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Accessibility
Interaction between Obesity and the **NFKB1** - 94ins/delATTG Promoter Polymorphism in Relation to Incident Acute Coronary Syndrome: A Follow Up Study in Three Independent Cohorts

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Abstract

**Introduction:** The NF-κB transcription factor family regulates several genes encoding pro-inflammatory and anti-inflammatory proteins in adipose tissues and in atherosclerotic plaques. The deletion variant allele of the **NFKB1** - 94ins/delATTG promoter polymorphism leads to lower transcript levels of the p50 subunit, and the variant allele has been associated with the risk of several inflammatory diseases as well as coronary heart disease where inflammation is important in the pathogenesis. The objective of this study was to explore the potential interaction between the **NFKB1** - 94ins/delATTG promoter polymorphism and general, abdominal, and gluteofemoral obesity in relation to the risk of incident acute coronary syndrome (ACS) in three large independent cohorts.

**Methods and Results:** The analyses were conducted in the Danish prospective study Diet, Cancer and Health and the two US based cohorts; Nurses’ Health Study and Health Professionals Follow-up Study. We conducted sex stratified analyses that included 1202 male and 708 female cases of incident ACS. We observed a positive association for general and abdominal obesity with risk of incident ACS, independent of genotype in both genders. Gluteofemoral obesity was negatively associated with ACS risk in women independent of genotype, whereas there was no clear association for men. We calculated the relative excess risk due to interaction (RERI) and observed a statistically significant excess risk among men jointly exposed to general or abdominal obesity and the variant allele of the **NFKB1** - 94ATTG polymorphism, whereas there was a tendency towards sub-additivity for gluteofemoral obesity. The excess risks in all analyses were, however, small and could not clearly be demonstrated in women.

**Conclusion:** The variant allele of the **NFKB1** - 94ins/delATTG promoter polymorphism did not substantially modify the association between obesity and incident ACS.


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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Obesity is associated with a higher risk of acute coronary syndrome (ACS) [1–4]. However, many obese do not experience ACS, and other factors may affect the implications of obesity. Adipose tissue is, in addition to being a storage organ for energy, an active endocrine organ that secretes both pro- and anti-inflammatory cytokines [5]. As obesity develops, the release of adipose tissue derived cytokines increases [6], and the resulting state of chronic low-grade inflammation may accelerate the...
atherosclerotic processes in the arterial wall, and thus lead to the increased risk of ACS[6–8].

Many genes encoding pro-inflammatory and some genes encoding anti-inflammatory proteins in both adipose tissues and atherosclerotic plaques are regulated by the NF-kB transcription factor family[6,9–11]. The NF-kB family consists of heterodimeric or homodimeric combinations of five subunits (p50, p100, RelA[p65], RelB and c-Rel), and different combinations have different target genes. The p50/p50 homodimer represses transcription of pro-inflammatory cytokines like TNFa and IL-12 and promotes transcription of the anti-inflammatory IL-10 [10,11].

The NFKB1 -94ins/delATTG promoter polymorphism is an insertion/deletion of four bases in the promoter region of the NFKB1 gene encoding the p50 subunit, and the variant allele containing the deletion produces lower transcript levels of the p50 subunit [12]. The NFKB1 - 94ins/delATTG promoter polymorphism is the only functional polymorphism in NFKB1 [12] and the polymorphism has been associated with both the inflammatory marker CRP and diseases where inflammation is important in the diseases pathogenesis including ACS[12–14]. The NFKB1 - 94ins/delATTG promoter polymorphism is not a single nucleotide polymorphism (SNP) and thus not detected directly by genome wide association scans. However, the NFKB1 - 94ins/delATTG promoter polymorphism is in high linkage disequilibrium with rs230529 and rs699030 ($r^2 = 0.741$ and 0.714, respectively) [15], but as indicated by the $r^2$ of 0.7 the SNPs were not in complete linkage disequilibrium with the NFKB1 - 94ins/delATTG promoter polymorphism.

The objective of this study was to explore the potential biologic interaction between the NFKB1 -94ins/delATTG promoter polymorphism and obesity in relation to the risk of incident ACS.

Results

Baseline characteristics of the study participants are presented in Table 1. Compared to the controls both male and female cases in DCH appeared on average older, had a shorter education, fewer were never smokers, and drank less alcohol. In NHS and HPFS, age and smoking status were matching factors, and thus no differences were observed, but as in DCH, cases drank less alcohol than controls. In all studies, cases had a higher prevalence of hypertension, diabetes mellitus and hypercholesterolemia (data not shown) provided similar results, and thus we do not believe that residual errors. However, all participants in NHS and HPFS were health workers, and the self reported measures have been validated, albeit there was a tendency towards underestimation of obesity measures [16]. Due to the subtle nature of atherosclerosis, reverse causation in relation to the anthropometric variables was a possibility, but we have previously in the total DCH cohort found similar associations for all anthropometric variables, when we conducted separate analyses in participants who experienced ACS within the first 2 years of follow up and in participants who experienced an ACS event after more than 2 years of follow up [3].

