# DISSERTATION

## Resistin and Colorectal Cancer Risk

## Resistin und Kolorektalkrebs-Risiko

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# Table of contents

# List of Tables

# List of Figures

# List of Abbreviations

## (in alphabetical order)

| Abbreviations | Full description |
|---|---|
| 95%CI | 95% Confidence Interval |
| ADSF | Adipose Tissue-Specific Secretory Factor |
| AHEI | Alternate Healthy Eating Index |
| BMI | Body Max Index |
| CAP1 | Adenylyl Cyclase-Associated Protein 1 |
| CD4+ | Cluster of Differentiation 4 Positive |
| CIN | Chromosomal Instability |
| corr coeff | Correlation Coefficient (either Pearson Correlation Coefficient or Spearman Rank Correlation Coefficient) |
| CRC | Colorectal Cancer |
| DAG | Directed Acyclic Graph |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| EPIC | European Prospective Investigation into Cancer and Nutrition |
| FFQ | Food Frequency Questionnaire |
| FIZZ3 | Found in an Inflammatory Zone |
| GWAS | Genome-Wide Association Studies |
| HDI | Human Development Index |
| HMW | High-Molecular-Weight |
| hsCRP | high-sensitivity C-Reactive Protein |
| IARC | The International Agency for Research on Cancer |
| ICD-10 | The 10th Revision of The International Classification of Diseases |
| ICE | Income, Crowding, Education |
| IL | Interleukin |
| MAPK | Mitogen-Activated Protein Kinase |
| MMP | Matrix Metalloproteinases |

| | |
|---|---|
| MR | Mendelian Randomization |
| MSI | Microsatellite Instability |
| NF-κB | Nuclear Factor-Kb |
| NGSP | National Glycohemoglobin Standardization Program Standardization |
| NHS | Nurses' Health Study |
| PBMC | Peripheral Blood Mononuclear Cells |
| PI3K | Phosphoinositide 3-Kinases |
| QC | Quality Control |
| RELM | Resistin-Like Molecules |
| rho | Spearman Rank Correlation Coefficient |
| ROM | Reactive Oxygen Metabolites |
| RR | Relative Risk |
| SCALLOP | Systematic and Combined Analysis of Olink Proteins |
| TLR4 | Toll-Like Receptor 4 |
| TNF-α | Tumor Necrosis Factor-A |
| VEGF | Vascular Endothelial Growth Factor |
| WC | Waist Circumference |
| WHI | Women's Health Initiative |
| WHO | World Health Organization |

## Abstract (English)

Colorectal cancer (CRC) is one of the most common types of cancer in terms of cancer incidence. Obesity, characterized by the accumulation of excess fat, is a well-established risk factor for CRC, but the mechanism is unclear. It has been suggested that adipokines, which are a group of cytokines and hormones released from adipose tissue, may play a role. Resistin was originally discovered as an adipokine in rodents, although studies in humans suggest that resistin may be more closely related to inflammation which is one of the pathways to CRC development. Resistin was also shown to promote angiogenesis, which may increase tumor growth. Based on these observations, it was hypothesized that resistin may have a potential role in the development of CRC. Case-control studies had observed higher resistin concentrations in patients with CRC compared to controls; yet, there is insufficient evidence to determine whether resistin is associated with risk of incident CRC.

This dissertation used data from a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC), which is a large, multi-center prospective cohort study. Incident cases of CRC observed during follow-up were matched to controls using risk set sampling to increase statistical efficiency. Resistin concentrations were measured in baseline blood samples. Conditional logistic regression models with adjustment for additional potential confounders identified from a directed acyclic graph were used to estimate the effect sizes for the association between resistin and CRC risk. Sensitivity analyses were performed to account for the possibility of a reverse relationship by excluding cases (and matched controls) diagnosed within 2 years from blood collection. Furthermore, the cross-sectional correlations between resistin and adiposity, inflammation, and metabolic markers were examined.

The results showed that higher circulating resistin concentrations were not significantly associated with CRC risk. No significant associations were observed in individuals followed-up longer than 2 years, whereas a positive significant association was observed in cases (and matched controls) diagnosed within 2 years from blood collection. Resistin was not correlated with adiposity measures, however, was statistically significantly correlated with hsCRP.

In conclusion, this dissertation does not support an association between resistin levels and CRC risk in a European population. Taking into account the observations in

earlier case-control studies, it is speculated that the significant positive association estimated within the first 2-year follow-up window may be due to a reverse influence of undiagnosed CRC on resistin levels. The findings on weak correlations with adiposity biomarkers are in line with several prior epidemiological studies, which overall question resistin as an adipokine in humans. The potential role of resistin in inflammation warrants further research.

# Zusammenfassung

Kolorektalkrebs (CRC) ist eine der häufigsten Krebsarten in Bezug auf die Krebsinzidenz. Adipositas, die durch die Akkumulierung von überschüssigem Fett gekennzeichnet ist, wurde als Risikofaktor für CRC identifiziert, wenngleich der Mechanismus nicht geklärt ist. Es wurde vorgeschlagen, dass Adipokine, d.h. eine Gruppe von Zytokinen und Hormonen, die von adipösem Fettgewebe freigesetzt werden, eine Rolle spielen könnten. Resistin wurde zuerst als Adipokin bei Nagetieren entdeckt, aber Studien am Menschen deuten darauf hin, dass Resistin enger mit Entzündungsprozessen verbunden sein könnte, die einen der Wege zur Entstehung von CRC darstellen. Daher wurde die Hypothese aufgestellt, dass Resistin eine mögliche Rolle bei der Entwicklung von CRC spielen könnte. In Fall-Kontroll-Studien wurden höhere Resistinkonzentrationen bei Patienten mit CRC im Vergleich zu Kontrollpersonen beobachtet; es gibt jedoch keine ausreichende Evidenz, um festzustellen, ob Resistin mit dem Risiko für die Entwicklung von CRC assoziiert ist.

In dieser Dissertation wurden Daten aus einer eingebetteten Fall-Kontroll-Studie verwendet, die im Rahmen der European Prospective Investigation into Cancer and Nutrition (EPIC), einer groß angelegten, multizentrischen prospektiven Kohortenstudie durchgeführt wurde. Die inzidenten Fälle von CRC, die während der Nachbeobachtungszeit auftraten, wurden Kontrollen zugeordnet, wobei zur Erhöhung der statistischen Effizienz eine Risikosatz-Stichprobe verwendet wurde. Die Resistinkonzentrationen wurden in Blutproben aus der Basisuntersuchung gemessen. Zur Schätzung der Effektgrößen für den Zusammenhang zwischen Resistin und CRC-Risiko wurden konditionale logistische Regressionsmodelle mit Adjustierung für zusätzliche potenzielle Confounder verwendet, die anhand eines gerichteten azyklischen Graphen ermittelt wurden. Sensitivitätsanalysen wurden durchgeführt, um die Möglichkeit eines umgekehrten Zusammenhangs (Einfluss von noch nicht diagnostiziertem CRC auf Resistinkonzentrationen) zu berücksichtigen, indem Fälle (und zugeordnet Kontrollen) ausgeschlossen wurden, bei denen die Diagnose innerhalb von 2 Jahren nach der Blutentnahme gestellt wurde. Außerdem wurden die Querschnittskorrelationen zwischen Resistin und Adipositas-, Entzündungs- und Stoffwechselmarkern untersucht.

Die Ergebnisse zeigten, dass höhere zirkulierende Resistinkonzentrationen nicht signifikant mit dem CRC-Risiko assoziiert waren. Bei Personen, die länger als 2 Jahre

nachbeobachtet wurden, wurde kein signifikanter Zusammenhang beobachtet, während bei der Analyse von Fällen, die innerhalb von 2 Jahren nach der Blutentnahme diagnostiziert wurden (und den zugeordneten Kontrollen), ein signifikant positiver Zusammenhang beobachtet wurde. Resistin korrelierte nicht mit Adipositasmarkern, war aber statistisch signifikant mit hsCRP korreliert.

Zusammenfassend zeigt diese Arbeit keinen Zusammenhang zwischen Resistinkonzentrationen und CRC-Risiko in einer europäischen Population. Unter Berücksichtigung der Ergebnisse früherer Fall-Kontroll-Studien wird spekuliert, dass die signifikante positive Assoziation, die innerhalb des ersten 2-Jahres-Follow-up-Fensters gefunden wurde, auf den umgekehrten Einfluss einer (noch) nicht diagnostizierten CRC auf die Resistinkonzentration zurückzuführen sein könnte. Die Ergebnisse der geringen Assoziation mit Adipositasmarkern stehen im Einklang mit mehreren früheren epidemiologischen Studien, in denen Resistins Rolle als Adipokin beim Menschen insgesamt in Frage gestellt wurde. Die mögliche Rolle von Resistin bei Entzündungen muss weiter untersucht werden.

# 1. Introduction

## 1.1. Motivations of the research questions in this dissertation

Colorectal cancer (CRC) is identified as the third most commonly diagnosed cancer in 2020.[1] There are an estimated 3.2 million cases globally in 2040.[2] Obesity, as defined by the World Health Organization (WHO), is "a condition of abnormal or excessive fat accumulation in adipose tissue", which can impair health.[3] The relationship between obesity and an increased risk of CRC is well-established but the exact mechanism behind this remains unclear.[4] It has been suggested that investigations into the potential relationship between obesity-related biomarkers and CRC development could provide a better understanding of the relationship between obesity and an increased risk of CRC.[4] Adipose tissue, especially visceral fat tissue, is a functional active endocrine organ that produces cytokines and hormones released directly into the bloodstream which are termed "adipokines".[5] Classic adipokines such as adiponectin and leptin are suggested as mediators in the association between obesity and CRC,[6-8] while novel adipokines such as resistin, omentin, fatty-acid binding protein 4 (FABP-4), and lipocalin could also potentially play a similar role in this association.[5,8-10] Of note, although resistin does not originate from adipocytes, it is produced by macrophage/monocyte fractions in the adipose tissues and is thus classified as an adipokine. Therefore, resistin has been proposed as a potential factor related to the development of CRC.[9-11] To address the research question regarding the association between resistin (and other adipokines) and risk of CRC, a scientific research proposal entitled "*Novel obesity-related biomarkers and risk of colorectal cancer: a nested case-control study in EPIC"* was submitted to the International Agency for Research on Cancer - World Health Organization (IARC-WHO). The proposal was approved, and measurements were carried out to provide data on several obesity-related biomarkers, including data on resistin, which were utilized in this dissertation. Surprisingly, although always being described as an adipokine,[12] several population-based studies have suggested that resistin is not such strongly correlated with adiposity measurements (body mass index (BMI)/ waist circumference (WC)), or classic adipokines (adiponectin and leptin) in humans. Nevertheless, even if resistin is not an adipokine, it may still link to CRC development through inflammation,[13-15] or angiogenesis,[16] because these are pathways of CRC development.[17,18] That motivated us to investigate the association between resistin concentrations and risk of CRC.

## 1.2. Resistin

### 1.2.1. Resistin discovery and differences between man and mice

Resistin, which is also referred to as ADSF (ADipocyte-Secreted Factor) or FIZZ3 (Found In an inflammatory Zone-3), was first described in 2001 by three independent research groups.[19-21] Steppan and colleagues classified resistin as an adipokine that was expressed and secreted by adipocytes in genetic and diet-induced obesity mice models and named after its role ("resistance to insulin").[19] Kim and colleagues reported a protein named ADSF that functioned as a feedback regulator of adipogenesis.[20] Holcomb and colleagues found a cysteine-rich secreted protein named FIZZ3 which was upregulated in murine ovalbumin-induced asthma.[21] Thus, resistin was proposed to implicate in insulin resistance and inflammation processes.[22] Since its discovery, many studies have been carried out to clarify the specific physiological roles of resistin. However, in contrast to mice, studies in humans have shown that resistin is primarily secreted by non-adipocyte inflammatory cells including macrophages, peripheral blood mononuclear cells (PBMCs), and bone marrow cells in humans;[23,24] and is widely expressed in normal human tissues such as bone marrow, lung, spleen, liver, adipose tissue, placenta, pancreas, kidney, and intestine.[23,25]

The differences in protein structure and position of the resistin-encoded gene between mice and humans (**Table 1**) may lead to differences in resistin's functions between species.[14] Notably, from genomic perspectives, two sequences of mouse resistin and human resistin vary greatly in their organizations.[26] This suggests that the effects of resistin may vary between rodents and humans.

**Table 1: Primary differences in resistin between humans and mice.** (Source: Literature search conducted solely by the dissertation's author. This table was not included in the dissertation-based publication).

| | Humans | Mice |
|---|---|---|
| Resistin protein molecular mass | 11 kDa - 11.3 kDa[27,28] (some molecular weight forms could be up to 45 kDa and 55 kDa, or 660 kDa.[29,30]) | 12.5 kDa[20] |
| Number of amino acids | 108[19] (the mature sequence) | 114[19] (94 amino acids in the mature sequence and 20 amino acids in the signal sequence[31]) |
| Amino acid level sequence identity | 59%[22,26] (the mature segments are 55% identical) | |
| mRNA level sequence identity | 64.4%[26] | |
| DNA level sequence identity | 46.7%[26]<br>Mice resistin genomic organization contains an extra intron of 2279 nucleotides which is absent in the human resistin; thus, mice resistin DNA sequence is nearly three times larger than the resistin DNA sequence in human counterpart.[26] | |
| Chromosome; position | 19[22]; 19p13.2 | 8[22]; 8 0.37 cM |
| Main source | Immune cells[23,24] | Adipocytes[19] |
| Biological functions | Inflammation | Insulin resistance |

Furthermore, resistin belongs to the family of RELMs (resistin-like molecules). The RELM family includes RELMα/FIZZ1, RELMβ/FIZZ2, RELMγ/FIZZ4, and resistin protein;[32] however, in humans, RELMα and RELMγ do not exist.[33,34] The existing isoforms in humans are resistin and RELMβ/FIZZ2.[35] The RELMβ/FIZZ2 isoform consisting of 111 amino acids is produced by epithelial, fibroblast, and smooth muscle cells at sites of vascular remodeling,[35] and is encoded by the RETNLB gene located in chromosome 3 at location: 3q13.13 [Accessed in Pubmed/protein with the GenBank number EAW79719.1 as the search term]. The function of the human RELMβ isoform is not in the scope of this dissertation.

### 1.2.2. Resistin properties and factors affect resistin levels in humans

The sequence of human resistin protein was extracted from the PubMed protein database by the GenBank ID: AAG59824.1 and is shown in **Figure 1**.

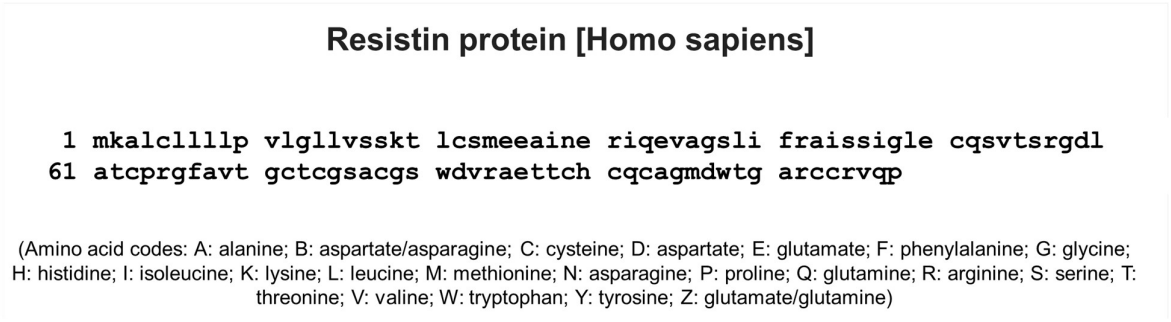


**Figure 1: The sequence of human resistin protein**. (Source: Pubmed protein database with GenBank: AAG59824.1 as the search term. Similar information can be found at: https://www.ncbi.nlm.nih.gov/protein/AAG59824.1)

It is important to note that evidence regarding resistin in humans and mice is often mixed. In this dissertation, I have focused solely on human populations. To this end, when I performed a literature search to provide context for the present dissertation, I considered only experimental (for example, in human cells) and epidemiological studies in humans pertaining to resistin and its relationship with factors representing adiposity, inflammation, and metabolism, and focused on the findings from population-based studies.

#### 1.2.2.1. Resistin and adiposity, the correlation between resistin and adipose measurements

Resistin has always been described as adipokine in literature.[9,10,13,16] Evidence suggests that resistin mRNA levels are detectable at extremely low levels in freshly isolated human adipocytes,[36] and lower levels in adipocytes isolated from adipose tissue than in the whole adipose tissue itself.[27] Resistin is detected in macrophages (represented by cluster of differentiation 4 positive (CD4+)) accumulated in the human obese visceral white adipose tissues, at least in obese individuals.[37,38] Particularly, resistin is produced by blood monocytes undergoing differentiation into macrophages during their migration into the stroma of adipose tissues.[23,39,40] Of note, resistin also originates from circulating human blood monocytes in the blood.[39] Nevertheless, at experimental tissue levels, resistin could be considered as an adipokine - a cytokine produced from adipose tissues.

Surprisingly, most population-based studies have found that resistin is not strongly correlated with adiposity measurements (BMI, WC) (**Table 2**). These studies have resulted in a mix of nonsignificant and significant results with weak correlations. For

example, the Nurses' Health Study (NHS)[41] and the Women's Health Initiative (WHI) Study[42] found a significant but weak correlation, while the Nutrition and Health of Aging Population in China study[43] found a significant correlation with a coefficient of almost zero, and the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study, as well as the Ansung cohort from the Korea Genome Epidemiology study[44] reported a zero correlation. Of note, as the purpose of this literature search is to establish the background of the present study, several considerations were taken into account. First, because the p-values of the correlation analysis are influenced by large sample sizes,[45] interpretations of the correlations between two variables merely based on the p-values are inadequate. Second, it is recommended that with exemplified data of 780 participants, an absolute correlation coefficient of 0.70 or higher suggests a strong correlation, between 0.50 and 0.70 suggests a moderate correlation, between 0.30 and 0.50 suggests a modest correlation, and below 0.30 suggests a weak correlation.[46] Third, estimates for correlation coefficients are stable when the sample size gets to 250.[47] Thus, in this dissertation, correlations between two variables were interpreted by considering both the p-values and the magnitudes of the correlation coefficient estimates. Of all the thresholds for correlation interpretation mentioned before, the value of 0.30 should be given the utmost attention since all the correlation coefficient estimates in the presented population-based studies are under 0.30. Furthermore, only population-based studies with sample sizes higher than 250 were included to assure stable correlation coefficients estimated.[47] Based on these pre-established criteria, there is justification to conclude that the correlation between resistin and adiposity measurements in the NHS and WHI study is weakly significant. Together with evidence from experimental studies, these statistically significant correlations should not be interpreted as biologically or clinically relevant correlations. Additional investigation into fat compartments by magnetic resonance imaging scans (including subcutaneous and visceral adipose tissue) indicated that the explained variance in plasma resistin concentrations accounted for by these compartments was merely 1% and suggested that resistin should not be considered an adipokine.[48]

Furthermore, population-based studies suggest that resistin is weakly significantly or even not significantly correlated with other adipokines (including adiponectin and leptin) (**Table 2**). Naïve comparisons of the correlations between adiposity measurements and biomarkers classified as adipokines showed that the correlations were somewhat lower for resistin than for other biomarkers. For example, in the WHI

study, although WC was weakly correlated with resistin (correlation coefficient (corr coeff) = 0.12 ($p<0.001$)), it was moderately correlated with leptin (corr coeff = 0.64 ($p<0.001$)) and modestly correlated with adiponectin (corr coeff = -0.35 ($p<0.001$)).[9] Moreover, in the NHS cohort, WC was weakly correlated with resistin (corr coeff =0.22 ($p<0.0001$)), but modestly correlated with adiponectin (corr coeff =-0.39 ($p<0.0001$)).[41] In the Ansung cohort of the Korea Genome Epidemiology study, WC was not significantly correlated with resistin (corr coeff =0.009 ($p>0.05$), but moderately correlated with leptin (corr coeff =0.53 ($p<0.01$)), and weakly significantly correlated with adiponectin (corr coeff =-0.21 ($p<0.01$)).[49] These findings could be explained by the different sources of production of resistin and other adipokines. While adiponectin and leptin, the primary adipokines, are exclusively identified in mature adipocytes, resistin is produced by mononuclear cells.[37] Thus, human resistin may link with the chronic low-grade sub-clinical inflammation properties of adipose tissues rather than with the accumulation of adipose tissues itself.[50]

#### 1.2.2.2. Inflammatory properties of resistin and correlations between resistin and inflammatory biomarkers

Resistin has been proposed for implicating inflammatory processes.[13,14] First, resistin potentially displays proinflammatory properties by strongly up-regulating the inflammatory cytokines (tumor necrosis factor-α (TNF-$\alpha$) and interleukin (IL)-6)).[13] In fact, Bokarewa and colleagues conducted a study in which human PBMCs obtained from patients with rheumatoid arthritis were cultured with various concentrations of resistin and observed an increase in the production of inflammatory cytokines such as TNF-$\alpha$, IL-6, and IL-1 in these PBMCs.[13] Second, inflammation could induce a significant increase in circulating resistin levels.[51-53] Indeed, resistin protein and mRNA expression increase in macrophages after TNF-$\alpha$ stimulation,[51] as well as in whole-blood monocytes after the administration of lipopolysaccharide - an inflammation-induced component.[52,53] Third, resistin may trigger an up-regulation of resistin mRNA itself by inducing a positive feedback mechanism.[15] In an experimental study, resistin was used to simulate PBMCs or macrophages, and levels of resistin measured again after the simulation were significantly higher than the simulated dose.[15] Thus, the proposed mode of action of resistin is by involving in a “vicious circle” of inflammation with a positive feedback mechanism (**Figure 1**),[13,15] and consequently contributing to perpetuating inflammatory reactions and severe systemic inflammation.

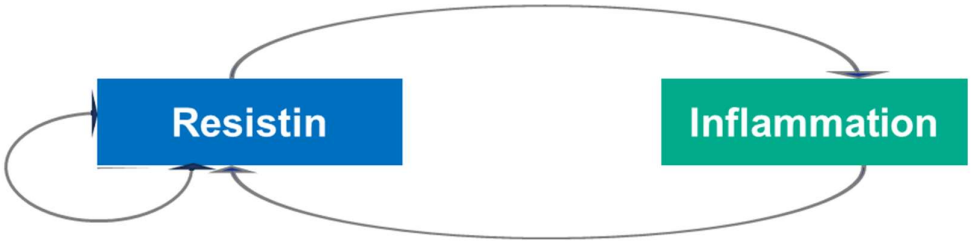


**Figure 2: A conceptual visualization illustrating the "vicious circle" relationship between resistin and inflammation, with a positive feedback loop formed by resistin.**
**(**Source: Own unpublished illustration.)

The consensus signaling pathway by which resistin expresses its inflammatory properties is the nuclear factor-κB (NF-κB) pathway. Although resistin receptors have not yet been explicitly defined, there is evidence pointing to toll-like receptor 4 (TLR4) or adenylyl cyclase-associated protein 1 (CAP1) being candidates for resistin receptors.[14] After binding to its receptors, resistin activates the inflammatory signaling pathway leading to the activation of pro-inflammatory genes in targeted tissues.[14,54]

Many population-based studies (in healthy people) have found a significant, yet weak correlation between resistin and inflammatory biomarkers including high-sensitivity C-reactive protein (hsCRP), TNF-α, and IL-6 (**Table 2**). As described in the previous section, both the absolute correlation coefficients (>0.30) and p-values (<0.05) were applied to distinguish between a modest significant correlation, a weak significant correlation, and a non-significant correlation. While the correlation was not particularly strong in these population studies, there is remarkable evidence from experimental studies that supports resistin's inflammatory properties as described earlier. It is important to note that the interpretation regarding whether the correlations are clinically significant can not be solely determined by statistics, but instead requires the use of clinical assessment.[55] As in the example of obesity, although there is a well-established link between obesity and low-grade pre-clinical inflammation, not all correlation coefficients between BMI and other inflammatory biomarkers in the listed population-based studies reached the value of 0.30,[9,42,56] (for example, in the NHS[56], correlations between BMI and hsCRP, IL-6, tumor necrosis factor-α receptor type 2 (TNF-αR2), (TNF not reported) were 0.39 (n=4403, p<0.0001), 0.29 (n=2661, p<0.01), 0.21 (n=2993, p<0.0001), respectively, and in the WHI[9,42], correlations between WC and hsCRP, IL-6, TNF-α were 0.30 (n=

4937, p not shown), 0.45 (n=834, p<0.001) and 0.14 (n=834, p<0.001), respectively). Thus, it may be incorrect to conclude that the inflammatory properties of resistin are statistically significant but clinically negligible based on these correlations between resistin and inflammatory biomarkers.

