

Overweight, Hypertension and Cardiovascular Disease: Focus on Adipocytokines, Insulin, Weight Changes and Natriuretic Peptides

Ekim Seven

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Tutors: Jørgen Jeppesen, Allan Linneberg and Lise Lotte Nystrup Husemoen.

Official opponents: Gunnar Gislason, Christian Torp-Pedersen and Olle Melander.

Correspondence: Department of Internal Medicine, Rigshospitalet – Glostrup Nordre Ringvej 57, 2600 Glostrup, Denmark

E-mail: ekim_seven@hotmail.com

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THE FOUR ORIGINAL PAPERS ARE

1. Seven E, Husemoen LL, Wachtell K, Ibsen H, Linneberg A, Jeppesen JL. Overweight, adipocytokines and hypertension: a prospective population-based study. *J Hypertens.* 2014;32:1488–1494.
2. Seven E, Husemoen LL, Wachtell K, Ibsen H, Linneberg A, Jeppesen JL. Five-year weight changes associate with blood pressure alterations independent of changes in serum insulin. *J Hypertens.* 2014;32:2231–2237.
3. Seven E, Husemoen LL, Ibsen H, Friedrich N, Nauck M, Wachtell K, Linneberg A, Jeppesen JL. Higher Serum Concentrations of N-Terminal Pro-B-Type Natriuretic Peptide Associate with Prevalent Hypertension Whereas Lower Associate with Incident Hypertension. *PLoS ONE.* 10(2): e0117864. doi:10.1371/journal.pone.0117864. eCollection 2015.
4. Seven E, Husemoen LL, Sehested TSG, Ibsen H, Wachtell K, Linneberg A, Jeppesen JL. Adipocytokines, C-reactive Protein, and Cardi-ovascular Disease: A Population-Based Prospective Study. *PLoS One.* 10(6):e0128987. doi: 10.1371/journal.pone.0128987. eCollection 2015.

INTRODUCTION

Hypertension remains one of the leading causes of renal and cardiovascular disease worldwide (CVD) [1]. Overweight caused by excessive growth of adipose tissue is a major risk factor for hyper-

tension [2], but the mechanisms by which overweight lead to hypertension are complex and incompletely understood [3]. Nevertheless, abnormalities in insulin and glucose metabolism, inflammation, the sympathetic nervous system (SNS), and the renin-angiotensin-aldosterone system (RAAS) have all been linked with overweight-related hypertension for decades [4-7]. With respect to body composition, it is the excess weight owing to fat tissue and not muscle tissue, which is associated with an increased risk of hypertension [8]. In this context, it has become clear that adipose tissue is not just a passive energy-conserving tissue, but acts as a highly active metabolic and endocrine organ that secretes numerous hormones and cytokines, collectively called adipocytokines [9]. Several studies have shown that many of these adipocytokines have pro-inflammatory and vascular effects, and therefore it has been suggested that these substances play a pivotal role in overweight-related disorders, including hypertension [10]. Of the many different hormones and cytokines secreted by adipose tissue, there has been particular focus on adiponectin [11], leptin [12] and the interleukins e.g. interleukin-6 (IL-6) and interleukins-8 of which IL-6 mainly stimulates liver C-reactive protein (CRP) production and secretion [13, 14]. Even though many different tissues produce IL-6, it has been estimated that around 30% of total circulating IL-6 originates from adipose under normal circumstances. Thus, the concentration of CRP in the blood is a good marker of the amount of adipose tissue in healthy adults [15]. Additionally, it is important to emphasize that these hormones and cytokines are generally closely associated with other metabolic variables that are linked to adiposity, such as serum insulin and plasma glucose levels [16].

In 2004, using data from the Framingham study, Wang and colleagues proved that overweight and obese persons have markedly lower plasma natriuretic peptide levels than normal weight persons [17]. Recently, the finding that obese individuals compared with normal weight individuals have decreased circulating natriuretic peptide (NP) levels, despite of their high blood pressure (BP) and salt intake have attracted attention [18]. This 'natriuretic handicap' has also been highlighted in genetic studies. Common genetic variants in the loci encoding NP precursors, all related to lower circulating NP concentrations, have been shown to be associated with higher systolic BP (SBP) and diastolic BP (DBP) [19].

Although weight loss causes considerable short-term BP-reduction [20], several reports indicate that the effect diminishes with time and concomitant difficulties in weight loss maintenance results in less pronounced long-term reductions [21, 22]. Consequently, most overweight individuals with hypertension need drug treatment to control their BP [2]. However, as antihypertensive drugs often cause side-effects and fail to control BP sufficiently, there is still room for improvements in the treatment of overweight-related hypertension [23]. Therefore, more knowledge on normal and abnormal BP regulation is needed for the development of better treatment strategies, including new antihypertensive medication, for overweight individuals with hypertension, and better preventive strategies for overweight individuals who have not developed hypertension yet.

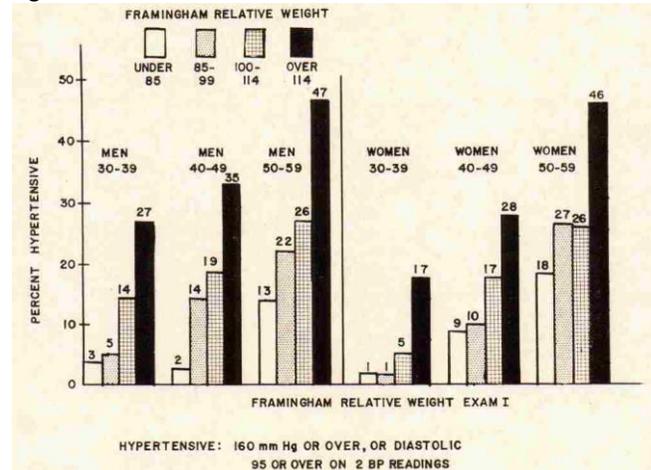
Hence, in an attempt to shed new light upon the mechanisms of overweight-related hypertension, this PhD programme was initiated. More specifically, to study the role of leptin, adiponectin, CRP, insulin, weight changes and the NP, brain natriuretic peptide (BNP), in a population based prospective setting. The data used in this thesis were acquired from the Inter99 study [24], which is particularly well suited to investigate the impact of adipocytokines on hypertension and CVD risk, because the Inter99 study, in addition to the traditional risk factors, includes several important potential confounders and mediators of overweight- and obesity-related diseases.

BACKGROUND

Obesity and Hypertension

Obesity and hypertension are two common diseases that frequently coexist [25, 26]. Epidemiological data has unarguably documented that the association between obesity and prevalent hypertension is more than can be expected by chance alone (figure 1) [27]. With the Framingham Study of Kannel et al. from 1967 being the first [28], prospective studies have also shown that obesity is an independent strong risk factor for the development of hypertension [29]. The magnitude of the obesity epidemic is illustrated by the most recent National Health and Nutrition Examination Survey, which indicates that approximately two thirds of US adults are presently classified as overweight (body mass index [BMI] ≥ 25 kg/m²) and one third as obese (BMI ≥ 30 kg/m²) [26]. In Europe, obesity has also reached comprehensive proportions, although there are significant regional differences [30]. Consequently, hypertension affects almost one third of the world population [31] and is regarded as the leading risk factor for the global disease burden [32]. Unfortunately, BP control is still described by the “rule of halves”; only half of hypertensive patient are diagnosed, only half of these received treatment, and despite the availability of effective antihypertensive medication, only half of these obtain optimal BP control [33].

