponectin and risk of stroke in apparently healthy populations, we also performed a meta-analysis on prospective studies.
Methods

**EPIC-Potsdam Study**

**Study Population**
EPIC-Potsdam study comprises 27,548 individuals from the general population of the Potsdam area (Germany). The association of plasma total-adiponectin with risk of IS was analyzed using a case-cohort design. The study-set included all incident IS cases that occurred during a mean follow-up of 8.2±2.2 years and a random subcohort (n=2500). When both IS and myocardial infarction (MI) occurred in the same participant (n=5), we only considered the first event. IS occurred before MI in 2 cases and MI before IS in 2 more. One participant had a stroke and MI on the same day; the MI was prioritized over stroke. After exclusion of individuals because of prevalent IS or MI, missing follow-up, or missing adiponectin or covariate information, the final study population comprised a subcohort of 2155 participants and 170 IS cases (11 being fatal and 27 belonging to the subcohort). A total of 605 participants were fasting for 28 hours before the blood draw. Informed consent was obtained from all study participants. The study was approved by the Ethical Committee of the state of Brandenburg, Germany.

**Biochemical Analyses**
Baseline plasma levels of biomarkers were measured at Tübingen University (Germany) in samples retrieved from frozen storage (2007–2008). Adiponectin was determined with an ELISA (Linco Research, St Charles, MO; intra-assay and interassay coefficients of variation, 0.1%–6.2% and 5.0%, respectively). High-density lipoprotein-cholesterol, triglycerides, high-sensitivity C-reactive protein, and creatinine were determined with the Siemens ADVIA 1650. N-terminal probrain-type natriuretic peptide was measured in all incident IS cases and in a random subsample of 1137 subcohort members (attributable to financial restrictions) at the Institute of Clinical Chemistry, University of Magdeburg (2012) with an electrochemiluminescence immunoassay on the Immulite analyzer (Siemens AG, Munich, Germany).

**Statistical Analysis**
Because women had higher total-adiponectin levels than men, baseline characteristics were cross-sectionally compared across sex-specific total-adiponectin tertiles based on the distribution among the subcohort, by age-adjusted ANOVA. Association of total-adiponectin with risk of IS was calculated as hazard ratios using Cox proportional-hazard regression, modified according to Prentice, using a robust estimator to compute the 95% confidence interval. Age was the underlying time metric. HRs were estimated per 5-µg/mL higher total-adiponectin and according to sex-specific tertiles of total-adiponectin in the subcohort. HRs were adjusted for MI (M1); further for common cardiovascular risk factors. After additional adjustment for prevalent IS or MI, no significant associations were found after adjustment for sex and common cardiovascular risk factors. After additional adjustment for potential mediators, including high-density lipoprotein-cholesterol, high-sensitivity C-reactive protein, and diabetes mellitus, adiponectin was directly associated with IS risk. Adjustment for creatinine, a crude estimate of renal function, yielded essentially similar HRs.

**Meta-Analysis of Prospective Studies**
Meta-analysis of Observational Studies in Epidemiology guidelines were applied (Table II in the online-only Data Supplement). Two investigators independently searched MEDLINE and EMBASE for prospective studies performed in healthy populations, with adiponectin as exposure (total or either of its circulating fractions) and stroke (total stroke or IS) as outcome, published until April 2013. Search terms used in MEDLINE were (Adiponectin[Mesh] or adiponectin or acrp30 or apm) and (Cerebrovascular Disorders[Mesh] or stroke or brain infarction) and (Longitudinal Studies[Mesh] or prospective[Mesh] or prospective or nested or cohort). The equivalent search was constructed for EMBASE. Reference lists of retrieved articles were hand-searched for additional studies. Data gathered included: first author, publication year, location, study design, race/ethnicity, number of participants, proportion of women, duration of follow-up, age, adiponectin assay, mean adiponectin levels and body mass index among noncases, number of cases, case ascertainment, variables controlled for and comparison used.

**Results**

**EPIC-Potsdam Study**

**Baseline Characteristics of Participants**
Baseline median (interquartile range) total-adiponectin levels in men and women were 5.6 (4.15–7.36) and 8.6 (6.32–11.40) µg/mL, respectively. Baseline characteristics of the subcohort across adiponectin tertiles are shown in Table 1 separately for men and women. Compared with those in the lower tertiles, men and women in the highest tertiles were slightly older and showed better cardiovascular profiles.

