PAPER II

Journal of Thrombosis and Haemostasis, **14**: 1561–1571 DOI: 10.1111/jth.13369

ORIGINAL ARTICLE

C-reactive protein, obesity, and the risk of arterial and venous thrombosis

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To cite this article: Horvei LD, Grimnes G, Hindberg K, Mathiesen EB, Njølstad I, Wilsgaard T, Brox J, Brækkan SK, Hansen JB. C-reactive protein, obesity, and the risk of arterial and venous thrombosis*J Thromb Haemost* 2016; **14**:1561–71.

Essentials

- We performed repeated measurements of C-reactive protein (CRP) and obesity in a cohort study.
- CRP was associated with risk of myocardial infarction and venous thromboembolism.
- CRP was a mediator for risk of myocardial infarction in obese men and women.
- CRP was a partial mediator for risk of venous thromboembolism in obese women, but not in men.

Summary. *Background:* Low-grade inflammation in obesity may be a shared pathway for the risk of venous thromboembolism (VTE) and myocardial infarction (MI). *Objectives:* To investigate the associations between repeated measurements of C-reactive protein (CRP) and the risks of MI and VTE, and to explore whether CRP mediated these risks in obese subjects. *Methods:* CRP and obesity measures were collected from 15 134 subjects who participated in one or more surveys of the Tromsø study in 1994–1995, 2001–2002, or 2007–2008. Incident VTEs and MIs were registered until 1 January 2011. Time-varying Cox regression models were used to calculate hazard ratios of MI and VTE according to categories of CRP and obesity measures. *Results:* There were 291 VTEs and 920 MIs during follow-up. High levels of CRP (≥ 3 mg L^{-1} versus < 1 mg L^{-1}) were associated with increased risks of MI (hazard ratio [HR] 1.73; 95% confidence interval [CI] 1.32–2.26) and VTE (HR 1.84; 95% CI 1.22–2.78) in women, but only with MI in men (HR 1.93; 95% CI 1.53–2.44). All obesity measures showed stronger associations with CRP in women than in men. In obese women (body mass index [BMI] of ≥ 30 kg m^{-2} versus < 25 kg m^{-2}), adjustment for CRP attenuated the risk estimate for VTE by 22%, whereas the incidence rates of VTE increased with combined categories of higher BMI and CRP. No association was found in men. *Conclusions:* Our findings suggest that low-grade inflammation, assessed by measurement of CRP, is associated with the risks of MI and VTE, and may be a shared pathway for MI and VTE in obesity.

Keywords: cardiovascular diseases; inflammation; obesity; risk factors; venous thrombosis.

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Received 28 January 2016
Manuscript handled by: M. Carrier
Final decision: F. R. Rosendaal, 27 April 2016

Introduction

Although obesity has consistently been shown to be a shared risk factor for myocardial infarction (MI) and venous thromboembolism (VTE) [1,2], it is not known to what extent the risk is mediated through the same underlying mechanism(s). Insulin resistance and its related cluster of atherosclerotic risk factors are key abnormalities linking obesity to arterial thrombosis [3]. However, several studies have shown that these cardiometabolic consequences of obesity have little impact on VTE risk [1,2,4].

Growing evidence suggests that inflammation plays a pivotal role in cardiovascular risk in obese subjects [5,6]. High-sensitivity C-reactive protein (CRP), which is a sensitive marker of inflammation, is robustly associated with an increased MI risk [7]. In obesity, visceral adipose tissue is infiltrated by macrophages, which secrete inflammatory substances such as tumor necrosis factor and interleukin-6 [8]. The regulation of CRP synthesis in the liver is thought to be driven by interleukin-6 [9], and inflamed

adipose tissue may even produce CRP [10]. Thus, the level of CRP is closely related to inflammation in adipose tissue [6].

In contrast to the consistent association between CRP and MI risk in prospective studies, conflicting results have been reported for the association between CRP and VTE risk [11–16]. Temporary fluctuations and changes in CRP levels over time may result in underestimation of the true association between exposure and outcome (regression dilution bias) [17], particularly in cohorts with long follow-up. Accordingly, this phenomenon may partly explain why no association between CRP and VTE has been reported in some cohorts with relatively long follow-up [11,18].

An approach to minimize the impact of regression dilution bias is to perform time-varying analyses. When modifiable variables are measured at different time points within the same individual during the study period, time-varying analysis will allow for changes in exposure status during follow-up. By taking repeated measurements into account, we aimed to investigate the associations between CRP and the risks of MI and VTE, and to explore whether CRP partly mediated these risks in obese subjects.