Figure 1:



Prevalence of hypertension by sex and age according to Framingham relative weight. All trends significant at 0.05 level. From Kannel et al. [28].

The most common anthropometric measure to assess the degree of obesity is BMI. BMI is calculated by dividing body weight (in kilograms) by height (in meters) squared. Although widely used, and highly correlated with body fat and BP, BMI as a measure of obesity, has some limitations [34]. For instance, at moderately increased values of BMI, an excess of fat is not always present, and conversely, “normal” BMI does not imply protection from metabolic consequences of obesity [35]. Another limitation of BMI is its inability to distinguish between upper- and lower-body obesity. Studies, led by the important observations of Vague in the 1940s and 1950s [36], have shown that upper-body obesity is stronger associated to CVD and death than lower-body obesity. Generally, men tend to deposit fat in the abdominal area, so called “android obesity”, whereas women tend to deposit fat in the gluteal and femoral regions referred to as “gynoid obesity”. Consequently, the measurement of waist/hip ratio or waist circumference (WC) as indexes of upper-body obesity correlates more closely with hypertension and CVD [37]. However, BMI is a simple, convenient and universally known measure, and therefore still widely used in epidemiological studies.

The Adipocytokines

In overweight-related hypertension, the study of adipose tissue, particularly of the adipocyte, seems natural and necessary. Adipocytes are also known as fat cells and their primary role is storing energy as fat. Besides the role of an energy conserving cell, the adipocyte also produces and secretes various numbers of cytokines, and is regarded as the biggest endocrine organ [38]. The most abundant of these hormones is adiponectin. Paradoxically, the level of adiponectin is inversely correlated to body fat percentage. Adiponectin increases insulin sensitivity and thereby lowers the risk for type 2 diabetes, and there is considerable evidence, including Mendelian randomization studies, that suggests a causal relationship [39]. Furthermore, especially in animal studies, it has been shown that adiponectin have anti-atherogenic and anti-inflammatory effects [11, 40], and epidemiological data suggests that adiponectin is an independent predictor of future CVD [41]. Additionally, it has been proposed that adiponectin may play a protective role against the development of hypertension [42],

nevertheless, a recent meta-analysis and systematic review warranted more prospective studies [43].

Leptin is another adipocyte-derived hormone. Its discovery goes back to the 1950s, where Ingalls et al. described a new mutant strain of obese mice (ob/ob) that were characterized by severe obesity from excessive eating and decreased energy expenditure [44]. Subsequent studies reported weight normalization in ob/ob mice when their circulation was connected to wild-type mice [45], suggesting that ob/ob mice were lacking a hormone involving energy balance. This hormone, later known as Leptin regulates energy balance through a wide range of mechanisms, including appetite and SNS, and is strongly positively correlated with the amount of body fat with r values reaching 0.85 [46]. Studies indicate that resistance to the anorexic effects of leptin, but not to the SNS effects, develops as its plasma concentration rises, a concept introduced by Correia et al. in 2002 [47]. Therefore, "leptin resistance" have also been suggested as a mediator of overweight-related hypertension, mainly caused by a systemic pressor effect following activation of the SNS [48], and both cross-sectional and prospective epidemiological studies have reported positive associations [16, 49].

Finally, excess visceral fat is associated with non-infectious inflammation and human studies have shown that portal vein IL-6 concentration, a central mediator of the acute-phase response, correlates directly with CRP concentrations ($r=0.544$, $P=0.005$) [14]. Furthermore, subcutaneous adipose tissue have been shown to release IL-6 in vivo, and significant correlation with BMI ($r=0.48$, $P<0.01$) have been reported [13]. Nevertheless, the relationship between low-grade chronic inflammation and the early development of hypertension remains unsettled [50], and prospective epidemiological studies have shown both positive and negative associations [51, 52].

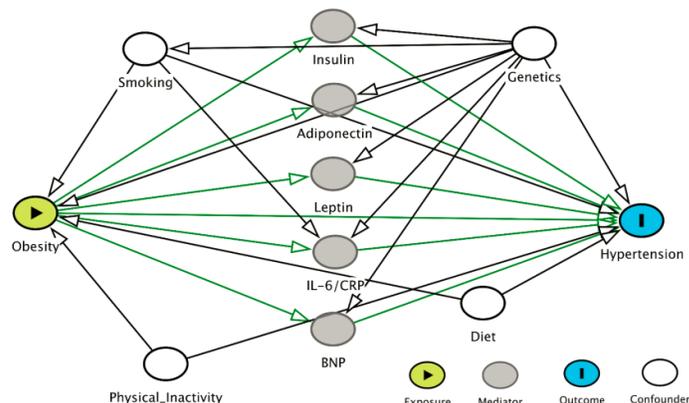
Insulin and Blood Pressure

In 1966, Welborn et al. [53], suggested that normoglycemic hypertensive patients with hyperinsulinemia were insulin resistant, and that this state of insulin resistance could contribute to the pathogenesis of hypertension. Reaven and colleagues refined the hypothesis in the late 80s, and highlighted the possible effect of insulin on the SNS, renal sodium retention and vascular smooth muscle hypertrophy [54, 55]. However, acute insulin infusion (within the physiological range) in healthy adults produced vasodilation and lowered DBP [56]. A couple of years later, it was shown that this vasodilator effect was mediated by nitric oxide and prostaglandins, at least among non-insulin-resistant individuals [57]. Furthermore, surgical treatment of patients with insulinoma and thereby correction of hyperinsulinemia did not result in BP changes [58]. Even though a large number of studies have examined the relationship between BP, the development of hypertension and insulin, the results remain inconclusive [59]. Nevertheless, it must be emphasized that insulin resistance characterizes hypertension, CVD and type II diabetes, and that the compensatory hyperinsulinaemia have been associated independently with all three clinical syndromes [60]. In this context, it has also been proposed that the BP lowering effect of weight loss could be mediated by increased insulin sensitivity [60].

Natriuretic Peptides and Hypertension

The cardiac NPs, atrial natriuretic peptide and BNP stimulate the discharge of sodium through the urine (natriuresis), promote vasodilatation and therefore, have a net BP lowering effect [61]. Thus, at any given time, a higher blood NP concentration is a robust marker of pressure induced cardiac stress, caused by, for example, hypertension, and high levels of the NPs are closely associated with both systolic and diastolic heart failure and poor outcome [62]. In contrast, in Mendelian randomization studies, genetically elevated serum concentrations of BNP (within the physiological range) [63], or the N-terminal fragment of proBNP (NT-proBNP), have been associated with a reduced risk of hypertension [19]. Furthermore, it has recently been shown that obese patient with newly diagnosed uncomplicated hypertension have lower-than-expected serum concentrations of BNP with regards to their high salt intake and high BP [18]. This natriuretic handicap, primarily seen in overweight and obese persons [17], could partially explain some of the mechanisms by overweight-related hypertension. Studies have shown that NT-proBNP levels are elevated in prevalent hypertension, especially when left-ventricular hypertrophy is present [64]. However, so far, even though several prospective studies have investigated the association between BNP and incident hypertension, no significant association has been found [65, 66]. Figure 2 illustrates possible causal pathways and classical confounders of obesity related hypertension.