#### 1.2.2.3. Resistin and metabolism, the correlation between resistin and metabolic biomarkers

Resistin has been found as a potential intermediary between obesity and insulin resistance from screens for targets of insulin-sensitizing drugs in animal models.[19] However, in humans, resistin's metabolic effects have been largely controversial, and it is difficult to determine whether it actively contributes to metabolic dysfunctions or springs from them.[57] Nevertheless, chronic inflammation and the relationship between resistin and inflammation create a vicious cycle that could increase the risk of metabolic dysfunctions.[58]

Circulating resistin may not be associated with insulin sensitivity in humans.[59] However, an observational study combining data from two prospective nested case-control studies in the Women's Health Study and Physicians' Health Study II found that elevated baseline resistin levels were associated with an elevated risk of type 2 diabetes during 10 years of follow-up even after excluding all cases occurring in the initial 3 years of follow-up and their matched controls to address the concern of underdiagnosed type 2 diabetes.[60] In contrast, a Mendelian Randomization (MR) study by Folkersen and colleagues which included large-sample-size genome-wide association studies (GWAS) did not show a significant association between genetically determined resistin levels and type 2 diabetes.[61] Nonetheless, the presence of an association between increased circulating resistin levels and an elevated risk of type 2 diabetes can not be ruled out.

Studies have reported controversial findings on the associations between increased circulating resistin levels and metabolic syndromes. A cross-sectional study sampled 6,637 participants from the "CDC de Canarias" cohort in Canary Island (Spain) found that elevated resistin concentrations were associated with unfavorable blood lipids, (for example, low high-density lipoprotein cholesterol (HDL-C) (≤40 mg/dL in men and ≤50 in women) and high low-density lipoprotein cholesterol (LDL-C) (≥160 mg/dL)).[62] In cross-sectional studies of Caucasian females with obesity, higher resistin levels were also associated with a higher percentage prevalence of metabolic syndrome (defined by the Adult Treatment Panel III criteria,[63] WHO,[64] the European Group for the Study of Insulin Resistance,[65] and the International Diabetes Federation[66]) which includes three

or more of the following conditions: hypertriglyceridemia, low HDL-C, high blood pressure, hyperglycemia, and central obesity.[67,68] Nevertheless, the MR study by Folkersen and colleagues did not find significant associations between resistin and type 2 diabetes, HDL-C, total cholesterol, triglycerides, hemoglobin A1c (HbA1c), fasting glucose, fasting insulin, glucose tolerance, insulin secretion, and insulin sensitivity.[61]

Importantly, population-based studies did not find statistically significant correlations between resistin and HbA1c, C-peptide, and HDL-C in individuals who were healthy and free of diabetes and cardiovascular diseases at baseline (**Table 2**). Furthermore, reactive oxygen metabolites (ROMs) are a group of molecules generated as by-products of normal oxygen metabolic processes in human bodies and increase when there is an imbalance between the production of free radicals and their elimination by protective antioxidants.[69] Since ROMs have been linked to the development of chronic inflammation,[69-71] which is a primary characteristic of resistin,[13,15] there may be a correlation between resistin and ROMs. Nevertheless, no population-based studies investigating the association between resistin and this biomarker at baseline have been found after conducting a PubMed search (on 24 April 2023) using the keywords 'resistin' and 'reactive oxygen metabolites'.

It should be noted that biomarkers data collected from the population-based studies were predominantly cross-sectional, where levels of resistin and metabolic biomarkers such as HbA1c, HDL-C, and C-peptide were measured from the same blood samples taken only once at baseline from healthy individuals, and the temporal directions of the relationship between resistin and these biomarkers are therefore unknown. Correlation even if it exists with a statistically significantly moderate or high correlation coefficient does not imply causation.[45]

#### 1.2.2.4. Factors related to resistin concentrations

So far, there have been no published reviews on factors related to resistin concentrations. Given that resistin could have both upstream and downstream effects of low-grade inflammation (resistin and inflammation are involved in a vicious circle),[13,15] any factor that exerts effects upstream of inflammation may be associated with resistin levels. In this regard, previous studies have suggested that aging is positively correlated with elevated levels of TNF-α, hsCRP, and IL-1;[72,73] sex (men, women, and menopausal status in women) is also a factor related to inflammation as the X chromosome itself may influence the immune response,[74] and sex hormones like testosterone and estrogen can

differently inhibit the generation and release of pro-inflammatory markers;[73] physical activity is inversely associated with inflammation;[72] while cigarette smoking, physical and emotional stress, and lower socioeconomic status are associated with significantly elevated levels of inflammatory cytokines.[73,75] Surprisingly, it is suggested that alcohol consumption may decrease low-grade inflammation;[72,76] diet has both negative and positive influences on inflammation, such as harmful effects of meals rich in glucose and fats can increase inflammation, while healthy eating patterns (for example, high consumptions of whole grains, vegetables, fruits, and fish) may reduce inflammation.[72] Gut microbiota changes may be a factor associated with inflammation, for example, a reduction in Turicibacter and an increase in Lactococcus were indicative of elevated inflammation.[77]

I performed searches on the PubMed database by combining the keywords "resistin" and each of the factors related to inflammation described above. As a result, resistin levels were found to be positively associated with age,[78-81] and to be higher in women than in men.[43,82] Furthermore, studies have reported higher resistin levels in individuals with less physical activity,[79-81] current smokers,[81,83] individuals who consume less alcohol,[43,79,83] and have a lower-income,[80] or lower social class defined by the ICE (Income, Crowding, Education) model.[84] Furthermore, data from NHS showed that persons in the upper resistin quartiles had lower healthy diet adherence measured by the Alternate Healthy Eating Index (AHEI) which covers the adherence to the basic pre-defined intake levels of fruits, vegetables, trans fat, cereal fiber, nuts/ soy, moderate alcohol use, multivitamin use for more than 5 years, and the ratios of white meat to red meat, of polyunsaturated to saturated fat.[41] Details of the AHEI scoring method have been described elsewhere.[85] Elevated resistin concentrations were reported to be inversely associated with adherence to the Mediterranean diet and monounsaturated fat intake, and positively associated with saturated fat intake.[62] Furthermore, there is a tendency for there to be an inverse relationship between resistin levels and fish intake and a positive relationship between resistin levels and meat intake.[86] The available evidence supporting the relationship between resistin and gut microbiota is limited.

While the evidence supporting the relationship between these factors and resistin levels is not particularly strong, all of the identified patterns of risk factors related to resistin levels are supported by evidence related to inflammation. Given the novel nature of resistin as a biomarker, all the identified risk factors were included in the directed acyclic graph (DAG) which was constructed to show the assumed relationship between

resistin and CRC risk and developed more than one DAG to address the uncertainty surrounding this relationship.

### 1.2.3. The measurement of resistin

Circulating resistin concentrations have been reported to be stable in humans during a period of 3-4 years with a high overall intraclass correlation of 0.95 over the repeatedly measured samples,[87] and thus, it was supported that single resistin measurement per individual in epidemiological studies could reflect long-term resistin exposure. Furthermore, human resistin molecular structure is also stable up to 90°C, and the partial loss of secondary structure (the primary structure is α-helical structure and the secondary structure is a β-sheet form) only occurs starting at the temperature of 105°C.[88]

In terms of the measured values, a typical range of circulating (serum) resistin concentrations in humans is 7 - 22 ng/ml.[89] Currently, there is a lack of agreement in the standardization of resistin levels measured across studies.[90] The variability in the measurements of resistin among studies may depend on assay methods.[90] There are typically two methods for resistin measurement including enzyme-linked immunosorbent assay (ELISA) and multiplex immunoassay which may be very highly correlated (the correlation coefficients between the measurements from the two assays for the same samples (corr coeff = 0.92)).[91] However, resistin concentrations measured by multiplex were approximately 1.4 times the concentrations determined by ELISA reported in a study.[91] Resistin presents in human blood with multiple molecular weight forms (the molecular weights range from 10 kDa to 20 kDa, and from 45 kDa to 55 kDa, encompassing sizes of 660 kDa), and different assay methods may detect these molecular weight forms differently.[29,30] Indeed, the distributions of resistin levels in controls in population-based studies were reported together with assay methods as follows: the WHI study, multiplex assay, median (interquartile range), 12.3 (9.8–15.6) ng/ml;[9] the EPIC-Potsdam study, ELISA assay, median and median absolute deviation, in women, 4.0 (1.2) ng/ml;[48] the Nutrition and Health of Aging Population in China study, multiplex assay, median (interquartile range), 8.60 (5.78–14.00) ng/ml. A study using ELISA assay by BioVender Laboratory Medicine (this method was later applied for our study) reported a median (interquartile range) of 3.1 (2.2-4.7) ng/ml among individuals who had received a prior diagnosis of being free from CRC or adenoma through colonoscopy.[92] Of note, the differences in single measured values of resistin concentrations across population-based studies may also be due to diverse populations

causing heterogeneity among studies (for example, different prevalence rates of inflammation-related conditions). Thus, comparing mean/median or pooling differences in resistin concentrations from studies with different assay systems may be challenging.[29] Resistin concentrations should be reported along with information on assays and the assays should be characterized by the resistin molecular weight forms.[29] Meta-analysis studies pooling mean different resistin levels using different assays should be performed with caution.

**Table 2: Correlations between pre-diagnostic resistin and adiposity biomarkers, other adipokines, inflammatory biomarkers, and metabolic biomarkers: A literature search of data from population-based studies.** (Source: Literature search conducted solely by the dissertation's author. This table was not included in the dissertation-based publication).

| Biomarkers | Correlation coefficients (p-value) | Sample size | Population, Country | Year | Assay of resistin | Ref |
|---|---|---|---|---|---|---|
| **Adiposity measures** | | | | | | |
| BMI | 0.22(<0.0001) [a, d] | 1922 | The Nurses' Health Study, USA | 2005 | ELISA | 41 |
| | 0.17 (<0.001) [b, c] | 4937 | Women's Health Initiative Study (WHI), USA | 2016 | multiplex assay | 42 |
| | 0.02 (0.58) [b, c, e] | 827 | The EPIC-Potsdam Study, Germany | 2008 | ELISA | 44 |
| | 0.10 (< 0.001) [a,c] | 1508 | The Finnish Health 2000 Survey, Finland | 2011 | in-house assay based on the DELFIA technique | 68 |
| | 0.04 (0.03) [a, e, f] | 3193 | The Nutrition and Health of Aging Population in China study, China | 2008 | a Bio-Rad Multiplex Suspension Array System | 43 |
| WC | 0.12 (<0.001) [a, d] | 834 | The Women's Health Initiative Study, USA | 2012 | multiplex assay | 9 |
| | 0.01 (0.85) [b, c, e] | 827 | The EPIC-Potsdam Study, Germany | 2008 | ELISA | 44 |
| | 0.09 (0.001)[a,c] | 1508 | The Finnish Health 2000 Survey, Finland | 2011 | in-house assay based on the DELFIA technique | 68 |
| | 0.07 (<0.001) [a, d, f] | 3193 | The Nutrition and Health of Aging Population in China study, China | 2008 | a Bio-Rad Multiplex Suspension Array System | 43 |
| | 0.009 (>0.05) [a, c] | 912 | The Korea Genome Epidemiology Study - Ansung cohort, South Korea | 2020 | multiplex assay | 49 |
| **Other adipokines** | | | | | | |
| Adiponectin | −0.12 (<0.0001) [a, d] | 1065 | The Nurses' Health Study, USA | 2010 | ELISA | 83 |
| | –0.004 (0.902) [a, d] | 834 | The Women's Health Initiative Study, USA | 2012 | multiplex assay | 9 |
| | -0.046 (0.011) [a, d] | 3044 | The Health ABC Study, USA | 2016 | ELISA (EDTA plasma) | 90 |
| | − 0.10 (>0.05) [b, c] | 620 | Female participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| | − 0.04 (>0.05) [b, c] | 510 | Male participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| | 0.14 (<0.001) [a, d, f] | 3193 | The Nutrition and Health of Aging Population in China study, China | 2008 | a Bio-Rad Multiplex Suspension Array System | 43 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Leptin | 0.14 (<0.001) [a, d] | 834 | The Women's Health Initiative Study, USA | 2012 | multiplex assay | 9 |
| | 0.056 (0.002) [a, d] | 3044 | The Health ABC Study, USA | 2016 | ELISA (EDTA plasma) | 90 |
| | 0.17 (<0.05) [b, c] | 620 | Female participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| | 0.03 (>0.05) [b, c] | 510 | Male participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| **Inflammatory biomarkers** | | | | | | |
| hsCRP | 0.22 (<0.0001) [a, d] | 1065 | The Nurses' Health Study, USA | 2010 | ELISA | 83 |
| | 0.19 (<0.05) [b, c] | 4937 | The Women's Health Initiative, USA | 2016 | a multiplex assay | 42 |
| | 0.184 (<0.001) [a, d] | 3044 | The Health ABC Study, USA | 2016 | ELISA (EDTA plasma) | 90 |
| | 0.12 (>0.05) [b, c] | 620 | Female participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| | 0.10 (>0.05) [b, c] | 510 | Male participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| | 0.12 (<0.001) [a, d, f] | 3193 | The Nutrition and Health of Aging Population in China study, China | 2008 | a Bio-Rad Multiplex Suspension Array System | 43 |
| | 0.222 (<0.01) [a, c] | 912 | The Korea Genome Epidemiology Study - Ansung cohort, South Korea | 2020 | multiplex assay | 49 |
| TNF-α | 0.20 (<0.001) [a, d] | 834 | The Women's Health Initiative Study, USA | 2012 | multiplex assay | 9 |
| | 0.26 (<0.001) [a, d] | 3044 | The Health ABC Study, USA | 2016 | ELISA (EDTA plasma) | 90 |
| | 0.14 (< 0.001) [a,c] | 1508 | The Finnish Health 2000 Survey, Finland | 2011 | in-house assay based on the DELFIA technique | 68 |
| IL-6 | 0.26 (<0.0001) [a, d] | 1065 | The Nurses' Health Study, USA | 2010 | ELISA | 83 |
| | 0.23 (<0.001) [a, d] | 834 | The Women's Health Initiative Study, USA | 2012 | multiplex assay | 9 |
| | − 0.07 (>0.05) [b, c] | 620 | Female participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| | 0.04 (>0.05) [b, c] | 510 | Male participants, The NU-AGE cohort, France, Italy, the Netherlands, Poland, and the United Kingdom | 2019 | biochemical assay | 93 |
| **Metabolic biomarkers** | | | | | | |
| HbA1c | 0.01 (>0.05) [a, d] | 1065 | The Nurses' Health Study, USA | 2010 | ELISA | 83 |
| | 0.096 (<0.001) [a, d] | 3044 | The Health ABC Study, USA | 2016 | ELISA (EDTA plasma) | 90 |
| | -0.09 (<0.001) [a, d, f] | 3193 | The Nutrition and Health of Aging Population in China' Study, China | 2002 | a Bio-Rad Multiplex Suspension Array System | 43 |
| | 0.065 (>0.05) [a, c] | 912 | The Korea Genome Epidemiology Study - Ansung cohort, South Korea | 2020 | multiplex assay | 49 |
| HDL-C | -0.107 (<0.001) [1a, 4d] | 3044 | The Health ABC Study, USA | 2016 | ELISA (EDTA plasma) | 90 |
| | -0.09 (<0.01) [b, c, e] | 827 | The EPIC-Potsdam Study, Germany | 2008 | ELISA | 44 |
| | 0.14 (0.001) [a,c] | 1508 | The Finnish Health 2000 Survey, Finland | 2011 | in-house assay based on the DELFIA® technique | 68 |
| C-peptide | 0.14 (<0.0001) [a, d] | 1065 | The Nurses' Health Study, USA | 2010 | ELISA | 83 |

*(ELISA: enzyme-linked immunosorbent assay; EDTA: ethylene diamine tetraacetic acid; BMI: Body mass index; WC: Waist circumference; hsCRP: high-sensitivity C-reactive protein; TNF-α: Tumor necrosis factor; IL-6: Interleukin 6; HbA1c: hemoglobin A1C; HDL-C: high-density lipoprotein cholesterol; Ref: reference).*

*[a]original resistin levels; [b]log-transformed resistin levels; [c]Pearson correlation; [d]Spearman rank correlation. [e]Age- and sex-adjusted correlation; [f]Age-, sex-, region-, and residence-adjusted correlation.*

*The participants in the population-based studies were described as follows:*

- *The Nurses' Health Study, USA:*[83] *Female registered nurses, aged 30 to 55 years, hailing from 11 US states.*
- *The Women's Health Initiative, USA:*[9,42] *postmenopausal women residing in the community of 40 US centers aged 50–79 years.*
- *The Health ABC Study, USA:*[90] *community-dwelling participants aged 70-79 years residing in areas surrounding Memphis, Tennessee, and Pittsburgh, Pennsylvania*
- *The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study, Germany:*[44] *community-dwelling participants aged 35-65 years for women and 40- 65 years for men.*
- *The Finnish Health 2000 Survey:*[68] *A study was conducted in 2000-2001, which sampled a two-stage, stratified cluster of 8,028 individuals representing the entire Finnish population aged 30 years and above.*
- *The NU-AGE cohort (The New Dietary Strategies Addressing The Specific Needs Of Elderly Population For An Healthy Ageing In Europe), France, Italy, the Netherlands, Poland, and the United Kingdom:*[93] *individuals aged between 65 and 79 years from the community who were free from significant and noticeable chronic illnesses.*
- *The Nutrition and Health of Aging Population in China study, China:*[43] *men and women ranging from 50 to 70 years of age, residing in both urban and rural regions of the northern (Beijing) and southern (Shanghai) areas.*
- *The Korea Genome Epidemiology Study - Ansung cohort, South Korea:*[49] *residents aged 40–69 years from an industrialized community 100 km south of Seoul (Ansung).*

## 1.3. Colorectal cancer (CRC)

### 1.3.1. CRC epidemiology

CRC holds the third position in terms of the most commonly diagnosed cancers and the second position in terms of the leading cause of cancer-related deaths worldwide,[94] with an estimated number of new CRC cases worldwide reaching 3.2 million in 2040.[2] Incidence rates are higher in higher Human Development Index (HDI) countries compared to lower HDI countries and are highest in European regions.[94]

Although CRC development is complex and multi-factorial, it occurs through various separate pathways: chromosomal instability (CIN), microsatellite instability (MSI), serrated, and inflammatory pathway.[17,18] Furthermore, angiogenesis occurs during the CRC cancer development through all the pathways by initiating an “angiogenic switching” phenomenon.[18,95] The timeline for tumorigenesis depends on the particular pathway involved.[18] For instance, the CIN or inflammatory pathways may take at least 10 years to decades, while the MSI pathway, which is relatively faster, can lead to tumor development within 1–3 years.[18,96]

### 1.3.2. Factors associated with CRC risk

While the three first pathways are characterized by the accumulation of genetic, epigenetic, and oncogene alterations; the inflammatory pathway involves other modifiable factors related to severe systemic inflammation.[17] According to the World Cancer Research Fund, the major risk factors for CRC development via the systemic inflammation pathway with strong evidence are inflammatory bowel disease, smoking, being overweight or obese, being tall, dietary factors (high intake of red meat, processed meat, excessive alcohol intake).[97] Factors that decrease the risk of CRC include being physically active, healthy dietary factors (the consumption of wholegrains, high dietary fiber foods, and dairy products), and the use of calcium supplements.[97] Other factors implicated in CRC development are age, sex, race, socioeconomic status, and family history of cancer, while gut microbiome was indicated as closely related to CRC.[98] Information on risk factors for CRC was used to create a DAG later in the method section.

To reiterate, as one of the major factors related to CRC,[97] obesity is the result of the excessive buildup of fat tissue and is marked by chronic low-grade inflammation, accompanied by the production of pro-inflammatory cytokines and hormones, which can promote systemic inflammation and contribute to the development of several diseases,

including CRC.[4] Adipokines which are cytokines secreted from adipose tissue, such as resistin and leptin, have also been implicated as potential mediators of the link between obesity and CRC development.[5]

### 1.4. Resistin and Colorectal cancer

As described in **1.1** and **1.2.1**, although not directly produced by adipocytes, adipose tissues, in general, may still play a role in resistin production by inducing inflammation. Indeed, although always being listed as an adipokine,[10,12] resistin and adiposity measurements (BMI/ WC) do not have a such strong correlation in humans (described in **1.2.2.1**, or **Table 2**). However, many studies have shown that even when dismissing the adipokine role of resistin, resistin may still link to CRC development by regulating the inflammatory process via the NF-kB inflammatory pathway (described in detail in section **1.2.2.2**). In addition, resistin could induce cell proliferation via the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling in the CIN pathway.[14] Of note, both the inflammatory pathway and CIN pathway were mentioned in section **1.3.1** as the main pathways to CRC development. Furthermore, evidence supported that resistin may involve in angiogenesis,[16] at least in a study of human coronary artery endothelial cells.[16] Resistin upregulates the expression of vascular-permeability-regulated-factor (vascular endothelial growth factor (VEGF), VEGFR-1, and VEGFR-2) and other angiogenesis-promoting factors (matrix metalloproteinases (MMP)-1 and MMP-2).[16] Since angiogenesis might occur at the initiation of cancer development or at the hypoxia-induced angiogenic switch point,[95] angiogenesis could also play a pathway from resistin to CRC development.[14]

In terms of epidemiological studies, seven studies estimated the magnitudes of the association between resistin and risk of, or odds of CRC.[9,11,92,99-102] Details of these studies were summarized in the original paper.[103] Among those, only one study was a prospective study in postmenopausal women within the WHI study which allowed for the collection of blood samples (and therefore the measurement of resistin) before the diagnosis of CRC ("pre-diagnostic resistin").[9] This WHI study suggested no association between baseline resistin and risk of CRC.[9] In contrast, results from retrospective case-control studies have shown higher levels of resistin in CRC patients compared to controls.[9,11,92,99-102] Resistin concentrations measured in these case-control studies could result from the presence of existing tumors. The odds ratio estimated derived from these retrospective case-control studies should be interpreted as higher levels of resistin were

associated with higher odds of CRC rather than with higher risk of CRC. As the WHI study population consisted entirely of women, the study findings can not be generalized to the general population, which includes both men and women.[103] Furthermore, subgroup analyses by tumor sites or other effect modifiers were not performed, thus, the WHI study has not provided any risk estimate of colon or rectal cancer as well as CRC risk estimated in subgroups by sex.[103] Therefore, evidence on circulating resistin concentrations and risk of CRC has been sparse.

### 1.5. Design considerations of epidemiological biomarker studies

In retrospective case-control studies, a significant issue is that the presence of CRC may have affected resistin levels instead of resistin being a risk factor for CRC. While the research question is whether increased resistin concentrations lead to an increased risk of CRC, point estimates from retrospective case-control studies were prone to temporal bias, and concluding resistin concentrations and “risk” of CRC may not be appropriate. Exposure assessment using conventional methods such as questionnaires enabled recollections recalled by study participants on the exposure information that occurred in the past; whereas, in studies where biomarkers are used as exposure, the information for exposure could not be retrospectively collected. In order to accurately estimate the risk of developing CRC, it is crucial to obtain data on resistin levels prior to the diagnosis of CRC (referred to as "pre-diagnosis resistin"), thus, the association between resistin and risk of CRC should be estimated from prospective studies such as cohort, case-cohort, or nested case-control studies.

