

1 **Genetic variation in the *ADIPOQ* gene, adiponectin concentrations and risk of**
2 **colorectal cancer – a Mendelian Randomization analysis using data from three**
3 **large cohort studies**

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19

20

1 **Abstract**

2 Higher levels of circulating adiponectin have been related to lower risk of colorectal cancer in
3 several prospective cohort studies, but it remains unclear whether this association may be causal.
4 We aimed to improve causal inference in a Mendelian Randomization meta-analysis using nested
5 case-control studies of the European Prospective Investigation into Cancer and Nutrition (EPIC,
6 623 cases, 623 matched controls), the Health Professionals Follow-up Study (HPFS, 231 cases,
7 230 controls) and the Nurses' Health Study (NHS, 399 cases, 774 controls) with available data on
8 pre-diagnostic adiponectin concentrations and selected single nucleotide polymorphisms (SNPs)
9 in the *ADIPOQ* gene. We created an *ADIPOQ* allele score that explained approximately 3% of
10 the interindividual variation in adiponectin concentrations. The *ADIPOQ* allele score was not
11 associated with risk of colorectal cancer in logistic regression analyses (pooled OR per score-unit
12 unit 0.97, 95% CI 0.91, 1.04). Genetically determined two-fold higher adiponectin was not
13 significantly associated with risk of colorectal cancer using the *ADIPOQ* allele score as
14 instrumental variable (pooled OR 0.73, 95% CI 0.40, 1.34). In a summary instrumental variable
15 analysis (based on previously published data) with higher statistical power, no association
16 between genetically determined two-fold higher adiponectin and risk of colorectal cancer was
17 observed (0.99, 95% CI 0.93, 1.06 in women and 0.94, 95% CI 0.88, 1.01 in men). Thus, our
18 study does not support a large causal effect of circulating adiponectin on colorectal cancer risk.
19 Due to the limited genetic determination of adiponectin, larger Mendelian Randomization studies
20 are necessary to clarify whether adiponectin is causally related to lower risk of colorectal cancer.

21

22 **Keywords:** adiponectin, *ADIPOQ*, colorectal cancer, Mendelian Randomization

23

24

1 **Background**

2 Obesity, in particular abdominal obesity is an established risk factor for the development of
3 colorectal cancer (1). Although the underlying biological mechanisms have not been fully
4 elucidated, it is widely accepted that the adipose tissue, particularly visceral adipose tissue, is an
5 active endocrine organ secreting various bioactive substances collectively named adipokines,
6 which may provide an important link between body fatness and colorectal cancer risk (2). In
7 contrast to many other adipokines, adiponectin expression is suppressed in obesity and plasma
8 concentrations are lower in obese than in lean individuals (3). Adiponectin has been suggested to
9 play a protective role in the development of cancer either directly through inhibition of cell
10 growth (e.g. via RAS signaling(4)) and induction of apoptosis, or indirectly through improved
11 insulin sensitivity and reduced inflammation(5). The association between circulating adiponectin
12 concentrations and risk of colorectal cancer has been investigated in several prospective cohort
13 studies, with mixed findings: Higher plasma adiponectin concentrations were associated with
14 lower risk of colorectal cancer (slightly stronger in women than men, but no statistically
15 significant sex-differences) in the European Prospective Investigation into Cancer and Nutrition
16 (EPIC) (6) and in the Health Professionals Follow-up study (HPFS), while no association was
17 observed in the Nurses' Health Study (NHS)(7). A meta-analysis of ten case-control or nested
18 case-control studies (not including the data from EPIC, NHS or HPFS) reported a statistically
19 significant two percent lower risk of colorectal cancer or adenoma for a 1 $\mu\text{g/mL}$ increment in
20 adiponectin in men whereas among women no association was observed(8).

21 To date, it remains unclear whether adiponectin plays a causal role in the development of colorectal
22 cancer not least because it cannot be excluded that residual confounding and/or reverse causation
23 bias might have introduced bias in observational associations (Figure 1). Mendelian
24 Randomization is a statistical approach that can improve causal inference(9). The principle is that
25 under the assumption of the random assortment of alleles at conception, genetic variants that are
26 associated with biomarker levels can be used as relatively unbiased proxies for biomarker
27 concentrations due to two advantages. First, since the genotype of an individual is determined at
28 gamete formation and cannot be altered later on (e.g. by disease onset), there is no possibility of
29 reverse causation(10). Second, the relationship between genetic variants and disease risk can be
30 assumed to be not confounded by lifestyle and behavioral factors that can confound the observed
31 association between circulating biomarkers and risk of disease. Therefore, using genetic variants
32 associated with circulating biomarker concentrations in a Mendelian Randomization approach may

1 provide insight into the underlying causal relationships by circumventing reverse causation and
2 residual confounding. In a pooled analysis from the Genetics and Epidemiology of Colorectal
3 Cancer Consortium (GECCO), which includes data from NHS, HPFS and eight other studies
4 comprising overall more than 7,000 colorectal cancer cases and approximately the same number
5 of controls, genetic variants in the gene encoding adiponectin (*ADIPOQ*) were not associated with
6 colorectal cancer risk(11). However, a simultaneous analysis of adiponectin concentrations,
7 *ADIPOQ* genetic variants and colorectal cancer was not conducted, because adiponectin plasma
8 levels were only available in a subset of included studies, namely NHS and HPFS. With a dataset
9 including individual participant data on genetic variants, biomarker concentration and disease
10 outcome, a traditional Mendelian Randomization analysis taking into account the actual strength
11 of the association between *ADIPOQ*-SNPs and adiponectin concentrations in the study population
12 can be performed, which has the advantage that instrumental variable assumptions can be directly
13 assessed (12, 13). The aim of our investigation was therefore to improve causal inference in the
14 association between circulating adiponectin and colorectal cancer risk using *ADIPOQ* genetic
15 variants in a Mendelian Randomization meta-analysis with individual participant data from the
16 EPIC, HPFS and NHS cohorts.