Materials and methods

Study population

Participants were recruited from the fourth, fifth and sixth surveys of the Tromsø study (conducted in 1994–1995, 2001–2002, and 2007–2008, respectively) [19]. The Tromsø study is a single-center prospective cohort study, in which inhabitants living in the municipality of Tromsø, Norway were invited to participate. Overall participation rates were high, ranging from 78% in Tromsø 4 to 66% in Tromsø 6. Participants were aged 25–89 years at study entry. A detailed description of study participation has been published elsewhere [19]. Subjects with a known pre-baseline history of VTE (n = 121) or MI (n = 978) were excluded from the study. Furthermore, subjects were excluded if they had missing values for CRP in all surveys (n = 306). Thus, our study population consisted of 14 981 unique subjects. Individuals who attended multiple surveys had exposure data updated at each survey, and contributed with one observation per survey, yielding 22 697 observations in total (Fig. 1). The study was approved by the Regional Committee of Medical and Health Research Ethics, and all participants gave their informed written consent to participate.

Measurements

Data were collected by physical examination, from non-fasting blood samples, and from self-administered questionnaires, and were updated at each survey. Height and weight were measured with subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg m^{-2}). Waist circumference (WC) was measured in centimeters at the umbilical line. Hip circumference (HC) was measured in centimeters at the widest point at the hips. The waist-to-hip ratio was calculated by dividing WC by HC. The waist-to-height ratio (WHtR) was calculated by dividing WC by body height in centimeters. Information on self-reported diabetes, physical activity and current smoking was collected from self-administered questionnaires. Physical activity was defined as exercise with sweat production and breathlessness for $\geq$ 1 h per week during leisure time. Non-fasting blood samples were collected from an antecubital vein. High-sensitivity CRP was analyzed in thawed aliquots after storage at − 70 °C (Tromsø 4) or − 20 °C (Tromsø 5 and Tromsø 6) with a particle-enhanced immunoturbidimetric assay on a Modular P (Tromsø 4 and Tromsø 6) or Hitachi 917 (Tromsø 5) autoanalyzer (Roche Hitachi, Mannheim, Germany), with reagents from Roche Diagnostics (Mannheim, Germany). Samples from Tromsø 4 were analyzed after 12 years of storage, and samples from Tromsø 5 and Tromsø 6 were analyzed in batches at the time of the surveys. The lower detection limit of the high-sensitivity CRP assay was 0.03 mg L^{-1}, and measurements of CRP lower than 0.03 mg L^{-1} were therefore set at this value. The analytical coefficient of variation for CRP levels between 0.1 mg L^{-1} and 20 mg L^{-1} was < 4%. Blood pressure, serum lipid levels and glycosylated hemoglobin were measured as previously described [20].

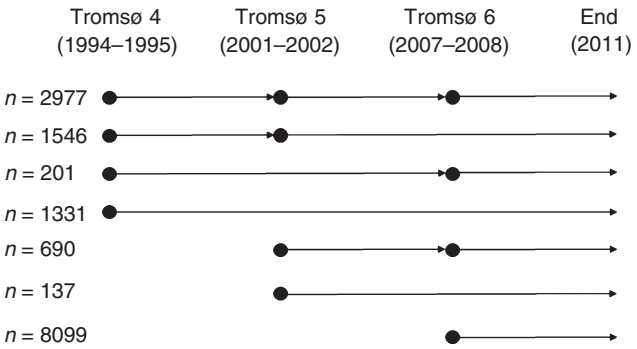

Fig. 1. Overview of study inclusion. Dots indicate participation at the survey, and arrows indicate observation periods. A total of 14 981 unique individuals were included in the study. Of these, 2977 individuals participated in all three surveys, 2437 participated in two of three surveys, and 9567 participated in only one survey.

Outcome assessment for VTE

All first lifetime events of VTE were identified by searching the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry at the University Hospital of North Norway (UNN) from the

date of enrollment in the Tromsø study (1994–1995) to 1 January 2011, as previously described [20]. All hospital care and relevant diagnostic radiology in the Tromsø municipality is provided exclusively by this hospital. The medical record for each potential VTE case was reviewed by trained personnel. A potential VTE event derived from the hospital discharge diagnosis registry or the radiology procedure registry was recorded when the presence of clinical signs and symptoms of deep vein thrombosis or pulmonary embolism were combined with objective confirmation tests (compression ultrasonography, venography, spiral computed tomography, perfusion–ventilation scan, pulmonary angiography, or autopsy), and resulted in a VTE diagnosis that required treatment. VTE cases from the autopsy registry were recorded when the death certificate indicated VTE as cause of death or a significant condition associated with death.