A cohort study is a type of research that follows a group of people over time and collects data on their exposure to risk factors and the development of specific outcomes or health conditions.[104] This research approach provides several advantages, including obtaining adequately accurate exposure data, reducing recall bias by collecting exposure information before disease diagnosis, and increasing generalizability.[104,105] However, as cancer is not a common outcome, there may be limited cases at the end of the follow-up period, thus, collecting biomarker data for all participants in a large cohort study is expensive and time-consuming.[104] Furthermore, biosamples become more valuable over time, especially decades after collection. Therefore, it is necessary to conduct more cost-effective and logistically feasible studies with smaller sample sizes which only result in an acceptable decrease in statistical power.

Two cost-effective alternatives to full cohort studies are the case-cohort study and the nested case-control study.[104] In case-cohort studies, controls are selected as a random subset or a stratified random sample of the total baseline cohort representing the cohort's exposure distribution.[105] Subcohorts can be selected prospectively (once achieving full assembly of the cohort) or retrospectively (at the end of follow-up after cases ascertainment).[105] Depending on grant funding periods, laboratory specimens could be processed first for the subcohort and later for cases or could be stored until the end of follow-up and processed after randomly assigning to analytic batches.[105] In nested case-control studies, controls are individuals who have not yet developed the disease at the time a case occurs selected from among the members of the cohort, and are matched with cases on several baseline characteristics such as age and gender using incidence density sampling approaches, and laboratory specimens can be processed at the same time for cases and matched controls.[105]

Novel biomarkers are discovered decades after the time of blood collection of cohort studies (for example, resistin was found in 2001 while the EPIC study was initiated in 1990). The samples used for the laboratory analysis of novel biomarkers were aliquoted, frozen, thawed, and refrozen for future use. Thus, the study design should be appropriate for analyzing several novel biomarkers (for example, resistin was analyzed together with several obesity-related biomarkers), each with a different required sample volume, with the use of the smallest amount of specimens without affecting the remaining specimens.[105] Importantly, technical variability due to laboratory processes may occur when samples are processed in different batches ("batch effects") (for example, the use of different lots of commercial kits between the first-analyzed samples and the last-analyzed samples). This is more likely to be controlled in the nested case-control than the case-cohort study (details are shown in **Table 3**). Indeed, it often requires a significant amount of effort and may not be feasible to analyze samples from the subcohort and cases in the same batches because the subcohort is selected to serve as a comparison group for several disease outcomes with different induction periods.[105] While in nested case-control studies, biomarkers are measured in the samples of cases and their corresponding controls together in the same batches. The issues of batch effects become more profound in laboratory analysis of additional cases ("subsequently ascertained

cases") in case-cohort studies because samples from these subsequently ascertained cases may be analyzed up to years after the analysis of samples from the subcohort. In nested case-control studies, the procedures for selecting controls and analyzing samples for subsequently ascertained cases are identical for the initially ascertained cases, thus, batch effects are not an issue with this type of study.

**Table 3: Advantages and disadvantages of a case-cohort study and a nested case-control study in light of novel biomarker studies.** The information provided in this table captures the key points discussed in the publication by Rundle et al. 2005.[105] This table was not included in the dissertation-based publication.

| | Case-cohort study | Nested cases control study |
|---|---|---|
| Control sections | Controls could be representatives of the larger cohort. No matching is performed at the time of control selection.<br>Data for the subcohort could be used for analyzing more than one outcome. | Controls may not accurately reflect the person-time experience of the entire cohort. The more factors matched, the less the sample size; also, matching creates a very clustered and complex data structure.<br>Controls share many risk factors as cases due to the matching and have a high possibility to become cases at the end of follow-up. |
| Time scale | Time scales are selected to be the most appropriate for conducting the analyses, but cases and the subcohort may have different time scales. | Controls are conventionally matched with cases based on their age. Cases and matched controls have the same length of follow-up, thus, any analysis of the data must incorporate these factors as the time scale. |
| For initially ascertained cases | Batch effects could be controlled ensuring that the samples of cases and their corresponding controls are processed together in the same batches. Storage effects and thawing-freezing cycle effects should be constrained in the statistical analyses.<br>Freeze-thaw effects could be problematic since the same subcohort can be utilized for multiple disease outcomes.[105] | Effects of long-term storage, freeze-thaw cycles, and the analytic batch could be controlled by matching processes.[105] The design is served best when exact matching (with other factors) does not lead to a loss of cases.[105] |
| For subsequently ascertained cases | Batch effects occur when analyzing subsequently ascertained cases years after the laboratory analysis of the subcohort and initially ascertained cases. Storage effect and thawing-freezing cycle effect could be statistically controlled but less efficient. | As the same as the initial ascertained cases and their matched controls. |
| Statistical analysis | Modified Cox regression which enables odds ratio to estimate the incidence rate ratio.<br>The models may even become more complicated to weigh the cases arising from the subcohort or not from the subcohort differently in prospective designs.[104] | Conditional logistic regression, stratified by matched pairs could enable the estimation of the odds ratio represented by the risk ratio. However, controls become cases that could be a problem.[105,106] |

## 1.6. Aims of this dissertation

The two objectives of this dissertation are as follows:

- Primary objective: to estimate the effect of resistin on CRC risk by estimating the confounding-adjusted effect size for the association between pre-diagnostic resistin concentrations and CRC risk in a prospective observational study.
- Secondary objective: to examine the strength of the cross-sectional relationship between resistin levels and factors representing adiposity, inflammation, and metabolism using measures of correlation.

# 2. Methods

## 2.1. The European Prospective Investigation into Cancer and Nutrition (EPIC) study

### 2.1.1. General description

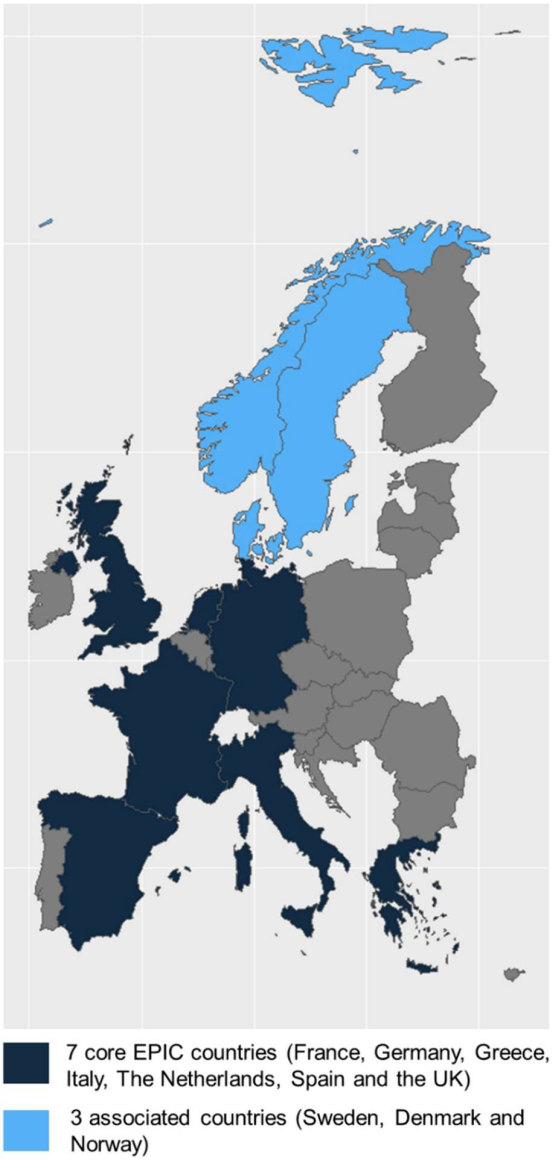


**Table 3: Map of countries participating in the EPIC study.** (Source: Own unpublished illustration created using the **'rnaturalearth'** and **'rnaturalearthdata'** packages in R. URL: github.com/ropensci/rnaturalearth)

Detailed information about the EPIC study can be found in the publication by Riboli and colleagues.[107] Briefly, the EPIC study is one of the largest cohort studies in the world, with 519,978 men and women aged generally from 35 to 70 years recruited during the period from 1992 to 2000. The study enrolled participants primarily from the general population across 23 centers in 10 European countries. These individuals were then followed up for several decades to observe disease outcomes. The EPIC study was initiated with seven core EPIC countries (France, Germany, Greece, Italy, The Netherlands, Spain, and the United Kingdom), and subsequently expanded to include three Scandinavian countries (Sweden, Denmark, and Norway) and the Naples center in Italy as associated EPIC cohorts (**Figure 3**).[107] Data on anthropometry, lifestyle factors, dietary exposure, and bio-samples were collected at baseline using standardized protocols and were harmonized in subsequent stages.

### 2.1.2. Baseline data collection

The collection of baseline data in EPIC has been described elsewhere,[107] and is here briefly summarized. The typical procedures for participant enrollment in the EPIC were:[107] (1) invitations were delivered either by mail or in person, and participants provided their consent through signature on informed consent forms upon their agreement, (2) participants filled out a diet questionnaire and a lifestyle questionnaire at home, along with self-reported anthropometry measurements in some centers, and (3) participants had an examination at the study center, including the retrieval of the two self-fulfilled questionnaires, anthropometric measurements, blood sample collection, and measurements of blood pressure. Some centers had procedures that deviate slightly from the typical procedure: in the centers in Spain, Ragusa (Italy), Denmark, and Malmo (Sweden), only the lifestyle questionnaire was mailed to the participants, and the dietary questionnaire was collected during the examination; and in study centers in Umea (Sweden), and Greece, participants had completed all questionnaires and measurements during the examination at the study center.

The study employed country-specific validated food frequency questionnaires (FFQ) to collect long-term dietary intake data. A quantitative FFQ consisting of 260 food items was administered in Italy, The Netherlands, Germany, Spain, France and Ragusa (south Italy), and Greece; and a semi-quantitative FFQ was used in the United Kingdom, Denmark, Norway, Naples in Italy, and Umeå (Sweden).[108] The questionnaires were mostly self-administered except for Italy (centers Ragusa, Naples), Spain, and Greece. In addition, 7-day records (diaries) were introduced in the United Kingdom and a 14-day record of hot meals was introduced in Sweden, Malmo.

The health and lifestyle questionnaire was used in the core centers of EPIC, while in the associated EPIC cohorts, an independent questionnaire was applied which broadly covered the same variables used in the core centers. Data on lifestyle and other health factors collected from the associated EPIC countries were standardized to align with data obtained from the core EPIC countries using a comprehensive re-coding scheme.[107]

Anthropometry measures were performed following a common protocol to directly measure the height, weight, and waist and hip circumference of all participants. Self-reported height and weight were additionally collected in French participants and merely measured in Norway and a group of participants in the Oxford cohort (the United

Kingdom). Where direct anthropometry measurement data were not available, they were estimated using self-reported anthropometry data.

Blood samples were obtained from 385,747 participants. In the core EPIC centers, Norway, and Naples (Italy), blood samples were aliquoted into straws and partially stored in the gaseous state of liquid nitrogen (at a temperature of -196ºC) in a central repository at IARC. In Sweden and Denmark, blood samples were stored in tubes with temperatures at -70ºC (Sweden), and -150ºC (Denmark).

### 2.1.3. Follow-up data

The description of follow-up approaches in EPIC has been described elsewhere,[107] and is here briefly summarized. Incident CRC cases were ascertained by linking records of patients with regional cancer registries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden, and the United Kingdom) or through an active follow-up procedure involving study participants or their closest-living-relative which were then confirmed by data from the records of health insurance and pathology registries (France, Germany, and Greece). A description of the active follow-up procedure could be found in detail in the publication of Bergmann and colleagues.[109] The accuracy of CRC incident data obtained through active follow-up procedures were comparable to that of data obtained from cancer registries,[109] indicating minimal disparities in CRC case ascertainment among countries,[110] and eliminating the need for standardization or harmonization.

## 2.2. The nested case-control study within EPIC

The description of the nested case-control study within the EPIC study has been described elsewhere,[107] and is here briefly summarized. Data for this dissertation consisting of 1,293 cases and 1,293 matched controls were based on a nested case-control study within the EPIC study.[103] The conceptual timeline of the present nested case-control study is presented in **Figure 4**. The closure dates of the study in each center ranged between December 2001 and December 2005. Cases were determined by the 10th Revision of the International Classification of Diseases (ICD-10) codes C18.0–C20. An incidence density sampling approach was used to select one control from the population at risk of CRC who were cancer-free at baseline to match with each case at the time when the index case was ascertained. Matching factors were study center, sex, age at blood collection (±0.5 to 2 years), blood collection date (±3 months), blood

collection clock time (±4 hours), and fasting hours before blood collection (<3, 3–6, and >6 hours).[111] Furthermore, women were additionally matched based on their menopausal status (premenopausal, perimenopausal, postmenopausal, or surgically menopausal). Among premenopausal women, further matching was done based on the phase of their menstrual cycle phase (follicular early/late, ovulatory or luteal early/late) and contraceptive use (no, yes, or unknown), while among postmenopausal women further matching was done based on the use of hormone replacement therapies (no, yes, or unknown). The matching variables in women were designed for various biomarker studies.

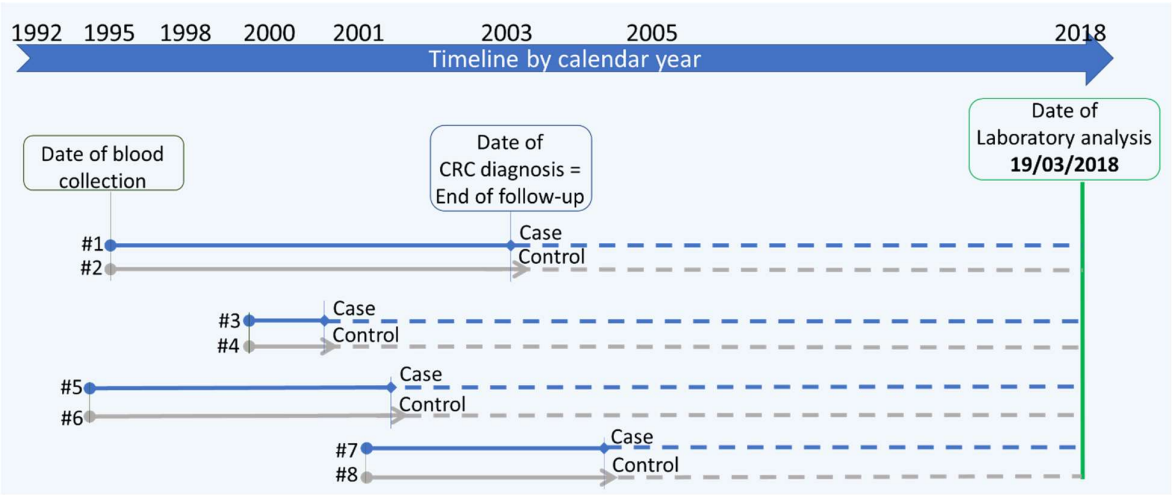


**Figure 4: Conceptual timeline in the present nested case-control study within the EPIC study**. The closure dates (or the latest dates of case ascertainment of the study) in each center ranged between December 2001 and December 2005. (Source: Own unpublished illustration.)

The measures of resistin used in this nested case-control study were reported in the original publication.[103] Briefly, human resistin was measured in all samples using ELISA assays from BioVendor Laboratory Medicine, Inc. in Brno, Czech. Coefficients of variations for the quality controls (QC) at high concentrations, low concentrations, and for an internal QC were all lower than 10.4%. Furthermore, the assays used to analyze the other biomarkers were reported as follows: CRP and HDL-C (high-sensitivity assay (Beckman-Coulter, Woerden, the Netherlands),[111] total adiponectin and high molecular weight (HMW) adiponectin levels (Multimeric ELISA assays (ALPCO Diagnostics, Salem, NH)),[6] leptin and soluble leptin receptor (ELISA assays (Biovendor)),[7] C-peptide (radioimmunoassay (Diagnostic System

Laboratories, Webster, TX)),[112] HbA1c (high-performance liquid chromatography method (Karolinska University Hospital, Stockholm),[113] ROM (Diacron kit (Grosseto, Italy)).[114] Interassay/ intraassay coefficients of variations for QCs at different concentrations of these biomarkers were all lower than 10.1%.[6,7,111-114]

## 2.3. Data availability and ethical approval

Data for this dissertation are stored at the Molecular Epidemiology Research at Max-Delbrück-Centrum für Molekulare Medizin Berlin-Buch (Robert-Rössle-Straße 10, 13125 Berlin, Deutschland). Due to ethical restrictions imposed by the EPIC Steering Committee, raw data cannot be freely shared with the public. Aggregated data are available to other researchers upon request, and inquiries should be sent to Prof. Dr. Tobias Pischon (Tobias.Pischon@mdc-berlin.de). Proposals for accessing the EPIC data should be submitted to IARC-WHO.

Since the present dissertation utilizes pre-collected data from EPIC, obtained with the approval from IARC-WHO as part of its parent research proposal, no additional ethical approval is required for this study. The publication[103] from which this dissertation is derived follows the recommended guidelines of the STROBE checklist. The STROBE checklist is included in this dissertation as a supplement to the publication[103].

## 2.4. Statistical analysis

### 2.3.1. Study population characteristic

The distributions of baseline variables were tabulated using the format of means (standard deviations) or median (quartiles ranges) for continuous variables, and frequencies (percentages) for categorical variables across resistin quartiles (Q1, Q2, Q3, Q4) as well as in cases and controls.[103] The difference in resistin levels between cases and controls for each matched pair was calculated and referred to as paired differences ($Resistin_{paired\ difference} = Resistin_{case} – Resistin_{control}$). The length of follow-up for each case was estimated as the time difference between the date of blood collection and the date of CRC diagnostic, and the length of storage time was defined as the time from blood collection to the date of laboratory analysis (19/03/2018). For controls who later became cases, the time intervals between the end of follow-up for being controls and the date of CRC diagnosis were estimated and reported.

### 2.3.2. Primary objective

The effect sizes of the association between resistin concentrations and CRC risk were estimated using conditional logistic regression models and stratified by countries and presented as RRs and 95%CIs; with the note that, odds ratios estimated from the conditional logistic regression models could be interpreted as RRs with the present study design. As the exposure in the models, resistin concentrations were analyzed by quartiles based on the distribution of the concentrations in controls and by log-transformed values by log2 that were later interpreted as an increase of log-transformed resistin concentrations corresponding to doubling resistin concentrations in the original scale (ng/ml). Rationales for using these continuous and categorical forms of resistin concentrations were mentioned in the original publication.[103]

**Covariate selection: The directed acyclic graph (DAG)**

A DAG[115] was created using the DAGitty web tool[116] (http://dagitty.net/) to visually represent the association between resistin and CRC risk and to summarily reflect the current knowledge about resistin and CRC risk which was mentioned in the introduction of the present dissertation. All of the major risk factors for developing CRC that were outlined in section **1.3.2** were summarized and included in the graph (**Figure 5**). Afterward, based on the literature review of the risk factors of CRC related to resistin levels that were discussed in section **1.2.2.4**, arrows were plotted to illustrate the relationships between resistin and these factors. Finally, the associations of each CRC-related factor and the others were extracted from a publication,[117] and reviewed manually if not available. The DAG showing the assumed effects of resistin on CRC risk is shown in **Figure 5** as follows:

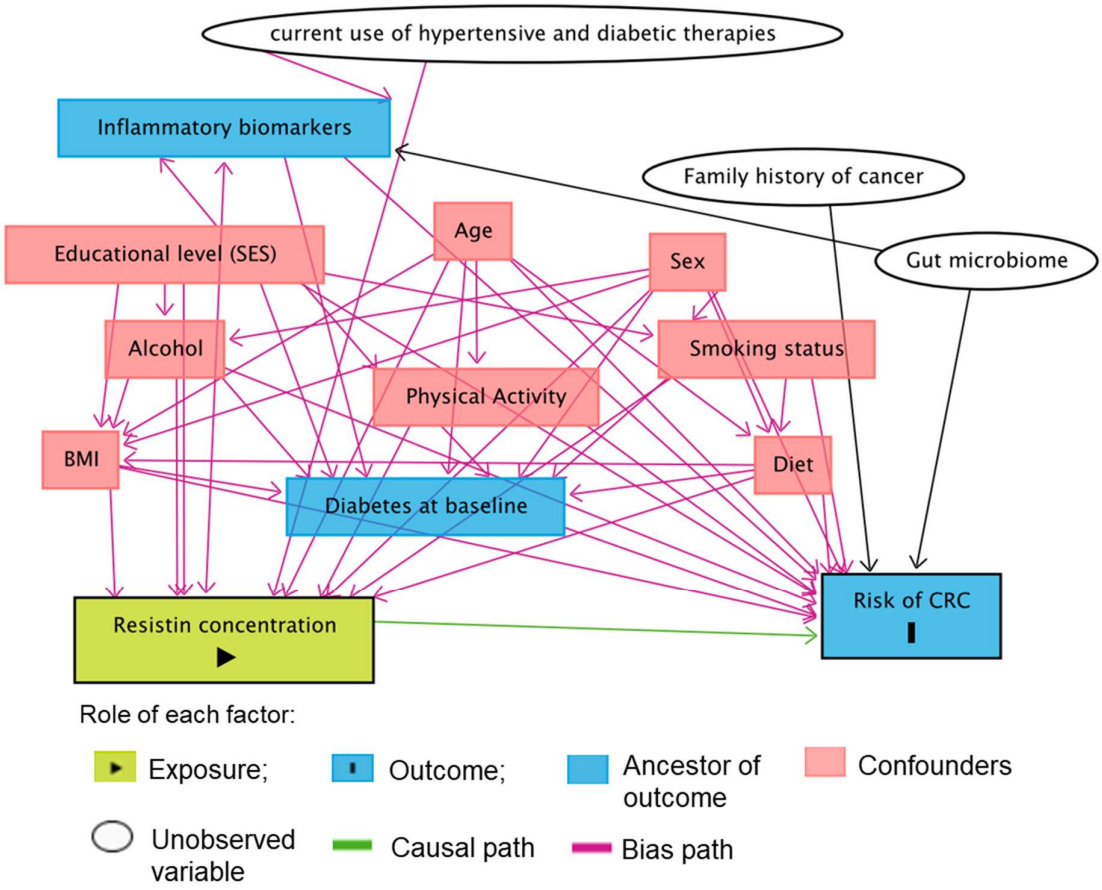


**Figure 5: Directed acyclic graph showing the association between pre-diagnostic resistin and risk of CRC.** Source: Own unpublished work. Education was used as a proxy for socioeconomic status. Dietary variables in the graph include intakes of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, vegetable,[98,118] and energy intake. Paths between all risk factors were from a publication,[117] and reviewed manually. With the uncertainties in the presence or absence of arrows between variables in the final proposed DAG, more than one prior DAG was plotted,[119] however, the final proposed DAG was only minorly altered when adding one and subtracting one pattern in the graph. Minor alterations were shown as follows:

(1) The path from BMI to resistin may not be present,[41,43,120] and eliminating the path from BMI to resistin would result in BMI becoming an ancestor of the outcome;
(2) The path from resistin to diabetes may be present,[60] or may not present,[61] however, the role of each factor does not change when adding the path from resistin to diabetes.
(3) Adding paths from other factors to inflammation biomarkers and/or gut microbiome without considering their relationship and assuming all variables are measured, does not alter the role of each factor in the DAG.

(Abbreviations: BMI: Body mass index; WC: Waist circumference; CRC: colorectal cancer).