**Adiponectin and Incident Stroke**
Table 2 shows HRs of incident IS per 5-µg/mL higher total-adiponectin, and across sex-specific tertiles of adiponectin. No significant associations were found after adjustment for sex and common cardiovascular risk factors. After additional adjustment for potential mediators, including high-density lipoprotein-cholesterol, high-sensitivity C-reactive protein, and diabetes mellitus, adiponectin was directly associated with IS risk. Adjustment for creatinine, a crude estimate of renal function, yielded essentially similar HRs. Adjustment for N-terminal probrain-type natriuretic peptide, a discerning marker for heart damage, only slightly attenuated the HRs (data not shown). The association between adiponectin and stroke did not differ by sex (P interaction=0.4) or age (P interaction=0.1) after adjustment for variables in M2 (Table III in the online-only Data Supplement). HRs remained similar after exclusion of 11 fatal cases of IS. Also, none of the other prespecified sensitivity analyses led to different results.
Meta-Analysis of Prospective Studies

Sixty-four hits from EMBASE and 82 from MEDLINE were retrieved. After exclusion of duplicates and of articles that did not meet the inclusion criteria (Figure I in the online-only Data Supplement), 9 articles11,13–20 reporting results for 8 independent study populations were eligible for inclusion. Nine studies,11,13–16,18–20 including EPIC-Potsdam, reported on total-adiponectin. The Cardiovascular Health Study11 and the Women’s Health Initiative Study17,18 reported also on HMW-adiponectin, with findings being similar to total-adiponectin. Therefore, our analysis includes risk estimates of total-adiponectin only. Thus, together with the EPIC-Potsdam study, ≤9 independent studies were meta-analyzed (Table 3) in total 259 participants and 2960 incident stroke cases.

| Table 1. Baseline Characteristics of the EPIC-Potsdam Subcohort by Sex-Specific Adiponectin Tertiles |
|--------------------------------------------------|--|---|--|---|
| **Men**                                          | **Women**                          |
| **n**                                            | 268 | 268 | 268 | 450 | 451 | 450 |
| Adiponectin mean (range), µg/mL                   | 3.5 (1.2–4.5) | 5.6 (4.6–6.6) | 9.4 (6.7–26.3) | ... | 5.3 (0.6–7.1) | 8.6 (7.2–10.2) | 13.5 (10.3–33.0) | ... |
| Age, y                                           | 51.1±0.49 | 52.3±0.49 | 53.0±0.49 | 0.005 | 47.7±0.44 | 49.0±0.44 | 50.0±0.44 | <0.001 |
| Education (%)                                     | ... | ... | ... | ... | ... | ... | ... | ... |
| Vocational school or less                         | 28.9 | 30.63 | 31.54 | 0.32 | 44.39 | 38.74 | 39.67 | 0.01 |
| Technical school                                  | 13.2 | 16.03 | 18.19 | 0.03 | 29.39 | 31.68 | 28.19 | 0.96 |
| University degree                                 | 58.0 | 53.34 | 50.27 | 0.01 | 26.22 | 29.58 | 32.14 | 0.005 |
| Physical activity <2 h/wk                         | 75.1 | 75.62 | 79.54 | 0.92 | 78.76 | 77.12 | 72.84 | 0.03 |
| Smoking status, %                                 | ... | ... | ... | ... | ... | ... | ... | ... |
| Current ≥20 cigarettes/d                          | 27.9 | 28.4 | 31.4 | 0.90 | 57.15 | 58.87 | 57.41 | 0.007 |
| Current <20 cigarettes/d                          | 34.2 | 38.6 | 34.6 | 0.86 | 14.55 | 17.32 | 20.53 | 0.13 |
| Former ≤5 y                                      | 9.3 | 7.2 | 8.5 | 0.93 | 6.47 | 8.02 | 6.83 | 0.95 |
| Former >5 y                                      | 16.6 | 16.8 | 14.7 | 0.66 | 17.11 | 12.67 | 13.08 | 0.005 |
| Never                                            | 12.0 | 9.0 | 10.7 | 0.86 | 4.7 | 3.1 | 2.2 | 0.95 |
| Alcohol intake, %                                 | ... | ... | ... | ... | ... | ... | ... | ... |
| 0 g/d                                            | 0 | 0 | 0 | ... | 0.0 | 0.0 | 0.0 | ... |
| >0–12 g/d                                        | 39.2 | 38.0 | 34.7 | 0.16 | 56.1 | 55.2 | 48.8 | 0.003 |
| >12–24 g/d                                       | 25.9 | 27.3 | 26.7 | 0.96 | 23.2 | 24.4 | 24.8 | 0.04 |
| >24 g/d                                          | 34.8 | 34.7 | 38.6 | 0.17 | 20.7 | 20.4 | 26.4 | 0.15 |
| Prevalent diabetes mellitus, %                   | 6.4 | 7.0 | 3.4 | 0.13 | 5.7 | 2 | 1.4 | 0.003 |
| Prevalent hypertension, %                        | 63.3 | 57.2 | 52.2 | 0.001 | 51.2 | 38.8 | 33.6 | <0.001 |
| Use of medication, %                             | ... | ... | ... | ... | ... | ... | ... | ... |
| Antihypertensive                                  | 18.8 | 22.1 | 17.3 | 0.57 | 26.3 | 14.5 | 10.9 | <0.001 |
| Lipid-lowering medication                        | 6.6 | 3.3 | 5.0 | 0.82 | 4.9 | 3.9 | 2.2 | 0.10 |
| Antidiabetic                                      | 3.6 | 3.3 | 2.4 | 0.33 | 3.6 | 0.9 | 0.4 | 0.002 |
| Body mass index, kg/m²                            | 27.3±0.21 | 26.99±0.21 | 25.73±0.21 | <0.001 | 27.4±0.20 | 25.4±0.20 | 24.4±0.20 | <0.001 |
| Waist circumference, cm                           | 95.6±0.58 | 94.8±0.58 | 91.2±0.58 | <0.001 | 85.3±0.50 | 80.3±0.49 | 76.7±0.50 | <0.001 |
| HDL-cholesterol, mg/dL                            | 43.4±0.74 | 45.8±0.74 | 52.0±0.74 | <0.001 | 50.7±0.62 | 56.1±0.62 | 62.1±0.62 | <0.001 |
| Triglycerides, mg/dL†                             | 130.7 | 106.5 | 88.4 | <0.001 | 100.2 | 83.8 | 65.8 | <0.001 |
| hs-CRP, mg/L                                      | (114.1–149.7) | (93.2–121.8) | (77.9–100.4) | (91.9–109.2) | (77.2–90.9) | (60.8–71.2) | ... | ... |
| Creatinine, mg/dL‡                                | 0.76 (0.65–0.89) | 0.63 (0.54–0.74) | 0.51 (0.44–0.60) | 0.004 | 1.22 (1.08–1.39) | 0.76 (0.67–0.86) | 0.60 (0.53–0.68) | <0.001 |
| NTproBNP, pg/mL§                                  | 55.9 (47.9–65.4) | 55.0 (47.0–64.4) | 66.7 (57.7–77.1) | 0.01 | 68.7 (63.2–74.6) | 64.7 (59.7–70.2) | 72.1 (66.8–77.9) | 0.13 |