Figure 2:



Possible pathways between obesity and hypertension/CVD.

AIMS AND HYPOTHESES

The overall aim of this thesis was to gain better understanding of the pathophysiology of overweight-related hypertension, BP-regulation and CVD from an epidemiological perspective.

Specific hypotheses for paper I–IV:

Paper I

Serum concentrations of adiponectin, leptin and CRP (used as a surrogate marker of IL-6), are independently associated with prevalent and incident hypertension. BMI is a strong and independent predictor of incident hypertension, even after adjustment for adiponectin, leptin and CRP.

Paper II

Five-year weight changes associate with BP alterations, even after adjustment for changes in lifestyle risk factors and serum insulin.

Paper III

NT-proBNP (used as a surrogate marker of active BNP), is positively associated with prevalent hypertension, but negatively associated with incident hypertension.

Paper IV

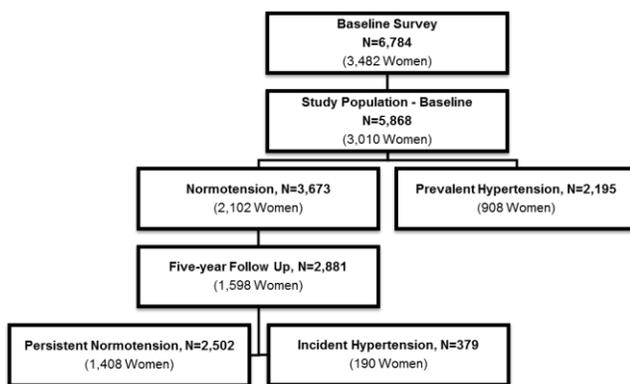
Adiponectin, leptin and CRP (used as a surrogate marker of IL-6), are independently associated with incident CVD.

MATERIALS AND METHODS

The Inter99 Study

The four papers presented in this thesis utilize data from the Inter99 study. A detailed description of the Inter99 study has been published previously [24]. Inter99 is a randomized, nonpharmacological intervention study for the prevention of ischemic heart disease (IHD) (CT00289237, ClinicalTrials.gov). The Inter99 study was initiated in 1999 and the cohort consists of all 61,301 individuals aged between 30 and 60 and living in 11 municipalities in the southern part of the former Copenhagen County. From this population, an age- and sex-stratified random sample comprising 13,016 individuals was drawn and invited for the intervention. In total, 6,906 persons attended the baseline investigation. One hundred and twenty-two participants were excluded either because drug abuse, alcoholism or because of linguistic problems, leaving 6,784 participants (participation rate of 52%) for the baseline examination. A flowchart of the study population, from paper I, is presented in figure 3. All participants gave a written consent before taking part in the study. The study was approved by the local ethics committee (KA 98155) and was registered at ClinicalTrials.gov (ID NCT00289237). The Inter99 study was conducted in accordance with the Declaration of Helsinki. The main results from the Inter99 study has just recently been published [67].

Figure 3:



Flowchart in Inter99, from Paper I.

Measurements

Before attendance at the center, the participants completed a comprehensive questionnaire [68, 69], in which information

about education, lifestyle, chronic diseases, and working conditions, among others were documented. Height was measured without shoes to the nearest centimeter. Weight with indoor clothing without shoes to the nearest 0.1 kg. BMI was calculated traditionally, as weight in kilograms divided by height in meters squared.

Persons without known diabetes underwent a 75 g oral glucose tolerance test with measurements of serum insulin and plasma glucose at three time points: 0, 30, and 120 minutes. Blood was drawn for measurements of fasting lipids, CRP, leptin, adiponectin and NT-proBNP among many others. At the pre-planned five-year follow-up examination, many, but not all, measurements were repeated.

Main Outcome of Interest: BP, Hypertension and CVD

BP was measured twice with a mercury sphygmomanometer (Mercurio 300, Speidel & Keller) with appropriate cuff size after 5 minutes of rest in the supine position. Trained staff performed all measurements. If SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg, the measurements were repeated twice to minimize the “white coat” effect with the two lowest values being recorded and the average of the recorded measurements was used. Hypertension was defined, in accordance with the European Society of Hypertension Guidelines [70], as use of antihypertensive medication or SBP ≥ 140 mm Hg or DBP ≥ 90 mm. Incident hypertension was defined as normotension at baseline and hypertension at the five-year follow-up visit. To correct for the use of antihypertensive medication in the quantitative BP analyses (paper II and paper III), the fixed Cui-Harrap adjustment method was used, and 10/5 mmHg was added to treated SBP/DBP, respectively [71].

Using various national registers three endpoints were defined in paper IV: a diagnosis of or death caused by ischemic heart disease (IHD) (ICD10: I20–25 and ICD8:410–414), a diagnosis of or death caused by stroke (ICD10: I60–69 and ICD8: 431, 433–434, and 436) and a combined endpoint, which included all the above-mentioned diagnosis. Information about CVD death was obtained from the Danish Registry of Causes of Death [72]. Information on diseases was obtained from the Danish National Patient Register [73].

STATISTICAL ANALYSES

Data were analysed using Statistical Analysis Software (SAS Institute, Cary, North Carolina, USA) version 9.3. All statistical tests were two-sided. $P < 0.05$ was chosen as significance level.

Descriptive Statistics, Paper I–IV

Data was presented as mean \pm standard deviation (SD) for normally distributed variables, as median (5th to 95th percentile) for skewed distributed variables, and frequency in percentage for categorical variables. Group comparisons were done with the ANOVA test for normally distributed variables, the chi-square test for categorical variables, and the Kruskal-Wallis test for skewed distributed variables. When necessary continuous variables with skewed distribution were logarithmically transformed to fulfill model assumptions.

In paper II paired t-test were performed to report five-year differences and the SAS LSMEANS statement was used to generate adjusted means (paper II and paper III).

Correlation Analyses, Paper III

Partial Spearman correlation analysis, adjusted for age and sex, were used to identify significant relationships between NT-proBNP and variables of interest.

Regression Analyses, Paper I–IV

Multiple logistic regression models, including c-statistics calculations (paper I and paper IV), were constructed to compute standardised odds ratios (OR) with 95% confidence interval (CI) with hypertension as outcome. In paper II, linear regression models were constructed to report Δ SBP and Δ DBP per Δ kilogram weight change. The validity of the multiple logistic regression models was checked by Hosmer and Lemeshow goodness-of-fit test. The linearity assumption was checked by adding the term squared and cubed to the model and checking for significance.