17

18 **Methods**

19

20 *Study population*

21 The three studies included in the present investigation were all nested case-control studies of large
22 prospective cohorts with long follow-up. In all nested case-control studies, colorectal cancer was
23 defined according to the International Statistical Classification of Diseases, Injury and Causes of
24 Death (ICD-10) as cancers of the colon (C18.0-C18.7), cancers of the rectum (C19-C20) and
25 tumors that were overlapping or unspecified (C18.8-C18.9). Blood samples were collected prior to
26 diagnosis and matched control participants were selected using incidence density sampling, i.e.
27 selection was performed among study participants who were alive and free of cancer (except non-
28 melanoma skin cancer) at the time of diagnosis of the colorectal cancer case.

29 The EPIC study is a large multicenter prospective cohort including more than 520,000 study
30 participants from 10 European countries who were aged between 35 and 70 years at recruitment
31 which took place from 1992 to 2000(14). Baseline examinations included anthropometric
32 measurements, standardized ascertainment of lifestyle characteristics and medical history

1 information as well as collection of blood samples. The EPIC study was approved by the ethical
2 review board of the International Agency for Research on Cancer (IARC, Lyon, France) and the
3 institutional review boards of each participating study center and informed consent was obtained
4 from all participants. Incident cancer cases including colorectal cancer cases were determined
5 through record linkage with local cancer registries in most countries (Denmark, Naples (Italy
6 except), the Netherlands, Norway, Spain, Sweden, United Kingdom, complete up to 2003). In some
7 countries (France, Germany, Naples (Italy), Greece, complete up to 2002) active follow-up was
8 organized by contact of participants or next of kin through mailed questionnaires, followed by
9 verification of self-reported cases by study physicians using health insurance data, data from cancer
10 and clinical registries as well as medical records provided by the treating physicians. In the present
11 analysis, colorectal cancer cases with available prediagnostic blood samples and DNA were
12 included. As has been described previously(6), the nested case-control design matched each case
13 to one control using incidence density sampling. Control participants were selected matched on age
14 at blood collection (2 months to 4-year intervals), study center, fasting status (<3, 3-6, or>6 hours)
15 as well as menopausal status and hormone use in women. The nested case-control study was
16 designed to be applicable for several biomarker studies, which explains inclusion of the latter
17 matching criteria which were not relevant for the present analysis. The number of cases and
18 matched controls included in the present study is 1,246 (623 cases, 623 matched controls) which
19 is 52% of the study size of the previous analysis on circulating adiponectin and risk of colorectal
20 cancer in EPIC (1,206 cases, 1,206 matched controls) (6). This difference is largely explained by
21 unavailability of DNA samples from the Danish EPIC centers due to local technical and
22 organizational issues.

23
24 The HPFS and NHS are two large US cohort studies, detailed descriptions of which are provided
25 elsewhere (15, 16). In brief, the HPFS started in 1986, including 51,529 men aged 40-75 years, and
26 the NHS started in 1976 and included 121,701 women aged 30-55 years. In both cohorts, study
27 participants provided information on medical history and lifestyle at recruitment. Since then,
28 follow-up questionnaires were administered biennially to collect and update medical and lifestyle
29 information and to elicit medical diagnoses. The follow-up rates in both cohorts exceeded 90% in
30 each 2-year cycle and the cumulative follow-up rate (percentage of potentially collected person-
31 years) was 94% in HPFS and 93% in NHS. Blood specimens were provided by 18,225 HPFS
32 participants (35%) between 1993 and 1995 and by 32,826 NHS participants (27%) between 1989

1 and 1990 by overnight courier. Details on the procedures of blood collection as well as handling
2 and storage of blood samples have been described previously(17, 18). Among the participants for
3 whom blood samples and DNA were available, 231 colorectal cancer cases were confirmed after
4 blood collection in HPFS (up to January 1st 2008) and 399 in NHS (up to October 1st 2008). For
5 each case up to two controls were randomly selected using incidence density sampling. The
6 majority of individuals included in the nested case-control studies were of Caucasian ancestry in
7 both HPFS (95.5%) and NHS (99.9%). All study participants provided informed consent and the
8 study protocol was approved by the Institutional Review Board of the Brigham and Women's
9 Hospital and the Harvard T.H. Chan School of Public Health.
10 The total number of participants in the present investigation is 2,880 (1,253 cases and 1,627
11 controls), including 1,246 in EPIC (623 cases and 623 controls), 461 in HPFS (231 cases, 230
12 controls) and 1,173 in NHS (399 cases, 774 controls).

13
14

15 *Adiponectin measurement*

16 Total circulating adiponectin concentration was measured using enzyme-linked immunosorbent
17 assays from ALPCO Diagnostics (Salem, New Hampshire) in the three studies (6, 7). Based on
18 quality control samples, interbatch coefficients of variation were 8.3% in EPIC and 8.6% in HPFS
19 and NHS. Adiponectin measurements in n=300 paired samples from HPFS showed high reliability,
20 with intraclass correlation coefficient of 0.85 when measured within the same persons one year
21 apart (19).

