Adiponectin and Risk of Stroke
Prospective Study and Meta-Analysis

Maria Arregui, PhD; Brian Buissje, PhD; Andreas Fritsche, MD; Romina di Giuseppe, PhD; Matthias B. Schulze, DrPH; Sabine Westphal, MD; Berend Isermann, MD; Heiner Boeing, MSPH, PhD; Cornelia Weikert, MD, MPH

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. Adiponectin has been suggested to exert anti-inflammatory, antiatherogenic, and insulin-sensitizing effects; to promote high-density lipoprotein-cholesterol formation; to reduce plasma triglyceride levels; and it has been inversely associated with carotid intima-media thickness. Total-adiponectin circulates in the blood stream as globular-adiponectin, and as full-length fractions 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, whereas the lower weight fractions have been associated with anti-inflammatory effects.

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.
Meta-Analysis of Prospective Studies

Meta-analysis of Observational Studies in Epidemiology guidelines were applied (Table I 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.

Effect estimates were extracted for the least and most adjusted models, with and without inclusion of presumed mediators. We contacted authors when data needed for the dose–response meta-analysis were incompletely reported. Study-specific dose–response associations were calculated per 5-µg/mL higher adiponectin concentration by using the generalized least squares for trend estimation method, to combine comparable estimates with random-effect meta-analysis. Heterogeneity between-study results was evaluated by using the F statistic. Publication bias was investigated by Egger test and by visual inspection of the funnel plot. An influential analysis was performed by omitting 1 study at a time. Statistical analyses were performed using SAS Enterprise Guide 4.3 (SAS Institute Inc, Cary, NC) and STATA SE version 12.1 (StataCorp, College Station, TX).

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.
Sixty-four hits from EMBASE and 82 from MEDLINE were retrieved. After exclusion of duplicates and 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

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest  Middle  Highest</td>
<td>PValue*</td>
</tr>
<tr>
<td>n</td>
<td>268  268  268</td>
<td>...</td>
</tr>
<tr>
<td>Adiponectin mean (range), µg/mL</td>
<td>3.5 (1.2–4.5) 5.6 (4.6–6.6) 9.4 (6.7–26.3)</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>51.1±0.49 52.3±0.49 53.0±0.49</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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 19259 participants and 2960 incident stroke cases.

Five studies were nested case–control14–18 and 4 were cohort11,13,19,20 studies. Study populations were from Europe (predominantly white),13,15,20 Japan,4 and the United States
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Incident stroke was ascertained by review of medical records or death certificates in all studies. Relative risks (RR) were provided as odds ratio14,16–18 or hazard ratios.11,13,15,19,20 Odds ratios were assumed to reasonably approximate the RR.

Table 3. Characteristics of Prospective Studies Reporting on the Association Between Adiponectin and Incident Stroke in Healthy Populations