**Final models**

As in the DAG of resistin and CRC risk, all observed factors presented as potential confounders in the DAG (**Figure 5**) were included in the maximally adjusted model (Model 3). As described earlier in **1.2.2.1**, resistin is not strongly correlated with BMI/WC in population-based studies, however, it may be still linked to the chronic low-grade subclinical inflammation of adipose tissues. We put forth two distinct scenarios, one suggesting the presence of a path connecting resistin and BMI/WC, while the other positing the non-existence of such a pathway. The first scenario was accounted for in the maximally adjusted model (BMI/WC presented as a confounder). For the second scenario, removing the pathway between resistin and BMI/WC would make BMI/WC an ancestor of the outcome. Hence, a model without adiposity measurements was performed (Model 2). Moreover, evidence for the paths between resistin and lifestyle and dietary factors has been scarce. To accommodate the possibility of these paths being non-existent, a model without adjustment for these factors was performed. Ultimately, three models were employed for analysis. Model 1 was conditioned on matching factors only. Model 2 was built upon Model 1 by including smoking status (never having smoked, formerly smoked, or currently smoking), education level (no formal education, primary school, technical/professional school, secondary school, or education beyond secondary school including university-level), alcohol abstainers (yes/no, while "yes" was defined as an intake under 0.3 grams/day), physical activity index (inactive, moderately inactive, moderately active, active), energy intake (kcal/day), and the intake estimated by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy, dietary fiber, fruit, vegetable, as additional adjusted factors. Model 3 added to Model 2 by incorporating two additional variables including BMI ($kg/m^2$) and the residuals of the regression of BMI on WC (to avoid multicollinearity).

**Missing data in covariates**

The numbers of missing observations in cases and controls for each covariate were as follows: all dietary variables (4 in cases and 0 in controls), waist circumference (77 in both cases and controls), smoking status (14 in cases and 15 in controls), education levels (43 in cases and 34 in controls), and physical activity index (16 in cases and 18 in controls). The missing data were assumed as missing completely at random and imputed as median-sex-specific values. Other methods for

dealing with missing data (complete case analysis, multiple imputations) were applied in sensitivity analyses.

**Subgroup analyses and sensitivity analyses**

Subgroup analyses were carried out using Model 3 by prespecified potential effect modifiers including tumor subsites (colon, rectum, and subsite of colon cancer (distal colon cancer, proximal colon cancer)) and sex (male, female, female with restriction to postmenopausal women to generate a comparable estimate to the previous prospective study,[9] the combination of sex and subsite, and the length of follow-up (>5 years, 2 to up to 5 years, ≤2 years). Further subgroup analyses by baseline obesity status (yes (BMI≥30)/ no (BMI<30)), BMI (<25, ≥25) (due to a low prevalence of obesity in this present study), fasting status (yes (>6 hours), no (≤6 hours)), baseline diabetes (yes/no), hsCRP (<3mg/l, ≥3mg/l), and C-peptide (<2 ng/ml, ≥2 ng/ml) were performed using unconditional logistic regression models with covariates in model 3 and additionally adjusted for matched variables.

Sensitivity analyses were performed by additionally adjusting for height, lifetime alcohol consumption, intensity and duration of smoking, baseline diabetes, and inflammatory indicator (hsCRP levels) to examine if the estimated effect sizes changed after these adjustments. Moreover, the analysis was repeated in the datasets (1) in which missing data were imputed by multiple imputation approach, (2) in which all missing data of covariates were deleted (complete case analysis), (3) excluding participants with extreme resistin levels (defined as “values outside of the range from Q1-1.5*(Q3-Q1) to Q3+1.5*(Q3-Q1)”,[121] and (4) excluding matched pairs in which controls became cases the end of follow-up as controls. All subgroup analyses were repeatedly performed after excluding participants with less than or equal to 2 years of follow-up. In order to check whether the matching process itself eliminated the confounding effect of the matching variables in the main conditional logistic regression models,[122] we also repeated analyses using the unconditional logistic regression model adjusting for all matching factors and covariates selected in Model 3. As an additional sensitivity analysis, a meta-analysis was conducted to determine a pooled RR by combining RRs from the present study with those from the WHI study[9] using the random-effects method with an inverse-variance approach.

### 2.3.3. Secondary objective

The correlation between baseline resistin levels and factors represented for adiposity, inflammation, and metabolism in cases and controls were measured using Spearman rank correlation adjusted for age and sex. Correlations in cases were re-estimated after excluding individuals who developed CRC within 2 years after recruitment. As a sensitivity analysis, further adjustment for BMI was performed to estimate the correlations between resistin and hsCRP. We estimated the Spearman partial rank correlation statistics between resistin and hsCRP in controls again by additionally stratifying by hsCRP (<3mg/l, ≥3mg/l), baseline obesity status (yes (BMI≥30)/no (BMI<30)), and baseline diabetes (yes/no). Fisher's z transformation of Spearman partial correlation statistics was used to test the null hypothesis of equal-zero correlation coefficients if needed. To account for the potential impact of conducting simultaneously several correlation tests, multiple testing corrections were performed to re-estimate the p-values using the rank-based Benjamini-Hochberg method.

All statistical tests were two-sided, and statistical significance was attributed to p-values below 0.05. In cases where multiple testing corrections were applied, a re-estimated p-value below 0.05 was considered statistically significant. All analyses were performed by using SAS Enterprise Guide version 8.3 (SAS Institute, Inc., Cary, North Carolina).

# 3. Results

## 3.1. Study population characteristics

All study participants' characteristics were described by quartiles of resistin (in controls) and by case-control status in the original publication.[103] Among participants who did not develop CRC, those in the higher quintiles of resistin levels were characterized by higher ages, a higher proportion of women, higher levels of hsCRP and C-peptide, a lower proportion of individuals with education beyond secondary school including university-level and fasting blood samples, lower alcohol consumption, and lower levels of HDL-C levels compared to those in lower quintiles of resistin levels. As compared to participants who did not develop CRC, those who developed CRC were characterized by a lower proportion of active physical activity, lower dietary fiber intake, higher BMI, WC, and circulating levels of HMW adiponectin, ROM, hsCRP, and HbA1c.[103] The ranges of resistin concentrations in cases and controls were 1.3 to 23.5 ng/ml and 1.0 to 34.4 ng/ml, respectively. Resistin concentrations were similar in cases (means (SD), 4.7 (2.0) ng/ml) and controls (4.7 (2.2) ng/ml).[103] There was no significant mean difference in resistin concentrations between cases and controls (paired difference = 0.07, 95%CI (-0.09; 0.22)) (data not shown). The mean length of follow-up for cases in the nested case-control study population was 4.8 (±2.7) years, with minimal and maximal lengths were 0.02 and 12.6 years. In the whole study population, blood samples were stored for an average of 22.6 (±1.4) years with a range from 16.0 to 15.6 years. Of note, with the incident density sampling approach to select controls, controls could theoretically become cases at a later time. In the present study, 8 controls later became cases with lengths of 0.75 to 8.48 years as time intervals between being controls and being cases, among them, only 2 individuals were diagnosed with CRC within 2 years since the end of follow-up when being controls.

## 3.2. Primary objective: The association between resistin and CRC risk

There was no statistical association between circulating resistin and risk of CRC (**Figure 6**). In model 3 with the maximal adjusted set of covariates, effect sizes for the association were $RR_{Q2\ vs\ Q1}$: 1.11 (95% CI, 0.88; 1.39), $RR_{Q3\ vs\ Q1}$: 1.21 (95% CI, 0.97; 1.53), $RR_{Q4\ vs\ Q1}$: 1.15 (95% CI, 0.91; 1.46), p-trend= 0.41, and $RR_{per\ doubling\ resistin\ concentration}$: 1.11 (95% CI, 0.94; 1.30); p = 0.22.[103] The observed effect sizes attenuated minimally in the 3 different models with resistin as a continuous or categorical. Of note, the linear

relationship between circulating resistin and risk of CRC was graphically shown by using a restricted cubic spline regression model and described previously in the original publication[103]. As additional results, the pooled RRs of the two published prospective studies (the WHI study[9] and the EPIC study[103]) for all study participants and the postmenopausal women group were 1.10 (95%CI: 0.93; 1.29) and 1.09 (95%CI: 0.86; 1.39), respectively.[103]

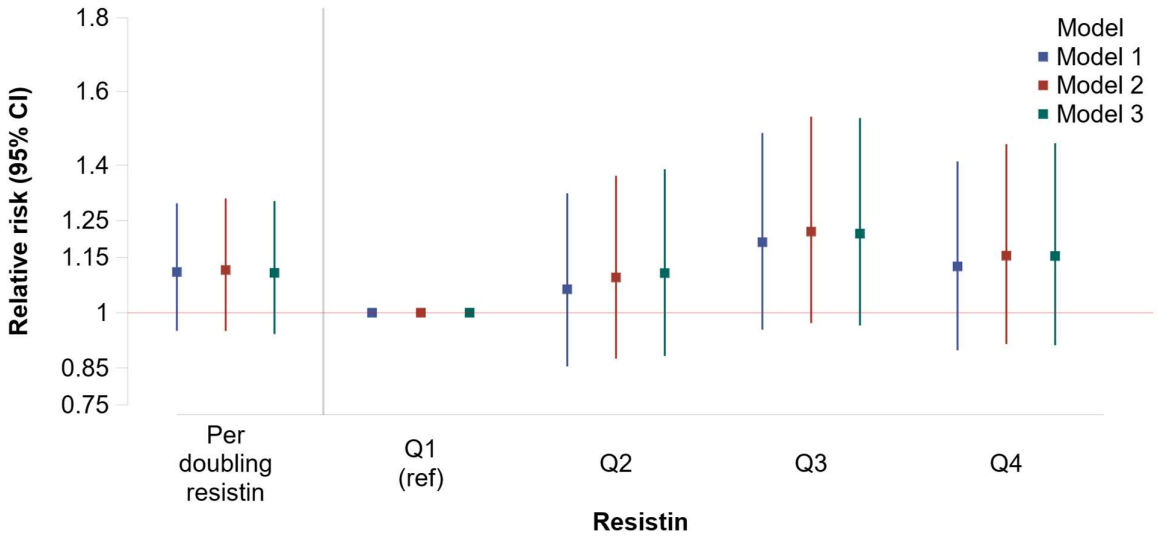


**Figure 6: Estimated effect sizes for the association between pre-diagnostic resistin concentrations and risk of CRC among 3 different conditional logistic regression models.** Three models were used. ***Model 1*** was conditioned on matching factors only (sex (men, women, and women with further matching for menopausal status, menstrual cycle phase, oral contraceptive, and hormone replacement therapy use), age at blood collection, blood collection date, blood collection clock time, and fasting hours before blood collection. ***Model 2*** was built upon Model 1 by including smoking status, education level, alcohol abstainers, physical activity index, energy intake, and the intake by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, vegetable as additional adjusted factors. ***Model 3*** added to Model 2 by incorporating two additional variables including body mass index and the residuals of the regression of body mass index on waist circumference (to avoid multicollinearity. This figure depicts the estimated effect sizes derived from published tables in Pham et al. 2022.[103]

Results of subgroup analyses by tumor subsite (colon, rectal, and subsite of colon cancer (distal colon cancer, proximal colon cancer)), sex (men, women, and postmenopausal women), and the combination of sex and subsite are shown in **Figure 7**. In general, resistin was not statistically significantly associated with risk of colon, rectal, distal colon, or proximal colon cancer. When stratifying by sex, a significantly higher risk of CRC was observed in women with higher resistin levels ($RR_{Q3vsQ1}$: 1.39, 95%CI: 1.00; 1.94), and postmenopausal women with higher resistin levels ($RR_{Q3vsQ1}$: 1.48 (1.01; 2.18). Furthermore, there was a significantly higher risk of rectal cancer in males ($RR_{\text{per doubling resistin concentration}}$:1.53, 95%CI: 1.01; 2.32). However, these significant associations did not remain after excluding participants with less than or equal to 2 years of follow-up (right-side of **Figure 7**).

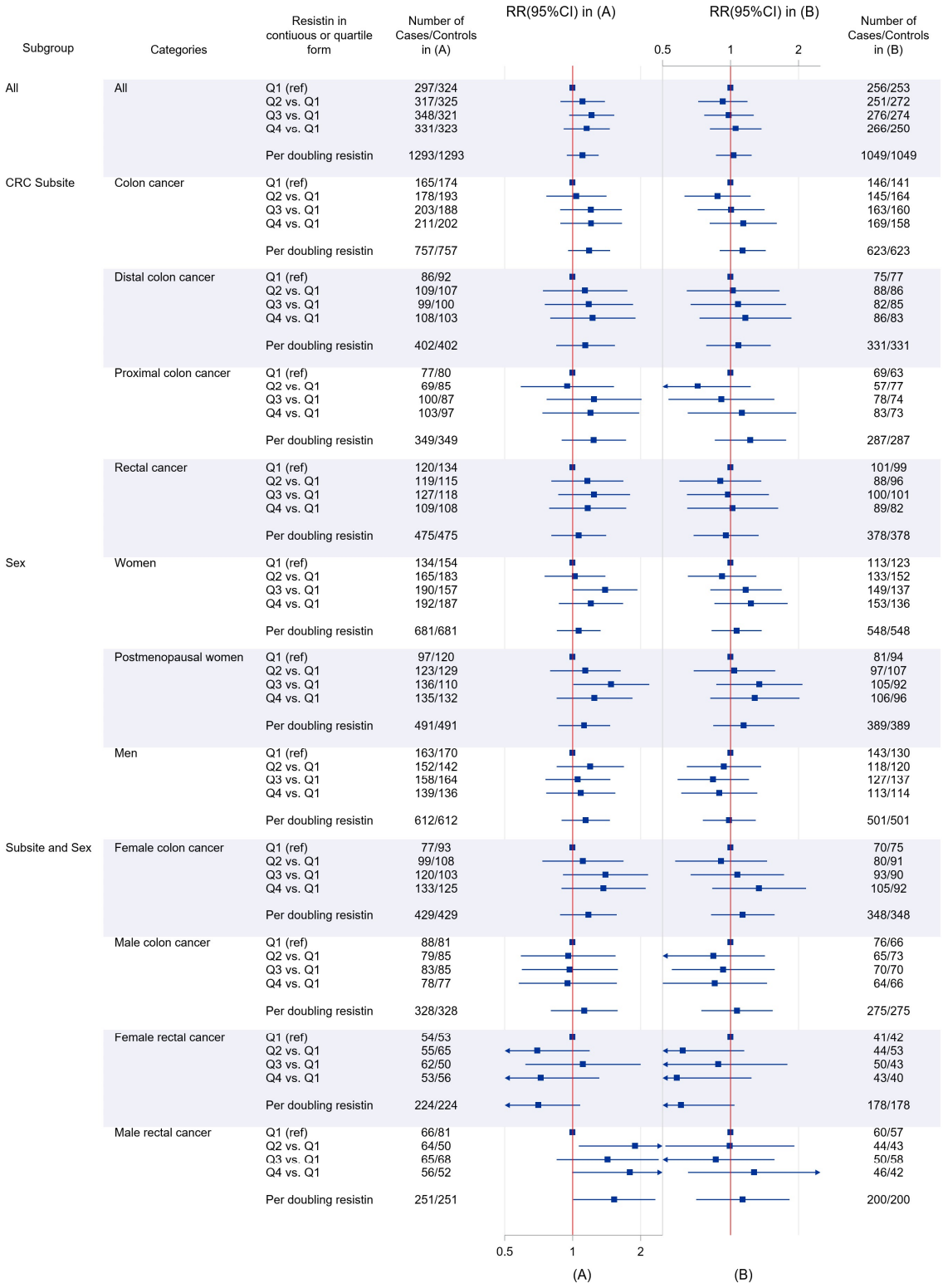


**Figure 7: Estimated effect sizes for the association between pre-diagnostic resistin concentrations and CRC risk by cancer subsite, sex, and the combination of cancer subsite and sex, are presented in (A): All participants; (B): Excluding participants with ≤2 years follow-up**. RR: Relative risk. Results were based on ***Model 3*** (conditional logistic

regression models) conditioned on matching factors (sex (men, women, and women with further matching for menopausal status, menstrual cycle phase, and oral contraceptive and hormone replacement therapy use), age at blood collection, blood collection date, blood collection clock time, and fasting hours before blood collection), and further adjusted for smoking status, education level, alcohol abstainers, physical activity index, energy intake, and the intake by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, vegetable, body mass index and the residuals of the regression of body mass index on waist circumference. This figure depicts the estimated effect sizes derived from published tables in Pham et al. 2022.[103]

In subgroup analyses by the length of follow-up (years), statistically significant measures of association were observed among participants diagnosed with CRC within 2 years after recruitment and their matched controls ($RR_{Q2\ vs\ Q1}$: 3.36, 95%CI (1.71; 6.62); $RR_{Q3\ vs\ Q1}$: 4.42, 95%CI (2.25; 8.71); $RR_{Q4\ vs\ Q1}$: 1.97, 95%CI (1.06; 3.64) and $RR_{per\ doubling\ resistin\ concentration}$: 1.44, 95%CI (0.97; 2.12)); whereas no such a significant association was observed among the participants diagnosed with CRC with later than 2 years of follow-up (**Figure 8**).

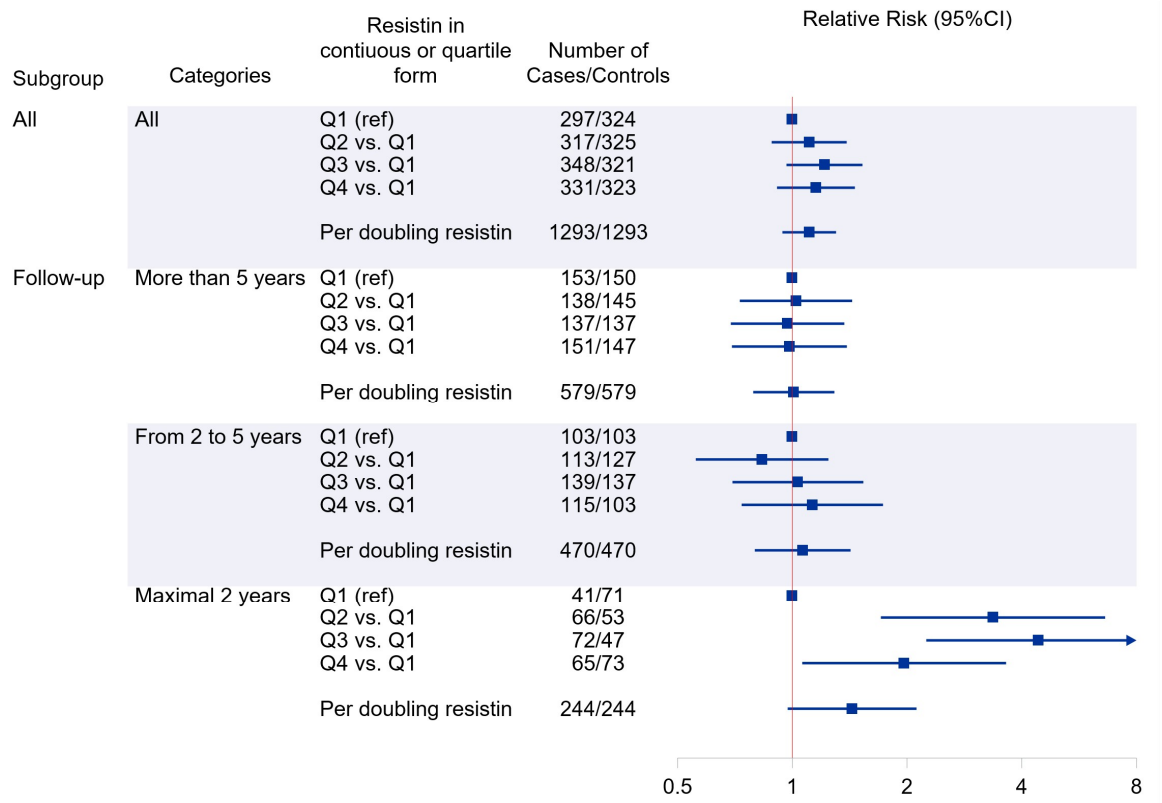


**Figure 8: Estimated effect sizes for the association between pre-diagnostic resistin concentrations and CRC risk by follow-up time.** Results were based on Model 3 (conditional

logistic regression models) conditioned on matching factors (sex (men, women, and women with further matching for menopausal status, menstrual cycle phase, and oral contraceptive and hormone replacement therapy use), age at blood collection, blood collection date, blood collection clock time, and fasting hours before blood collection), and further adjusted for smoking status, education level, alcohol abstainers, physical activity index, energy intake, and the intake by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, vegetable, body mass index and the residuals of the regression of body mass index on waist circumference. This figure depicts the estimated effect sizes derived from published tables in Pham et al. 2022.[103]

Results (RRs and 95%CI) of the subgroup analyses by baseline obese status (BMI≥30, <30), BMI groups (BMI≥25, <25), baseline fasting status, baseline diabetes, hsCRP (≥3mg/l, <3mg/l), C-peptide (≥2ng/ml, <2ng/ml) are shown in **Figure 9**. In general, there was no statistically significant between resistin and CRC risk in different subgroups of participants, except for a significant association observed in the participants with BMI <25, in participants with baseline fasting blood samples (both categorical and continuous forms of resistin), and in participants did not have diabetes at baseline. The significant associations in subgroups by BMI and baseline diabetes did not remain in the analysis excluding participants with less than or equal to 2 years of follow-up. However, there was still a significant association among participants with baseline fasting blood samples ($RR_{Q2\ vs\ Q1}$: 1.43, 95%CI (0.87; 2.36); $RR_{Q3\ vs\ Q1}$: 1.91, 95%CI (1.14; 3.20); $RR_{Q4\ vs\ Q1}$: 1.42, 95%CI (0.85; 2.38) and $RR_{per\ doubling\ resistin\ concentration}$: 1.45, 95%CI (1.00; 2.09) even after excluding participants with less than or equal to 2 years of follow-up.