22

23 *SNP selection and genotyping*

24 In EPIC, a set of tagging SNPs covering variations in the *ADIPOQ* gene in populations of
25 European ancestry was selected using HapMap 22/phase II CEPH population data (Utah residents
26 with northern and western European descent) applying stringent criteria (minor allele frequency
27 >5% and pairwise $r^2 \geq 0.8$). A total number of 15 SNPs were genotyped using TaqMan
28 methodology (genotype call rates >99.2% for all the assays), of which one (rs7649121) was not
29 in Hardy-Weinberg equilibrium in control participants ($p < 0.0001$) and therefore was excluded
30 from analysis. In HPFS and NHS SNPs in the *ADIPOQ* gene were selected based on previous
31 evidence from genome-wide association studies (GWAS) on circulating adiponectin
32 concentrations (20-23). Additional SNPs in adiponectin-related genes that have been associated

1 with colorectal cancer risk were genotyped (24-26). A total of 19 SNPs were genotyped using
2 Illumina HumanOmniExpress as part of the GECCO project (11). Missing SNPs were imputed to
3 HapMap II release 24. All genotyped SNPs were in Hardy-Weinberg equilibrium in control
4 participants. Eight *ADIPOQ* SNPs were available in all three included studies (rs1063539,
5 rs16861194, rs822394, rs17300539, rs17366568, rs17366743, rs266729, rs1501299) and minor
6 allele frequencies were comparable.

7

8 *Statistical analysis*

9 We created an *ADIPOQ* allele score that was used to derive Mendelian Randomization estimates
10 using two different approaches: Firstly, we analyzed the *ADIPOQ* allele score in relation to
11 colorectal cancer risk. Secondly, we applied an instrumental variable approach, simultaneously
12 incorporating *ADIPOQ* genetic variation and plasma adiponectin concentrations, to model the
13 association between genetically determined circulating adiponectin and colorectal cancer risk.
14 While the first approach, which is considered as an equivalent to the intention-to-treat analysis in
15 a randomized controlled trial (27), can only test for the existence of a causal association, the
16 second approach aims at estimating the magnitude of a causal association (e.g. risk estimate per
17 2-fold higher genetically determined adiponectin) (28).

18

19 *ADIPOQ* allele score

20 The weighted *ADIPOQ* allele score was constructed by summing alleles that have been
21 associated with higher adiponectin with genome-wide significance in a previous meta-analysis of
22 GWAS on adiponectin levels, using their GWAS-coefficients as weights (20). Only SNPs not
23 highly correlated ($r^2 < 0.8$) were included in the score. For NHS and HPFS, the included SNPs
24 were rs17300539, rs17366568, rs266729, rs1501299 and rs6810075. The score in EPIC was built
25 using the same SNPs, except that for rs6810075 the proxy SNP rs182052 was used. To examine
26 whether the *ADIPOQ* allele score is independent of potentially confounding factors, we
27 compared baseline characteristics in each study across score categories (approximate tertiles).

28

29 The associations between the individual *ADIPOQ* SNPs as well as the *ADIPOQ* allele score and
30 adiponectin concentrations were examined using linear regression models with robust variance in
31 control participants (29). Adiponectin concentrations were naturally log-transformed (because of
32 skewed distribution) and we calculated the estimated relative change in percent in adiponectin per

1 minor allele (with genotypes coded 0, 1 or 2 according to the number of variant alleles) or per
2 score unit, respectively. In addition, R^2 and F-values as measures of instrument strength are
3 presented. In the previous publication by Song et al. (11) the association between *ADIPOQ* SNPs
4 and plasma adiponectin concentrations was presented for HPFS and NHS, but the here included
5 colorectal cancer controls were only a small subset of the individuals included in that analysis.

6 *Association between ADIPOQ allele score and colorectal cancer*

7 The association between the *ADIPOQ* allele score (per score-unit) in relation to risk of colorectal
8 cancer was calculated in each study. In EPIC, we used conditional logistic regression
9 conditioning on the matching variables and calculating odds ratios (ORs) and 95% confidence
10 intervals that approximate incidence rate ratios and can be interpreted as relative risks. In HPFS
11 and NHS, we used unconditional logistic regression adjusted for matching variables (age at blood
12 draw and date of blood draw) to estimate relative risks. In sensitivity analyses, we restricted the
13 logistic regression models to individuals with Caucasian ancestry (n=16 excluded in HPFS and
14 n=1 excluded in NHS). Because multivariable adjustment is per definition not required in
15 Mendelian Randomization studies, only minimally adjusted (conditional logistic regression
16 conditioned on the matching variables or unconditional logistic regression adjusted for matching
17 factors) estimates are presented. We pooled the study-specific results using a meta-analytic
18 approach with random effects (30), thereby also assessing potential heterogeneity across studies.