Outcome assessment for MI

Adjudication of hospital and out-of-hospital events was performed by an independent endpoint committee, and was based on data from hospital and out-of-hospital journals, autopsy records, and death certificates. The national 11-digit identification number allowed linkage to national and local diagnosis registries. Cases of incident MI were identified by linkage to the discharge diagnosis registry at the UNN with a search for ICD 9 codes 410–414 in the period 1994–1998, and thereafter ICD 10 codes I20–I25. The hospital medical records were retrieved for case validation. Modified World Health Organization MONICA/MORGAM [21] criteria for MI were used, and included clinical symptoms and signs, findings in electrocardiograms, values of cardiac biomarkers, and autopsy reports when applicable. Furthermore, linkage to the National Causes of Death Registry at Statistics Norway allowed identification of fatal incident cases of MI that occurred as out-of-hospital deaths, including deaths that occurred outside of Tromsø, as well as information on all-cause mortality. Information from the death certificates was used to collect relevant information on the event from additional sources such as autopsy reports and records from nursing homes, ambulance services, and general practitioners.

Statistical analyses

Follow-up time and risk estimates were calculated separately for VTE and MI. For each participant, person-years of follow-up were accrued from the date of survey attendance to the date when the event of interest (e.g. VTE or MI) occurred, to the date on which the participant died or officially moved from the municipality of Tromsø, or up to the end of the study period (1 January 2011). Subjects who attended more than one survey contributed with one observation period per attended survey, and both exposure and confounder information was updated at each survey (Fig. 1). Observations of a CRP level of > 10 mg L^{-1} ($n = 1\,011$) were excluded from further analysis to avoid possible acute-phase reactions. Subjects who died ($n = 1\,385$) or moved from the municipality of Tromsø ($n = 743$) during the follow-up period were censored from the date of death or migration, respectively.

Statistical analyses were carried out with STATA (version 13; Stata Corporation, College Station, TX, USA) and R (version 3.03 for Windows). Normality of the independent variables was assessed by visual inspection of probability plots and histograms of the residuals. CRP showed a right-skewed distribution, and was log-transformed when used as a continuous variable. Pearson correlation coefficients were calculated between CRP and the anthropometric measures.

Cox proportional hazards regression models were used with age as the time-scale [22]. CRP was categorized into low-risk (< 1.0 mg L^{-1}), intermediate-risk (1.0–3.0 mg L^{-1}) or high-risk (> 3.0 mg L^{-1}) groups, according to the American Heart Association and Centers for Disease Control and Prevention (AHA/CDC) guidelines for cardiovascular risk [23]. We calculated sex-specific incidence rates and hazard ratios (HRs) with 95% confidence intervals (CIs) for MI and VTE, using the low-risk group as reference, in models adjusted for smoking, and models adjusted for smoking, physical activity, and BMI.

Next, we estimated HRs with 95% CIs of MI and VTE across predefined categories of BMI and WHtR. BMI and WHtR were chosen for these analyses because they showed the highest correlations with CRP in women and men, respectively. For BMI, overweight was defined as a BMI of 25–29.9, and obesity as a BMI of ≥ 30 [24], whereas WHtR was dichotomized with boundary ratio set at 0.5 [25]. In order to address the impact of CRP on the relationship between obesity and the two outcomes, we performed analyses in a model adjusted for smoking (Model 1), and then subsequently added CRP to the model (Model 1 + CRP). By using bootstrapping, we tested whether CRP mediated the associations between obesity measures and the two outcomes. We generated 10 000 replicate samples with replacement, and calculated the 95% CIs of the change in HRs when CRP was added to the models. Percentage changes in HRs were calculated by use of the formula ([HR with CRP adjustment − HR without CRP adjustment]/[HR without CRP adjustment − 1]) × 100%. CIs were calculated from the 2.5th and 97.5th percentiles of the replicate sample distributions [26]. Finally, we included a model in which we adjusted for smoking, physical activity, CRP, and obesity-related atherosclerotic risk factors (systolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, HbA_{1C}, and self-reported diabetes mellitus) (Model 2).