Shown are mean±SE or geometric mean (95% confidence interval), unless otherwise indicated. EPIC indicates European Prospective Investigation into Cancer; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; and NTproBNP, N-terminal probrain-type natriuretic peptide.

*P value based on modeling adiponectin as a continuous variable. All variables other than age are adjusted for age.
†Based on 235 men or 370 women in fasting status.
‡Based on 2112 observations.
§Based on 1137 observations.
(multiple ethnic groups),\textsuperscript{11,17–19} and included men,\textsuperscript{13,15,20} women,\textsuperscript{17,18} or both.\textsuperscript{11,14,16,19} Mean age ranged from 47 to 74\textsuperscript{11} and follow-up time from 4.9 to 27\textsuperscript{13} years. Adiponectin was measured in serum\textsuperscript{17} or plasma.\textsuperscript{11,13–16,18–20} Incident stroke was ascertained by review of medical records or death certificates in all studies. Relative risks (RR) were provided as odds ratio\textsuperscript{14,16–18} or hazard ratios.\textsuperscript{11,13,15,19,20} Odds ratios were assumed to reasonably approximate the RR.

<table>
<thead>
<tr>
<th>Table 3. Characteristics of Prospective Studies Reporting on the Association Between Adiponectin and Incident Stroke in Healthy Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Author, Year of Publication</td>
</tr>
<tr>
<td>Soderberg, 2004\textsuperscript{14}</td>
</tr>
<tr>
<td>Matsumoto, 2008\textsuperscript{16}</td>
</tr>
<tr>
<td>Khalili, 2010\textsuperscript{13}</td>
</tr>
<tr>
<td>Rajpathak, 2011\textsuperscript{19}</td>
</tr>
<tr>
<td>Prugger, 2012\textsuperscript{15}</td>
</tr>
<tr>
<td>Gardener, 2013\textsuperscript{10}</td>
</tr>
<tr>
<td>Kizer, 2013\textsuperscript{11}</td>
</tr>
<tr>
<td>Wannamethee, 2013\textsuperscript{17}</td>
</tr>
<tr>
<td>Arregui, 2013, current study</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; IS, ischemic stroke; NR, not reported; and TS, total stroke. 
*Based on a method using monoclonal antibodies and recombinant human adiponectin.
Studies evaluated adiponectin in quartiles, quintiles, and as a continuous variable, either untransformed or log-transformed (Table 4). Outcomes were total stroke, IS, or both. For 1 study that reported RR only for a subgroup of smokers, we calculated the crude odds ratio from the reported contingency table including participants. One study provided separate RR for men and women (personal correspondence), and these were included in the analysis as separate RR. For another study, we used a fixed-effects meta-analysis to combine the RRs corresponding to before and after the knot of the spline-regression. Before and after adjustment for potential mediators, adiponectin was significantly related to stroke risk only in 1 and 3 studies, including EPIC-Potsdam, respectively.