19 *Instrumental variable analysis*

20 For the joint analysis of adiponectin concentrations, genetic variants of the *ADIPOQ* gene and
21 colorectal cancer risk, we performed an instrumental variable analysis using two-stage regression.
22 In the first stage, adiponectin concentrations were predicted based on the *ADIPOQ* allele score by
23 means of linear regression. In order to avoid potential bias (31), the first stage regression was
24 performed only in control participants and genetically determined adiponectin was predicted for
25 the total study population including participants without measured adiponectin. In the second
26 stage, a logistic regression of colorectal cancer on the predicted adiponectin concentrations was
27 performed in each study. In EPIC, the second stage was a conditional logistic regression
28 appropriate for the matched design, whereas in HPFS and NHS, the second stage was an
29 unconditional logistic regression adjusted for matching factors. For HPFS and NHS, we restricted
30 instrumental variable analyses to individuals of Caucasian ancestry in sensitivity analyses. The
31 risk estimates resulting from the instrumental variable analysis display the association between 2-

1 fold genetically determined higher adiponectin in relation to risk of colorectal cancer. Pooled
2 associations were determined using random effects model and potential heterogeneity was
3 assessed. We tested whether the causal risk estimates of the individual SNPs included in the
4 *ADIPOQ* allele score were of similar magnitude using an over-identification test. Finally, to
5 increase statistical power, we performed a summary instrumental variable analysis using
6 published data (13). Parameters for the association between the SNPs included in the *ADIPOQ*
7 allele score and circulating adiponectin were taken from GWAS data (20) and parameters for the
8 association between the SNPs and colorectal cancer were derived from the analysis in GECCO
9 (11). Because summary instrumental variable analysis can be biased when correlated SNPs are
10 included, we omitted rs266729 from this analysis, because it is in linkage disequilibrium with
11 rs6810075 (r^2 0.5).

12 All statistical tests are two-sided with significance at the 5% level. Instrumental variable analyses
13 were performed using the STATA SE 12 (StataCorp, College Station, Texas, USA). Summary
14 instrumental variable analyses were performed with a publicly available R-Studio application
15 (Foundation for Statistical Computing, Vienna, Austria). All other analyses were performed
16 using SAS (for EPIC data: SAS Enterprise Guide 4.3; for HPFS and NHS data: SAS 9.3; SAS
17 Institute Inc., Cary, North Carolina, USA).

18

19 **Results**

20 Baseline characteristics of study participants in EPIC, HPFS and NHS are displayed in table 1. In
21 EPIC and HPFS, incident colorectal cancer cases had a statistically significantly higher body
22 mass index (BMI) and waist circumference than controls at baseline, whereas in NHS, these
23 anthropometric measures did not differ between case and control participants. In EPIC, colorectal
24 cancer cases consumed more alcohol and red and processed meat than control participants,
25 whereas in the US cohorts, no such differences were observed. Other potentially confounding
26 factors including physical activity and fiber intake did not differ remarkably between cases and
27 controls in any study. In EPIC and HPFS, but not in NHS, adiponectin concentrations were lower
28 in cases than in control participants in univariate comparisons.

29 Of the 14 *ADIPOQ* SNPs available for analysis in EPIC, five SNPs were statistically significantly
30 associated with circulating adiponectin (Table 2). Each unit of the GWAS-based *ADIPOQ* allele

1 score was associated with 6.5 % (95% CI 3.6, 9.4) higher adiponectin. The *ADIPOQ* allele score
2 explained 3.2% of the interindividual variation in adiponectin concentrations (F-value 20.2). In
3 HPFS, three of the 19 genotyped SNPs were statistically significantly associated with adiponectin
4 concentrations (Table 3). Each unit of the *ADIPOQ* allele score was associated with 7.7 % (95%
5 CI 0.9, 14.9) higher adiponectin (F-value 5.0). In NHS, six of the 19 available SNPs were
6 statistically significantly associated with circulating adiponectin. The *ADIPOQ*-score was
7 associated with 7.7 % (95% CI 4.1, 11.3) higher adiponectin concentrations and explained 3.6%
8 of the interindividual variation in circulating adiponectin (F-value 14.2).

9 Potentially confounding factors assessed in the three cohorts did not differ remarkably across
10 categories (approximate tertiles) of the external *ADIPOQ*-score (all P-values >0.05, supplemental
11 table 1).

12 The *ADIPOQ* allele score was not statistically significantly associated with risk of colorectal
13 cancer (pooled OR per score-unit 0.97, 95% CI 0.91, 1.04) in logistic regression analysis (table
14 4). This result was not altered when logistic regression analyses were restricted to Caucasian
15 individuals in HPFS and NHS (pooled OR 0.97, 95% CI 0.91, 1.04). In the instrumental variable
16 analysis taking measured adiponectin and *ADIPOQ* genetic variation in our study population
17 simultaneously into account (table 5), using the *ADIPOQ* allele score as instrumental variable,,
18 genetically determined two-fold higher adiponectin was not statistically significantly associated
19 with lower risk of colorectal cancer (pooled OR 0.73, 95% CI 0.40, 1.34). These associations
20 were not altered by restriction to Caucasians in HPFS and NHS (pooled OR 0.73, 95% CI 0.41,
21 1.33). Over-identification tests suggested that the effects of all SNPs included in the *ADIPOQ*
22 allele score were similar with respect to colorectal cancer risk in all three studies (P-values were
23 0.64 in EPIC, 0.81 in HPFS and 0.52 in NHS).

24 In the summary instrumental variable analysis using published data (GWAS on adiponectin(20);
25 associations of *ADIPOQ*-SNPs with colorectal cancer published by the GECCO consortium(11)),
26 no association between genetically determined higher adiponectin and risk of colorectal cancer
27 was observed (OR per 2-fold higher adiponectin 0.99, 95% CI 0.93, 1.06 in women and 0.94,
28 95% CI 0.88, 1.01 in men) using the four SNPs rs1730539, rs17366568, rs1501299 and
29 rs6810075 as instruments.

30 **Discussion**

1 In this Mendelian Randomization analysis using data from three nested case-control studies of
2 large prospective cohorts, we did not find evidence for a causal contribution of high adiponectin
3 levels to lower risk of colorectal cancer. However, adiponectin concentrations were genetically
4 determined only to a limited extent, which limited statistical power for our Mendelian
5 Randomization analysis.