To assess the joint effect of high BMI and a high CRP level on the risks of the outcomes (i.e. MI and VTE), the

other eight constellations of BMI and CRP were compared with normal weight and a low CRP level (BMI of < 25 and CRP level of < 1.0 mg L^{-1}). We calculated incidence rates and HRs with 95% CIs in models adjusted for smoking.

Statistical interactions between anthropometric measures, CRP and the other variables in the models were tested, and no interactions were found. The proportional hazards assumption was verified by evaluating the parallelism between the curves of the log–log survivor function for different categories of the variables.

Results

The mean age at inclusion was 59 ± 12 years (range, 25–87 years) in men and 60 ± 12 years (range, 25–89 years) in women. There were 920 incident MIs and 291 incident VTEs during a median follow-up of 3.1 years (range, 0.01–16.3 years). Of these subjects, 26 experienced MI and VTE during follow-up. For MI, the overall crude incidence rates per 1000 person-years were 12.3 (95% CI 11.2–13.3) in men and 6.1 (95% CI 5.5–6.7) in women. The corresponding figures for VTE were 2.7 (95% CI 2.2–3.2) and 2.8 (95% CI 2.4–3.2).

The characteristics of the study population are shown in Table 1. The distribution of CRP and traditional atherosclerotic risk factors was similar between genders. The correlation coefficients between obesity measures and CRP are shown in Table 2. The associations between obesity measures and CRP were stronger for women than for men, and BMI ($r = 0.24$ in men and $r = 0.40$ in women) and WHtR ($r = 0.29$ in men and $r = 0.40$ in women) showed the highest correlations with CRP. Correlations between the different anthropometric measures are shown in Table S1.

Risk estimates for MI and VTE across AHA/CDC risk categories of CRP are shown in Table 3. High CRP levels (≥ 3 mg L^{-1} versus < 1 mg L^{-1}) were significantly associated with an increased MI risk in men (HR 1.93; 95% CI 1.53–2.44) and in women (HR 1.73; 95% CI 1.32–2.26). Women with high CRP levels had an almost two-fold increased VTE risk (HR 1.84; 95% CI 1.22–2.78) as compared with those with low CRP levels, whereas no association between CRP and VTE risk was found in men. Further adjustment for BMI attenuated the risk estimates for both MI and VTE.

HRs of MI across predefined risk categories of BMI and WHtR are shown in Table 4. Among men, 17% had a BMI of ≥ 30 and 80% had an WHtR of ≥ 0.5. Obese subjects of both genders, according to these criteria, had a higher MI risk, with the strongest associations being seen in men. The risk estimates were substantially attenuated (22–45%), and most profoundly in women, after addition of CRP to the adjustment models (Table 4). Additional adjustment for atherosclerotic risk factors led to further attenuation of the HRs.

Table 1 Study population characteristics

	Men (n = 6941)	Women (n = 8193)
No. of observations*	10 072	12 625
Age (years), mean ± SD	58.9 ± 11.6	59.6 ± 11.9
BMI (kg m^{-2}), mean ± SD	26.8 ± 3.6	26.4 ± 4.5
Overweight (BMI of 25–29.9), % (n)	51.2 (5157)	38.5 (4863)
Obesity (BMI of ≥ 30), % (n)	16.6 (1670)	19.1 (2417)
Waist circumference (cm), mean ± SD	97.1 ± 10.3	87.7 ± 12.0
Hip circumference (cm), mean ± SD	103.3 ± 6.4	103.1 ± 8.9
Waist-to-hip ratio, mean ± SD	0.94 ± 0.07	0.85 ± 0.07
Waist-to-height ratio, mean ± SD	0.55 ± 0.06	0.54 ± 0.08
CRP (mg L^{-1}), median (IQR)	1.9 (0.7–2.4)	1.9 (0.6–2.4)
Systolic blood pressure (mmHg), mean ± SD	141 ± 20	138 ± 25
Total cholesterol (mmol L^{-1}), mean ± SD	6.0 ± 1.2	6.2 ± 1.3
HDL cholesterol (mmol L^{-1}), mean ± SD	1.4 ± 0.4	1.6 ± 0.4
Triglycerides (mmol L^{-1}), mean ± SD	1.7 ± 1.1	1.4 ± 0.8
Diabetes mellitus, % (n)	1.8 (158)	1.8 (197)
Smoking, % (n)	25.1 (2510)	24.7 (3085)

BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; SD, standard deviation. *Subjects contributed with one observation per attended survey.