The Figure (A) shows the pooled multivariable-adjusted RR without adjustment for mediators. Initially, we observed moderate heterogeneity across study results ($I^2=52.2\%$; $P=0.027$). This was mainly driven by the smallest study, which after exclusion reduced the $I^2$ statistic to 3.2% ($P=0.41$) and yielded a pooled RR of 0.99 (95% confidence interval, 0.96–1.01) per 5-µg/mL higher adiponectin. Similar to EPIC-Potsdam, combining RR additionally adjusted for potential mediators

### Table 4. Relative Risks of Stroke According to Adiponectin Levels in Prospective Studies Performed in Healthy Populations

<table>
<thead>
<tr>
<th>Study, Year</th>
<th>Comparison, µg/mL</th>
<th>Model</th>
<th>Outcome, Relative Risk (95% CI)</th>
<th>Controlled Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soderberg, 2004</td>
<td>Top vs bottom quartile; men: ≥17.3 vs &lt;8.3; women: ≥27.6 vs &lt;14.5</td>
<td>M1</td>
<td>TS, men: 0.89 (0.50–1.60); women: 0.77 (0.41–1.44)</td>
<td>Sex, age, date/type of health survey, region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>TS, men: 1.04 (0.56–1.94); women: 0.82 (0.43–1.55)</td>
<td>M1+BMI, smoking, hypertension</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>TS, men: 1.08 (0.56–2.08); women: 0.85 (0.44–1.63)</td>
<td>M2+cholesterol, diabetes mellitus</td>
</tr>
<tr>
<td>Matsumoto, 2008</td>
<td>Top vs bottom quartile; &lt;5.6 vs ≥12.4</td>
<td>M1</td>
<td>TS: 0.67 (0.40–1.11); IS: 0.49 (0.26–0.92)</td>
<td>Age, sex, community</td>
</tr>
<tr>
<td>Khalili, 2010</td>
<td>Top (median, 16.57) vs bottom (median, 2.37) quintile</td>
<td></td>
<td>IS: 0.98 (0.65–1.47)</td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Rajpathak, 2011</td>
<td>Top (median, 46.0) vs bottom (median, 14.8) quartile</td>
<td>M1</td>
<td>IS: 0.77 (0.59–1.01)</td>
<td>Age, race/ethnicity, date of study enrollment, follow-up time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>IS: 0.81 (0.61–1.08)</td>
<td>M1+BMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>IS: 1.16 (0.82–1.63)</td>
<td>M2+current smoking, physical activity, nonsteroidal anti-inflammatory drug use, hypertension medication use, SBP, history of coronary and artery diseases, HDL-cholesterol, triglycerides, diabetes mellitus, WC</td>
</tr>
<tr>
<td>Prugger, 2012</td>
<td>Per SD-increase (11.6 µg/mL)</td>
<td>M1</td>
<td>IS: 2.05 (1.38–3.05)</td>
<td>Age, study center, date of examination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>IS: 1.99 (1.29–3.07)</td>
<td>M1+SBP, antihypertensive treatment, smoking, alcohol drinking</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>IS: 2.18 (1.37–3.48)</td>
<td>M2+total-cholesterol, HDL-cholesterol, WC, diabetes mellitus, hs-CRP</td>
</tr>
<tr>
<td>Gardener, 2013</td>
<td>Top (median, 33.6) vs bottom (median, 4.6) quartile</td>
<td>M1</td>
<td>TS: 1.14 (0.79–1.61)</td>
<td>Age, sex, race/ethnicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>TS: 1.47 (0.96–2.22)</td>
<td>M1+smoking, hypertension, diabetes mellitus, LDL-cholesterol, HDL-cholesterol, triglycerides, WC, moderate alcohol use, moderate-heavy physical activity, previous cardiac disease history</td>
</tr>
<tr>
<td>Kizer, 2013</td>
<td>Per SD (7.9) increase</td>
<td>M1</td>
<td>IS, &lt;20 µg/mL: 0.97 (0.82–1.15), ≥20 µg/mL: 1.14 (0.97–1.36)</td>
<td>Age, sex, race</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>IS, &lt;20 µg/mL: 0.91 (0.76–1.09), ≥20 µg/mL: 1.13 (0.95–1.34)</td>
<td>M1+BMI, income, education, center, smoking status, alcohol use, SBP, antihypertensive medication, estrogen replacement therapy, eGFR, aspirin use, health status, albumin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>IS, &lt;20 µg/mL: 1.07 (0.88–1.30), ≥20 µg/mL: 1.15 (0.96–1.37)</td>
<td>M2+subclinical CVD, pre/diabetes mellitus, LDL-cholesterol, HDL-cholesterol, triglycerides, hs-CRP</td>
</tr>
<tr>
<td>Wannamethee, 2013</td>
<td>Top vs bottom quartile; ≥10.8 vs &lt;4.3</td>
<td>M1</td>
<td>TS: 0.74 (0.49–1.12)</td>
<td>Age, BMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>TS: 0.73 (0.48–1.10)</td>
<td>M1+diabetes mellitus, angina, atrial fibrillation, smoking, social class, alcohol intake, physical activity, lung function, SBP, use of antihypertensive drugs</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; CI, confidence interval; CVD, cardiovascular diseases; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HMW, high-molecular weight; hs-CRP, high-sensitivity C-reactive protein; IS, ischemic stroke; LDL, low-density lipoprotein; M, model; SBP, systolic blood pressure; TS, total stroke; and WC, waist circumference.