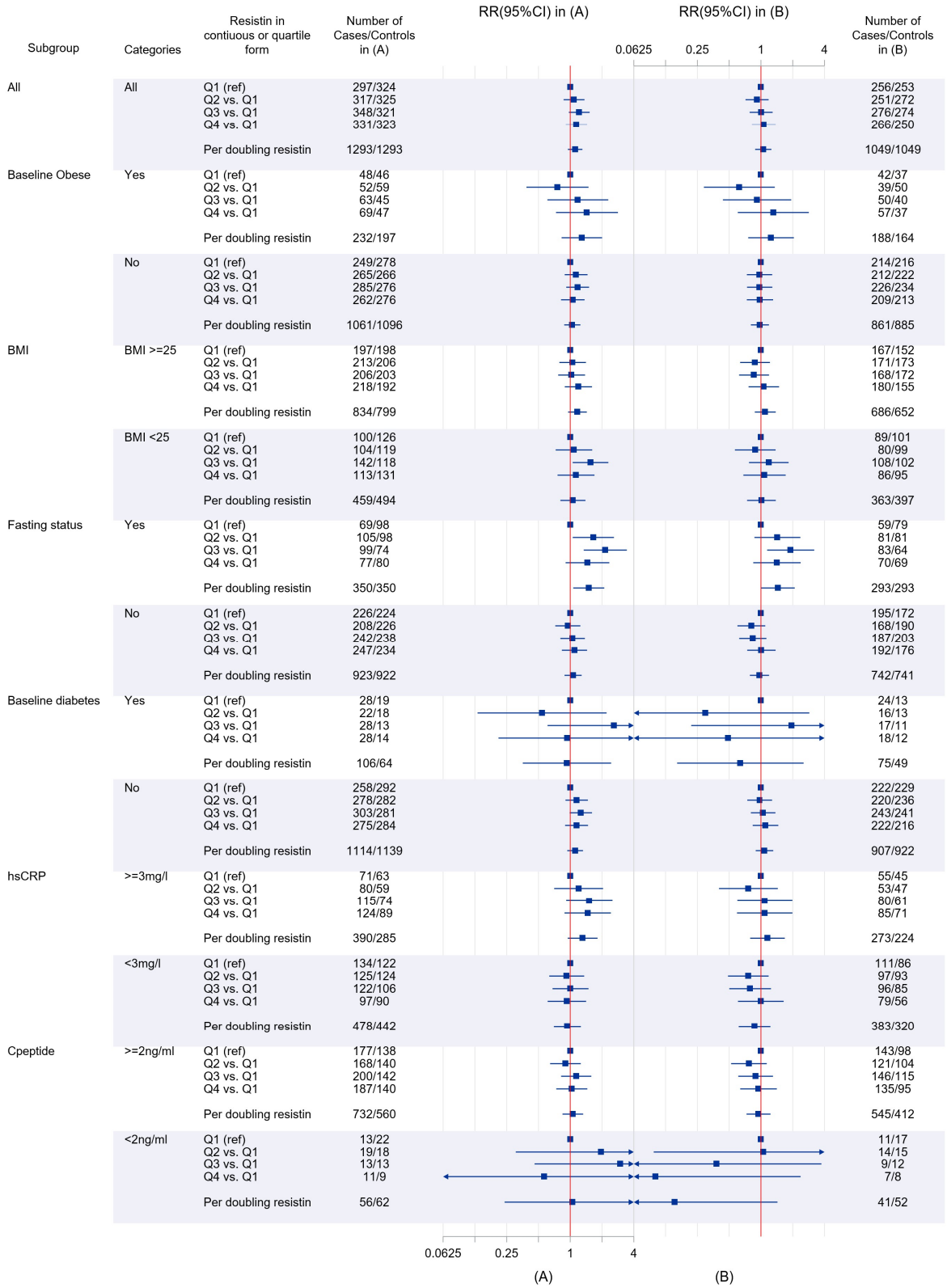


**Figure 9: Estimated effect sizes for the association between pre-diagnostic resistin concentrations and CRC risk by baseline obese status, BMI, fasting status, baseline diabetes, hsCRP, and C-peptide, are presented in (A): All participants; (B): Excluding**

**participants with ≤2 years follow-up.** RR: Relative risk. Results for all participants were based on ***Model 3*** as described in the method. Results for all other subgroups were based on unconditional logistic regression models adjusted for study center, sex (men, women, and women further adjusted for a new categorical variable with multiple categories as combinations of categories of menopausal status, menstrual cycle phase, and hormone replacement therapy usage), age at blood collection, blood collection date, blood collection clock time, and fasting hours before blood collection, smoking status, education level, alcohol abstainers, physical activity index, energy intake, and the intake by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, vegetable, body mass index and the residuals of the regression of body mass index on waist circumference. This figure depicts the estimated effect sizes derived from published tables in Pham et al. 2022.[103]

There was no considerable change in the estimated effect sizes of the association after additionally adjusting for each of the following factors: height, lifetime alcohol consumption, intensity and duration of smoking, baseline diabetes, and hsCRP levels.[103] Further sensitivity analyses are shown in **Figure 10**. We observed no significant association between resistin and risk of CRC after excluding participants with extreme resistin levels (from 8.38 to 34.41 ng/ml); excluding matched pairs in which controls later become cases; when performing complete case analyses; and when performing multiple imputations. Notably, the estimated effect sizes for the association were similar when missing data were imputed by the median-sex-specific imputation (single imputation) and with multiple imputations (effect sizes for multiple imputations: $RR_{Q2\ vs\ Q1}$: 1.11; 95%CI (0.88; 1.39); $RR_{Q3\ vs\ Q1}$: 1.21, 95%CI (0.96; 1.53); $RR_{Q4\ vs\ Q1}$: 1.16, 95%CI (0.91; 1.46) and $RR_{per\ doubling\ resistin\ concentration}$: 1.11, 95%CI (0.94; 1.31).

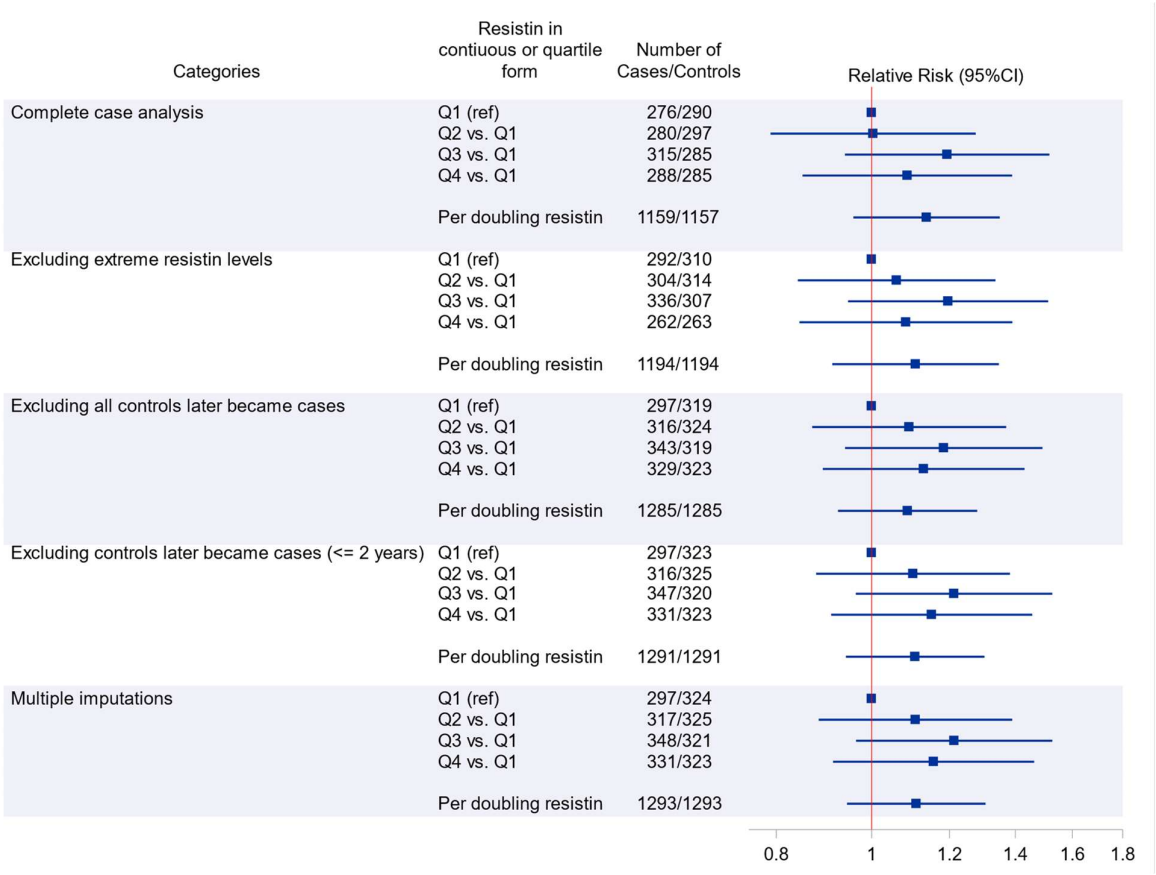


**Figure 10: Estimated effect sizes for the association between pre-diagnostic resistin concentrations and CRC risk in sensitivity analyses.** Results for complete case analysis (listwise deletion of 134 cases and 136 controls) were based on unconditional logistic regression models adjusted for study center, sex (men, women, and women further adjusted for a new categorical variable with multiple categories as combinations of categories of menopausal status, menstrual cycle phase, and hormone replacement therapy usage), age at blood collection, blood collection date, blood collection clock time, and fasting hours before blood collection, smoking status, education level, alcohol abstainers, physical activity index, energy intake, and the intake by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, and vegetable, body mass index and the residuals of the regression of body mass index on waist circumference. Results for other analyses were based on ***Model 3*** as described in the method. This figure depicts the estimated effect sizes derived partially from published tables in Pham et al. 2022.[103]: the effect sizes estimated from complete case analysis and analysis after excluding extreme resistin levels were derived from the publication[103], while the effect sizes estimated from other analyses have not been published before.

Analysis by unconditional logistic models suggested that estimated effect sizes for the association were similar to the conditional logistic models used as the primary analysis models (effect sizes from the fully-adjusted unconditional logistic models: $RR_{Q2 \text{ vs } Q1}$: 1.09; 95%CI (0.86; 1.37); $RR_{Q3 \text{ vs } Q1}$: 1.21; 95%CI (0.96; 1.52); $RR_{Q4 \text{ vs } Q1}$: 1.14; 95%CI (0.90; 1.44) and $RR_{\text{per doubling resistin concentration}}$: 1.10; 95%CI (0.94; 1.29).

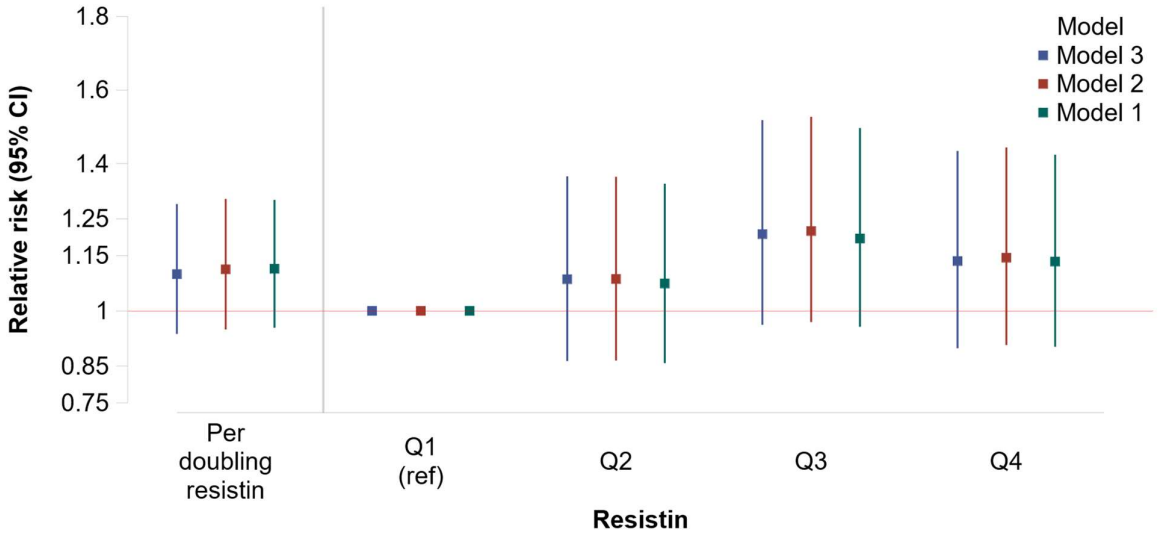


**Figure 11: Estimated effect sizes for the association between pre-diagnostic resistin concentrations and risk of CRC among 3 different unconditional logistic regression models**. Three models were used**.** ***Model 1*** was conditioned on matching factors only (sex (men, women, and women with further matching for menopausal status, menstrual cycle phase, oral contraceptive, and hormone replacement therapy use), age at blood collection, blood collection date, blood collection clock time, and fasting hours before blood collection. ***Model 2*** was built upon Model 1 by including smoking status, education level, alcohol abstainers, physical activity index, energy intake, and the intake by grams per day of alcohol, red meat, processed meat, fish/shellfish, dairy products, dietary fiber, fruit, and vegetable as additional adjusted factors. ***Model 3*** added to Model 2 by incorporating two additional variables including body mass index and the residuals of the regression of body mass index on waist circumference (to avoid multicollinearity). This figure was created from original data and has not been published before.

### 3.3. Secondary objective: The correlation between resistin and adiposity measurements, adipokines, and inflammatory biomarkers

In controls, resistin was not significantly correlated with adiposity measurements (BMI and WC); adipokines (adiponectin, HWV adiponectin, leptin, and soluble leptin), and metabolic biomarkers (C-peptide, HbA1c, ROM) (**Table 4).** There was a significant correlation between hsCRP and resistin in subgroups of cases and controls even after multiple testing corrections (Benjamini-Hochberg method); HDL-C was weakly significantly correlated with resistin in controls but not in cases. The correlation between hsCRP and resistin was stronger in participants who later became cases than in controls; however, all correlation coefficients were insufficient to even infer that a modest significant correlation exists. In the sensitivity analysis where the correlation between resistin and hsCRP was adjusted for age at blood collection, sex, and BMI, we observed little to no change in the correlation (corr coeff in controls and cases, rho=0.13, 95%CI (0.06; 0.20); rho=0.22, 95%CI (0.16; 0.29), respectively).

**Table 4: Spearman partial rank correlation coefficients and p-values between resistin and adiposity measurements, adipokines, inflammatory and metabolic biomarkers, adjusted for age at blood collection and sex.**

| | Controls | | Cases | | Cases >2 years follow-up | |
|---|---|---|---|---|---|---|
| | **rho** | **95%CI** | **rho** | **95%CI** | **rho** | **95%CI** |
| **Adiposity** | | | | | | |
| BMI | -0.02 | (-0.07 ; 0.04) | 0.02 | (-0.03 ; 0.07) | 0.04 | (-0.03 ; 0.10) |
| Waist circumference | -0.03 | (-0.09 ; 0.03) | 0.05 | (-0.01 ; 0.11) | 0.05 | (-0.01 ; 0.12) |
| **Adipokines** | | | | | | |
| Adiponectin (µg/ml) | -0.08 | (-0.16 ; -0.003) | -0.04 | (-0.11 ; 0.03) | -0.05 | (-0.13 ; 0.03) |
| HMW Adiponectin (µg/ml) | -0.07 | (-0.15 ; 0.01) | -0.06 | (-0.13 ; 0.01) | -0.07 | (-0.15 ; 0.01) |
| Leptin (ng/ml) | 0.002 | (-0.07 ; 0.08) | 0.02 | (-0.05 ; 0.09) | 0.04 | (-0.04 ; 0.12) |
| sOB-R (ng/ml) | -0.02 | (-0.10 ; 0.05) | -0.01 | (-0.08 ; 0.06) | -0.03 | (-0.11 ; 0.06) |
| **Inflammation** | | | | | | |
| CRP (mg/l) | 0.12* | (0.04 ; 0.19) | 0.21* | (0.15 ; 0.27) | 0.20* | (0.13 ; 0.27) |
| **Metabolism** | | | | | | |
| C-peptide (ng/ml) | 0.09 | (0.01 ; 0.17) | 0.09 | (0.02 ; 0.16) | 0.08 | (0.00 ; 0.16) |
| HbA1c (NGSP standardization) (%) | -0.05 | (-0.13 ; 0.03) | 0.02 | (-0.05 ; 0.09) | -0.02 | (-0.10 ; 0.06) |
| HDL-C (mmol/l) | -0.12* | (-0.19 ; -0.04) | -0.07 | (-0.14 ; -0.01) | -0.10 | (-0.17 ;-0.02) |
| ROM (Carratelli units) | 0.07 | (0.00 ; 0.15) | 0.11* | (0.04 ; 0.17) | 0.09 | (0.02 ; 0.17) |

* p-value <0.05 after multiple testing corrections by the Benjamini-Hochberg method. (Abbreviation: rho: Spearman rank correlation coefficient; BMI: Body Mass Index; High molecular weight: HMW; sOB-R: Soluble leptin receptor; CRP: C-reactive protein; HbA1c: hemoglobin A1c; NGSP: National Glycohemoglobin Standardization Program; HDL-C: High-

density lipoprotein cholesterol; ROM: Reactive oxygen metabolites). This table represents a modification of a published version in Pham et al. 2022[103] for the group of controls, and shows unpublished results for the groups of cases and cases >2 years follow-up.

Furthermore, results from subgroup analyses by hsCRP (<3mg/l, ≥3mg/l), baseline obese status (yes (BMI≥30)/no (BMI<30)), and baseline diabetes (yes/no) in controls are presented in **Table 5**. With this analysis, we observed a modestly significant correlation between resistin and hsCRP in obese controls (rho= 0.37); and between resistin and hsCRP in controls with baseline diabetes (rho=0.59). We observed somewhat higher correlation coefficients in obese compared to non-obese controls, in diabetic compared to non-diabetic controls, and in controls with high hsCRP (≥3 mg/l) compared to those with low hsCRP (<3 mg/l).

**Table 5: Spearman partial rank correlation coefficients and p-values between resistin and hsCRP in sensitivity analysis and subgroup analyses in controls.**

| Stratifying variable | | Sample size | rho | P value for rho | P value for rho (BH correction) | P values comparing corr coeffs between groups |
|---|---|---|---|---|---|---|
| Baseline obese status | | | | | | |
| | Yes | 111 | 0.37 | 6.40E-05 | 0.0002 | 0.005 |
| | No | 616 | 0.09 | 0.02 | 0.03 | |
| C-reactive protein (mg/l) - hsCRP | | | | | | |
| | ≥3 mg/l | 285 | 0.11 | 0.06 | 0.07 | 0.13 |
| | <3 mg/l | 442 | -0.002 | 0.96 | 0.96 | |
| Diabetes | | | | | | |
| | Yes | 41 | 0.59 | 3.66E-05 | 0.0002 | 0.0004 |
| | No | 651 | 0.09 | 0.017 | 0.03 | |

(All correlation coefficients were adjusted for age at blood collection and sex. The Benjamini-Hochberg method was used as a multiple-testing correction method. Abbreviation: rho: Spearman rank correlation coefficient). This table was created from original data and has not been published before.

# 4. Discussion

## 4.1. Summary of the findings

As reported in our publication[103] which is a part of this dissertation, findings from this dissertation showed that pre-diagnostic resistin concentrations were not associated with risk of CRC, colon, or rectal cancer. In subgroup analysis by length of follow-up, we observed significant estimates of the association among cases diagnosed with CRC within 2 years after recruitment and their matched controls; while no significant association among cases diagnosed with CRC later than 2 years after recruitment and their matched controls. The significant associations identified in some subgroups (risk of rectal cancer in males and risk of CRC in women, in postmenopausal women, in participants with BMI<25, and in participants without diabetes at baseline) were observed in the entire sample but did not persist after excluding participants with less than or equal to 2 years of follow-up. However, the association found in a subgroup of participants with fasting blood samples remained significant regardless of follow-up time. No statistically significant association was observed in other subgroup analyses. Furthermore, resistin was not significantly correlated with BMI, WC, adiponectin, HMW adiponectin, leptin, soluble leptin receptor, C-peptide, and HbA1c in both cases and controls. Resistin was weakly positively correlated with ROM in participants who later became cases, and weakly negatively correlated with HDL-C in controls; however, the magnitude of correlation coefficients was weak to infer any correlation. The correlation between resistin and hsCRP was stronger in participants who later became cases compared to controls, and modestly stronger in the obese controls compared to non-obese controls, and in diabetic controls compared to non-diabetic controls.

The measured resistin values in the present study were comparable to those measured in other studies with the same assay and laboratory vendor (ELISA, BioVender Laboratory Medicine, Brno, Czech Republic).[48,92] Directly comparing resistin concentrations in the present study with the previous prospective study in the WHI cohort[9] is not reasonable since different assays had been used.

## 4.2. Pre-diagnostic resistin and CRC risk

As reported in our publication[103] which is a part of this dissertation, no statistically significant associations were observed between pre-diagnostic resistin concentrations and risk of CRC in this nested case-control within the EPIC study.[103] The results were

consistent with the findings from WHI among menopausal women ($RR_{Q4vsQ1}$=1.04 (95% CI: 0.72; 1.50)).[9] Furthermore, all the significant associations found in a subgroup of women, male (risk of rectal cancer), BMI<25, and participants without diabetes at baseline were no longer present after excluding participants with less than or equal to 2 years of follow-up. Interestingly, a significant association among participants with fasting blood samples (for both $RR_{Q3vsQ1}$ and $RR_{\text{per doubling resistin levels}}$) was observed both before and after excluding participants with less than or equal to 2 years of follow-up. Fasting status was one of the matching variables between cases and controls in the present study; thus, potential confounding effects caused by fasting status were minimized (and adjusting for fasting status in unconditional logistic regression models did not change the main results). We also observed that control participants with non-fasting blood samples had somewhat higher age-and-sex-adjusted mean levels of resistin than those with fasting blood samples (data not shown). Several publications did not confirm fasting status as a factor influencing resistin levels.[82,123,124] For example, in a study by Murphy and colleagues, each participant had four repeated blood samples, including an overnight fasting sample, and three postprandial blood samples 1, 3, and 6 hours after meal completion, the intraclass correlation coefficient value between the four-time points was 0.81, 95% CI: 0.71; 0.87, suggesting that resistin levels were stable over fasting status.[124] Importantly, the WHI[9] included all overnight fasting participants and presented no statistically significant association between resistin and CRC risk. Of note, the subgroups of fasting participants presented in our study with only ~27% study population. We are not aware of any discernible characteristics, except for resistin levels as described earlier, that would suggest differences between groups of participants with fasting vs non-fasting blood samples. The reduced sample size may increase the likelihood of random fluctuations and multiple testing problems may lead to false positive results. Thus, the significant estimate could be due to chance. Nevertheless, the presence of small associations can not be ruled out in the present study.

Furthermore, as reported in our publication[103] which is a part of this dissertation, no statistically significant association between pre-diagnostic resistin and risk of CRC was found in participants diagnosed with CRC later than 2 years after recruitment and their matched controls while significant measures of association were observed in participants diagnosed with CRC within 2 years after recruitment and their matched controls. A scatter plot depicting resistin concentrations against time to CRC diagnosis in

cases indicated that time to CRC did not influence resistin concentrations among cases, however, there might be a potential pattern of higher resistin levels in samples collected closer to CRC diagnosis in cases diagnosed within two years before CRC diagnosis (data not shown). Those participants diagnosed with CRC within 2 years after recruitment may have had but not yet been diagnosed with CRC at the time of blood collection. It is important to mention that higher levels of resistin in CRC patients compared to controls were found in several case-control studies.[9,11,92,99-102] Therefore, it is speculated that the significant measures of association observed in participants with less than or equal to 2 years of follow-up may be due to the existing tumors that reversely influence resistin levels by creating a severe systemic inflammatory condition. Here we proposed a possibility of a reverse relationship between resistin and CRC in a subgroup of participants who had their resistin measured near the time of CRC diagnosis, which requires further comprehensive investigations (for example, study design establishes the temporal relationship with statistical methods incorporating resistin as an outcome variable). We intentionally refrained from referring to "effect estimates" in this subgroup and used the "measures of association" instead. Nevertheless, the temporal sequence of exposure and outcome in the present study is presumed to be well-established. Resistin was measured with a mean of 4.8 years before CRC diagnosis, while it is known that resistin is quite stable in human bodies,[87] it may reflect a "steady stable stage" of inflammation in humans that occurs prior to the CRC development and that stage serves as the context under investigation. Thus, the present EPIC study[103] and the WHI study[9] are two well-designed prospective studies jointly suggesting that pre-diagnostic resistin concentrations are not associated with CRC risk.

## 4.3. Correlation between pre-diagnostic resistin and adiposity measurements, adipokines, and inflammatory biomarkers

### 4.3.1. Resistin and adiposity measurements

In the present study, resistin was not significantly correlated with any adiposity measurements (BMI (rho= -0.02, p=0.52), WC (rho= -0.03, p=0.28)). Alternatively, the NHS (corr coeff with BMI= 0.22, p<0.0001),[41] and the WHI study (corr coeff with BMI= 0.17 p<0.001, with WC= 0.12 (p<0.001),[9,42] both reported weakly significant correlations between resistin and adiposity measures. Our estimations were close to the results from another study using data from the Finnish Health 2000 Survey (European population).[68] As mentioned in **1.2.2.1**, all these statistically significant correlations should not be

assumed to have biological or clinical relevance. Nevertheless, further investigation is required to comprehend the factors related to the disparities in correlation measures among studies.