In paper IV, multivariate Cox regression analyses were performed to determine the association between baseline levels of adiponectin, leptin, and CRP with CVD. Estimates were presented as hazard ratios (HR). The log-rank test was used to compare event-free survival, and Kaplan-Meier curves for event-free survival were constructed. We used age as underlying time axis and delayed entry where participants entered the analysis at their baseline age, and they exited the analysis at their event or censoring age. If participants experienced multiple outcome events, only the first event was included. The linearity assumption was checked by adding the term squared and cubed to the model and checking for significance. The proportional hazards assumption was checked visually.

Population Attributable Risk and Net Reclassification Improvement (NRI)

In paper I, for public health decision-making purposes, the PAR of overweight and obesity (the exposure) regarding development of hypertension (the disease) was calculated. PAR is the proportion of disease occurrence in the population that would be eliminated if the exposure was eliminated. PAR was calculated by subtracting the incidence in the unexposed from the incidence in the total population (exposed and unexposed), and the percentage (PAR%) was obtained by dividing the PAR by the incidence in the total population and then multiplying the product by 100 [74].

In paper IV, in order to evaluate the predictive performance of the different models NRI and Integrated Discrimination Improvement was calculated by the use of a SAS macro as proposed by Pencina et al. [75].

MAIN RESULTS

Detailed results of the four papers are available in the corresponding papers I–IV, and in the respective online data supplements (paper I and paper II). A summary of the main results are provided below:

Paper I

We hypothesized that adiponectin, leptin and the IL-6 product, CRP, would be independent predictors of incident hypertension and thereby strengthen their candidature as intermediates in overweight-related hypertension. Furthermore, we hypothesized that BMI, as an anthropometric measure of obesity level, would be independently associated with both prevalent and incident hypertension, even after adjustment for adiponectin, leptin and

CRP. Finally, we speculated that the PAR of overweight and obesity regarding incident hypertension was considerable.

Table 1:

	Prevalent Hypertension (OR 95% CI)	
	Model 1	Model 2
Leptin	1.69 (1.58–1.82)*	1.18 (1.06–1.32)†
Adiponectin	0.91 (0.86–0.97)†	0.94 (0.87–1.02)
CRP	1.34 (1.26–1.42)*	1.16 (1.07–1.26)*
Incident Hypertension (OR 95% CI)		
Leptin	1.39 (1.21–1.60)*	1.24 (1.01–1.54)‡
Adiponectin	0.95 (0.84–1.07)	0.93 (0.80–1.08)
CRP	1.11 (0.99–1.25)	0.98 (0.84–1.14)

Association between plasma levels of leptin, adiponectin and C-reactive protein and hypertension.

Data are presented as odds ratios (OR) with 95% confidence intervals (CI) for one standard deviation increase in log-transformed levels of the candidate intermediate. Model 1: age- and sex-adjusted. Model 2: Model 1 + mutually adjusted for leptin, adiponectin, C-reactive protein (CRP), and alcohol intake, smoking, physical activity, dietary habits, educational level, parental history of hypertension, total cholesterol, triglycerides, insulin, HbA1c, and further adjusted for baseline heart rate, systolic- and diastolic blood pressure regarding incident hypertension. * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$.

Among 5,868 adults (51.3% women, mean age 46) we recorded 2,195 prevalent and 379 incident cases of hypertension (figure 2). In models including leptin, CRP, adiponectin, sex, age, lifestyle risk factors, lipids, insulin, hemoglobin A1c (HbA1c), and in the incident model also baseline heart rate and BP, only leptin of the three candidate intermediates was significantly associated with both prevalent and incident hypertension (table 1).

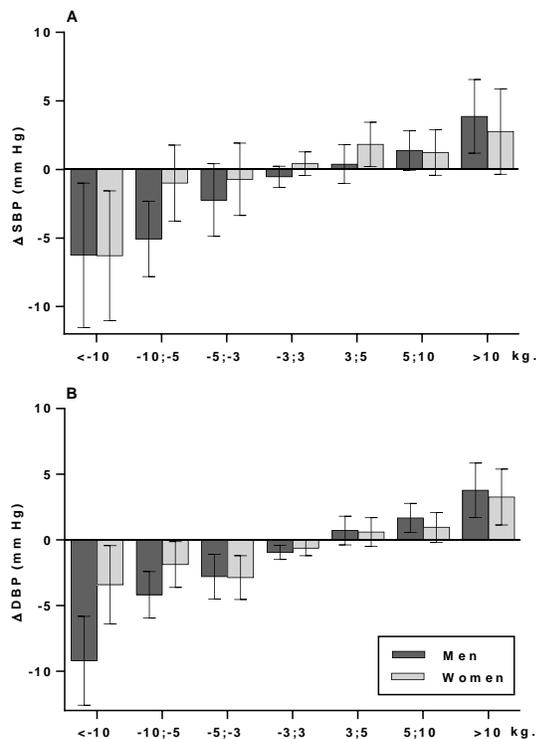
BMI was robustly associated with IHT. Thus, comparing the lowest with the highest quintile of sex-specific BMI levels, an almost two-fold increased risk in incident hypertension (OR=1.89, 95% CI 1.10–3.25, $P=0.023$) in the fully adjusted model was observed.

Finally, the calculated unadjusted PAR for overweight was 31%. If we used OR instead of relative risk and adjusted for sex, age, smoking habits, alcohol consumption, and physical activity level we obtained similar results with a PAR of 33%. In other words, hypothetically, one third of cases with incident hypertension could have been prevented if the study population had been completely unexposed to overweight and obesity.

Paper II

In this paper, we studied five-year changes in weight, BP and insulin. Our hypothesis was that five-year weight changes would be associated with BP alterations independent of changes in serum insulin and lifestyle risk factors. We had a full set of data on 3,443 participants. Based on their five-year weight change, participants were divided into three groups: weight loss, weight stable and weight gain. We observed that weight changes were associated with BP alterations and had a substantial impact on both fasting and two-hour post-glucose serum insulin levels. However, in our multivariable regression analyses, additional adjustments for insulin values only attenuated the associations between weight changes and BP minimally. The observed changes displayed a dose-response pattern (figure 4).

Figure 4:



Mean five-year change in blood pressure according to weight change and sex.

Additionally, we found significant interaction between Δ weight and sex with both Δ SBP ($P=0.042$) and Δ DBP ($P=0.002$), and interaction regarding baseline BMI level and Δ SBP ($P=0.008$). The associations were significantly stronger in overweight men as presented in table 2.

Finally, we could not help but notice that the percentage of former smokers in the weight gain group increased noticeably during the 5-years, as reported previously by Pisinger et al. [76], suggesting that smoking cessation could partly explain weight gain in this group and possibly the subsequent increase in BP. This finding emphasizes the importance of weight control, especially in lifestyle intervention studies that includes a smoking cessation arm.