<table>
<thead>
<tr>
<th>First Author, Year of Publication</th>
<th>Study, Country</th>
<th>Study Design</th>
<th>Mean Age, Y</th>
<th>No. Participants (% Women)</th>
<th>Mean Follow-up, Y</th>
<th>Outcome, No. Cases</th>
<th>Adiponectin Assay</th>
<th>Adiponectin Fraction, Mean Among Noncases, µg/mL</th>
<th>Mean BMI Among Noncases, kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soderberg, 200414</td>
<td>MONICA-Västerbotten Intervention Program, Sweden</td>
<td>Nested case–control</td>
<td>54.9</td>
<td>828 (43.1)</td>
<td>4.9</td>
<td>TS, 276</td>
<td>Double-antibody radioimmunoassay (Linco Res.)</td>
<td>Men: 11.5; women: 18.2</td>
<td>25.9</td>
</tr>
<tr>
<td>Matsumoto, 200816</td>
<td>Jichi Medical School Cohort Study, Japan</td>
<td>Nested case–control</td>
<td>66</td>
<td>746–809 (≈49)</td>
<td>9.7</td>
<td>TS, 179; IS, 116</td>
<td>Solid phase ELISA (Otsuka Pharmaceutical Co Ltd)</td>
<td>Men: 6.73; women: 10.09</td>
<td>22.6</td>
</tr>
<tr>
<td>Khalili, 201013</td>
<td>Malmo Preventive Project, Sweden</td>
<td>Cohort</td>
<td>47</td>
<td>3512 (0)</td>
<td>27</td>
<td>IS, 373</td>
<td>In-house time-resolved immunofluorometric assay* (Aarhus Denmark)</td>
<td>5.72</td>
<td>24.8</td>
</tr>
<tr>
<td>Rajpathak, 201119</td>
<td>Women’s Health Initiative, USA</td>
<td>Nested case–control</td>
<td>68.7</td>
<td>1944 (100)</td>
<td>15–20</td>
<td>IS, 972</td>
<td>Milliplex Human Adipokine Panel A</td>
<td>27.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Prugger, 201218</td>
<td>Prospective Epidemiological Study on Myocardial Infarction, France and Ireland</td>
<td>Nested case–control</td>
<td>55.5</td>
<td>240 (0)</td>
<td>&lt;10</td>
<td>IS, 80</td>
<td>Human CVD panel-1 multiplex immunomassay (Linco Res.)</td>
<td>13.5</td>
<td>26.6</td>
</tr>
<tr>
<td>Gardener, 201319</td>
<td>Northern Manhattan Study, USA</td>
<td>Cohort</td>
<td>69</td>
<td>2900 (63)</td>
<td>10</td>
<td>TS, 269</td>
<td>Sandwich ELISA (Merodia)</td>
<td>11.4</td>
<td>28.0</td>
</tr>
<tr>
<td>Kizer, 201315</td>
<td>Cardiovascular Health Study, USA</td>
<td>Cohort</td>
<td>74.4</td>
<td>3290 (63.0)</td>
<td>10.5</td>
<td>IS, 492</td>
<td>Immunoassay (Millipore)</td>
<td>12.3</td>
<td>26.8</td>
</tr>
<tr>
<td>Wannamethee, 201320</td>
<td>British Regional Heart Study, UK</td>
<td>Cohort</td>
<td>68.4</td>
<td>3411 (0)</td>
<td>9</td>
<td>TS, 192</td>
<td>ELISA (R&amp;D Systems)</td>
<td>6.77</td>
<td>26.8</td>
</tr>
<tr>
<td>Arregui, 2013, current study</td>
<td>EPIC-Potsdam, Germany</td>
<td>Nested case–control</td>
<td>50.1</td>
<td>2325 (62.7)</td>
<td>8.2</td>
<td>IS, 190</td>
<td>ELISA (Linco Res.)</td>
<td>8.03</td>
<td>26.1</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 ($F=52.2\%$, $P=0.027$). 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>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 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>Matsumoto, 2008</td>
<td>Top vs bottom quartile; &lt;5.6 vs ≥12.4</td>
<td>M1 IS: 0.67 (0.40–1.11); IS: 0.49 (0.26–0.92)</td>
<td>Age, sex, community</td>
</tr>
<tr>
<td>Khalil, 2010</td>
<td>Top (median, 16.57) vs bottom (median, 2.37) quintile</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 IS: 0.77 (0.59–1.01)</td>
<td>Age, race/ethnicity, date of study enrollment, follow-up time</td>
</tr>
<tr>
<td>Prugger, 2012</td>
<td>Per SD-increase (11.6 µg/mL)</td>
<td>M1 IS: 2.05 (1.38–3.05)</td>
<td>Age, study center, date of examination</td>
</tr>
<tr>
<td>Gardener, 2013</td>
<td>Top (median, 33.6) vs bottom (median, 4.6) quartile</td>
<td>M1 TS: 1.14 (0.79–1.61)</td>
<td>Age, sex, race/ethnicity</td>
</tr>
<tr>
<td>Kizer, 2013</td>
<td>Per SD (7.9) increase</td>
<td>M1 IS: 1.01 (0.63–1.63)*</td>
<td>Age, sex, race</td>
</tr>
<tr>
<td>Wannamethee, 2013</td>
<td>Top vs bottom quartile; ≥10.8 vs &lt;4.3</td>
<td>M1 TS: 0.74 (0.49–1.12)</td>
<td>Age, BMI</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.
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 ($I^2=63.1\%$; $P=0.006$). After omitting the smallest study, the $P$ 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 2 and 3 studies. 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 disease\(^\text{11}\) and cardiovascular death\(^\text{21,24}\) 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.\(^\text{25}\) In the EPIC-Potsdam study, we excluded participants with a clinical history of major cardiovascular disease events at baseline. We also lagged 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.\(^\text{26}\) 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.\(^\text{27}\) Nevertheless, total and HMW-adiponectin have shown to be highly correlated,\(^\text{11}\) and recent findings do not support HMW-adiponectin to be more strongly related to risk of stroke\(^\text{11}\) or coronary heart disease.\(^\text{28}\) 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.\(^\text{29}\)