In the present study, resistin was not significantly correlated with classic adipokines such as adiponectin and leptin. This finding was in line with findings from other population-based studies including the Nutrition and Health of Aging Population in China study,[43] the NU-AGE cohort study,[93] the WHI Study,[9] and the NHS.[41,83] Although not being quantified in the present study, the correlations between classic adipokines and BMI/WC were reported in other studies using EPIC data.[6,7] While resistin was not statistically correlated with BMI/WC (as per the description above), other classic adipokines were significantly correlated with adiposity measurements.[6,7] For example, the correlations between adiponectin with BMI (corr coeff = -0.27, $p<0.0001$) and with WC (corr coeff = -0.29, $p<0.0001$);[6] leptin with BMI (corr coeff = 0.60, $p<0.0001$) and with WC (corr coeff = 0.54, $p<0.00001$);[7] and soluble leptin receptor with BMI (corr coeff = −0.43, $p<0.0001$) and with WC (corr coeff = −0.38, $p<0.0001$) were all significant with modest or moderate correlation coefficients.[7] These findings could potentially support the conclusion that resistin may not play a role as an adipokine. However, resistin may still be related to the chronic low-grade sub-clinical inflammation properties of adipose tissues (discussed in **4.3.2**).

### 4.3.2. Resistin and inflammatory biomarkers

In the present study, we found a weak positive significant correlation between resistin and hsCRP in controls. It was modestly significant in some subgroups. The finding was consistent with other population-based studies although the magnitudes of correlation between resistin and hsCRP in the present study (rho=0.12, $p<0.01$) was lower compared to other population-based studies to some extent (**Table 2**, the NHS[41,83] (corr coeff = 0.22, $p<0.0001$); the WHI[42] (corr coeff = 0.19, $p<0.05$)).

In our study, the correlation between resistin and hsCRP was somewhat stronger in participants who later became cases as compared to controls. This was not analyzed in the previous WHI study.[9] Furthermore, the correlation between resistin and hsCRP was significantly stronger in controls who were obese or diabetic compared to those who were not obese or diabetic. The substantial correlation between resistin and hsCRP among obese people may contribute to explain for the lower magnitude of the correlation

between resistin and hsCRP in our study and the NHS or WHI study. The prevalence of obesity, and mean baseline BMI in controls or in groups with no cancer at baseline were reported in population-based studies as follows: the present study (EPIC)[103]: 16.59%, 26.7± 4.1, respectively; the NHS study[41]: not reported, 28.13±6.06, respectively; the WHI study[42]: 24.1%, 27.7±5.6. Thus, the prevalence of obesity was somewhat lower in this nested case-control study within the EPIC study comparing those reported by the WHI[41], and NHS study[42]. Nevertheless, findings of the correlation between resistin and hsCRP in obese people suggested that resistin's proinflammatory properties may not be fully independent of overall obesity.

We assumed that the correlation between resistin and hsCRP depends on the inflammatory conditions based on the following six key considerations elaborated upon as follows: (1) in this study, individuals who later became cases had significantly higher baseline hsCRP levels than those who later did not become cases (means of hsCRP, 2.7 mg/l ((Q1; Q3, 1.0; 4.8) and 2.2 mg/l ((Q1; Q3, 0.9; 4.4), respectively);[103] (2) this correlation was somewhat higher in controls with higher hsCRP (≥3 mg/l) compared to controls with lower hsCRP (<3 mg/l); (3) obesity is a chronic low-grade sub-clinical inflammation condition,[4] which may lead to increased resistin levels and perpetuate the inflammation cycle[14] and we observed significantly stronger correlation between resistin and hsCRP in persons who were obese compared to not obese; (4) diabetes is a metabolic dysfunction which may be accompanied by chronic inflammation,[58] and we observed a higher correlation between resistin and hsCRP in diabetic compared to non-diabetic control participants; (5) we only presented data for the three conditions (high hsCRP, obese, and diabetes), however, many publications have reported the higher correlation between resistin and hsCRP as well as other inflammatory biomarkers in individuals who had inflammatory-related conditions.[13,125-129] For example, the higher correlation was observed in women with coronary heart disease (corr coeff, rho= 0.23, p=0.002) compared to healthy women (corr coeff=-0.09, p=0.21).[125] Substantial significant correlation between resistin and other inflammatory biomarkers were reported in individuals with rheumatoid arthritis (rho with IL-6= 0.36, p=0.014),[13] atherosclerosis (corr coeff with TNF-R2 = 0.31, p<0.001),[126] spontaneous basal ganglia hemorrhage (corr coeff with hsCRP = 0.51, p<0.001),[127] obstructive sleep apnoea syndrome (corr coeff with IL-6 = 0.61, p<0.001),[128] and systemic lupus erythematosus (corr coeff with TNF-α = 0.39, p=0.02; corr coeff with IL-8 = 0.54, p=0.0005);[129] and, (6) as presented in the introduction,

findings from experimental studies have suggested that resistin may involve in an inflammatory vicious cycle with a positive feedback loop.[13-15,51-53] Here, inflammatory conditions may positively influence resistin levels and vice versa, and generate high resistin levels, particularly in individuals with higher hsCRP levels, with obesity, or with diabetes and other inflammatory conditions at baseline.

The assumption mentioned above is uncertain and may require caution to avoid overinterpretation of the correlations in subgroup analyses, because sample sizes of the obese control group (n=111), and the diabetic control group (n=41) were all small while the recommended sample size to achieve a stable correlation estimate is 250.[47] Although much literature support this assumption, additional adjustment for hsCRP did not change the estimated risk of CRC compared to the main analysis (adjusted for hsCRP, $RR_{\text{per doubling resistin concentration}}$= 1.01, 95%CI (0.81; 1.26), p=0.94 (data not shown)). As discussed in **4.2**, in the present study, resistin may be manifested a low-grade "steady stable stage" of inflammation rather than conditions as in the presence of tumors; and all of the inflammatory conditions may be accompanied by elevated resistin levels described above could induce this "steady stable state" conditions. In this study, the "steady stable state" conditions were not too different between cases and controls given by the blood samples were collected 4.8 years before CRC diagnosis in individuals later become cases. Nonetheless, it is worth mentioning that any post-hoc or subgroup analyses performed on the correlations in this study were merely used to provide direction and support generating hypotheses for further research.

### 4.3.3. Resistin and metabolism biomarkers

Findings in the present study were consistent with other population-based studies[43,44,49,83,90] that resistin was weakly associated with HbA1c and C-peptide. Resistin was significantly weakly correlated with low HDL-C levels in our study which was in line with findings from the Finnish Health 2000 Survey.[68] In terms of genetics, SNP-related resistin levels in the SCALLOP consortium (Systematic and Combined AnaLysis of Olink Proteins)[61], including, rs7746716 and rs2239619 were related to LDL-C and total cholesterol in other GWAS studies.[130,131] If further research can establish a temporal association between resistin and HDL-C, it may serve as a significant connection between inflammation, dyslipoproteinemia, and ultimately atherosclerosis. In terms of ROM, we observed no substantial correlation between resistin and ROM, however, as

this correlation was first examined in the present study, it is important to conduct further research to confirm this result.

Of note, only 55-60% study population (~600-700 participants for each group of cases and controls) had data on these metabolic biomarkers (C-peptide, HbA1c, HDL-C, ROM). In this study, there was a somewhat higher prevalence of metabolic disorders in cases compared to controls. The prevalence of baseline diabetes (self-reported or/and HbA1c≥6.5% (NGSP standardization) in this study population (cases (8.20%) and controls (4.95%)) were somewhat higher than those reported for the EPIC population (3.6%) by the InterAct Project[132]. Furthermore, in the present study, cases and controls had the prevalence of low HDL-C (defined as HDL-C <1.0 mmol/L in men, <1.2 mmol/L in women[133]) was 26.68% and 16.37%, respectively; the prevalence of low C-peptide (defined as C-peptide <2 ng/ml[134]) was 9.98% and 8.46%, respectively (while only 2 cases and 0 control had a C-peptide level <0.2 nmol/l or 0.6 ng/ml indicating complete lack of insulin[134]); the prevalence of heavy oxidative stress (defined as ROM >400 Carratelli units[135]) was 49.14% and 40.39%, respectively. Thus, as discussed above, resistin may indicate a low-grade inflammation stage or “steady state inflammation conditions”; and although resistin levels were not significantly different between individuals who later developed CRC and those who did not; the somewhat higher prevalence of metabolic disorders in cases compared to controls may support our “steady state inflammation conditions” hypothesis in the investigation into the association between resistin and CRC risk.

### 4.4. Strengths and limitations of the studies

The present study has strengths as the data were from the EPIC study which has a prospective design, a long follow-up time, and adequate data on variables. It facilitated the estimation of CRC risk and decreased differential misclassification bias by using pre-diagnostic resistin data. Furthermore, it had a large enough sample size to provide sufficient power to detect the association of interest. For analyzing several upcoming novel biomarkers proposed decades after blood sample collection, the nested case-control study serves as a well-designed study avoiding batch effect, freeze-and-thaw cycles, and storage time.[105]

In terms of the exposure assessment, laboratory analysis of a nested case-control study typically was processed by assigning cases and matched controls in the same batches to reduce the random noise or bias by batch effects.[105] Samples of participants

who contributed information more than once (for example, controls later became cases) should be analyzed multiple times, each in the same batches with their matching counterparts.[105] Although, in this study, all controls were only measured once (at the first batches); only 8/1293 controls became cases, and only 2 controls became cases within 2 years after the ending of being controls. Including or excluding these controls became cases did not change the main results, thus, batch effects were not a concern of this study. There were no issues of concern regarding the effects of storage or freeze-and-thaw cycles on the biological samples. Indeed, in this study, cases and controls were matched by age at blood collection leading to the similar storage duration in the two groups, blood samples were stored for a mean time of 22.6 (±1.4) years and several freeze-and-thaw cycles until laboratory analysis. Furthermore, we did not detect any significant change in resistin concentrations over time based on the zero-slope line we observed in the scatter plot of resistin concentrations versus storage time (data not shown). Thus, in this study, the likelihood of degradation of resistin during storage periods was minimized, and even if it occurs, it should have been the same in cases and non-cases, and eventually, the changes in resistin levels did not result in any fluctuations in the estimated effect sizes.

Additionally, although the present study may encounter certain difficulties with matching in nested-case control studies, these challenges are not expected to pose a significant issue. First, the controls selected to be matched with cases on multiple factors in this study may not be representative of the entire EPIC cohort. However, we did not observe clear differences in correlations between resistin and many biomarkers in our study and other population studies (**Table 2**). Second, this also makes controls have similar characteristics and risk factors as cases, and are likely to become cases later; however, as described above, only a small number of controls (8 in the total of 1293) became cases during follow-up. Third, the matching process also creates very complex datasets that could lead to biased estimates if matching factors were intermediate variables, factors influenced by the disease, or variables highly correlated with exposure with no influence on the disease making a valid biomarker appear less efficient at discriminating between cases and controls (false positives) (in such case, resistin levels are similar in cases and controls).[106] Nevertheless, in this study, none of the matching factors played roles in the manner described. Fourth, although conditional logistic regression has become a standard for a nested case-control study with

matching processes,[106] many researchers have questioned if the matching itself in a nested case-control study design could entirely account for residual confounding of the matched factors,[122] and if the use of unconditional logistic regression models adjusting for these factor amends to the conditional logistic regression models.[122] Nevertheless, the use of conditional logistic regression models was justified for the present study. This is because, the matching was performed on multiple factors at the individual level, resulting in one case and one control being placed in one stratum, creating multiple strata and eventually resulting in problems of sparse data. With such sparse data, unconditional logistic regression needs a large number of dummy variables representing the strata created by matching factors, leading to biased odds ratio estimates.[122] Therefore, conditional logistic regression is necessary to produce better statistical precision than unconditional logistic regression. Furthermore, effect estimates from the main conditional logistic regression were similar to the unconditional logistic regression models adjusting for matching factors ($RR_{\text{per doubling resistin level}}$ in the fully-adjusted unconditional logistic regression model, 1.10, 95%CI (0.94; 1.29)), indicating that residual confounding by matching factors were controlled by conditioning the matching factors in conditional logistic regression. Of note, to enable the implementation of unconditional logistic regression models and avoid the extreme condition where matching pairs generating 1293 strata representing by 1292 dummy variables, we created categorical variables covering all matching factors with the minimum strata (for example, groups of age, groups of clock time and date of blood collection time, groups of the combination of sex and all women-related matching factors).

Furthermore, several limitations in the present study have been described in the original publication.[103] First, the generalizability of the present study should be restricted to populations sharing similar characteristics as the present study population: men and women from the European population. Second, non-differential biases may be introduced in this study due to one single measurement of resistin per individual at baseline. Indeed, defining the true exposure as the mean measure over the relevant time period can be challenging to achieve in population-based studies, which often involve ambitious data collection efforts.[136] However, a single measurement of resistin levels may be subjected to within-person variation over time, leading to nondifferential misclassification which has the potential to introduce biases towards the null value of one in the point estimates.[136] Resistin data in this study were not repeatedly measured, however, previous studies have

shown that resistin levels remained stable in the human body over a period of 3-4 years,[87] suggesting that one single measurement of resistin measuring with a mean of roughly 5 years before CRC diagnosis in cases as in our study could represent for the steady inflammation stage that enables the study of CRC development. Third, as described earlier, resistin in the present study emerged in the context of the low-grade “steady stable stage” inflammatory condition, this condition could be caused by other underlying comorbidities (for example, type 2 diabetes mellitus, obesity, rheumatoid arthritis, atherosclerosis, hemorrhage, obstructive sleep apnea syndrome, systemic lupus erythematosus, and chronic kidney disease). However, data on these comorbidities were not available for further analysis. Nevertheless, there was no statistically significant association between resistin levels and the risk of hsCRP even after additional adjustments for inflammatory biomarkers/conditions including hsCRP/obesity, or after doing subgroup analyses by hsCRP/obesity.

### 4.5. Implications for practice and/or future research

We acknowledged that the presence of small associations between resistin and CRC risk can not be ruled out. Therefore, we conducted a MR study using data from the most up-to-date and largest genetic consortia to estimate the risk of CRC through genetic approaches. This MR study entitled "***Genetically determined circulating resistin concentrations and risk of colorectal cancer: a two-sample Mendelian Randomization study***" can provide robust effect estimates that complement the findings from the present prospective observational studies. The paper of this MR study has been submitted to a scientific peer-reviewed journal at the time of this dissertation submission. Furthermore, since the present study was solely in the European population, future studies need to validate findings from the present study in different population groups (for example, the Asian population). Future research should focus to elucidate the reverse relationship between resistin and CRC, several overarching questions exemplifying this trajectory are “does the presence of CRC (exposure) causally influence resistin levels (outcome)”, and “whether resistin can serve as a target for diagnostic, prognostic, or therapeutic of CRC”. Epidemiological studies with repeated measurements of resistin may contribute to tackling the within-person variation which may cause biases in the present study. Additionally, it should be noted that while different assays used to measure resistin captured different resistin molecular weight forms (section **1.2.3**), thus, may impact resistin measurements, the sensitivity, and specificities in differentiating CRC cases and controls, no publications on this matter have come to our attention. Future research should therefore aim to address these questions and establish standardized measurement procedures for resistin levels. Additionally, in this dissertation, we put forth the hypothesis that the correlation between resistin and hsCRP may vary depending on the conditions of inflammation. Moreover, we suggested that resistin's proinflammatory properties may not be entirely independent of overall obesity, future studies should examine and validate this hypothesis to further advance our understanding of the complex interplay between resistin, inflammation, and obesity. The strength of the cross-sectional relationship between resistin and HDL-C as well as resistin and ROM should be estimated in different populations.

Additionally, resistin with inflammatory and angiogenetic properties in humans may involve in both CRC development and progression. However, there have been no studies investigating its impact on survival after CRC. To address this gap, we have conducted a

study entitled "***Pre-diagnostic circulating resistin concentrations and mortality among individuals with colorectal cancer in the European Prospective Investigation into Cancer and Nutrition study***".[137] Upon submission of the dissertation, the paper of this study was submitted, and under review.

Finally, to enhance future original research, potentially high-quality systematic reviews should be implemented to provide general knowledge about resistin in humans that distinguishes it from the rodent versions. This is because during performing the literature review for this dissertation, I detected many papers composed of the text "Human resistin is a 12.5 kDa cysteine-rich peptide with a mature sequence consisting of 108 amino acids" which was cited from review papers.[138] This statement seems to be a mixing up of the descriptions of resistin protein in mice and humans. Indeed, in the paper which reported the discovery of resistin in 2001, a molecular mass of 12.5 kDa of a 114 amino acid protein (sometimes described as a mature sequence with 94 amino acids) was calculated for murine resistin.[20] The human resistin molecule with 108 amino acid mass was measured later in 2003 by other research groups,[27,28] showing that human resistin is a molecular mass of ~11 kDa band for resistin extracted from adipose tissues,[27] or a mass of 11.3 kDa for purified human recombinant resistin.[28] This highlights the fact that many papers in the field of resistin research failed to clearly distinguish between findings regarding resistin in mice and those in humans.

# 5. Conclusions

In this dissertation, we did not find any evidence of an association between pre-diagnostic resistin concentrations, assessed roughly five years prior to CRC diagnosis in CRC cases and assessed at the same time in their matched controls who remained CRC-free during follow-up, and CRC risk in European men and women. Findings from this EPIC study and the WHI study jointly suggested no association between pre-diagnostic resistin and CRC risk. The significant measures of the association in individuals diagnosed within 2 years of recruitment and their matched controls could be due to the reverse influence of existing but not yet diagnosed CRC tumors on resistin levels. Although a weakly significant correlation between hsCRP and resistin was observed, there may be still a link between resistin and inflammation which is not clinically negligible; the correlation between resistin and hsCRP may depend on the conditions of inflammation and suggests that resistin's proinflammatory properties may not be totally independent of overall obesity. The connection between resistin and dyslipoproteinemia is still unclear. These findings highlight the potential relationship between resistin, inflammation, and obesity as well as dyslipoproteinemia, which warrants further investigation to fully comprehend the underlying mechanisms.

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# Statutory Declaration

"I, Thi Thu, Pham, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic, "**Resistin and Colorectal Cancer Risk**" ("**Resistin und Kolorektalkrebs-Risiko**"), independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons. Figures and tables were generated by myself. The opening and closing drawing were painted by myself.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made Together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

# Declaration of your own contribution to the top-journal publication for a PhD or MD/PhD degree

Thi Thu Pham contributed the following to the below listed publications:

**Publication 1:**

**Pham TT**, Nimptsch K, Aleksandrova K, Jenab M, Reichmann R, Wu K, Tjønneland A, Kyrø C, Schulze MB, Kaaks R, Katzke V. Pre-diagnostic circulating resistin concentrations are not associated with colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition study. Cancers. 2022 Nov 9;14(22):5499.

Contribution:

Thi Thu Pham joined the writing group on behalf of the EPIC study, including Tobias Pischon, Katharina Nimptsch, Krasimira Aleksandrova, and Mazda Jenab. The paper is a part of the project "*Novel obesity-related biomarkers and risk of colorectal cancer: a nested case-control study in EPIC*" proposed by the writing group to IARC, the data were transferred from IARC to the writing group.

Thi Thu Pham together with her supervisors, Tobias Pischon and Katharina Nimptsch, conceptualized the study. She conducted the literature search on the topic of resistin and CRC risk, build the method by evaluating confounders, selecting statistical models, and planned all statistical analyses by herself which were then reviewed by her supervisors, Tobias Pischon and Katharina Nimptsch.

Once data were available, she programmed the statistical analyses for the data by SAS® Enterprise Guide® 8.3 (SAS Institute Inc., Cary, NC, USA) and analyzed the respective results which were then validated by Katharina Nimptsch. Thi Thu Pham independently generates all figures and tables in the manuscript. As a result, she independently created Table 1, Table 2, Table 3, Supplementary Table S1, and Supplementary Table S2. Furthermore, she created Supplementary Figure S1 with support from Robin Reichmann (Krasimira Aleksandrova's lab) – one of the co-authors. Thi Thu Pham created Supplementary Tables S3 based on the review literature as her own work.

Moreover, Thi Thu Pham contributed to the interpretation of the results and wrote the original draft of the manuscript, with the review of her supervisors, and close support from Krasimira Aleksandrova, and Mazda Jenab as writing group members. She received comments and compiled input from the coauthors (listed in the article and revised the manuscript accordingly to compose the manuscript for submission). She finalized the manuscript. She further independently submitted the paper and actively corresponded to the peer-review process, she drafted the response to peer reviewers and revised, and performed the proofreading of the manuscript for final publication.

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Signature, date and stamp of first supervising university professor / lecturer

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Signature of doctoral candidate

# Excerpt from Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2021** Selected Editions: SCIE,SSCI
Selected Categories: **"ONCOLOGY"** Selected Category Scheme: WoS
**Gesamtanzahl: 246 Journale**

| Rank | Full Journal Title | Total Cites | Journal Impact Factor | Eigenfaktor |
|---|---|---|---|---|
| | [illegible] | [illegible] | [illegible] | [illegible] |
| 60 | Cancers | 56,338 | 6.575 | 0.07275 |
| 61 | Oncogenesis | 4,572 | 6.524 | 0.00664 |
| 62 | CANCER SCIENCE | 21,270 | 6.518 | 0.02009 |
| 63 | Cancer Cell International | 11,032 | 6.429 | 0.01119 |
| 64 | MOLECULAR CANCER RESEARCH | 12,521 | 6.333 | 0.01158 |
| 65 | Molecular Therapy-Oncolytics | 2,599 | 6.311 | 0.00406 |

# Printing copy(s) of the publication(s)

Pham T-T, Nimptsch K, Aleksandrova K, Jenab M, Reichmann R, Wu K, Tjønneland A, Kyrø C, Schulze MB, Kaaks R, Katzke V, Palli D, Pasanisi F, Ricceri F, Tumino R, Krogh V, Roodhart J, Castilla J, Sánchez M-J, Colorado-Yohar SM, Harbs J, Rutegård M, Papier K, Aglago EK, Dimou N, Mayen-Chacon A-L, Weiderpass E, Pischon T. Pre-Diagnostic Circulating Resistin Concentrations Are Not Associated with Colorectal Cancer Risk in the European Prospective Investigation into Cancer and Nutrition Study. *Cancers*. 2022;14(22):5499.

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# Pre-Diagnostic Circulating Resistin Concentrations Are Not Associated with Colorectal Cancer Risk in the European Prospective Investigation into Cancer and Nutrition Study

**Thu-Thi Pham [1,2], Katharina Nimptsch [1], Krasimira Aleksandrova [3,4], Mazda Jenab [5], Robin Reichmann [3], Kana Wu [6], Anne Tjønneland [7,8], Cecilie Kyrø [7], Matthias B. Schulze [9,10], Rudolf Kaaks [11], Verena Katzke [11], Domenico Palli [12], Fabrizio Pasanisi [13], Fulvio Ricceri [14,15], Rosario Tumino [16], Vittorio Krogh [17], Jeanine Roodhart [18], Jesús Castilla [19,20], Maria-Jose Sánchez [20,21,22,23], Sandra Milena Colorado-Yohar [20,24,25], Justin Harbs [26], Martin Rutegård [27,28], Keren Papier [29], Elom K. Aglago [30], Niki Dimou [5], Ana-Lucia Mayen-Chacon [5], Elisabete Weiderpass [31] and Tobias Pischon [1,2,32,33,*]**

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**Simple Summary:** Resistin has been proposed to link to cancer development via inflammatory processes. Prior case-control studies suggest higher post-diagnosis resistin concentrations in CRC cases compared to controls. Here, we found no association between pre-diagnostic circulating resistin concentrations and the risk of CRC; however, we observed a marginally significant association among cases (and their matched controls) diagnosed with CRC within the first two years of follow-up, whereas no such association was observed among cases (and their matched controls) diagnosed with CRC after two years of follow-up. We speculate that resistin is more likely a marker of existing tumors than a risk factor of CRC.