Table 2:

	Δ SBP, mm Hg (95%CI)	Δ DBP, mm Hg (95%CI)
All, n=3,443	0.27* (0.2 to 0.4)	0.27* (0.2 to 0.3)
Women, n=1,683	0.21† (0.1 to 0.3)	0.20* (0.1 to 0.3)
- BMI <25 kg/m ² , n=1,017	0.03 (-0.2 to 0.2)	0.17‡ (0.0 to 0.3)
- BMI ≥25 kg/m ² , n=666	0.31* (0.1 to 0.5)	0.19* (0.1 to 0.3)
Men, n=1,760	0.36* (0.2 to 0.5)	0.36* (0.3 to 0.5)
- BMI <25 kg/m ² , n=677	0.28‡ (0.0 to 0.5)	0.36* (0.2 to 0.5)
- BMI ≥25 kg/m ² , n=1,083	0.40* (0.3 to 0.5)	0.38* (0.3 to 0.5)

Changes in SBP and DBP in mmHg per kilogram weight change stratified by sex and weight class.

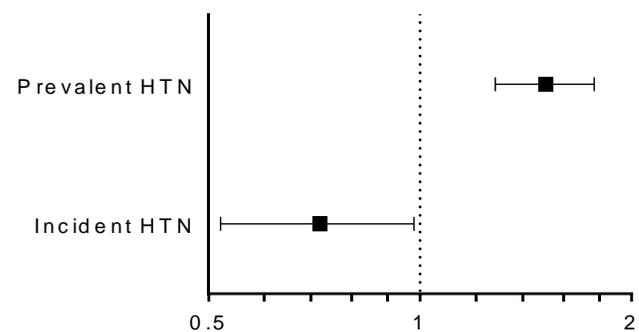
All estimates are adjusted for sex, age, family history of hypertension, baseline BMI, baseline SBP and DBP, baseline lifestyle risk factors and five year changes, fasting insulin, two-hour post-glucose insulin, Δ fasting insulin and Δ two-hour post-glucose. CI, confidence interval. * $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$.

Paper III

Overweight and obese individuals tend to have lower than anticipated circulating concentrations of NPs considering their high BP. This led us to hypothesize, that among normotensive individuals higher levels of NT-proBNP (within the physiological range) would be associated with a lower risk for incident hypertension, indicating that lower levels could be causally related to the development of hypertension.

At baseline, among 5,307 participants with a complete set of data, we recorded 1979 cases with prevalent hypertension and 324 cases of incident hypertension on follow-up five years later. Overweight participants had significantly lower baseline serum NT-proBNP concentrations (median [5th to 95th percentile]; 36 pg/mL [10 to 144] versus 45 pg/mL [12 to 148], $P < 0.001$) in spite of significantly higher SBP (mean \pm SD; 124 ± 10 mm Hg versus 119 ± 10 mm Hg, $P < 0.001$) and DBP (78 ± 7 mm Hg versus 75 ± 7 mm Hg, $P < 0.001$) compared with normal weight participants.

Figure 5:



Odds ratios for hypertension according to baseline N-terminal pro-B-type natriuretic peptide in Inter99.

Comparison of participants with baseline values above the 80th percentile with those below the 80th percentile, in the fully adjusted model.

In multivariate logistic regression models, for all participants, one SD increase in log transformed NT-proBNP concentration was associated with 21% higher risk of prevalent hypertension (OR=1.21, 95% CI 1.13–1.30, $P < 0.001$), and 14% lower risk of incident hypertension (OR=0.86, 95% CI 0.76–0.98, $P = 0.020$). The associations are illustrated in a forest plot (figure 5). The plot shows OR for hypertension for participants with NT-proBNP values above the 80th percentile compared with those below the 80th percentile in fully adjusted model.

Therefore, we can conclude that higher serum concentrations of NT-proBNP associate with prevalent hypertension whereas lower concentrations associate with incident hypertension.

Paper IV

In this study, we investigated the associations of adiponectin, leptin and CRP with incident CVD. We defined three endpoints: a diagnosis of or death caused by IHD, a diagnosis of or death caused by stroke and combined endpoint, which included all the above-mentioned diagnosis. Participants with prior CVD were excluded from this study. Among 6,502 participants with a mean follow-up

time of 11.4 years, 527 participants experienced one or multiple events. As expected, well-established CVD risk factors differed significantly between cases and controls. We used Cox multivariate regression analyses to report HRs, and constructed different models to adjust for various CVD risk factors, including adjustments for the Framingham Risk CVD Score variables (table 3).

Table 3:

	Hazard Ratio (95% CI)		
	Model 1	Model 2	Model 3
Adiponectin	0.94 (0.86–1.03)	1.00 (0.92–1.11)	0.97 (0.87–1.08)
Leptin	1.17* (1.07–1.28)	1.02 (0.93–1.13)	0.97 (0.85–1.12)
CRP	1.33* (1.22–1.45)	1.18* (1.08–1.30)	1.19* (1.07–1.33)

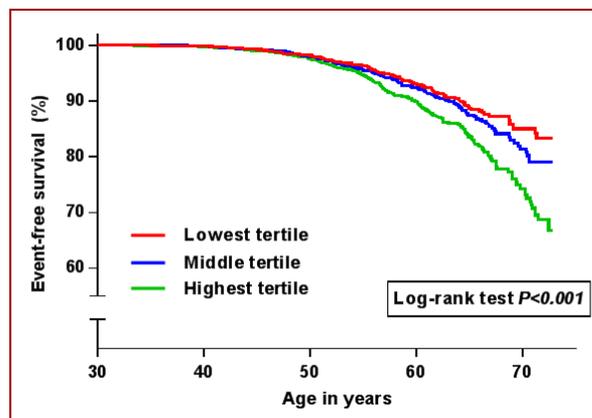
Associations of Baseline Adiponectin, Leptin and CRP with the Combined Endpoint in Inter99.

Data are presented as Hazard Ratio per one standard deviation increase in log transformed leptin level. Model 1: Adjusted for sex, age and intervention group. Model 2: Model 1 + total cholesterol, HDL-cholesterol, smoking status, systolic blood pressure, treat-ment for hypertension and baseline diabetes. Model 3: Model 2 + BMI, HOMA-IR, eGFR, CRP and adiponectin. *P<0.001

In sex-and age adjusted models both leptin and CRP showed significant associations with CVD, however, only CRP remained significantly positive associated with the combined endpoint, IHD and stroke in all models (table 3).

Finally, in figure 6 we present a Kaplan-Meier plot for baseline CRP level in sex-specific tertiles, plotted against event-free survival time. As seen, the curves start to separate before the age of 60 with those participants in the highest CRP-tertile having the worst outcome.

Figure 6:



Kaplan-Meier Curves for Event-free Survival Stratified by Sex-specific Tertiles of Baseline CRP in Inter99.