*Based on 2091 participants.
Overall (I−squared = 52.2%, p = 0.027)

Khalili, 2010
Rajpathak, 2011
Matsumoto, 2008
Prugger, 2012
Gardener, 2013
Kizer, 2013
Wannamethee, 2013
Prugger, 2012
Söderberg, 2004 (women)

Overall (I−squared = 63.1%, p = 0.006)

Söderberg, 2004 (men)
Söderberg, 2004 (women)
Matsumoto, 2008
Khalili, 2010
Rajpathak, 2011
Prugger, 2012
Gardener, 2013
Kizer, 2013
Wannamethee, 2013
Arregui 2013, current study

Figure. Most completely adjusted relative risks (RRs) of stroke per 5-μg/mL higher circulating adiponectin in individual studies and combined across studies in dose–response random-effects meta-analysis before (A) and after (B) adjustment for potential intermediate factors.
available for 8 independent studies (Figure [B]) yielded a direct association between adiponectin and stroke (RR, 1.08; 95% confidence interval, 1.01–1.15) per 5-µg/mL higher adiponectin. However, also here we observed moderate between-study heterogeneity (I²=63.1%; P=0.006). After omitting the smallest study, the I² became 45.1% (P=0.08) and the pooled RR 1.05 (95% confidence interval, 1.00–1.11). The lack of symmetry of the funnel plot as well as Egger test (P<0.05) suggested lack of smaller studies reporting an inverse association between adiponectin and stroke.

Discussion
In EPIC-Potsdam, higher levels of total-adiponectin were associated with better metabolic profiles. In contrast, total-adiponectin tended to be positively associated with IS risk. Interestingly, this association became stronger and significant after controlling for metabolic markers that have been suggested to be intermediates. Although (putative) overadjustment may have produced spurious results, unknown pathways underlying the increased risk cannot be excluded. Results of our meta-analysis based on 9 independent prospective studies extend those from 2 recent meta-analyses on the relationship between adiponectin and stroke, which included only 26 and 3 studies.23 Our meta-analysis indicated lack of association between adiponectin and stroke risk. If anything, adiponectin was directly related to stroke risk after controlling for metabolic factors that favorably correlate with adiponectin.

A trend toward increased risk of coronary heart disease11 and cardiovascular death21,24 associated with higher adiponectin despite better metabolic profiles has also previously been observed in studies in older populations. One possible explanation is that the progression of cardiovascular diseases may lead to adiponectin resistance.23 In the EPIC-Potsdam study, we excluded participants with a clinical history of major cardiovascular disease at baseline. We also assessed the statistical analysis by 4 years to account for latency, which did not alter our results. It has also been suggested that the presence of diseases prompting to a hypercatabolic state may induce compensatory increased adiponectin levels.26 Our findings, however, did not substantially change after excluding prevalent cases of several chronic diseases or controlling for creatinine, and adjusting for N-terminal probrain-type natriuretic peptide only slightly attenuated the risk estimates. Also, the better metabolic profiles associated with higher total-adiponectin would argue against these explanations. We focused on total-adiponectin and not on HMW-adiponectin, which is thought to be the most biologically active form.27 Nevertheless, total and HMW-adiponectin have shown to be highly correlated,11 and recent findings do not support HMW-adiponectin to be more strongly related to risk of stroke11 or coronary heart disease.28 Adiponectin levels increase with age, and this could be a critical factor in assessing the associations between adiponectin and cardiovascular end points. However, age did not modify the association between adiponectin and incident stroke in our study population, which age was mostly between 35 and 65 years. Finally, it has been suggested that very high levels of adiponectin could have harmful effects by means of the activation of complement mechanisms.29

Limitations of our prospective study include that a single assessment of adiponectin may be susceptible to within-individual variation. Adiponectin concentrations, however, have shown to be quite stable over time.30 Furthermore, only a third of the study population was in fasting status. However, adiponectin values did not differ according to fasting status, and adjusting for it did not affect our risk estimates.