6
7 In a genetic association meta-analysis, the minor alleles of three *ADIPOQ* SNPs (rs1501299,
8 rs2241766, rs266729) were associated with colorectal cancer risk (32), but associations were only
9 seen in Asians and not in Caucasians. Individual SNPs at the *ADIPOQ* loci, including those
10 incorporated in the *ADIPOQ* allele score in the present study (rs17300539, rs17366568,
11 rs17366743, rs1501299, rs3774261 (the proxy SNPs rs2241766 was used in GECCO),
12 rs6810075, rs266729, rs6773957, rs6444175, rs1063538) were unrelated to risk of colorectal
13 cancer in GECCO (11). In the same study, the allele-sum of 16 SNPs that have been related to
14 higher adiponectin concentrations in previous GWAS were combined in a genetic score, which
15 was not related to colorectal cancer risk in women (OR per ten-allele increment 1.08, 95% CI
16 0.95, 1.22) or men (OR 1.01, 95% CI 0.90, 1.13). In contrast, in a two-sample Mendelian
17 Randomization meta-analysis (33) using the *ADIPOQ* SNP rs2241766 as instrumental variable, 1
18 mg/L genetically determined higher adiponectin was associated with a 20-40% higher risk of
19 colorectal cancer.

20 The strength of our study is the ability to jointly investigate adiponectin, genetic variation in the
21 *ADIPOQ* gene and risk of colorectal cancer. In contrast to a two-sample Mendelian
22 Randomization design, a full sample design, where genetic information and intermediate
23 phenotype data (i.e. measured adiponectin concentration) are available in the same study
24 participants, generally requires less assumptions and allows for systematic evaluation of
25 instrumental variable assumptions (34). Thus, we were able to estimate the strength of the
26 association between the *ADIPOQ* SNPs as well as the *ADIPOQ* allele score and adiponectin
27 concentrations in our sample, thereby showing that the first Mendelian Randomization
28 assumption was fulfilled. Furthermore, we showed that potentially confounding lifestyle factors
29 did not vary substantially across categories of the instrumental variable, i.e. the second
30 Mendelian Randomization assumption was also satisfied (35). It should be noted that only
31 potentially confounding factors measured in the three studies could be investigated, thus, it

1 cannot be entirely excluded that unmeasured confounders varied by categories of the *ADIPOQ*
2 allele score. Assessment of the third Mendelian Randomization assumption (instrumental variable
3 is associated with the outcome only through the intermediate exposure of interest, i.e. no
4 pleiotropy) is not as straightforward, but the use of multiple SNPs as instrumental variables argue
5 against unknown pleiotropy given that all genetic variants have a similar effect on the outcome,
6 which was confirmed in our samples by the non-significant over-identification test results.
7 Furthermore, the genetic variants used as instrumental variables were restricted to the *ADIPOQ*
8 gene and therefore likely act directly on the adiponectin trait, which argues also against
9 pleiotropic effects (27).

10 However, our study has also several limitations: Given the limited genetic determination of
11 adiponectin concentrations, our sample sizes from three nested case-control studies of
12 prospective cohorts was limited to derive robust causal estimates. The *ADIPOQ*-score applied
13 here explained only a low proportion (2.9%-3.6%) of the interindividual variation in adiponectin
14 concentrations. With this genetic instrument and our sample size, the minimal OR that could have
15 been detected with 80% statistical power was 0.61 per standard deviation in adiponectin, which is
16 a stronger association than has been observed in most observational studies (8). Even with the
17 relatively large sample size in GECCO (7,020 cases, 7,631 controls) no association between a
18 genetic score of variants associated with adiponectin and colorectal cancer was detected(11).
19 Furthermore, in our summary data instrumental variable analysis, genetically determined higher
20 adiponectin was not associated with colorectal cancer risk (minimal detectable OR with 80%
21 power: 0.76 per standard deviation in adiponectin). A much larger sample size (n=33,960 cases,
22 33,960 controls) would be necessary to detect a moderate effect (e.g. OR 0.89 per 1 SD as
23 observed in EPIC(6)) of adiponectin and colorectal cancer. Therefore, with our study and the
24 summary instrumental variable analysis based on GECCO, we cannot rule out causality in the
25 association between circulating adiponectin and risk of colorectal cancer. Although it has been
26 suggested that the 2-stage instrumental variable estimator may result in biased estimates under
27 case-control sampling, it has been shown to be unbiased under the null hypothesis of no causal
28 effect as in the present study (36).

29 In conclusion, this Mendelian Randomization meta-analysis using data from three nested case-
30 control studies of prospective cohorts does not support a large causal effect of circulating
31 adiponectin on colorectal cancer risk. This lack of association may be related to the limited

1 genetic determination of adiponectin and the limited sample size. Therefore, larger Mendelian
2 Randomization studies are necessary to clarify whether adiponectin is causally related to lower
3 risk of colorectal cancer.

4

5 Potential conflict of interest

6 Andrew T. Chan declared consultancies for Bayer Healthcare and Pfizer Inc. Ruth Travis
7 declared salary and support for EPIC-Oxford from Cancer Research UK (paid by the University
8 of Oxford), support for prostate cancer research project on metabolomics from the World Cancer
9 Research Fund as well as support for EPIC-Oxford from the UK Medical Research Council. All
10 other authors declared no potential conflict of interest.