DISCUSSION

Methodological Considerations

At this point, some general methodological considerations seem appropriate. The main problem with observational studies is that the researcher has no control of the composition of the control groups and cannot randomize the distribution of individuals. This can create bias, mask cause-and-effect relationships and even suggest associations where there are none [77]. However, regardless of the limitations, an observational study allows a useful insight into a phenomenon and sidesteps the ethical and practical

difficulties of setting up a large and cumbersome research project and observational studies have made many contributions to the understanding of various CVDs [78]. Compared to cross-sectional studies, the prospective cohort studies (e.g. Framingham Heart Study) have several advantages. The longitudinal tracking of participants in prospective studies make observed changes more accurate, can establish sequences of events, and particularly in the field of medicine, the design allows researchers to uncover predictors of certain diseases [79]. Nevertheless, in any observational study, the possibility that unmeasured confounders bias the intended comparison persists. In addition, observational studies are limited in their inability to demonstrate causality [80]. To cope with this issue various statistical approaches are used. Logistic regression is especially useful for analyses of categorical observational data when adjustment is needed to reduce the potential bias resulting from the differences in the groups being compared [81]. However, the various models used when performing multivariate adjustments must be very carefully constructed, to avoid overadjustment bias [82]. It is of utmost importance to distinguish between a confounding variable and an intermediating variable. A confounding variable is a factor that correlates with both the dependent (the disease) and the independent (the exposure) variable without representing a step in the causal chain, whereas, an intermediate variable is a factor that represents a step in the causal chain between the independent variable and the dependent variable. In any model, it is appropriate to adjust for a confounder variable, but inappropriate to adjust for an intermediate variable [82]. By adjusting the confounding effect is removed, but only if, the underlying causal pathways are correctly specified. Regarding hypertension, which is a rather complex disease with multifactorial etiology [83] the causal pathways cannot always easily be recognized which makes model construction rather difficult (figure 2).

To complicate things further, there are occasions where adjustment can cause biases rather than decreasing biases. For example, the selection of confounding variables in observational studies is often based on baseline differences between study groups. Frequently, adjustments for well-known prognostic factors are omitted because of large P-values, conversely, adjustment for non-confounding variables included because of small P-values, for example intermediate variables. However, if the confounding variable(s) omitted is conditionally dependent on a second variable, which does differ between groups and therefore is included in the model, valuable information is lost and wrong conclusions can be drawn. Hence, a P-value-guided adjustment strategy can actually introduce bias rather than decreasing it. Therefore, selection of confounding variables starts with identifying risk factors for the outcome [84] based on current knowledge of the medical literature.

Finally, it should be mentioned that the effect size of any biomarker (e.g. the adipocytokines) are markedly influenced by the precision of the measurements and the short-term changeability of the biomarker. In contrast, BMI or WC can be measured very precisely and changes very slowly over time. Therefore, BMI, or any other anthropometric measurement, has great a priori statistical advantage compared with highly fluctuating plasma biomarkers, when it comes to providing prognostic information [50]. The following example illustrates the problem. The high-sensitive CRP measurements in the Inter99 study was initially done at

Tethys Bioscience (Emeryville, California, USA) using an ultrasensitive molecular counting technology platform [85]. A couple of years later, using the same blood samples, high-sensitive CRP measurements were repeated by the Institute of Clinical Chemistry and Laboratory Medicine in Greifswald Germany, using nephelometry on a Dimension VISTA (Siemens Healthcare Diagnostics, Eschborn, Germany) platform. A total of 5,611 participants had both measurements done. Even though the measurements correlated strongly ($r=0.86$, $P<0.001$), using a paired t-test the high-sensitive CRP measurements from Tethys Bioscience were on average 20% lower (95% CI 19–21, $P<0.001$) than the measurements from Greifswald; median [5th to 95th percentile]; 0.94 mg/L [0.15 to 7.83] versus 1.11 mg/L [0.25 to 7.81], respectively. It is difficult to imagine that the BMI measurements would differ by an average of 20%, even if they were done in two different countries by different laboratories on the same day.

Adipocytokines and Hypertension, Paper I

Our positive results concerning leptin being associated with incident hypertension in this paper are in agreement with three of four previously published prospective cohort studies [49, 86, 87]. However, one study including 748 middle-aged white participants did not find any association [88]. In that study all outcomes was adjusted for baseline BMI, because the authors found a significant positive correlation with BMI ($r=0.53$, $P<0.0001$). This is somewhat debatable, as it should be remembered that leptin is regarded as an intermediate between being overweight (the exposure) and hypertension (the outcome). BMI is therefore not a confounder in the classical sense as it represents the exposure, and therefore acts as the first step in the causal chain [89]. Nevertheless, one understands the need to adjust for BMI, in order to isolate the effect of leptin, to study the BP of individuals with comparable BMIs and different levels of leptin. In our study, the associations with hypertension were also markedly attenuated when we adjusted for BMI as a continuous variable, as expected, one might say. However, the associations remained significant when we adjusted for BMI categories; furthermore, the association between leptin and prevalent hypertension was also present analyzed by BMI-strata. In that context, the current study, besides including a substantial number of participants, is also remarkable for the comprehensive multivariable adjustment for potential confounders, including lifestyle risk factors, lipids and both insulin and HbA1c, which further strengthens the possibility of a causal relationship.

Similarly, leptin's candidacy as a mediator of overweight-induced hypertension has gained support from genetic studies. In recent years, genome wide association based studies have appeared as a unique alternative to explore simultaneously a large number of genomic loci for associations with a certain phenotypic trait. A study by Söber et al. [90] targeted 160 candidate BP regulation genes, and found among very few associations, evidence of sizeable association between BP and single-nucleotide polymorphisms in both the leptin gene and the leptin receptor gene. Overall, based on these epidemiological and genetic findings, we believe, that the statement of leptin being independently associated with and a possible causal risk factor for hypertension is justified.

Hypoadiponectinemia have also been linked to the development of hypertension. Animal studies have for instance shown that adiponectin replenishment reduces BP in obese hypertensive

mic [91], and in vitro studies of endothelial cells have proven that adiponectin induces nitric oxide production [92]. Nevertheless, in our study we did not find any association between low levels of adiponectin and prevalent nor incident hypertension in the adjusted models. A recent systematic review and meta-analysis [43], based on 48 studies of which only five were prospective [49, 93-96], suggested that plasma adiponectin level is a biomarker and possible mediator in overweight-related hypertension. Besides the inclusion of very few prospective studies, with a total prospective sample size of 3,161 persons, the review was notable for many small studies with high level of unexplained between study heterogeneity. Two of the five prospective studies [93, 94] and many of the cross-sectional studies were conducted in populations with Asian origin, so ethnic differences may exist. The largest of the former adiponectin studies published by Asferg et al. including the last author in our group [49], in a cohort of 920 participants, did neither find a positive association. In that study, all models were likewise adjusted for leptin. Once again, given the size of our study and the comprehensive adjustment for potential confounders, we believe that our conclusion, of circulating adiponectin not being linked to hypertension is valid. Nonetheless, we have to acknowledge that we cannot completely rule out a BP lowering effect of increased adiponectin secretion. Thus, studies in both humans and animals have shown that adiponectin secreted locally (in a paracrine manner) seems to be a physiological modulator of local vascular tone by increasing nitric oxide bioavailability [97, 98].