The meta-analysis included studies that differed in population characteristics, methods, variables controlled for, and outcomes considered (total stroke/IS). However, we did not find that these factors influenced results across studies. Although we observed a potential risk for publication bias, it may be unnecessary to evaluate the existence of preferential publication when very few studies reported significant results.31

Summary
This prospective study and meta-analysis shows that circulating total-adiponectin does not relate to risk of stroke. If anything, adiponectin relates directly to stroke risk after controlling for metabolic factors that correlate favorably with adiponectin. The number of studies on HMW-adiponectin is limited, and no studies on other adiponectin fractions are available. Therefore, it remains unclear whether specific molecular-weight fractions of adiponectin may influence risk of stroke.

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Disclosures
None.

References


Adiponectin and Risk of Stroke: Prospective Study and Meta-analysis
Maria Arregui, Brian Buijsse, Andreas Fritsche, Romina di Giuseppe, Matthias B. Schulze, Sabine Westphal, Berend Isermann, Heiner Boeing and Cornelia Weikert

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Online Data Supplement

Supplementary Table I. Association of circulating total-adiponectin with incident myocardial infarction: the EPIC-Potsdam Study

<table>
<thead>
<tr>
<th>HR (95% CI) per 5-µg/ml higher total-adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI cases (n)</td>
</tr>
<tr>
<td>Model 1*</td>
</tr>
<tr>
<td>Model 2 †</td>
</tr>
<tr>
<td>Model 3 ‡</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR: hazard ratio, MI myocardial infarction. * Model 1: Derived from Prentice-weighted Cox proportional-hazards regression models, with age as underlying time variable, stratified by age at baseline. † Model 2: as model 1 with further adjustments for waist circumference, smoking status (never smoker, former smoker, current smoker <20 cigarettes per day, current smoker ≥20 cigarettes per day), sports activity (<2 h/week, ≥2 h/week), education (vocational school or less, technical school, university), alcohol consumption (men: 0, >0-12, >12-24, >24; women: 0, >0-6, >6-12, >12 g/day), and prevalent hypertension. ‡ Model 3: As model 2 with further adjustments for fasting status (yes/no), prevalent diabetes, HDL-cholesterol, triglycerides, and hs-CRP.
Supplementary table II. MOOSE Checklist

<table>
<thead>
<tr>
<th>Reported on section (page)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reporting of background</strong></td>
<td></td>
</tr>
<tr>
<td>Problem definition</td>
<td>Introduction (2)</td>
</tr>
<tr>
<td>Hypothesis statement</td>
<td>Introduction (2)</td>
</tr>
<tr>
<td>Description of study outcomes</td>
<td>Introduction (2)</td>
</tr>
<tr>
<td>Type of exposure</td>
<td>Introduction (2)</td>
</tr>
<tr>
<td>Type of study designs used</td>
<td>Introduction (2)</td>
</tr>
<tr>
<td>Study population</td>
<td>Introduction (2)</td>
</tr>
<tr>
<td><strong>Reporting of search strategy</strong></td>
<td></td>
</tr>
<tr>
<td>Qualifications of searchers</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
</tr>
<tr>
<td>Search strategy, including time period used in the synthesis and key words</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
</tr>
<tr>
<td>Effort to include all available studies</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
</tr>
<tr>
<td>Databases and registries searched</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
</tr>
<tr>
<td>Search software used</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
</tr>
<tr>
<td>Use of hand searching</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
</tr>
<tr>
<td>List of citations located and</td>
<td>Supplementary figure</td>
</tr>
</tbody>
</table>
those excluded, including justification

- Duplicates excluded: n = 42
- Was not a prospective study: n = 1
- Adiponectin was not the exposure: n = 43
- Stroke was not the outcome: n = 24
- Participants had already suffered a stroke: n = 6
- Not healthy populations: n = 17
- Review or meta-analysis: n = 4
- Duplicity of study-population: n = 1

Method of addressing articles published in languages other than English

No language restrictions were applied, however no relevant studies in languages other than English were retrieved

Method of handling abstracts and unpublished studies

No limits on type of publication or publication status were applied

Description of any contact with authors

Authors of three studies were approached twice in written form to inquire additional information regarding risk estimates