Table 2 Pearson's correlation coefficients (r) for the association between C-reactive protein (CRP)* and anthropometric measures

	Men		Women	
Anthropometric measure	Pearson's r	P	Pearson's r	P
Body mass index	0.24	< 0.001	0.40	< 0.001
Waist circumference	0.27	< 0.001	0.38	< 0.001
Hip circumference	0.13	< 0.001	0.33	< 0.001
Waist-to-hip ratio	0.28	< 0.001	0.27	< 0.001
Waist-to-height ratio	0.29	< 0.001	0.40	< 0.001

*CRP log-transformed.

Obesity was associated with higher risk estimates for VTE in both genders (Table 5). However, the effects of adjusting for CRP were more pronounced in women, with attenuation of the risk estimates by 16–22%, as compared with 4–7% in men. Further adjustment for atherosclerotic risk factors had a minor impact on the HRs. Corresponding analyses were also performed separately for provoked and unprovoked VTE. There were no significant differences between provoked and unprovoked VTE (12–18% versus 14% attenuation in analysis including both genders), but the numbers of events in the subgroups were too low to give reliable results.

Table 3 Crude incidence rates (IRs) and hazard ratios (HRs) with 95% confidence intervals (CIs) of myocardial infarction and venous thromboembolism across American Heart Association and Centers for Disease Control and Prevention risk categories of C-reactive protein (CRP)

		Myocardial infarction				Venous thromboembolism			
CRP	*n**	Events	IR† (95% CI)	Model 1‡ HR (95% CI)	Model 2§ HR (95% CI)	Events	IR† (95% CI)	Model 1‡HR (95% CI)	Model 2§ HR (95% CI)
Both genders (sex-adjusted)									
< 1 mg L^{-1}	9318	214	5.6 (5.0–6.4)	Reference	Reference	87	2.0 (1.6–2.5)	Reference	Reference
1–2.9 mg L^{-1}	9278	416	9.6 (8.7–10.6)	1.35 (1.15–1.58)	1.27 (1.07–1.51)	136	3.1 (2.6–3.7)	1.29 (0.99–1.69)	1.21 (0.89–1.63)
≥ 3 mg L^{-1}	4101	263	13.4 (12.1–15.4)	1.82 (1.53–2.17)	1.67 (1.37–2.03)	68	3.5 (2.7–4.4)	1.41 (1.02–1.94)	1.24 (0.86–1.79)
Men									
< 1 mg L^{-1}	3958	136	7.6 (6.5–9.0)	Reference	Reference	41	2.3 (1.7–3.1)	Reference	Reference
1–2.9 mg L^{-1}	4356	264	13.6 (12.0–15.3)	1.46 (1.19–1.80)	1.34 (1.08–1.67)	60	3.0 (2.3–3.9)	1.11 (0.74–1.65)	1.06 (0.69–1.62)
≥ 3 mg L^{-1}	1758	155	19.3 (16.5–22.6)	1.93 (1.53–2.44)	1.66 (1.29–2.13)	22	2.6 (1.7–4.0)	0.93 (0.55–1.57)	0.83 (0.47–1.47)
Women									
< 1 mg L^{-1}	5360	105	4.2 (3.5–5.1)	Reference	Reference	46	1.8 (1.4–2.4)	Reference	Reference
1–2.9 mg L^{-1}	4922	152	6.4 (5.5–7.5)	1.20 (0.93–1.54)	1.19 (0.90–1.58)	76	3.2 (2.5–4.0)	1.46 (1.01–2.12)	1.39 (0.91–2.11)
≥ 3 mg L^{-1}	2343	108	9.6 (8.0–11.6)	1.73 (1.32–2.26)	1.80 (1.32–2.46)	46	4.1 (3.0–5.4)	1.84 (1.22–2.78)	1.69 (1.04–2.75)

*Observations. †Crude IRs per 1000 person-years. ‡Age as time-scale, adjusted for smoking. §Age as time-scale, adjusted for smoking, physical activity, and body mass index.

Sex-specific crude incidence rates (per 1000 person-years) of MI and VTE across combined categories of BMI and CRP are shown in Fig. 2. For MI, subjects of both genders with the highest CRP levels and highest BMIs had the highest incidence rates. In women, there was a clear additive effect of high BMI and CRP level on VTE incidence rates (Fig. 2), whereas in men there was no such pattern. The corresponding HRs with 95% CIs according to the combined categories are shown in Table S2.