The role of inflammation in hypertension is very complex and a subject of much debate [6]. CRP has repeatedly been associated with BP and prevalent and incident hypertension [99], but whether a causal link exists is uncertain [100]. While there is an apparent association between CRP levels and prevalent hypertension, and it has been shown that elevated CRP precedes BP elevation [101], adjustment for confounding factors (e.g. BMI, smoking among others) attenuates the effect to such a degree, that it can be concluded that elevated CRP levels reflects overweight induced inflammation [15, 102] and other potential confounders. Furthermore, in Mendelian randomization studies, where different genotypes are directly related to the outcome and not influenced by confounding factors, polymorphisms associated with higher CRP levels are not associated with higher BP levels, suggesting that the association between CRP and BP outcomes is not causal [100, 102]. Accordingly, in our study we showed that CRP was positively associated with prevalent hypertension even after multivariate adjustment, however, in the prospective part the association became none significant in the sex- and age-adjusted model, and completely abolished in the multivariate adjusted model.

Finally, and in accordance with the medical literature [52], we showed that BMI was a robust independent predictor of hypertension. In fact, the calculated PAR was 31%. Interestingly, BMI was associated with incident hypertension after adjustment for baseline leptin and all other variables of interest in the study, indicating that other pathophysiological pathways related to variables not included in our study are profoundly involved in the pathogenesis of overweight-related hypertension [3].

Weight Changes, BP and Insulin, Paper II

The coexistence of hypertension and insulin resistance can be regarded as a cause-effect relationship [54] (insulin resistance cause

hypertension or vice versa) or as a non-causal relationship, representing two independent consequences of the same metabolic disorder or as being associated with a specific phenotype [103]. Obviously, since being overweight or obese is such a strong risk factor for the development of hypertension and for insulin resistance [3], we thought it was natural to examine whether five-year weight changes associated with BP alterations and insulin changes and whether these changes were independent of each other. We observed substantial changes in both fasting and 2-hour post-glucose serum insulin levels and BP with even modest weight changes. Our main hypothesis was supported by the data; weight loss was associated with BP reduction and weight gain with BP elevation, and the data additionally suggested that the observed BP and weight changes were independent of changes in life-style risk factors and insulin changes. Interestingly, the observed associations were strongest for men with BMI at least 25 kg/m² and almost non-existent for normal weight women. Even though the BP alterations in our study seemed modest, the clinical relevance of the findings is supported by a meta-analysis by the Prospective Studies Collaboration Group [104]. They concluded that a 2 mm Hg reduction in SBP results in approximately 10% lower stroke mortality and 7% lower IHD and other vascular diseases and that there was a continuous relationship with risk at least as far as 115/75.

Although a large number of studies have examined the relationship between insulinemia and hypertension our study is remarkable for several reasons. Firstly, the study is population based with a high number of participants. Secondly, and probably most importantly, we had insulin measurements both at baseline and at the five-year follow-up examination, which provided us with the unique opportunity to assess whether the BP alterations were independent of insulin changes. Therefore, we believe that our results contribute to the evidence indicating that hyperinsulinemia does not play a major direct role in the early development of hypertension, and that the coexistence of these entities are primarily based on excess body weight per se. Furthermore, we believe that weight loss intervention has the potential for considerable public health impact as it clearly improves BP- and insulin profile.

NT-proBNP and Hypertension, Paper III

Being overweight or obese is characterized by a state of fluid overload, which is caused by sodium retention, eventually leading to increased cardiac output and rise in BP [3]. The NPs induce natriuresis and promote vasodilatation and hence a BP lowering effect [61]. Paradoxically, several reports have underscored lower circulating concentrations of BNP and other NPs in the presence of excess weight [17, 18]. Furthermore, Mendelian randomization studies have shown that genetically elevated serum concentrations of BNP (within the physiological range), which results in life-long exposure independent of comorbidity, associates with reduced risk of hypertension [19]. Accordingly, this so-called “natriuretic handicap”, which is predominant in obese persons, could possibly explain a substantial part of the link between obesity and hypertension. However, it should be remembered, that when the hypertensive condition progresses and becomes more severe, the concentration of the NPs will start to increase as a consequence of myocardial stretch and strain, caused by an increase in total peripheral resistance due to vasoconstriction and

chronic volume overload, and ultimately, if antihypertensive treatment is not begun, heart failure [105].

Our study is the first prospective study to report that lower serum concentrations of NT-proBNP are associated with an increased risk of incident hypertension. Unsurprisingly, we also found that higher serum concentrations of NT-proBNP were associated with higher risk of prevalent hypertension. The associations were present even after adjustments for a wide range of anthropometric, lipid, pulmonary, metabolic and renal risk factors. Even though we did not find a significant interaction with BMI level, the associations seemed stronger among the overweight participants of our study. Thus, based on our data and the medical literature in general, and especially the genetic studies, we believe that it is reasonable to assume that at least part of the overweight-related hypertension is mediated by low circulating NP concentrations.

Adipocytokines and CVD, Paper IV

Several animal and human studies have proposed that the adipocytokines play a pivotal role in overweight and obesity-related disorders such as atherosclerosis, hypertension, and type II diabetes, which could lead to overt CVD [40, 106]. For example, Öhman et al. have shown that transplantation of visceral adipose tissue into apolipoprotein E-deficient mice causes a marked acceleration of atherosclerosis by increased production of pro-inflammatory factors [107]. Nevertheless, people are not mice, and epidemiological adiponectin and leptin studies have produced conflicting results. More specifically regarding adiponectin, three recently published independent meta-analyses, could not find any evidence suggesting that lower circulating adiponectin concentrations were associated to increased risk for CVD [108-110].

Data relating leptin to CVD has also been inconsistent, and the positive associations reported have been largely dependent on BMI [111]. However, a recently published systematic review and meta-analysis, including eight original papers with a total of 21,064 participants and 2,053 CVD events, reported a borderline significant positive association after adjustment for age, sex, lipids, and BP in men [112].

The medical literature has been consistent in linking elevated CRP levels, regardless of origin, to CVD [113, 114]. However, since genetically elevated CRP concentrations seem unrelated to the development of CVD, a direct causal relation is unlikely [115, 116]. The close association of CRP and CVD could be due to retro-causality, hence it is the inflammation caused by atherosclerotic plaques, in asymptomatic individuals, which stimulates liver-CRP production. In our study, the IL-6 product CRP was the only substance that was consistently significantly associated with the outcome in all models. It is noteworthy that CRP predicted risk after adjustment for a wide range of risk factors, including estimates of insulin resistance and HbA1c. Neither adiponectin nor leptin showed significant association in the adjusted models. Elevated CRP levels in obesity primarily originate from the production of IL-6 in adipose tissue [13]. Since adjustment for CRP decreased the BMI associated CVD risk markedly, our data indirectly suggest that IL-6 originating from fat tissue could play a role in overweight and obesity-related CVD.

Finally, in the context of leptin and CVD, the possibility of over-adjustment bias needs to be briefly discussed. Over-adjustment bias is defined as control for an intermediate variable on a causal path from exposure to outcome [82]. In the Inter99 study,

we have previously found that leptin, but not adiponectin or CRP, could play a mediating role in overweight-induced hypertension [89]. Therefore, adjustment for BP, which represent an intermediate variable on the path from leptin (exposure) to CVD (outcome), could be considered as over-adjustment bias when relating leptin to CVD risk.