We adjusted for several potential confounders related to the associations between obesity and ACS, but did not include hypertension, diabetes mellitus and hypercholesterolemia in the analyses of the presented results as they can be seen as intermediate variables. Thus, their inclusion in multivariate analyses would restrict the outcome to associations through other pathways; however, analyses without these adjustments could lead to confounding from other causes to these intermediate variables. Crude analyses (Table S2), adjusted analyses (Table 3, 4 and 5) and analyses with additional adjustment for hypertension, diabetes mellitus and hypercholesterolemia (data not shown) provided similar results, and thus we do not believe that residual confounding explains our results.

General Discussion

The variant allele containing the deletion NFKB1-94ins/delATTG promoter polymorphism leads to lower levels of the p50 subunit, and this affects both the availability of the anti-inflammatory p50/p50 NF-κB homodimer and the pro-inflammatory p50/p65 NF-κB heterodimer. However, the combined effect of relatively low levels of both p50/p50 and p50/p65 will be pro-inflammatory, as low p50 levels intuitively will affect the concentration of p50/p50 more than the concentration of p50/p65. Furthermore, the p50/p65 heterodimer blocks binding sites for the p50 subunit, and thus, the level of the p50/p65 heterodimer will be relatively closer to normal, due to the
**Materials and Methods**

**Study Populations**

The present study was based on three independent cohorts: the Danish prospective study Diet, Cancer and Health (DCH), and two US based cohorts; the Nurses’ Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). The Diet, Cancer and Health study was approved by the National Committee on Health Research Ethics (journal nr. (KF) 01-345/93) and the Danish Data Protection Agency. Written informed consent was obtained from all participants to search information from medical registers.

All studies have previously been described in detail [17–19], and thus only a brief description is presented here.

**Diet, cancer and health.** From November 1993 to May 1997, all men and women aged 50–64 years, born in Denmark, living in the greater Copenhagen or Aarhus areas, and with no previous cancer diagnosis registered in the Danish Cancer Registry were invited to participate in Diet, Cancer and Health (DCH). The study was approved by the National Committee on Health Research Ethics (journal nr. (KF) 01-345/93) and the Danish Data Protection Agency. Written informed consent was obtained from all participants to search information from medical registers.

To preserve biological material the present study was conducted as a case-cohort design based on a sex-stratified subcohort drawn...
randomly from the entire cohort. After case validation, we excluded participants with an ACS diagnosis prior to baseline, and participants for whom information on one or more variables were missing.

All anthropometric data were collected by trained technicians. Height was measured to the nearest 0.5 cm with the participants standing without shoes. Weight was measured using a digital scale with the participants wearing light clothing and recorded to the nearest 0.1 kg. Waist circumference (WC) was recorded to the nearest 0.1 cm. Hip circumference (HC) was measured to the nearest 0.5 cm. Waist and hip circumferences were lower (426/679 and 382/821, respectively).

Blood samples were collected from each participant at baseline, and lymphocytes were isolated and frozen within 2 h. For genotyping, DNA was isolated from frozen lymphocytes from cases and participants in the subcohort and the NFKB1-94 ins/del promoter polymorphism was determined as previously described.[14,20–22]. Controls were included in each run, and repeated genotyping of a random 10% subset yielded 100% identical genotypes.[14].

Information on confounders was obtained at baseline through questionnaires including socio-demographic factors, lifestyle, medication and prevalent disease.

In Diet, Cancer and Health, ACS was defined as unstable angina pectoris and myocardial infarction (ICD-8:410–410.99 and ICD-10: I20.0, I21.0–I21.9). Potential cases were identified by linkage to the Danish National Patient Registry [23] and the Danish Causes of Death Registry using the Danish Civil Registration System, in which every Danish citizen is identified by a unique 10 digit number. All incident cases between baseline and ultimo 2003 were validated by review of medical records in accordance with the guidelines of the American Heart Association and the European Society of Cardiology for use in epidemiology [24]. Furthermore, participants with a sudden cardiac death diagnosis (ICD 8:427.27 or ICD 10: I46.0–I46.9) were accepted as cases, if the cardiac arrest at validation was believed to have been caused by ACS.