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.\(^\text{30}\) 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.\(^\text{31}\)

**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.

**Acknowledgments**

We are grateful to W. Fleischhauer for case ascertainment; E. Kohlsdorf for data management; A. Bury, S. Herbert, and E. Eiden for biochemical analyses; and to Drs Süderberg (Umeå University Hospital, Sweden) and Prugger (Paris Cardiovascular Research Centre, University of Paris Descartes, France) for kindly providing additional data for the meta-analysis.

**Sources of Funding**

This study was supported by German Federal Ministry of Education; German Federal Ministry of Science (01 EA 9401); European Union (SOC 95201408 05F02 and SOC 98200769 05F02); and German Cancer Aid (70-2488-Ha I).

**Disclosures**

None.

**References**


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Stroke. 2014;45:10-17; originally published online November 7, 2013;
doi: 10.1161/STROKEAHA.113.001851

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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

<table>
<thead>
<tr>
<th>MI cases (n)</th>
<th>HR (95% CI) per 5-µg/ml higher total-adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>237</td>
<td>1.09 (0.85-1.39)</td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.27 (1.02-1.60)</td>
</tr>
<tr>
<td>Model 2 †</td>
<td>1.54 (1.24-1.90)</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, 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>Reporting of background</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Problem definition</td>
<td>Introduction (2) A meta-analysis was conducted to assess the consistency of the association between adiponectin and risk of stroke in apparently healthy populations.</td>
</tr>
<tr>
<td>Hypothesis statement</td>
<td>Introduction (2) The favourable cardiovascular effects attributed to adiponectin could have a beneficial impact on the risk to develop stroke.</td>
</tr>
<tr>
<td>Description of study outcomes</td>
<td>Introduction (2) Risk to develop stroke</td>
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<td>Type of exposure</td>
<td>Introduction (2) Total-adiponectin</td>
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<tr>
<td>Type of study designs used</td>
<td>Introduction (2) Prospective studies</td>
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<td>Study population</td>
<td>Introduction (2) Apparently healthy populations</td>
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<table>
<thead>
<tr>
<th>Reporting of search strategy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualifications of searchers</td>
<td>Methods-Systematic-review and meta-analysis (5) Two independent investigators</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) Prospective studies conducted in healthy populations, with adiponectin as exposure (total or either of its circulating fractions) and stroke (total or ischemic) 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: ‘adiponectin’/syn OR ‘acrp30’/syn OR ‘apm’/syn AND (‘stroke’/syn OR ‘brain infarction’/syn OR ‘cerebrovascular’) AND (prospective OR longitudinal OR nested OR cohort) AND [humans]/lim AND [embase]/lim</td>
</tr>
<tr>
<td>Effort to include all available studies</td>
<td>Methods-Systematic-review and meta-analysis (5) In case of missing important information, authors were contacted</td>
</tr>
<tr>
<td>Databases and registries searched</td>
<td>Methods-Systematic-review and meta-analysis (5) MEDLINE and EMBASE databases</td>
</tr>
<tr>
<td>Search software used</td>
<td>Methods-Systematic-review and meta-analysis (5) MEDLINE and EMBASE databases were searched by means of their available on-line Web-based interfaces</td>
</tr>
<tr>
<td>Use of hand searching</td>
<td>Methods-Systematic-review and meta-analysis (5) Reference lists of retrieved papers were hand-searched for additional studies</td>
</tr>
<tr>
<td>List of citations located and</td>
<td>Supplementary figure I Records identified through database searching: 146 (MEDLINE: n = 64; EMBASE: n = 82)</td>
</tr>
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</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