**Abstract:** Resistin is a polypeptide implicated in inflammatory processes, and as such could be linked to colorectal carcinogenesis. In case-control studies, higher resistin levels have been found in colorectal cancer (CRC) patients compared to healthy individuals. However, evidence for the association between pre-diagnostic resistin and CRC risk is scarce. We investigated pre-diagnostic resistin concentrations and CRC risk within the European Prospective Investigation into Cancer and Nutrition using a nested case-control study among 1293 incident CRC-diagnosed cases and 1293 incidence density-matched controls. Conditional logistic regression models controlled for matching factors (age, sex, study center, fasting status, and women-related factors in women) and potential confounders (education, dietary and lifestyle factors, body mass index (BMI), BMI-adjusted waist circumference residuals) were used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for CRC. Higher circulating resistin concentrations were not associated with CRC (RR per doubling resistin, 1.11; 95% CI 0.94–1.30; $p$ = 0.22). There were also no associations with CRC subgroups defined by tumor subsite or sex. However, resistin was marginally associated with a higher CRC risk among participants followed-up maximally two years, but not among those followed-up after more than two years. We observed no substantial correlation between baseline circulating resistin concentrations and adiposity measures (BMI, waist circumference), adipokines (adiponectin, leptin), or metabolic and inflammatory biomarkers (C-reactive protein, C-peptide, high-density lipoprotein cholesterol, reactive oxygen metabolites) among controls. In this large-scale prospective cohort, there was little evidence of an association between baseline circulating resistin concentrations and CRC risk in European men and women.

**Keywords:** pre-diagnostic resistin; colorectal cancer; risk; prospective; inflammation

## 1. Introduction

Resistin is a polypeptide consisting of 108 amino acids named after "resistance to insulin" that belongs to the "resistin-like molecules" family [1]. It was initially reported in rodents as a protein primarily secreted by adipocytes and plays a role in obesity-induced insulin resistance. Higher resistin concentrations have been found in obese as compared to non-obese animal models [1]. In humans, reports on the correlation between circulating resistin concentrations and adipose tissue mass have been inconsistent [2–6], and resistin was found to be predominantly produced by macrophages and monocytes, rather than adipocytes [2,3]. The production and upregulation of resistin occur during monocyte–macrophage differentiation [3] and resistin then promotes an M1-like (pro-inflammatory) phenotype in macrophages [7]. Increases in adipose tissue mass in humans may be accompanied by infiltration of macrophages and monocytes, which release resistin and may thereby affect multiple cell types and tissues [3,8]. Human resistin is relevant for inflammatory processes [8,9]. By various mechanisms, resistin receptor binding may lead to an upregulation of inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) [8–10], to promote NF-kB activation [7], whereas resistin expression is also upregulated in the presence of inflammatory cytokines by a positive feedback loop, which may result in a vicious cycle and perpetuate inflammatory conditions [7,10,11]. Other than inflammation, resistin could promote the expression of adhesion molecules and growth

factors that may promote angiogenesis [8,9]. Thus, resistin emerges as an important marker implicated in the development of cancer [8].

Colorectal cancer (CRC) is the third most common and second most fatal cancer worldwide [12]. The global burden of CRC is expected to increase by 60% by 2030 [13]. While European, Australia/New Zealand, and Northern American regions have both the highest CRC cancer incidence and mortality rate, the majority regions of Africa and South Central Asia have the lowest incidence rate [12]. Thus, CRC incidence has been suggested to correlate with socioeconomic development and may be linked to western lifestyles [12,13]. Furthermore, incidence of CRC is closely related to multiple factors, including a family history of colon polyps or inflammatory bowel diseases, socioeconomic status, lifestyle and dietary factors, and the gut microbiome, which shares the underlying mechanism related to inflammation, angiogenesis, and insulin resistance [14,15].

As both inflammation and angiogenesis are related to CRC, it was speculated whether resistin may also be relevant to the risk of CRC [8,16]. In fact, higher resistin levels have been found in CRC patients compared to healthy individuals in case-control studies [17]. However, it is unclear whether higher resistin levels are a cause or consequence of CRC development. Prospective studies with pre-diagnostic resistin concentrations are necessary to investigate whether higher resistin concentrations are associated with a higher risk of developing CRC. To date, there has been only one prospective study using data from 1224 postmenopausal women (427 CRC cases) in the Women's Health Initiative (WHI), which found no significant associations between resistin and CRC risk [5]. However, different associations between inflammation and the risk of CRC have been observed in men versus women [18], suggesting that the association between resistin concentrations and the risk of CRC may differ by sex. Furthermore, that study did not separate the association by tumor subsite. Given that inflammation is more strongly related to colon cancer than to rectal cancer [19], an examination by tumor subsite is important to better understand the impact of resistin as an exposure.

Therefore, we conducted a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) study to investigate the association between pre-diagnostic resistin concentrations and CRC risk and to examine whether the association differed by cancer subsite or sex.

## 2. Materials and Methods

### *2.1. The European Prospective Investigation into Cancer and Nutrition Study*

#### 2.1.1. Study Population

The EPIC study is a large, ongoing cohort study, details of which have been extensively reported elsewhere [20]. Briefly, the EPIC study was initiated in 1990 with the collaboration of 23 centers in 10 European countries, including Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden, and the United Kingdom. Study participants' enrollment in all EPIC centers had been completed in 2000, with 519,978 participants aged 35 to 70 years. In the current nested case-control study, data from Greece were not included due to administrative reasons.

#### 2.1.2. Assessments of Anthropometry, Lifestyle Factors, and Dietary Exposure

Weight and height were measured for all participants in all EPIC centers by trained observers, except in France, Oxford, and Norway [21]. In France and Oxford, weight and height were measured in a part of the population, whereas self-reported weight and height were available for all participants. For part of the Oxford cohort, linear regression models were used to predict sex- and age-specific values from individuals with both measured and self-reported body measures. For France, only the measured values were used. In Norway, only self-reported weight and height data were available. Waist circumference was measured in all centers except in Norway and Umeå, Sweden.

Lifestyle characteristics were collected via questionnaires at recruitment including questions on education, tobacco smoking status, consumption of alcoholic beverages,

physical activity according to the validated Cambridge physical activity index, and for women, menstrual status, use of contraception, and hormone replacement therapy [20]. The Cambridge physical activity index describes four levels of physical activity (inactive, moderately inactive, moderately active, and active) based on recreational activity time in occupation, cycling, and other physical activities [22]. Questionnaires on lifestyle variables were developed and used independently in Denmark, Sweden, Norway, and the Naples center in Italy, while they were previously standardized in other countries. All lifestyle variable codes from different questionnaires were standardized to the core EPIC lifestyle questions using a comprehensive recoding scheme afterward. Baseline dietary exposure (including, among others, consumption of red meat, processed meat, dietary fiber, fruit, vegetable, dairy, fish, and shellfish intake, as well as alcohol consumption) was assessed on all EPIC participants by using locally adopted instruments, including food frequency questionnaires, diet history logs, and a combined method, and was used to estimate long-term usual dietary intake.

#### 2.1.3. Blood Collection

Biological samples including plasma, serum, leukocytes, and erythrocytes were collected at baseline from most participants, with the exception of those in France, the UK, Bilthoven (The Netherlands), and Norway, where only a proportion of the participants were invited for blood sampling [20]. For most EPIC centers, half of the blood samples were stored locally, and half were transported to the central repository of the International Agency for Research on Cancer (IARC) to be stored in straws in the vapor phase of liquid nitrogen at −196 °C. Blood samples were all stored locally in freezers at −70 °C in Sweden and nitrogen vapor at −150 °C in Denmark.

#### 2.1.4. Follow-Up for Cancer Incidence

Incident CRC cases were identified via regional cancer registries in Denmark, Italy, The Netherlands, Norway, Spain, Sweden, and the United Kingdom and by a combination of methods in France and Germany. The combination of methods included a direct follow-up procedure through study participants or their next of kin, and confirmation of tumors from a review of health insurance records and pathology registries. For centers that applied a combination of methods, individuals' end of follow-up was considered the last known contact, date of diagnosis, or date of death, whichever came first.

### *2.2. The Nested Case-Control Study*

#### 2.2.1. Study Design

Incident CRC cases were defined as EPIC participants who developed CRC after recruitment and before the closure dates. CRC was defined as a combination of tumors of the colon (the 10th Revision of the International Classification of Diseases (ICD-10) codes C18.0–C18.7), tumors that were overlapping or unspecified (C18.8–C18.9), and tumors of the rectum (C19–C20). The closure dates for the nested case-control study of the present analysis ranged from December 2001 to December 2005.

The control selection process was based on an incidence density sampling approach. One control was selected for each case from among a sample of those who were at risk at the time of diagnosis of the index case with available blood samples and was matched (1:1) by recruitment center, sex, age at recruitment (±2 years), date of blood collection (±3 months), time of day of blood collection (±4 h), fasting status at blood collection (<3, 3–6, and >6 h), and menopausal status (premenopausal, postmenopausal, perimenopausal, or surgically postmenopausal) for women. Premenopausal women were matched on phases of menstrual cycles and use of oral contraceptives, and postmenopausal women were matched on current hormonal replacement therapy use.

#### 2.2.2. Laboratory Analysis

Serum resistin concentrations were measured using human resistin ELISA assays (BioVendor Laboratory Medicine, Inc.; Brno, Czech Republic). Measurements were performed according to the manufacturer's protocols. The mean inter-assay coefficients of variation of the laboratory analysis were 7.4% and 6.6% for quality-control high concentrations (18.3 ng/mL) and low concentrations (5.01 ng/mL), respectively. The mean inter-assay coefficient of variation was <10.4% for all pooled serum quality controls. Measurements and inter-assay coefficients of variation in adiponectin and high-molecular weight (HMW) adiponectin [23], leptin [24], soluble leptin receptor [24], reactive oxygen metabolites (ROM) [25], high-density lipoprotein cholesterol (HDL-C), C-peptide [26], high-sensitivity C-reactive protein (hsCRP) [27], and glycated hemoglobin A1c (Hba1c) [28] have been described previously.

#### 2.2.3. Final Dataset, Handling of Missing Data

The current analysis was based on data from participants in the EPIC CRC nested case-control study, for which plasma samples were available for laboratory analysis. Resistin concentrations were successfully analyzed in 1383 CRC cases and 1375 controls. After excluding participants without their matching pairs, a total of 1293 first-incident CRC cases and their matched controls were included in the current study.

Waist circumference measurements were missing in 77 matched case sets (6.0%), including 16 and 61 case sets from Norway and Umeå-Sweden, respectively. For 4 CRC cases, data on alcohol consumption, energy intake, and consumption of red meat, processed meat, dietary fiber, fruit, vegetable, dairy, fish, and shellfish intake were missing. Data for other variables were missing in cases and controls as follows: fasting status (20/21), smoking status (14/15), highest education level (43/34), physical activity index (16/18), and diabetes (73/90). We checked the distribution of resistin concentrations in participants with missing and no missing data on these variables and observed no differences. Together with the arbitrary missing data patterns generated by the PROC MI procedure in SAS® Enterprise Guide® 8.3 (SAS Institute Inc., Cary, NC, USA), we assumed that the data were missing completely at random. Hence, missing data were imputed by sex-specific medians for discrete variables and sex-specific modes for categorical variables.

### *2.3. Statistical Analysis*

Quartile cut-off points of resistin concentrations were derived from controls and applied to the whole study population. We then analyzed the study population characteristics descriptively by case-control status and by quartiles of resistin concentrations. We calculated means (standard deviation (SD)) or medians (and quartiles) depending on the distribution of the variables.

Spearman partial correlation coefficients (and corresponding *p*-values) controlling for age and sex were estimated to assess the correlations between baseline resistin levels and adiposity measurements, other adipokines (adiponectin and leptin), as well as metabolic and inflammatory biomarkers (hsCRP, C-peptide, HDL-C, and ROM) in controls. A correlation coefficient lower than 0.30 was considered as little if any correlation [29].

Conditional logistic regression models were used to estimate RRs and 95% CI of CRC across quartiles of resistin concentrations. Since cases and controls were selected using an incidence density sampling protocol, the odds ratios estimate the incidence rate ratios, which can be interpreted as RRs of the association. Potential confounders were selected as covariates for the models. Higher intakes of fiber and dairy products are established protective factors for CRC, while higher intakes of red meat, processed meat, and alcohol are risk factors [14,15]. Low intakes of vegetables and fish have been associated with a higher risk of CRC [14,15]. Since these variables may also affect resistin concentrations [30], we adjusted for these variables in our regression models. BMI and waist circumference are risk factors for CRC [15], however, the relationship between obesity and resistin is not entirely clear [4,6,31]. Therefore, three models were used to estimate the association.

Model 1 was only conditioned on the matching variables. Model 2 was conditioned on matching variables and additionally adjusted for smoking status (never, former, or current smoker), education (none, primary school, technical/professional school, secondary school, or longer education), alcohol abstainers (defined as under 0.3 g/day; yes/no), alcohol consumption (gram/day), physical activity index (inactive, moderately inactive, moderately active, active), energy intake (kcal/day), and dietary intakes of red meat (gram/day), processed meat (grams/day), dietary fiber (grams/day), fruit intake (grams/day), vegetable intake (grams/day), dairy intake (grams/day), and fish and shellfish (grams/day). Model 3 was additionally adjusted for BMI (kg/m$^2$) and residuals of BMI-adjusted waist circumference (derived from a regression model with BMI as an independent variable and waist circumference as a dependent variable to avoid multicollinearity). Additional adjustment for height, alcohol intake during lifetime, smoking intensity and duration, or diabetes at baseline (defined as self-reported diabetes diagnosis or HbA1c concentrations $\geq$ 6.5% at baseline) did not alter the risk estimates appreciably and results are hence not shown. Resistin concentrations were log-transformed to approximate a normal (Gaussian) distribution and included as a continuous variable in conditional logistic regression models [32]. We also analyzed the associations between resistin concentrations and the risk of CRC by modeling restricted cubic polynomial splines with knots at the 5th, 35th, 65th, and 95th percentiles of resistin distribution. We tested for non-linearity using a likelihood ratio test to compare full multivariable-adjusted conditional logistic regression models, including both the linear and cubic spline term, and reduced multivariable-adjusted conditional logistic regression models with only the linear term. The test for non-linearity was not significant in the main analysis ($p$ = 0.35) or the subgroups, except for the subgroup of men ($p$ = 0.03). Results of non-linearity tests did not change implicitly after excluding participants diagnosed with CRC within 2 years after recruitment. Thus, using both log-transformed and quartile scales was considered sufficient for better capturing the relationship between resistin concentrations and the risk of CRC [32].

The associations between resistin concentrations and the risk of CRC were further assessed according to tumor anatomical subsite (colon, rectum), sex (female, male, as well as with restriction to postmenopausal women [5]), the combination of subsite and sex, by the length of follow-up ($\leq$2 years, 2–5 years, >5 years), BMI (<25, $\geq$25), hsCRP (<3 mg/L, $\geq$3 mg/L), baseline diabetes (yes/no), fasting status ($\leq$6 h, >6 h), and C-peptide (<2 ng/mL, $\geq$2 ng/mL). Sensitivity analyses were carried out by repeating all of the analyses with the exclusion of participants with less than 2 years of follow-up, and participants with extreme resistin levels (defined as concentrations of 1.5 times the interquartile range below the first and above the third quartile [33]). Analyses were also restricted to participants with no missing covariates' data (complete case analyses).

We finally pooled the relative risk estimate from our main analysis with the relative risk from the published result from the WHI [5] using a random-effects meta-analysis with an inverse variance method. Heterogeneity was assessed using Cochran's Q-statistic test and inconsistency index (I2).

Minimal detectable RRs for binary exposure were estimated for the second, third, or fourth quartiles as compared to the first quartile in matched case-control using the software "Power" version 2.10 (Channing laboratory, Boston, MA, USA) [34]. With 1293 study participants for each RR estimate, 80% power, and alpha = 0.05, the minimum detectable RR is 1.37 under the assumption of no correlation of exposure in matched pairs, and 1.42 with a recommended correlation of exposure between cases and matched controls of 0.2.

All statistical tests were two-sided, and $p$-values less than 0.05 were considered statistically significant. All analyses were performed using SAS® Enterprise Guide® 8.3 (SAS Institute Inc., Cary, NC, USA) and R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

The mean follow-up time between recruitment and CRC diagnosis in cases for the current study was 4.8 ± 2.7 years. Cases and controls had similar distributions of the matching factors (Supplementary Table S1). On average, cases at baseline were less physically active, had a higher BMI, a higher waist circumference, and a slightly lower dietary fiber intake compared to controls (Supplementary Table S1). Cases also had higher circulating levels of HMW adiponectin, ROM, hsCRP, and HbA1c than controls. Resistin concentrations at baseline were similar in incident CRC cases (4.7 ± 2.0 ng/mL) and controls (4.7 ± 2.2 ng/mL).

The main characteristics of controls in the population according to the quartiles of resistin are presented in Table 1. Participants with higher resistin concentrations were more likely to be women, have a higher age, a lower proportion of university-level education, lower alcohol intake, higher hsCRP, higher C-peptide, and lower HDL-C, while less likely to be in fasting status at blood collection. Interestingly, we observed higher levels of resistin in women compared to men (4.82 ± 2.48 vs. 4.53 ± 1.84 ng/mL, $p = 0.01$), and in participants with less than or equal to 6 h of fasting before blood collection compared to those with more than 6 h of fasting (4.79 ± 2.39 vs. 4.38 ± 1.56 ng/mL, $p \leq 0.001$).

**Table 1.** Characteristics of controls at baseline (n = 1293), by quartiles of resistin concentration in the nested case-control study, European Prospective Investigation into Cancer and Nutrition, 1992–2005.

| | Quartiles of Resistin Concentration | | | |
|---|---|---|---|---|
| | **Q1 (N = 324)** | **Q2 (N = 325)** | **Q3 (N = 321)** | **Q4 (N = 323)** |
| Resistin quartile ranges (ng/mL) | ≤3.47 | 3.47< to ≤4.28 | 4.28< to ≤5.42 | 5.42< to ≤34.41 |
| Age at blood collection, years, mean (SD) [a] | 57.6 (6.9) | 57.5 (7.2) | 58.1 (6.7) | 59 (7.1) |
| Women, n (%) [a] | 154 (47.5) | 183 (56.3) | 157 (48.9) | 187 (57.9) |
| Postmenopausal women, baseline, n (%) [a] | 123 (38.0) | 135 (41.5) | 116 (36.1) | 141 (43.7) |
| Fasting (>6 h), n (%) [a] | 98 (30.2) | 98 (30.2) | 74 (23.1) | 80 (24.8) |
| BMI, kg/m$^2$, mean (SD) | 26.2 (3.5) | 26.6 (4.1) | 26.3 (3.8) | 26.2 (3.7) |
| Waist circumference, cm, mean (SD) [b] | 89.2 (12.5) | 88.4 (12.2) | 88.8 (11.8) | 87.7 (12.6) |
| Diagnosed diabetes at baseline (self-reported or HbA1C ≥ 6.5%), n (%) | 19 (5.9) | 18 (5.5) | 13 (4.0) | 14 (4.3) |
| Current smoker, n (%) | 84 (25.9) | 86 (26.5) | 68 (21.2) | 85 (26.3) |
| University degree or higher education level, n (%) | 68 (21) | 52 (16) | 51 (15.9) | 58 (18) |
| Physically moderately active, or active, sex-specific, n (%) | 178 (54.9) | 176 (54.2) | 168 (52.3) | 195 (60.4) |
| Alcohol abstainers (<0.3 g/day), n (%) | 38 (11.7) | 45 (13.8) | 43 (13.4) | 51 (15.8) |
| Alcohol consumption, g/day, median (Q1–Q3) [b] | 10.6 (3.2–28.1) | 8.5 (1.6–23.5) | 7.7 (1.7–19.9) | 6.0 (1.0–16.8) |
| Energy intake, Kcal/day, median (Q1–Q3) [b] | 2084 (1608–2478) | 2010 (1638–2404) | 2052 (1719–2511) | 1997 (1590–2464) |
| Red meat, g/day, median (Q1–Q3) [b] | 43.4 (24.4–75.3) | 43.0 (25.3–69.9) | 49.6 (27.4–76.5) | 47.7 (24.2–74.9) |
| Processed meat, g/day, median (Q1–Q3) [b] | 24.6 (13.0–42.6) | 23.3 (12.5–45.9) | 28.4 (16.8–44.3) | 23.2 (12.9–42.6) |
| Dietary fiber, g/day, median (Q1–Q3) [b] | 23.6 (18.0–28.6) | 22.8 (18.0–27.5) | 23.2 (18.0–27.8) | 22.1 (17.9–26.7) |
| Fruit intake, g/day, median (Q1–Q3) [b] | 191.5 (106.4–314.3) | 195.2 (108.2–330.2) | 176.9 (98.2–279.8) | 191.5 (109.3–318.4) |
| Vegetable intake, g/day, median (Q1–Q3) [b] | 160.9 (104.3–230.8) | 166.8 (103.8–248.1) | 148.1 (96.0–221.6) | 157.9 (97.4–241.5) |
| Dairy intake, g/day, median (Q1–Q3) [b] | 278.9 (155.2–435.3) | 310.6 (175.0–451.0) | 318.2 (171.2–495.9) | 305.9 (161.5–484.5) |
| Fish and shellfish, g/day, median (Q1–Q3) [b] | 29.0 (13.4–52.3) | 29.7 (16.4–51.8) | 29.4 (14.2–49.3) | 30.0 (13.6–52.4) |
| Adiponectin, µg/mL, median (Q1–Q3) [b] | 7.3 (5.2–10.1) | 7.3 (5.5–9.7) | 6.9 (5.0–9.2) | 7.2 (5.3–9.6) |
| HMW adiponectin, µg/mL (Q1–Q3) [b] | 3.7 (2.4–5.7) | 3.8 (2.4–5.4) | 3.6 (2.2–5.1) | 3.7 (2.4–5.2) |
| Leptin, ng/mL (Q1–Q3) [b] | 6.3 (3.4–14.1) | 9.4 (5.1–19.2) | 7.7 (3.5–16.6) | 6.9 (3.6–17.0) |
| Soluble leptin receptor, ng/mL, mean (SD) [b] | 23 (8.9) | 21.9 (7.9) | 22.7 (8.6) | 23.6 (15.8) |
| ROM, Carratelli units, mean (SD) [b] | 381 (71.8) | 382.4 (69.4) | 379.1 (64.2) | 397.4 (71.7) |
| hsCRP, mg/L, median (Q1–Q3) [b] | 1.9 (0.8–3.7) | 2.2 (0.9–4.0) | 2.1 (0.9–4.2) | 2.9 (1.3–5.8) |
| C-peptide, ng/mL, median (Q1–Q3) [b] | 3.6 (2.5–5.9) | 3.7 (2.6–5.6) | 4.0 (2.9–6.3) | 4.3 (2.9–5.9) |
| HDL-C, mmol/L, median (Q1–Q3) [b] | 1.5 (1.2–1.8) | 1.5 (1.2–1.7) | 1.4 (1.2–1.7) | 1.4 (1.1–1.7) |
| HbA1c, %, mean (SD) [b] | 5.8 (0.6) | 5.8 (0.8) | 5.7 (0.6) | 5.7 (0.5) |

[a] Matching variable. [b] Among users only. Abbreviations: Q1–Q3, the first quartile (the 25th percentile) to the third quartile (the 75th percentile); SD, standard deviation; BMI: body mass index; HMW, high-molecular weight; ROM, reactive oxygen metabolites; HDL-C, high-density lipoprotein cholesterol (HDL-C); hsCRP, high-sensitivity C-reactive protein; HbA1c, glycated hemoglobin A1c.

Among controls, after adjustment for age and sex, resistin concentrations were weakly inversely correlated with adiponectin and HDL-C, and weakly positively correlated with C-peptide and hsCRP (correlation coefficient (r) < 0.15). Resistin was not statistically significantly correlated with BMI, waist circumference, leptin, soluble leptin receptors, reactive oxygen metabolites, or HbA1c concentrations (Table 2).