A total of 80,996 men and 79,729 women were invited, and 27,148 men (34%) and 29,863 women (37%) consented to participate. The sex stratified cohort sample consisted of 1869 participants who had become cases.

The nurses' health study and the health professionals follow-up study. The Nurses’ Health Study (NHS) was established in 1976 at the Channing Laboratory of the Brigham and Women’s Hospital, Boston, US. The study included married, female, registered nurses aged 30–55 years residing in one of 11 US states.

The Health Professionals Follow-up Study (HPFS) was established at the Harvard School of Public Health in 1986 with methods similar to that described for the NHS. It is a longitudinal

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet, Cancer and Health</th>
<th>US cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n = 922)</td>
<td>Women (n = 775)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>26.4 (23;31)</td>
<td>27.0 (23;32)</td>
</tr>
<tr>
<td>BMI grouped according to WHO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Normalweight</td>
<td>33%</td>
<td>27%</td>
</tr>
<tr>
<td>Obese</td>
<td>51%</td>
<td>52%</td>
</tr>
<tr>
<td>Obese</td>
<td>16%</td>
<td>22%</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>95 (85;108)</td>
<td>97 (87;112)</td>
</tr>
<tr>
<td>WC grouped according to WHO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female &lt;88 cm</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Female ≥88 cm</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Male &lt;102 cm</td>
<td>74%</td>
<td>65%</td>
</tr>
<tr>
<td>Male ≥102 cm</td>
<td>26%</td>
<td>35%</td>
</tr>
<tr>
<td>Hip Circumference (cm)</td>
<td>100 (93;108)</td>
<td>101 (93;110)</td>
</tr>
<tr>
<td>NFKB1-94ATTG polymorphism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype/Wildtype</td>
<td>40% (371)</td>
<td>35% (268)</td>
</tr>
<tr>
<td>Wildtype/Variant</td>
<td>45% (418)</td>
<td>48% (372)</td>
</tr>
<tr>
<td>Variant/Variant</td>
<td>14% (133)</td>
<td>17% (135)</td>
</tr>
</tbody>
</table>

Note: Sample size in NHS and HPFS with data available on waist and hip circumference was lower (426/679 and 382/821, respectively).
study among male health workers in the United States aged 40–75 years at enrollment.

In both studies, the participants have received follow-up questionnaires biennially to update information on lifestyle factors, body weight, and newly diagnosed illnesses. Self-reported information on height and weight was obtained at baseline, and self-reported measures of WC and HC only were obtained once in 1986/1987 and covariate data were derived from questionnaires administered at blood draw (1990 in the NHS and 1994 in the HPFS), with missing information substituted from previous questionnaires [16,18]. Similarly, blood samples were collected from participants free of cardiovascular disease and cancer. Whole blood samples were centrifuged and stored in cryotubes as plasma,uffy coat, and red blood cells. DNA was extracted from theuffy coat fraction of centrifuged blood with the QLAmp Blood Kit (Qiagen, Chatsworth, CA, USA). The genotypes were determined by Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA, USA) as previously described [14,25].

Obesity and NF-kB Interaction in Relation to ACS

Table 3. RR* with 95% confidence interval in brackets for the combined effect of general obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diet, Cancer and Health</th>
<th>HPFS</th>
<th>Metaanalysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt/wt</td>
<td>var/var</td>
<td>wt/wt</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25 kg/m²</td>
<td>1.00 (ref.)</td>
<td>1.14 (0.85; 1.53)</td>
<td>1.10 (0.73; 1.66)</td>
</tr>
<tr>
<td>25–29 kg/m²</td>
<td>1.15 (0.86; 1.52)</td>
<td>1.40 (1.07; 1.84)</td>
<td>1.71 (1.24; 2.37)</td>
</tr>
<tr>
<td>&gt;29 kg/m²</td>
<td>1.49 (1.08;2.05)</td>
<td>1.61 (1.20;2.18)</td>
<td>1.98 (1.35;2.91)</td>
</tr>
</tbody>
</table>

Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).

1Adjusted for age, smoking status, alcohol consumption, physical activity, and educational level. Women also adjusted for menopausal status.

1Adjusted for age, smoking status, alcohol consumption, and physical activity. Women also adjusted for menopausal status and hormone replacement therapy.

*RR estimated by Cox proportional hazards regression in DCH and logistic regression in NHS and HPFS.

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For the present study, we used nested case-control designs. Blood was donated from 32,826 Nurses and 10,224 HPFS participants. Between blood draw and June 2004, 512 female incident cases of ACS were identified in NHS, and 454 male cases were identified in HPFS. Using risk-set sampling, controls were selected randomly and matched in a 2:1 ratio on age, smoking, and month of blood return, among participants who were free of cardiovascular disease at the time ACS was diagnosed in the case patient [27].