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1 **Overview of figures and tables**

2

3

4 Figure 1. Directed acyclic graph (DAG) for Mendelian Randomization Study on adiponectin and
5 colorectal cancer risk.

6 Table 1 Baseline characteristics of study participants in EPIC, HPFS and NHS

7 Table 2 Association between all ADIPOQ SNPs genotyped in EPIC and plasma adiponectin
8 levels in control participants (n=623)

9 Table 3 Association between all ADIPOQ SNPs genotyped in NHS and HPFS and plasma
10 adiponectin levels in control participants (n=167 in HPFS, n=510 in NHS)

11

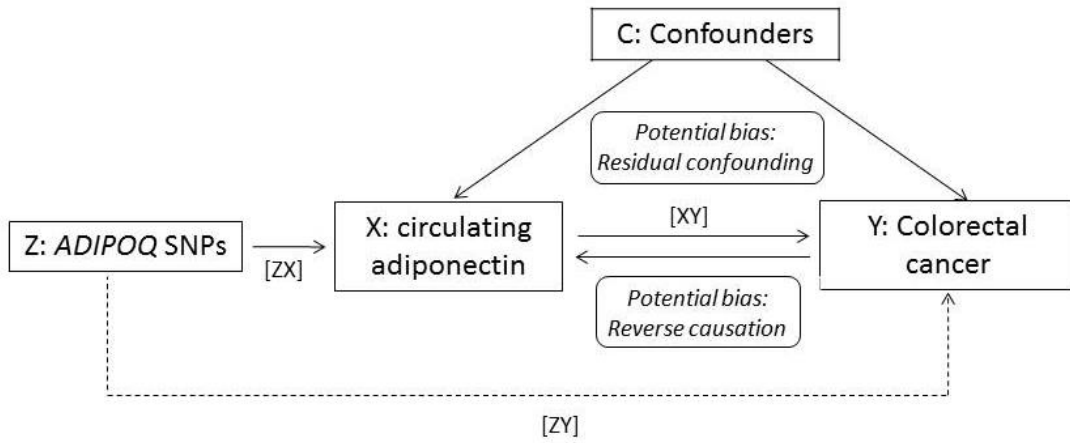
12 Table 4. Association between internal and external ADIPOQ-scores in EPIC, HPFS, and NHS

13

14 Table 5. Instrumental variable estimation of the association between genetically determined
15 adiponectin concentrations with risk of colorectal cancer in EPIC, HPFS, and NHS

16

1



2

3 **Figure 1.** Directed acyclic graph (DAG) for Mendelian Randomization Study on adiponectin and
4 colorectal cancer risk. X: modifiable exposure of interest; Y: outcome; C: confounder(s); Z: instrumental
5 variable. NOTE: The effect of Z on Y should be mediated only through X (no pleiotropy), therefore this
6 line is dashed. Associations [ZX] and [ZY] are used to estimate the causal effect of a biomarker on an
7 outcome circumventing residual confounding and reverse causation.

8

Table 1 Baseline characteristics of study participants in EPIC, HPFS and NHS

	EPIC (n=1,246) [§]			HPFS (n=461) [§]			NHS (n=1173) [§]		
	Controls (n=623)	Cases (n=623)	P ^a	Controls (n=230)	Cases (n=231)	P ^b	Controls (n=774)	Cases (n=399)	P ^b
Female sex, n (%)	289 (46.4)	289 (46.4)	*	0 (0)	0 (0)		771 (100)	398 (100)	
Age at blood collection, years, mean (SD)	58.3 (8.2)	58.3 (8.2)	*	65.6 (8.9)	66.1 (8.8)	*	59.1 (6.7)	59.2 (6.7)	*
Current smoking, n (%)	124 (19.9)	122 (19.6)	0.88	12 (5.4)	8 (3.6)	0.35	90 (11.7)	48 (12.1)	0.85
Caucasian ethnicity, n (%)	n.a.	n.a.	n.a.	226 (98.3)	219 (94.8)	0.04	773 (99.9)	399 (100)	0.47
Physical activity (MET-hours/week), mean (SD)	89.1 (52.1)	90.4 (54.8)	0.59	34.5 (29.2)	35.8 (41.3)	0.70	16.4 (19.8)	16.5 (19.2)	0.88
Body mass index, kg/m ² , mean (SD)	26.4 (3.8)	27.1 (4.4)	0.001	25.2 (3.3)	26.0 (3.1)	0.004	25.4 (4.4)	25.3 (4.3)	0.72
Waist circumference, cm, mean (SD)	89.3 (12.3)	91.9 (13.1)	<0.0001	94.2 (9.4)	96.8 (8.4)	0.004	79.5 (10.7)	80.3 (10.9)	0.41
Alcohol intake, g/day, median (IQR)	6.4 (1.0-21.1)	7.8 (0.8-22.6)	0.02	7.0 (1.8-15.8)	6.9 (0.9-18.7)	0.86	1.1 (0.0- 6.9)	1.8 (0.0- 8.5)	0.07
Fiber, g/day, median (IQR)	21.8 (17.7-27.0)	21.5 (16.8-27.5)	0.77	22.7 (18.6-28.8)	22.2 (18.4-27.2)	0.34	18.1 (15.1-21.3)	17.7 (15.2-21.1)	0.34
Red and processed meat, g/day, median (IQR)	69.1 (45.4-101.5)	72.3 (49.4-108.8)	0.02	63.4 (33.5-98.1)	64.5 (37.5-105.5)	0.20	52.4 (33.4-81.2)	55.8 (33.4-91.7)	0.23
Total adiponectin (µg/mL), median (IQR) ^c	6.3 (4.8- 8.7)	5.9 (4.3- 8.2)	0.01	5.6 (3.9- 8.2)	5.3 (3.5- 7.2)	0.21	8.5 (6.0-11.0)	8.5 (6.0-11.5)	0.64