### Reporting of methods

<table>
<thead>
<tr>
<th>Description of relevance or appropriateness of studies</th>
<th>Methods-Systematic-review and meta-analysis (5)</th>
<th>Only prospective studies conducted in healthy populations, with adiponectin as exposure (total or either of its circulating fractions) and incident stroke (total or ischemic) as outcome, published until April 2013 were included</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rationale for the selection and coding of data</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
<td>Data extracted were relevant to the population characteristics, study design, exposure and outcome. Data gathered included: first author, publication year, location, study-design, race/ethnicity, number of participants, proportion of women, duration of follow-up, age, adiponectin assay, mean adiponectin levels and BMI among non-cases, number of cases, case ascertainment, variables controlled for and comparison used. Effect estimates were extracted for the least, and most (where possible two models: with and without inclusion of presumed mediators) completely adjusting models</td>
</tr>
<tr>
<td>Documentation of how data were classified and coded</td>
<td>Methods-Systematic-review and meta-analysis (4)</td>
<td>Two investigators independently searched MEDLINE and EMBASE databases for relevant studies, and extracted the necessary data from them to fill in a standardized table of characteristics in a spreadsheet</td>
</tr>
<tr>
<td>Assessment of confounding</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
<td>From each study information regarding possible sources of confounding, such as study-design, race/ethnicity, proportion of women, duration of follow-up, age, assay used to asses adiponectin, mean adiponectin levels and BMI among non-cases, number of cases, case ascertainment, variables controlled for and comparison used was extracted. Also, effect estimates were extracted for the least, and most (where possible two models: with and without inclusion of presumed mediators: HDL, hs-CRP, triglycerides, diabetes) completely adjusting models</td>
</tr>
<tr>
<td>Assessment of study quality</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
<td>The influence of each study on the pooled estimate and I² was assessed by omitting 1 study at a time</td>
</tr>
<tr>
<td>Assessment of heterogeneity</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
<td>Heterogeneity between studies was evaluated by using the $I^2$ statistic</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------------------------------------------------</td>
</tr>
<tr>
<td>Description of statistical methods in sufficient detail to be replicated</td>
<td>Methods-Systematic-review and meta-analysis (5)</td>
<td>Study-specific dose-response associations were calculated per 5-µg/ml higher adiponectin concentration by means of the generalized least squares for trend estimation method, to allow for the combination of comparable estimates with random-effect meta-analysis. Heterogeneity between studies was evaluated by using the $I^2$ statistics. Publication bias was investigated by Egger's test besides funnel plot's visual inspection. The influence of each study on the pooled estimate and on $I^2$ was assessed by omitting 1 study at a time</td>
</tr>
<tr>
<td>Provision of appropriate tables and graphics</td>
<td>Tables 3-4 Figure 1</td>
<td><strong>Table 3.</strong> Characteristics of prospective studies investigating the association of adiponectin and incident stroke in apparently healthy populations <strong>Table 4.</strong> Relative Risks of stroke according to adiponectin levels in prospective studies conducted in healthy populations <strong>Figure 1.</strong> Relative risks and 95% confidence intervals of stroke per 5-µg/mL-increase of adiponectin across studies. RR for most adjusted models before (A) and after (B) potential intermediates</td>
</tr>
<tr>
<td>Reporting of results</td>
<td></td>
<td><strong>Figure 1.</strong> Relative risks and 95% confidence intervals of stroke per 5 µg/mL-increase of adiponectin across studies. RR for most adjusted models without (A) and with (B) potential intermediates</td>
</tr>
<tr>
<td>Graphic summarizing individual study estimates and overall estimate</td>
<td>Figure 1</td>
<td><strong>Table 3.</strong> Characteristics of prospective studies investigating the association of adiponectin and incident stroke in apparently healthy populations</td>
</tr>
<tr>
<td>Table giving descriptive information for each study included</td>
<td>Table 3</td>
<td><strong>Figure 1.</strong> Relative risks and 95% confidence intervals of stroke per 5-µg/mL-increase of adiponectin across studies. RR for most adjusted models without (A) and with (B) potential intermediates</td>
</tr>
<tr>
<td>Results of sensitivity testing</td>
<td>Results-Systematic-review and meta-analysis (7-8) Figure 1</td>
<td>Sensitivity analysis revealed that the high heterogeneity observed was mainly driven by the smallest study.</td>
</tr>
<tr>
<td>Indication of statistical uncertainty of findings</td>
<td>Results-Systematic-review and meta-analysis Figure 1</td>
<td>95% confidence intervals were presented with all summary estimates</td>
</tr>
<tr>
<td>Reporting of discussion</td>
<td></td>
<td>The asymmetry of the funnel plot and Egger's test results (P&lt;0.05) suggested lack of smaller studies reporting an an inverse association between adiponectin and stroke.</td>
</tr>
<tr>
<td>Quantitative assessment of bias</td>
<td>Results-Systematic-review and meta-analysis (8)</td>
<td>Duplicates excluded: n = 42 Was not a prospective study: n = 1 Adiponectin was not the exposure: n = 43 Stroke was not the outcome: n = 24 Participants had already suffer a stroke: n = 6 Not healthy populations: n = 17 Review or meta-analysis: n = 4 Articles excluded due to duplicity of study population: n = 1</td>
</tr>
<tr>
<td>Justification for exclusion</td>
<td>Supplementary figure I</td>
<td></td>
</tr>
<tr>
<td>Assessment of</td>
<td>Results-</td>
<td>We discussed the potential reasons for the observed</td>
</tr>
<tr>
<td>Quality of Included Studies</td>
<td>Systematic Review and Meta-analysis (7)</td>
<td>Heterogeneity. There was strong diversity among studies in terms of populations, methodology, variables controlled for and outcomes considered, however it did not seem to strongly influence results across studies, as they were mostly quite consistent. Sensitivity analysis revealed that the high heterogeneity observed was mainly driven by the smallest study.</td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
<td>Reporting of Conclusions</td>
<td>Summary (9)</td>
<td>There was large diversity among studies in terms of populations, methodology, variables controlled for and outcomes considered (TS/IS). Although we did not find that these factors influenced results across studies, this may be better investigated in a future larger meta-analysis.</td>
</tr>
<tr>
<td>Generalization of the Conclusions</td>
<td>Summary (10)</td>
<td>Current evidence does not support a relationship between total adiponectin and risk of stroke. The number of studies on HMW-adiponectin and no studies reported on other adiponectin fractions. Therefore, it remains unclear whether specific molecular-weight fractions of adiponectin may influence risk of stroke.</td>
</tr>
<tr>
<td>Guidelines for Future Research</td>
<td>Summary (9)</td>
<td>More studies on specific molecular weight fractions of adiponectin may shed further light on the role of adiponectin and risk of stroke.</td>
</tr>
<tr>
<td>Disclosure of Funding Source</td>
<td>Sources of Funding (10)</td>
<td>German Federal Ministry of Education; German Federal Ministry of Science (01 EA 9401); European Union (SOC 95201408 05F02 and SOC 98200769 05F02). German Cancer Aid (70-2488-Ha 1).</td>
</tr>
</tbody>
</table>
**Supplementary table III.** Association of circulating total-adiponectin with incident ischemic stroke: the EPIC-Potsdam study. Analysis stratified by sex and by age using 60y as cut-point.