Discussion

We aimed to investigate the impact of chronic low-grade inflammation, as assessed by repeated measurements of CRP during follow-up, as a potential common pathway for the risks of arterial and venous thrombosis in obese subjects. All obesity measures showed stronger correlations with CRP in women than in men. Serum CRP levels were associated with increased MI risk in both genders, and the risk estimates for MI in obese subjects were attenuated by 22–45% after adjustment for CRP. In women, high CRP levels were associated with VTE risk, and in obese women the risk was moderately (16–22%) attenuated after adjustment for CRP. Our findings suggest that chronic low-grade inflammation may be a shared pathway for obesity-related MI and VTE, but that its impact is less in VTE, particularly in men.

Population-based studies have shown that CRP is consistently associated with obesity [27,28], and the ability of CRP to predict cardiovascular disease has been confirmed in > 20 cohorts [7]. However, previous results regarding the association between CRP and VTE risk have given discrepant results. There is growing evidence in support of our hypothesis that regression dilution bias resulting from intraindividual variation in inflammatory markers camouflages a true, but moderate, impact of low-grade inflammation on VTE risk in studies with long-term follow-up. First, several prospective cohorts, including previous findings from the Tromsø study, which based their results merely on baseline CRP measures, reported no association between CRP and VTE risk [11,14,18]. Second, the otherwise robust association between CRP and VTE reported in the HUNT study was not present in subjects with > 3 years between blood sampling and the VTE event [29]. However, other cohorts with long follow-up have demonstrated an almost two-fold increased VTE risk [12,30].

Our findings suggest a sex-specific relationship between CRP, obesity, and VTE risk. First, we showed that all obesity measures showed stronger associations with CRP in women than in men. Second, the association between obesity measures and VTE risk was moderately attenuated after adjustment for CRP in women, but not in men. Accordingly, no association was found between CRP and VTE risk in the Physicians' Health Study, which included

Table 4 Sex-specific crude incidence rates (IRs) and hazard ratios (HRs) with 95% confidence intervals (CIs) for myocardial infarction in obese subjects, before and after adjustment for C-reactive protein (CRP)

	*n**	Events	IR† (95% CI)	Model 1‡ HR (95% CI)	Model 1‡ + CRP HR (95% CI)	Absolute attenuation of HR after inclusion of CRP in the model§	Percentage attenuation of HR after inclusion of CRP in the model	Model 2¶ HR (95% CI)
Both genders (sex-adjusted)								
BMI of < 25	8590	302	7.5 (6.7–8.4)	Reference	Reference	–	–	Reference
BMI of 25–29.9	10 020	412	8.9 (8.1–9.8)	1.22 (1.05–1–41)	1.13 (0.97–1.32)	0.09 (0.07–0.14)	41	1.02 (0.86–1.22)
BMI of ≥ 30	4087	206	11.0 (9.6–1.3)	1.64 (1.37–1.96)	1.41 (1.17–1.70)	0.23 (0.16–0.32)	36	1.12 (0.90–1.40)
WHtR of < 0.5	5777	151	5.2 (4.4–6.1)	Reference	Reference	–	–	Reference
WHtR of ≥ 0.5	16 920	769	10.1 (9.4–10.9)	1.60 (1.34–1.91)	1.43 (1.20–1.72)	0.17 (0.13–0.27)	28	1.17 (0.95–1.40)
Men								
BMI of < 25	3245	169	10.9 (9.4–12.7)	Reference	Reference	–	–	Reference
BMI of 25–29.9	5157	282	12.3 (10.9–13.4)	1.33 (1.10–1.62)	1.24 (1.02–1.51)	0.09 (0.06–0.14)	27	1.11 (0.88–1.38)
BMI of ≥ 30	1670	104	15.1 (12.5–18.3)	1.71 (1.33–2.20)	1.48 (1.15–1.91)	0.23 (0.14–0.34)	32	1.20 (0.89–1.62)
WHtR of < 0.5	1803	61	6.9 (5.4–8.9)	Reference	Reference	–	–	Reference
WHtR of ≥ 0.5	8269	494	13.5 (12.3–14.8)	1.93 (1.47–2.52)	1.73 (1.32–2.27)	0.20 (0.11–0.30)	22	1.40 (1.03–1.92)
Women								
BMI of < 25	5345	133	5.3 (4.5–6.3)	Reference	Reference	–	–	Reference
BMI of 25–29.9	4863	130	5.6 (4.7–6.7)	1.00 (0.78–1.28)	0.93 (0.73–1.19)	0.07 (0.03–0.12)	Not applicable	0.84 (0.64–1.12)
BMI of ≥ 30	2417	102	8.6 (7.1–10.5)	1.46 (1.12–1.91)	1.26 (0.96–1.67)	0.20 (0.08–0.33)	43	0.98 (0.71–1.37)
WHtR of < 0.5	3974	90	4.4 (3.6–5.4)	Reference	Reference	–	–	Reference
WHtR of ≥ 0.5	8651	275	7.0 (6.2–7.8)	1.29 (1.02–1.65)	1.16 (0.90–1.49)	0.13 (0.06–0.23)	45	0.94 (0.70–1.26)