Study Limitations

Some general limitations in the Inter99 study concern all of the four papers in this thesis. First of the low participation rate (52%) in the main study is concerning. Toft et al [117] dealt with this topic, and they found that bad health, unhealthy lifestyle, and perceived susceptibility to disease among other things were important mediators of participation in the study. Consequently, we suspect that participants in the Inter99 study were at least as unhealthy as the background population, and most likely more, possibly biasing our results. Additionally, it is a limitation that the Inter99 population was almost exclusively Caucasian, making it problematic to generalize the results to other populations. As a final general point, the studies conducted were observational hence; causality can naturally not be established.

Regarding specific limitations for the different studies, these are discussed in details in the corresponding papers.

CONCLUSION

This PhD thesis provides information to the scientific community regarding overweight-related hypertension and CVD from an epidemiological perspective. Based on our findings the main conclusions are summarized as follows:

Paper I:

Leptin, but not adiponectin or CRP, may play a mediating role in overweight-induced hypertension. However, as BMI was a strong independent predictor of hypertension, other factors than leptin must be involved in the pathogenesis of overweight-related hypertension.

Paper II:

Five-year weight changes associated with BP alterations independent of changes in insulin and lifestyle.

Paper III:

Higher serum concentrations of NT-proBNP (within the physiological range) associated with PHT whereas lower concentrations associated with IHT. This suggests that a lower amount of circulating BNP, resulting in diminished vasodilation and natriuresis, could be involved in the pathogenesis of hypertension in its early stages.

Paper IV:

Among the adipocytokines, adiponectin, leptin and CRP (as a pseudo-marker for IL-6) only CRP was significantly associated with CVD and decreased the BMI-associated CVD risk substantially, indicating that IL-6 could play a role as mediator of overweight- and obesity-related CVD.

PERSPECTIVES

The rise in obesity among both adults and children is a severe health crisis worldwide. In this thesis, besides confirming raised

BMI as a major independent risk for the development of hypertension, several biochemical abnormalities among hypertensive individuals have been elucidated. Ultimately, the goal is to develop better individualized prevention strategies, risk stratification models to pinpoint exactly those individuals at higher risk, and eventually, personalized medical treatment (for example therapeutic agents that elevate NP concentrations in hypertensive individuals with low circulating levels of NPs), to avoid unnecessary side effects and gain maximum effect. Even though the studies in this thesis are interesting, they were purely observational, and can therefore only describe associations. To document true cause-effect relationship randomized interventional studies are required. A relatively new method to investigate whether a factor is causally related to a variable is Mendelian randomization, which takes advantage of the random assortment of genes that occurs during gamete formation. Under some assumptions, this provides an unbiased method of assessing whether a specific substance, which has a genetic component, is causally related to a certain risk factor or vice versa. Thus, in our case, to assess whether genotypes, which specifically increases or lowers plasma levels of the various biomarkers e.g. leptin, adiponectin or NT-proBNP, influence BP levels independent of other risk factors. The conduction of additional Mendelian randomization studies could therefore be a constructive and less resource-demanding approach to obtain further knowledge than full-scale intervention trials.

LIST OF ABBREVIATIONS

BMI = Body mass index
BNP = Brain natriuretic peptide
BP = Blood pressure
CI = Confidence intervals
CRP = C-reactive protein
CVD = Cardiovascular disease
DBP = Diastolic blood pressure
HbA1c = Hemoglobin A1c
IHD = Ischemic heart disease
IL-6 = Interleukin-6
NP = Natriuretic peptides
NRI = Net reclassification improvement
NT-proBNP = N-terminal pro-brain-type natriuretic peptide
OR = Odds ratio
PAR = Population attributable risk
RAAS = Renin-angiotensin-aldosterone system
SBP = Systolic blood pressure
SD = Standard deviation
SNS = Sympathetic nervous system
WC = Waist circumference

SUMMARY

Background

Hypertension is one of the leading causes of cardiovascular disease (CVD) worldwide. Overweight and obesity are major risk factors for hypertension. The mechanisms linking these two diseases are incompletely understood, but abnormalities in several different pathways including insulin and glucose metabolism, inflammation, the sympathetic nervous system and the renin-angiotensin-aldosterone system have been known for decades. Lately, the attention has shifted toward the endocrine function of adipose tissue, which among others secrete adiponectin, leptin and interleukin-6 (IL-6), which stimulates liver CRP production. These substances have all been regarded as candidate intermediates between adiposity and the development of hypertension.

Furthermore, the so-called “natriuretic handicap” which characterizes obesity, has also attracted a great deal of attention as a possible pathway.

Primary Hypotheses

- The adipocytokines, adiponectin, leptin and CRP (used as a surrogate marker of IL-6) are independently associated with prevalent and incident hypertension.
- Five-year weight changes associate with BP alterations, even after adjustment for changes in lifestyle risk factors and serum insulin.
- NT-proBNP (used as a surrogate marker of active BNP) is positively associated with prevalent hypertension, but negatively associated with incident hypertension.
- The adipocytokines, adiponectin, leptin and CRP (used as a surrogate marker of IL-6), are independently associated with incident CVD.

Methods

The Inter99 study provided data for this thesis. In brief, Inter99 is a randomized, non-pharmacological intervention study for the prevention of ischemic heart disease. The study included approximately 6,700 participants from the background population, who were thoroughly examined at baseline. Various measurements, including blood samples, were done at baseline and 5-year follow-up. Data about cardiovascular events were gathered from national registers.

Results

Paper I:

In the prevalent model including leptin, CRP, adiponectin, sex, age, lifestyle risk factors, lipids, insulin, hemoglobin A1c, and in the incident model which also included baseline heart rate and blood pressure, only leptin of the three candidate intermediates was significantly associated with both prevalent and incident hypertension.

Paper II:

Five-year weight changes were associated with blood pressure alterations and had a substantial impact on both fasting and two-hour post-glucose serum insulin levels. However, in multivariable regression analyses, additional adjustments for insulin values only attenuated the associations between weight changes and blood pressure minimally.

Paper III:

Higher serum concentrations of NT-proBNP associated with prevalent hypertension whereas lower concentrations associated with incident hypertension.

Paper IV:

Among 6,502 participants with a mean follow-up time of 11.4 years, 527 participants experienced one or multiple cardiovascular events. Among adiponectin, leptin and CRP, only CRP were significantly positive associated with CVD in all models.

Conclusion

Regarding the pathophysiology of overweight-related hypertension and CVD, our results indicate that:

- Leptin is possibly an independent risk factor for the development of hypertension.
- Albeit weight loss improves insulin-profile, the effect of insulin on blood pressure changes seems minimal, indicating that insulin does not play a major direct role in the early development of hypertension.
- A deficiency of the natriuretic peptides, resulting in reduced vasodilation and natriuresis, could be involved in the pathogenesis of hypertension in its early stages.
- Since adjustment for CRP decreased the BMI associated CVD risk markedly, our data indirectly suggest that IL-6 originating from fat tissue could play a role in overweight and obesity-related cardiovascular disease.

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