SD, standard deviation, IQR, inter-quartile range, MET, metabolic equivalent of task

^aP-values for the difference between cases and controls were determined by Mc Nemar's test for variables expressed as %, by student's paired t-test for variables expressed as means, and by Wilcoxon's signed rank test for variables expressed as medians

^b P-values for the difference between cases and controls were determined by Chi2-test for variables expressed as %, analysis of variance (general linear model) for variables expressed as means, and by the non-parametric Kruskal-Wallis test for variables expressed as medians

° Adiponectin measurement was not available in n=16 controls and n=7 cases in EPIC, in n=63 controls and n=56 cases in HPFS, and in n=264 controls and n=112 cases in NHS; some study participants had missing values for the here displayed diet and lifestyle factors: in EPIC, there were missing values on physical activity (n=69 controls, n=67 cases) and waist circumference (n=65 controls, n=65 cases); in HPFS, there were missing values on smoking status (n=7 controls, n=6 cases), waist circumference (n=25 controls, n=46 cases), alcohol (n=4 controls, n=5 cases), fiber (n=1 case) or red and processed meat (n=4 controls, n=5 cases) intake; in NHS, there were missing values on alcohol (n=8 controls, n=1 cases), fiber (n=11 controls, n=2 cases) and red and processed meat (n=8 controls, n=1 case) intake.

Table 2. Association between ADIPOQ SNPs genotyped in EPIC, *ADIPOQ* allele score and plasma adiponectin levels in control participants

Men and women (n=623)						
SNP		MAF	Relative change (95% CI), % ^a	p _{trend}	F-Value	R ² (%)
rs1063539	G>C	13%	0.9 (-6.2; 8.6)	0.81	0.1	0.0
rs16861194	A>G	8%	-4.4 (-12.9; 4.8)	0.34	1.0	0.2
rs12495941	G>T	37%	0.1 (-5.0; 5.5)	0.96	0.0	0.0
rs822391	T>C	19%	5.2 (-1.7; 12.6)	0.14	2.2	0.4
rs822394	C>A	17%	4.1 (-2.9; 11.6)	0.26	1.3	0.2
rs17300539 ^b	G>A	9%	18.2 (8.5; 28.8)	<0.0001	14.5	2.3
rs17366568 ^b	G>A	11%	-12.3 (-19.0; -5.0)	<0.0001	10.3	1.7
rs17366743	T>C	3%	20.6 (3.0; 41.2)	0.02	5.4	0.9
rs182052 ^{b, c}	G>A	35%	-2.8 (-7.8; 2.5)	0.29	1.1	0.2
rs266729 ^b	C>G	27%	-1.6 (-7.1; 4.2)	0.59	0.3	0.1
rs1501299 ^b	G>T	28%	6.3 (0.3; 12.5)	0.04	4.3	0.7
rs2241766	T>G	13%	2.3 (-5.1; 10.3)	0.56	0.3	0.1
rs3774261	G>A	41%	5.3 (0.1; 10.9)	0.05	3.9	0.6
rs3821799	C>T	47%	0.0 (-4.9; 5.2)	0.99	0.0	0.0
<i>ADIPOQ</i> allele score			6.5 (3.6; 9.4)	<0.0001	20.2	3.2

MAF: Minor allele frequency; 95% CI, 95% confidence interval

^a Percent change in adiponectin concentrations per copy of minor allele (effect allele) or score unit, estimated in univariable linear regression models.

^b incorporated in *ADIPOQ* allele score for EPIC

^c proxy SNP for rs6810075

in bold: statistically significant associations (p<0.05)

1 Table 3. Association between all *ADIPOQ* SNPs genotyped in HPFS and NHS, *ADIPOQ* allele score and plasma adiponectin levels in
2 control participants