BMI, body mass index; WHtR, waist-to-height ratio. *Observations. †Crude IRs per 1000 person-years. ‡Age as time-scale, adjusted for smoking. §Absolute attenuation of HR with 95% CI after inclusion of CRP in the model (i.e. change in HR from Model 1 to Model 1 + CRP). ¶Age as time-scale, adjusted for smoking, systolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, HbA_{1C}, self-reported diabetes mellitus, physical activity, and CRP.

Table 5 Sex-specific crude incidence rates (IRs) and hazard ratios (HRs) with 95% confidence intervals (CIs) for venous thromboembolism in obese subjects, before and after adjustment for C-reactive protein (CRP)

	*n**	Events	IR† (95% CI)	Model 1‡ HR (95% CI)	Model 1‡ + CRP HR (95% CI)	Absolute attenuation of HR after inclusion of CRP in the model§	Percentage attenuation of HR after inclusion of CRP in the model	Model 2¶ HR (95% CI)
Both genders (sex-adjusted)								
BMI of < 25	8590	82	2.0 (1.6–2.5)	Reference	Reference	–	–	Reference
BMI of 25–29.9	10 020	138	2.9 (2.5–3.5)	1.45 (1.10–1.91)	1.40 (1.06–1.86)	0.05 (− 0.01 to 0.11)	11	1.17 (0.85–1.63)
BMI of ≥ 30	4087	71	3.7 (3.0–4.7)	1.73 (1.25–2.39)	1.62 (1.15–2.26)	0.09 (− 0.03 to 0.27)	15	1.59 (1.07–2.36)
WHtR of < 0.5	5777	49	1.7 (1.3–2.2)	Reference	Reference	–	–	Reference
WHtR of ≥ 0.5	16 920	242	3.1 (2.8–3.6)	1.63 (1.19–2.23)	1.55 (1.12–2.13)	0.08 (− 0.02 to 0.19)	13	1.48 (1.01–2.16)
Men								
BMI of < 25	3240	33	2.1 (1.5–3.0)	Reference	Reference	–	–	Reference
BMI of 25–29.9	5172	68	2.9 (2.3–3.7)	1.51 (0.99–2.31)	1.49 (0.97–2.29)	0.02 (− 0.05 to 0.10)	4	1.36 (0.83–2.20)
BMI of ≥ 30	1680	22	3.1 (2.0–4.7)	1.65 (0.96–2.86)	1.61 (0.92–2.81)	0.04 (− 0.13 to 0.23)	6	1.78 (0.93–3.39)
WHtR of < 0.5	1803	16	1.8 (1.1–2.9)	Reference	Reference	–	–	Reference
WHtR of ≥ 0.5	8269	107	2.9 (2.4–3.5)	1.44 (0.85–2.46)	1.41 (0.82–2.41)	0.03 (− 0.08 to 0.16)	7	1.30 (0.70–2.42)
Women								
BMI of < 25	5323	49	1.9 (1.5–2.6)	Reference	Reference	–	–	Reference
BMI of 25–29.9	4866	70	3.0 (2.4–3.8)	1.40 (0.97–2.03)	1.33 (0.91–1.94)	0.07 (− 0.03 to 0.17)	18	1.03 (0.66–1.61)
BMI of ≥ 30	2414	49	4.1 (3.1–5.4)	1.78 (1.19–2.68)	1.61 (1.05–2.47)	0.17 (− 0.08 to 0.42)	22	1.50 (0.90–2.49)
WHtR of < 0.5	3974	33	1.6 (1.1–2.3)	Reference	Reference	–	–	Reference
WHtR of ≥ 0.5	8651	135	3.4 (2.9–4.0)	1.74 (1.18–2.57)	1.62 (1.08–2.41)	0.12 (− 0.05 to 0.30)	16	1.60 (0.99–2.59)

BMI, body mass index; WHtR, waist-to-height ratio. *Observations. †Crude IRs per 1000 person-years. ‡Age as time-scale, adjusted for smoking. §Absolute attenuation of HR with 95% CI after inclusion of CRP in the model (i.e. change in HR from Model 1 to Model 1 + CRP). ¶Age as time-scale, adjusted for smoking, systolic blood pressure, total cholesterol, HDL cholesterol, triglycerides, HbA_{1C}, self- reported diabetes mellitus, physical activity, and CRP.