After exclusions of participants for whom information was missing on the NFKB1 genotype, body mass index and covariate data, the NHS comprised 471 cases of incident ACS and 922 controls, and the HPFS comprised 427 cases and 875 controls. However, information on WC and HC was not available for all participants, and thus analyses regarding WC and HC only included 426 cases and 679 controls in NHS and 302 cases and 821 controls in HPFS.
**Table 4.** RR* with 95% confidence interval in brackets for the combined effect of abdominal obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MEN</th>
<th>HPFS</th>
<th>Metaanalysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 94 cm</td>
<td>1.00(ref.)</td>
<td>1.09(0.84;1.42)</td>
<td>1.00(ref.)</td>
</tr>
<tr>
<td>94–102 cm</td>
<td>1.04(0.78;1.38)</td>
<td>1.29(0.99;1.69)</td>
<td>1.56(1.14;2.15)</td>
</tr>
<tr>
<td>&gt; 102 cm</td>
<td>1.37(0.98;1.91)</td>
<td>1.55(1.12;2.15)</td>
<td>2.00(1.35;2.95)</td>
</tr>
<tr>
<td>WOMEN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 80 cm</td>
<td>1.00(ref.)</td>
<td>0.92(0.57;1.48)</td>
<td>0.84(0.42;1.69)</td>
</tr>
<tr>
<td>80–91 cm</td>
<td>1.69(0.99;2.89)</td>
<td>1.68(1.03;2.76)</td>
<td>0.96(0.47;1.95)</td>
</tr>
<tr>
<td>&gt; 91 cm</td>
<td>2.07(1.09;3.95)</td>
<td>3.53(1.96;6.35)</td>
<td>5.50(2.53;12.0)</td>
</tr>
</tbody>
</table>

Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).
1 Adjusted for age, smoking status, alcohol consumption, physical activity, educational level, and hip circumference. Women also adjusted for menopausal status.
2 Adjusted for age, smoking status, alcohol consumption, physical activity, and hip circumference. Women also adjusted for menopausal status and hormone replacement therapy.
*RRR estimated by Cox proportional hazards regression in DCH and logistic regression in NHS and HPFS.
doi:10.1371/journal.pone.0063004.t004

**Statistical Analyses**

In accordance with the case-cohort design and to allow for delayed entry of the participants, the incidence rate ratios in DCH were analyzed by weighted Cox's proportional hazards regression models with age as the underlying time variable [28,29]. The observation time was censored by death of other causes than ACS, emigration or study end at December 31st 2003. Due to the nested case-control design and to allow for potential intermediate variables in analyses of anthropometry and ACS, hypertension, diabetes mellitus, and hypercholesterolemia are possible intermediate variables in analyses of anthropometry and ACS, and therefore, they were not included in the main analyses.

Deviation from an additive model was explored as the relative excess risk due to interaction (RERI) and calculated as suggested by Rothman [30] and Hosmer and Lemeshow [31].

To combine the estimates from the three study populations, we calculated the weighted average of the log rate ratios with weights according to the study-specific variance. A similar model on original scale was used to calculate the combined estimates of the relative excess risk due to interaction.

The proportionality assumptions of the Cox proportional hazards models were evaluated graphically by log-minus-log plots. All analyses were performed using Stata version 11.2 (StataCorp LP, College Station, TX) and SAS version 9 (SAS Institute Inc., Cary, NC).
Table S1. RR* with 95% confidence interval in brackets for the combined effect of obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome. Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).

Table S2. Crude analyses. RR * with 95% confidence interval in brackets for the combined effect of obesity and the NFKB1-94ATTG polymorphism in relation to acute coronary syndrome. Diet, Cancer and Health, Health Professionals Follow-up Study (HPFS), and Nurses Health Study (NHS).

Acknowledgments
The authors would like to thank the Danish Cancer Society, the Danish Heart Foundation, the Danish Obesity Research Centre (DanORC), Gene-diet Interactions in Obesity (GENDINOB), and the Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome (HEPADIP). We would like to thank Hardeep Ranu and Pati Soule from the DF/HCC Genotyping Core for genotyping.

Author Contributions
Conceived and designed the experiments: JGS EBS KO AT UV ER MKJ TIAS. Performed the experiments: JGS KO AT UV. Analyzed the data: JGS KO TLB UV AT ER MKJ. Contributed reagents/materials/analysis tools: JGS KO AT UV ER MKJ. Wrote the paper: JGS EBS TLB AT UV ER TIAS KO MKJ.

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