<table>
<thead>
<tr>
<th>HR (95% CI) 5-µg/ml-increase in total-adiponectin</th>
<th>Sex strata</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases/non-cases (n)</td>
<td>90/786</td>
<td>80/1342</td>
<td></td>
</tr>
<tr>
<td>Model 1*</td>
<td>0.97 (0.69-1.37)</td>
<td>1.09 (0.85-1.40)</td>
<td></td>
</tr>
<tr>
<td>Model 2†</td>
<td>0.93 (0.66-1.31)</td>
<td>1.17 (0.92-1.50)</td>
<td></td>
</tr>
<tr>
<td>Model 3‡</td>
<td>1.15 (0.76-1.73)</td>
<td>1.30 (1.02-1.67)</td>
<td></td>
</tr>
<tr>
<td>Age strata</td>
<td>Age≤60 y</td>
<td>Age&gt;60 y</td>
<td></td>
</tr>
<tr>
<td>Cases/non-cases (n)</td>
<td>97/1753</td>
<td>73/375</td>
<td></td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.08 (0.81-1.44)</td>
<td>1.02 (0.75-1.38)</td>
<td></td>
</tr>
<tr>
<td>Model 2†</td>
<td>1.18 (0.89-1.56)</td>
<td>1.00 (0.74-1.36)</td>
<td></td>
</tr>
<tr>
<td>Model 3‡</td>
<td>1.23 (0.92-1.66)</td>
<td>1.36 (0.94-1.96)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio. * Model 1: Derived from Prentice-weighted Cox proportional-hazards regression models, with age as underlying time variable and stratified by age at baseline. † Model 2: As model 1 with further adjustments for waist circumference, smoking status (never smoker, former smoker, current smoker <20 cigarettes per day, current smoker ≥20 cigarettes per day), sports activity (<2 h/week, ≥2 h/week), education (vocational school or less, technical school, university), alcohol consumption (men: 0, >0-12, >12-24, >24; women: 0, >0-6, >6-12, >12 g/day), and prevalent hypertension. ‡ Model 3: As model 2 with further adjustments for fasting status (yes/no), prevalent diabetes, HDL-cholesterol, triglycerides, and hs-CRP.
Supplementary figure I. Flow diagram of the selection of studies in the meta-analysis

Records identified through database searching: n = 146
  • MEDLINE: n = 64
  • EMBASE: n = 82

Records after exclusion of duplicates: n = 104

Duplicates excluded: n = 42

Records excluded: n = 95
  • Was not a prospective study: n = 1
  • Adiponectin was not the exposure: n = 43
  • Stroke was not the outcome: n = 24
  • Participants had already suffered a stroke: n = 6
  • Not healthy populations: n = 17
  • Review or meta-analysis: n = 4

Full-text articles assessed for eligibility: n = 9

Studies included in qualitative synthesis: n = 9

Excluded due to duplicity of study population: n = 1

Studies included in meta-analysis including EPIC-Potsdam: n = 9

EPIC-Potsdam study