**Table 2.** Age- and sex-adjusted Spearman partial rank correlations between resistin and adiposity measurements, other adipokines, as well as metabolic and inflammatory biomarkers in controls (n = 1293), the European Prospective Investigation into Cancer and Nutrition Cohort (1992–2005).

| | Number of Participants | Correlation Coefficient (r) | *p*-Value |
|---|---|---|---|
| BMI, kg/m$^2$ | 1293 | −0.02 | 0.52 |
| Waist circumference, cm | 1216 | −0.03 | 0.28 |
| Adiponectin, µg/mL | 651 | −0.08 | 0.04 |
| HMW adiponectin, µg/mL | 650 | −0.07 | 0.08 |
| Leptin, ng/mL | 651 | −0.002 | 0.96 |
| Soluble leptin receptor, ng/mL | 651 | −0.02 | 0.56 |
| ROM, Carratelli units | 723 | 0.07 | 0.05 |
| CRP-hs, mg/L | 727 | 0.12 | <0.01 |
| C-peptide, ng/mL | 622 | 0.09 | 0.02 |
| HDL-C, mmol/L | 726 | −0.12 | <0.01 |
| HbA1c, % | 606 | −0.05 | 0.24 |

r: correlation coefficient; BMI, body mass index; HMW, high-molecular weight; ROM, reactive oxygen metabolites; HDL-C, high-density lipoprotein cholesterol (HDL-C); hsCRP, high-sensitivity C-reactive protein; HbA1c, glycated hemoglobin A1c.

Resistin concentrations were not statistically significantly associated with the risk of CRC. Thus, compared to quartile one, the RRs in quartiles were, quartile two, 1.11; 95% CI: 0.88–1.39, quartile three, 1.21; 95% CI: 0.97–1.53, and quartile four, 1.15; 95% CI: 0.91–1.46, *p*-trend = 0.41. In the continuous scale, RR for doubling resistin concentrations was 1.11; 95% CI: 0.94–1.30; $p$ = 0.22 (Table 3, model 3). No statistically significant relationship between resistin and CRC was observed when the associations were further explored by tumor site (colon or rectum) (Table 3) and right-sided and left-sided colon cancer (Supplementary Table S2).

**Table 3.** Relative risk (RR) and 95% confidence interval (95% CI) estimated for the association between resistin concentrations and the risk of colorectal cancer in the EPIC study data (1992–2005) in conditional logistic regression models.

| | Quartile Form | | | | | Continuous Form | |
|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | *p*-Trend [a] | Doubling Resistin Concentrations [b] | *p*-Value |
| **Resistin quartile ranges (ng/mL)** | ≤3.47 | 3.47< to ≤4.28 | 4.28< to ≤5.42 | 5.42< to ≤34.41 | | | |
| Colorectal Cancer | | | | | | | |
| No. cases/controls | 297/324 | 317/325 | 348/321 | 331/323 | | 1293/1293 | |
| Model 1 | ref | 1.06 (0.85–1.32) | 1.19 (0.95–1.49) | 1.13 (0.90–1.41) | 0.46 | 1.11 (0.95–1.30) | 0.19 |
| Model 2 | ref | 1.10 (0.88–1.37) | 1.22 (0.97–1.53) | 1.15 (0.91–1.46) | 0.38 | 1.12 (0.95–1.31) | 0.18 |
| Model 3 | ref | 1.11 (0.88–1.39) | 1.21 (0.97–1.53) | 1.15 (0.91–1.46) | 0.41 | 1.11 (0.94–1.30) | 0.22 |
| Colon Cancer | | | | | | | |
| No. cases/controls | 165/174 | 178/193 | 203/188 | 211/202 | | 757/757 | |
| Model 1 | ref | 0.97 (0.73–1.30) | 1.16 (0.86–1.56) | 1.11 (0.83–1.50) | 0.62 | 1.14 (0.94–1.40) | 0.19 |
| Model 2 | ref | 1.01 (0.75–1.37) | 1.17 (0.86–1.59) | 1.15 (0.84–1.56) | 0.67 | 1.15 (0.93–1.42) | 0.19 |
| Model 3 | ref | 1.04 (0.76–1.41) | 1.20 (0.88–1.65) | 1.20 (0.88–1.65) | 0.53 | 1.18 (0.95–1.47) | 0.13 |

**Table 3.** *Cont.*

| | Quartile Form | | | | | Continuous Form | |
|---|---|---|---|---|---|---|---|
| | **Q1** | **Q2** | **Q3** | **Q4** | ***p*-Trend** [a] | **Doubling Resistin Concentrations** [b] | ***p*-Value** |
| **Resistin quartile ranges (ng/mL)** | ≤3.47 | 3.47< to ≤4.28 | 4.28< to ≤5.42 | 5.42< to ≤34.41 | | | |
| Rectal Cancer | | | | | | | |
| No. cases/controls | 120/134 | 119/115 | 127/118 | 109/108 | | 475/475 | |
| Model 1 | ref | 1.15 (0.81–1.64) | 1.20 (0.85–1.71) | 1.13 (0.78–1.64) | 0.76 | 1.07 (0.82–1.39) | 0.62 |
| Model 2 | ref | 1.17 (0.81–1.68) | 1.26 (0.88–1.82) | 1.20 (0.81–1.76) | 0.64 | 1.09 (0.83–1.43) | 0.55 |
| Model 3 | ref | 1.16 (0.80–1.68) | 1.25 (0.86–1.80) | 1.17 (0.79–1.73) | 0.69 | 1.06 (0.80–1.41) | 0.66 |

Model 1: Conditioned on matching factors only: age, sex, study center, time of the day at blood collection, and fasting status. Women were further matched by menopausal status, phase of the menstrual cycle, and use of oral contraceptives at blood collection, and postmenopausal women were matched by hormone replacement therapy use. Model 2: Model 1 + smoking status, education, alcohol consumption, alcohol abstainers, physical activity index, energy intake, red meat, processed meat, dietary fiber, fruit intake, vegetable intake, dairy intake, fish, and shellfish intake. Model 3: Model 2 + body mass index (BMI), and residuals of BMI-adjusted waist circumference. [a] *p*-values for trend derived from models with the median resistin concentration within quartiles as a continuous variable. [b] Models with continuous log-transformed resistin concentrations by log 2.

Sex-stratified analyses showed no significant association between resistin and CRC (Table 4). The test for interaction by sex was not statistically significant in all study participants ($p = 0.86$), colon cancer patients and their pairs ($p = 0.72$), and rectal cancer patients and their pairs ($p = 0.10$). However, among men, doubling resistin concentrations were related to 1.53-fold the risk of rectal cancer (95% CI: 1.01–2.33).

In stratified analyses, there was no association between resistin with CRC among persons with BMI < 25 kg/m$^2$ or BMI ≥ 25 kg/m$^2$ (Table S2), with or without baseline diabetes, C-peptide ≥ 2 ng/mL or <2 ng/mL (data not shown). Among persons with hsCRP ≥ 3 mg/L, persons with higher compared to those with lower resistin concentrations had higher relative risks of CRC, but these associations were not statistically significant (RR per doubling resistin in persons with hsCRP ≥ 3 mg/L, 1.31; 95% CI: 0.94–1.81). No association was observed for doubling resistin concentrations among individuals with hsCRP < 3 mg/L (Table S2). We observed an increased risk of CRC associated with resistin among those with more than 6 h of fasting as compared to those with less than 6 h of fasting (RR per doubling, 1.45; 95% CI: 1.03–2.03; $p = 0.03$).

When we restricted the main analysis to cases and matched controls who were diagnosed within the first 2 years of follow-up, we found that participants in the highest as compared to the lowest quartile of resistin concentrations had a 1.97-fold risk of CRC (95% CI: 1.06–3.64; *p*-trend < 0.001); RR per doubling of resistin, 1.44; 95% CI: 0.97–2.12; Supplementary Table S2. In contrast, when excluding cases and matched controls that were diagnosed within the first 2 years of follow-up, the RR in the highest versus lowest quartile was 1.05; 95% CI: 0.81–1.37; *p*-trend = 0.79; RR per doubling, 1.03; 95% CI: 0.86–1.24; Supplementary Table S2.

The main results were not substantially different when performing complete case analyses or when excluding participants with extreme resistin levels (Supplementary Table S2). All findings from subgroup analyses (including analyses by the combination of sex and tumor subsite, by fasting status) were no longer statistically significant when participants who were diagnosed within the first two years of follow-up were excluded (data not shown). In this analysis, the relative risk of rectal cancer per doubling resistin concentrations among men was 1.13; 95% CI: 0.70–1.82.

When we combined our results with those published from the WHI, the pooled RRs of the highest versus the lowest quartile of resistin concentrations were 1.10; 95% CI: 0.93–1.29 for all study participants combined and 1.09; 95% CI: 0.86–1.39 for postmenopausal women. No significant heterogeneity was found (I2 = 0.0%, $p = 0.77$, identical in both meta-analyses).

**Table 4.** Relative risk (RR) and 95% confidence interval (95% CI) estimated for the association between resistin concentrations and the risk of colorectal cancer stratified by sex and subsite in the EPIC study data (1992–2005) in conditional logistic regression models.

| | Quartile Form | | | | | Continuous Form | |
|---|---|---|---|---|---|---|---|
| | **Q1** | **Q2** | **Q3** | **Q4** | ***p*-Trend** [a] | **Doubling Resistin Concentrations** [b] | ***p*-Value** |
| Resistin quartile ranges (ng/mL) | ≤3.47 | 3.47< to ≤4.28 | 4.28< to ≤5.42 | 5.42< to ≤34.41 | | | |
| Sex | | | | | | | |
| Women | | | | | | | |
| No. cases/controls | 134/154 | 165/183 | 190/157 | 192/187 | | 681/681 | |
| RR (95% CI) | ref | 1.02 (0.75–1.40) | 1.39 (1.00–1.94) | 1.21 (0.87–1.67) | 0.17 | 1.06 (0.85–1.33) | 0.59 |
| Postmenopausal women | | | | | | | |
| No. cases/controls | 97/120 | 123/129 | 136/110 | 135/132 | | 491/491 | |
| RR (95% CI) | ref | 1.14 (0.79–1.63) | 1.48 (1.01–2.18) | 1.25 (0.85–1.84) | 0.25 | 1.12 (0.86–1.47) | 0.38 |
| Men | | | | | | | |
| No. cases/controls | 163/170 | 152/142 | 158/164 | 139/136 | | 612/612 | |
| RR (95% CI) | ref | 1.20 (0.85–1.69) | 1.05 (0.76–1.47) | 1.08 (0.76–1.54) | 0.77 | 1.14 (0.89–1.46) | 0.29 |
| Sex and Tumor subsite | | | | | | | |
| Colon cancer women | | | | | | | |
| No. cases/controls | 77/93 | 99/108 | 120/103 | 133/125 | | 429/429 | |
| RR (95% CI) | ref | 1.11 (0.73–1.68) | 1.40 (0.91–2.16) | 1.37 (0.89–2.11) | 0.37 | 1.17 (0.88–1.57) | 0.28 |
| Colon cancer men | | | | | | | |
| No. cases/controls | 88/81 | 79/85 | 83/85 | 78/77 | | 328/328 | |
| RR (95% CI) | ref | 0.96 (0.59–1.55) | 0.97 (0.60–1.59) | 0.95 (0.58–1.57) | 1.00 | 1.13 (0.80–1.58) | 0.50 |
| Rectal cancer women | | | | | | | |
| No. cases/controls | 54/53 | 55/65 | 62/50 | 53/56 | | 224/224 | |
| RR (95% CI) | ref | 0.70 (0.41–1.19) | 1.11 (0.62–2.00) | 0.72 (0.40–1.31) | 0.29 | 0.71 (0.46–1.08) | 0.11 |
| Rectal cancer men | | | | | | | |
| No. cases/controls | 66/81 | 64/50 | 65/68 | 56/52 | | 251/251 | |
| RR (95% CI) | ref | 1.89 (1.06–3.36) | 1.43 (0.85–2.40) | 1.8 (0.99–3.24) | 0.12 | 1.53 (1.01–2.33) | 0.05 |

Results were based on conditional logistic regression models conditioned on matching factors (age, sex, study center, time of the day at blood collection, and fasting status, women were further matched by menopausal status, phase of the menstrual cycle, and use of oral contraceptives at blood collection, and postmenopausal women were matched by hormone replacement therapy use) and adjusted for smoking status, education, alcohol consumption, alcohol abstainers, physical activity index, energy intake, red meat, processed meat, dietary fiber, fruit intake, vegetable intake, dairy intake, fish and shellfish intake, body mass index (BMI), and residuals of BMI-adjusted waist circumference. [a] *p*-values for trend derived from models with the median resistin concentration within quartiles as continuous variables. [b] Models with continuous log-transformed resistin concentrations by log 2.

## 4. Discussion

In this nested case-control study, we found no statistically significant associations between pre-diagnostic circulating resistin concentrations and the risk of CRC. Furthermore, resistin concentrations were not correlated with measures of adiposity and leptin, weakly inversely correlated with adiponectin and HDL-C, and weakly positively correlated with C-peptide and hsCRP.

To our knowledge, the association between circulating resistin and CRC risk has to date been investigated only once previously in a prospective study, within the WHI, which reported a relative risk of 1.04 (95% CI: 0.72–1.50) among postmenopausal women when comparing the highest versus lowest quartile of resistin concentrations [5]. This is consistent with our findings, which extend the evidence to a general European population of men and women. Indeed, we found no heterogeneity when combining our results with those published by the WHI. These data overall suggest that higher pre-diagnostic resistin concentrations are not associated with a higher risk of CRC. In subgroup analyses, we found that higher resistin concentrations were associated with a higher risk of rectal cancer in men and in individuals with more than 6 h of fasting before the blood collection in the current study. However, the results of this subgroup analysis should be interpreted cautiously in light of the multiple tests and small sample sizes for each subgroup. In addition, the association was no longer significant when we excluded participants with less than two

years of follow-up. Of note, we observed higher levels of resistin in women compared to men, and in non-fasting compared to fasting participants only in controls, but not in cases. In line with our observations, higher resistin levels in women compared to men have been observed previously [35]; however, except for the non-significant associations in postmenopausal women [5], we are not aware of any comparable studies investigating a relationship between higher resistin levels and the risk of CRC stratifying by sex, tumor site, or the combination of sex and tumor site. Furthermore, in contrast to our findings, several previous studies suggested that circulating resistin levels are not influenced by fasting status [35,36]. Taken together, we cannot rule out the possibility of weak associations among these groups.

Previous case-control studies have reported higher resistin levels in CRC patients (at or after diagnosis) compared to healthy individuals [17,37,38]. Of note, only six (out of thirteen) observational studies reported results from multivariable-adjusted regression models rather than unadjusted case-control comparisons (Table S3) [5,37–41]. All these studies showed that participants with CRC had higher resistin concentrations than those without CRC. The results of these retrospective studies warrant prudent interpretation because resistin levels were measured in post-diagnostic blood samples; therefore, elevated resistin levels are likely a result of existing tumors [40,42]. Interestingly, we found a significant association between higher resistin concentrations and a higher risk of CRC in participants diagnosed with CRC within two years after enrollment and their matched controls, whereas there was no such association among persons with more than two years of follow-up. Although individuals who reported prevalent cancers at baseline were excluded from our analysis, we speculate whether some individuals may have had undiagnosed cancer at baseline, which may have led to higher resistin concentrations in these individuals. In contrast, the primary results from our study show that pre-diagnostic resistin concentrations are not related to CRC risk.

Resistin was originally described as an adipokine that purportedly links obesity and cancer via inflammation in humans, and insulin resistance in animals [8]. However, in our study, we found no correlation between resistin and BMI as well as waist circumference. Consistent with our findings, previous analyses using different samples from the EPIC-Potsdam study, which were collected at baseline (1994–1998) [43] or between 2010 and 2013 [6], also suggested no such association. Furthermore, we previously performed an analysis on healthy individuals in whom body fat was assessed using magnetic resonance imaging (MRI) scans [6]. In that study, subcutaneous and visceral adipose tissue accounted for only 1% of the explained variance in plasma resistin concentrations [6]. Several previous studies, including population-based studies, reported comparably weak ($r < 0.2$) but statistically significant correlations between resistin concentrations and BMI [6,30,44], or waist circumference [5,6,44]. Taken together, the current evidence suggests that there is no substantial correlation between resistin and BMI or waist circumference in humans. Our current study confirmed findings from other studies that showed that resistin was also not substantially correlated with other adipokines secreted by adipose tissues, such as adiponectin or leptin ($r < 0.15$) [5]. These findings strengthen the point that the resistin–obesity relationship is different in humans and animals, and do not support the role of circulating resistin as a biomarker of adipose tissue mass in humans.

We found null or weak correlations between resistin and other metabolic and inflammatory biomarkers among controls, which are consistent with most results published in population-based studies. The findings are consistent with previous findings from cross-sectional studies regarding HbA1c [30,45], HDL-C [44], C-peptide [30,45], and hsCRP [30,45]. In addition, previous studies found that resistin was weakly correlated with other inflammatory molecules such as TNF-α, IL-6, insulin [5,30], and insulin resistance measured by homeostasis model assessment-insulin resistance (HOMA-IR) [46]. However, in a small retrospective case-control study (40 CRC cases, 40 controls), significant correlations between resistin and inflammatory and metabolic biomarkers such as hsCRP and HDL-C were found in CRC patients, but not in the control group [40]. Furthermore,

we observed a null association between resistin concentrations and CRC risk in subgroup analyses by BMI, hsCRP, C-peptide, and baseline diabetes. Taken together, based on these lines of evidence we speculate that high levels of resistin in CRC patients are likely a result of existing tumors being accommodated by inflammation.

The current study has several strengths, including its prospective design, long follow-up time before CRC diagnosis, and a large number of cases. The current study included participants from several European countries, which improves the generalizability of the results. The current study had some limitations. First, resistin was measured in blood samples that had been stored over a longer period of time. The stability of resistin measured in frozen blood samples, however, was confirmed by a high overall intraclass correlation (0.95) over samples stored for 4 years, 2 years, and 1 year at −70 °C, suggesting that resistin levels may not be affected by long-term frozen storage [47]. The measured values of resistin in our data are consistent with those of other studies using the same assay (ELISA) and vendor (BioVendor) in similar study participants [6] or controls [41]. Second, a single measurement of biomarker levels at baseline may not reflect the long-term exposure to biomarkers. However, a previous study showed that there was no significant difference but high reliability (intraclass correlation coefficient 0.70) between resistin concentrations in blood samples collected at baseline and one year after [36], which suggested that a single measurement of circulating resistin is reasonable to represent long-term exposure. Third, although we included only CRC incident cases in our study, we cannot exclude the possibility that some participants had existing but as yet undiagnosed cancer at the time of recruitment. To overcome this issue, we performed analyses excluding participants with a follow-up time of less than two years and the results did not change appreciably. Fourth, as a collaborative endeavor from 10 European countries, some assessment and follow-up methods as well as blood sample storage methods differed across centers in the EPIC study. A calibration study as well as a comprehensive re-coding scheme were applied to standardize the data [20]. Furthermore, it has been shown that CRC case ascertainment was not different between passive and active follow-up methods [48]. We found no significant inter-assay variation of resistin concentrations between plates. Thus, these subtle differences are unlikely to have impacted our analysis.

## 5. Conclusions

In conclusion, higher pre-diagnostic resistin concentrations were not associated with a higher risk of CRC in men and women. Furthermore, our study suggests that resistin concentrations are null or weakly correlated with general or abdominal obesity measures, or metabolic and inflammatory biomarkers. Our findings suggest that pre-diagnostic circulating resistin concentrations are not associated with CRC risk, however, the presence of weak associations cannot be ruled out.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers14225499/s1, Figure S1: Association of pre-diagnostic resistin concentrations and the risk of CRC. Table S1: Characteristics of 1293 colorectal cancer cases and the 1293 matched controls in the nested case-control study, European Prospective Investigation into Cancer and Nutrition, 1992–2005. Table S2: Association of resistin concentrations and the risk of colorectal cancer in subgroup analyses. Table S3: Characteristics of the studies investigating the relationship between circulating resistin concentrations and the risk of colorectal cancer.

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**Institutional Review Board Statement:** The EPIC study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethical Review Board of the International Agency for Research on Cancer (IARC) and the ethical committees of the participating centers.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Raw data cannot be made freely available because of restrictions imposed by the Ethical Committee that do not allow open/public sharing of data of individuals. However, aggregated data are available for other researchers upon request. Requests should be sent to Tobias Pischon (tobias.pischon@mdc-berlin.de).

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## STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

| Section and Item | Item No. | Recommendation | Reported on Page No. |
|---|---|---|---|
| **Title and Abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 1-2 |
| **Introduction** | | | |
| Background/Rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 3-4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study Design | 4 | Present key elements of study design early in the paper | 4-5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 4-5 |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | |
| | | *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls | 6 |
| | | *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed | |
| | | *Case-control study*—For matched studies, give matching criteria and the number of controls per case | 6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6-7 |

| Section and Item | Item No. | Recommendation | Reported on Page No. |
|---|---|---|---|
| Data Sources/ Measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5-6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7-8 |
| Study Size | 10 | Explain how the study size was arrived at | |
| Quantitative Variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 8 |
| Statistical Methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 8 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 8 |
| | | (*c*) Explain how missing data were addressed | 7-8 |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | 8 |
| | | (*e*) Describe any sensitivity analyses | 8 |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | NA |
| | | (b) Give reasons for non-participation at each stage | 5-6 |
| | | (c) Consider use of a flow diagram | NA |
| Descriptive Data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 18 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 7 |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | |
| Outcome Data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | 8; 18 |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | |

| Section and Item | Item No. | Recommendation | Reported on Page No. |
|---|---|---|---|
| Main Results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 8 |
| | | (*b*) Report category boundaries when continuous variables were categorized | 10; 21 |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | NA |
| Other Analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10; 22; 25 |
| **Discussion** | | | |
| Key Results | 18 | Summarise key results with reference to study objectives | 11 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 12 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12 |
| **Other Information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 14 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.**

# Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my dissertation for reasons of data protection.

# Publication list


1. **Pham TT**, Nimptsch K, Aleksandrova K, Jenab M, Reichmann R, Wu K, Tjønneland A, Kyrø C, Schulze MB, Kaaks R, Katzke V. Pre-diagnostic circulating resistin concentrations are not associated with colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition study. Cancers. 2022 Nov 9;14(22):5499. [Journal impact factors: 6.575 (JCR 2021)]
2. Hoang T, Myung SK, **Pham TT**, Kim J, Ju W. Comparative efficacy of targeted therapies in patients with non-small cell lung cancer: a network meta-analysis of clinical trials. Journal of clinical medicine. 2020 Apr 9;9(4):1063. [Impact factors 2018: 5.688]
3. Hoang T, Myung SK, **Pham TT**, Park B. Efficacy of crizotinib, ceritinib, and alectinib in ALK-positive non-small cell lung cancer treatment: a meta-analysis of clinical trials. Cancers. 2020 Feb 25;12(3):526. [Journal impact factors: 6.162 (JCR 2018)]
4. **Pham TT**, Lee ES, Kong SY, Kim J, Kim SY, Joo J, Yoon KA, Park B. Night-shift work, circadian and melatonin pathway related genes and their interaction on breast cancer risk: evidence from a case-control study in Korean women. Scientific Reports. 2019 Jul 29;9(1):1-9. [Journal impact factors: 4.011 (JCR 2018)]
5. **Pham TT**, Hwang M, Lee ES, Kong SY, Jung SY, Lee S, Kim J, Ha M, Kim SY, Park B. Night-shift work and risk of breast cancer in Korean women. Clinical Epidemiology. 2019 Aug 21:743-51. [Journal impact factors: 3.178 (JCR 2018)]
6. **Pham TT**, Park B. Alcohol use disorder and health-related quality of life in Korean night-shift workers: A cross-sectional study using the KNHANES 2007-2015 data. Plos one. 2019 Apr 1;14(4):e0214593. [Journal impact factors: 2.766 (JCR 2017)]

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**May 2023,**

**Thu Thi Pham**