Men (n=167), HPFS							Women (n=510), NHS						
SNP	MAF	Rel. change (95% CI), % ^a	p _{trend}	F-Value	R ² (%)		SNP	MAF	Rel. change (95% CI), % ^a	p _{trend}	F-Value	R ² (%)	
rs1063539	G>C	15%	-5.6 (-20.0; 11.5)	0.50	0.5	0.3	rs1063539	G>C	12%	-1.4 (-9.4; 7.4)	0.75	0.1	0.0
rs16861194	A>G	9%	-1.5 (-19.0; 19.8)	0.88	0.0	0.0	rs16861194	A>G	5%	-9.1 (-19; 1.9)	0.10	2.7	0.5
rs7615090	T>G	5%	-7.4 (-29.0; 20.8)	0.57	0.3	0.2	rs7615090	T>G	6%	-7.3 (-18; 4.8)	0.23	1.5	0.3
rs822394	C>A	15%	-2.3 (-16.3; 13.9)	0.76	0.1	0.1	rs822394	C>A	17%	6.1 (-1.3; 14.0)	0.11	2.6	0.5
rs17300539 ^b	G>A	9%	13.5 (-5.8; 36.6)	0.19	1.8	1.1	rs17300539^b	G>A	8%	13.3 (2.8; 25.0)	0.01	6.3	1.2
rs17366568 ^b	G>A	8%	-9.7 (-26; 10.1)	0.32	1.0	0.6	rs17366568^b	G>A	7%	-18.5 (-26.8; -9.2)	<0.0001	13.8	2.7
rs17366743	T>C	3%	0.9 (-26.4; 38.3)	0.96	0.0	0.0	rs17366743	T>C	3%	2.5 (-11.7; 18.9)	0.75	0.1	0.0
rs6810075^b	T>C	33%	-11.8 (-21.2; -1.2)	0.03	4.6	2.7	rs6810075 ^b	T>C	31%	-5.0 (-10; 0.7)	0.09	3.0	0.6
rs6773957	G>A	41%	9.2 (-2.6; 22.3)	0.13	2.3	1.4	rs6773957	G>A	38%	6.5 (0.9; 12.5)	0.02	5.2	1.0
rs822354	G>A	36%	7.6 (-4.1; 20.9)	0.22	1.5	0.9	rs822354	G>A	33%	1.4 (-4.2; 7.4)	0.62	0.2	0.1
rs6444175	G>A	28%	11.4 (-0.7; 25.0)	0.07	3.3	2.0	rs6444175	G>A	27%	7.6 (1.2; 14.5)	0.02	5.5	1.1
rs266717	T>C	49%	-3.2 (-13.3; 8.0)	0.56	0.3	0.2	rs266717	T>C	47%	-3.8 (-8.8; 1.5)	0.16	2.0	0.4
rs1426810	A>G	37%	0.7 (-9.8; 12.4)	0.91	0.0	0.0	rs1426810	A>G	40%	3.9 (-1.7; 9.9)	0.17	1.9	0.4
rs1342387	T>C	43%	2.9 (-7.9; 15.0)	0.62	0.3	0.2	rs1342387	T>C	45%	-4.4 (-9.5; 1.0)	0.11	2.5	0.5
rs12733285	C>T	30%	-2.7 (-13.7; 9.8)	0.66	0.2	0.1	rs12733285	C>T	30%	-4.1 (-9.6; 1.7)	0.16	2.0	0.4
rs266729^b	C>G	24%	-12.6 (-22.8; -1.2)	0.03	4.6	2.7	rs266729 ^b	C>G	26%	-4.4 (-10; 1.8)	0.16	2.0	0.4
rs1501299^b	G>T	26%	13.4 (1.0; 27.4)	0.04	4.4	2.6	rs1501299^b	G>T	27%	8.6 (2.2; 15.5)	0.01	7.0	1.4
rs1063538	C>T	41%	9.2 (-2.6; 22.3)	0.13	2.3	1.4	rs1063538	C>T	38%	6.6 (0.9; 12.5)	0.02	5.2	1.0
rs3774262	G>A	15%	-6.0 (-20.0; 10.5)	0.46	0.6	0.3	rs3774262	G>A	11%	-0.7 (-9.0; 8.4)	0.88	0.0	0.0
<i>ADIPOQ</i> allele score			7.7 (0.9; 14.9)	0.03	5.0	2.9	<i>ADIPOQ</i> allele score			7.7 (4.1; 11.3)	<0.0001	18.8	3.6

3 MAF: Minor allele frequency; 95% CI, 95% confidence interval

4 ^a Percent change in adiponectin concentrations per copy of minor allele (effect allele) or score unit, estimated in univariable linear
5 regression models.

6 ^b incorporated in *ADIPOQ* allele score for HPFS/NHS

7 **in bold**: statistically significant associations (p<0.05)

8

1 Table 4. Association between *ADIPOQ* allele score and colorectal cancer risk in EPIC, HPFS, and NHS

2

	No. Cases/No. Controls	Relative change % ^a	OR	(95% CI)	P _{trend}	P _{het.}
EPIC ^b	623/623	6.5	0.95	(0.87, 1.03)	0.23	
HPFS ^c	231/230	7.7	1.00	(0.85, 1.17)	0.97	
NHS ^c	399/774	7.7	0.98	(0.88, 1.09)	0.73	
Pooled	1253/1627		0.97	(0.91, 1.04)	0.43	0.86

3
4 ^a Per score unit, estimates based on univariate linear regression in controls

5 ^b Conditional logistic regression, controlling for matching factors (age at blood collection, study center, fasting status, menopausal status
6 and hormone use in women)

7 ^c Unconditional logistic regression, adjusted for matching factors (age at blood draw, date of blood draw)

8 OR, odds ratio; 95% CI, 95% confidence interval; p_{het.}, P value for heterogeneity by study

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13 Table 5. Instrumental variable estimation of the association between genetically determined adiponectin concentrations with risk of
14 colorectal cancer in EPIC, HPFS, and NHS (*ADIPOQ* allele score as instrumental variable)

15

	No. Cases/No. Controls	IV-OR	(95% CI)	P _{trend}	P _{het.}
EPIC	623/623	0.61	(0.25, 1.49)	0.28	
HPFS	231/230	0.90	(0.21, 3.84)	0.89	
NHS	399/774	0.83	(0.31, 2.22)	0.71	
Pooled	1253/1627	0.73	(0.40, 1.34)	0.31	0.86

16 IV-OR, instrumental variable odds ratio; 95% CI, 95% confidence interval; p_{het.}, P value for heterogeneity by study

17 IV-OR derived from two-stage regression. First stage was a linear regression. In EPIC, the second stage was a conditional logistic
18 regression controlling for matching factors (age at blood collection, study center, fasting status, menopausal status and hormone use in

- 1 women); in HPFS and NHS, the second stage was an unconditional logistic regression adjusting for matching factors (age at blood draw,
- 2 date of blood draw)
- 3
- 4 p_{het} , P value for heterogeneity by study
- 5