Method of handling abstracts and unpublished studies

Description of any contact with authors

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

No limits on type of publication or publication status were applied

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

**Reporting of methods**

**Description of relevance or appropriateness of studies**
Methods-Systematic-review and meta-analysis (5)

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

**Rationale for the selection and coding of data**
Methods-Systematic-review and meta-analysis (5)

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

**Documentation of how data were classified and coded**
Methods-Systematic-review and meta-analysis (4)

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

**Assessment of confounding**
Methods-Systematic-review and meta-analysis (5)

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 assess 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

**Assessment of study quality**
Methods-Systematic-review and meta-analysis (5)

The influence of each study on the pooled estimate and $I^2$ was assessed by omitting 1 study at a time
### Assessment of heterogeneity

<table>
<thead>
<tr>
<th>Methods-Systematic-review and meta-analysis (5)</th>
<th>Heterogeneity between studies was evaluated by using the I² statistic</th>
</tr>
</thead>
</table>

### Description of statistical methods in sufficient detail to be replicated

- **Methods-Systematic-review and meta-analysis (5)**
- 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 I² 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 I² was assessed by omitting 1 study at a time.

### Provision of appropriate tables and graphics

- **Tables 3-4**
- **Figure 1**
- **Table 3.** Characteristics of prospective studies investigating the association of adiponectin and incident stroke in apparently healthy populations
- **Table 4.** Relative Risks of stroke according to adiponectin levels in prospective studies conducted in healthy populations
- **Figure 1.** 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

### Reporting of results

#### Graphic summarizing individual study estimates and overall estimate

- **Figure 1**
- **Table 3**
- **Figure 1.** 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

#### Table giving descriptive information for each study included

- **Table 3**
- **Table 3.** Characteristics of prospective studies investigating the association of adiponectin and incident stroke in apparently healthy populations

#### Results of sensitivity testing

- **Results-Systematic-review and meta-analysis (7-8)**
- **Figure 1**
- Sensitivity analysis revealed that the high heterogeneity observed was mainly driven by the smallest study.

#### Indication of statistical uncertainty of findings

- **Results-Systematic-review and meta-analysis**
- **Figure 1**
- 95% confidence intervals were presented with all summary estimates

### Reporting of discussion

#### Quantitative assessment of bias

- **Results-Systematic-review and meta-analysis (8)**
- The asymmetry of the funnel plot and Egger’s test results (P<0.05) suggested lack of smaller studies reporting an an inverse association between adiponectin and stroke.

#### Justification for exclusion

- **Supplementary figure I**
- 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

#### Assessment of

- **Results-**
- We discussed the potential reasons for the observed
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.

**Reporting of conclusions**

| Consideration of alternative explanations for observed results | Summary (9) | 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. |
| Generalization of the conclusions | Summary (10) | 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. |
| Guidelines for future research | Summary (9) | More studies on specific molecular weight fractions of adiponectin may shed further light on the role of adiponectin and risk of stroke. |
| Disclosure of funding source | Sources of funding (10) | 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). |
**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>Age ≤60 y</th>
<th>Age &gt;60 y</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases/non-cases (n)</strong></td>
<td>Men</td>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>90/786</td>
<td>80/1342</td>
<td></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><strong>Cases/non-cases (n)</strong></td>
<td>Age ≤60 y</td>
<td>Age &gt;60 y</td>
<td></td>
</tr>
<tr>
<td>97/1753</td>
<td>73/375</td>
<td></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
- Duplicates excluded: n = 42
- Records after exclusion of duplicates: n = 104
- Full-text articles assessed for eligibility: n = 9
  - 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
- Studies included in qualitative synthesis: n = 9
- Excluded due to duplicity of study population: n = 1
- EPIC-Potsdam study
- Studies included in meta-analysis including EPIC-Potsdam: n = 9