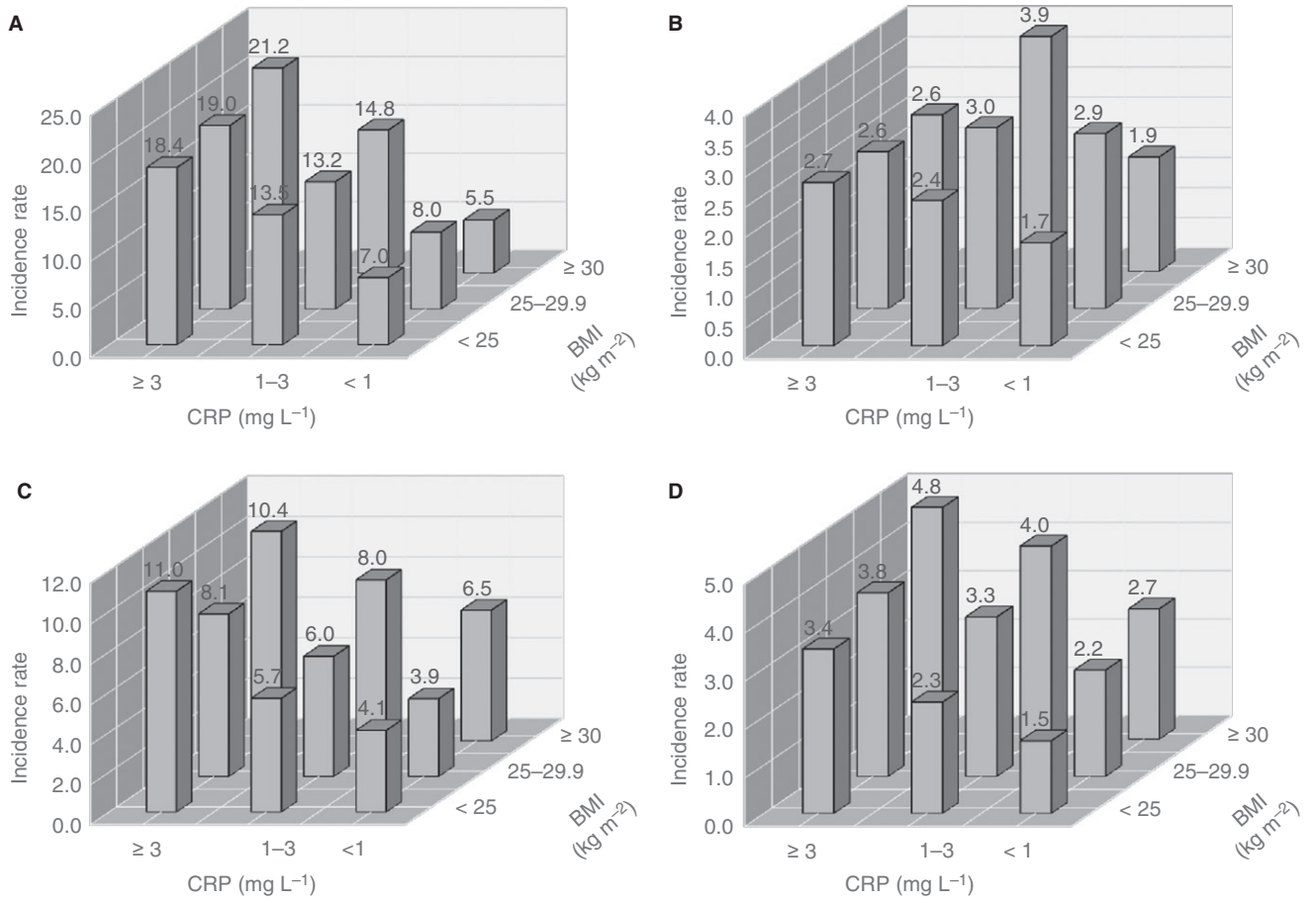

Fig. 2. Sex-specific crude incidence rates per 1000 person-years of myocardial infarction (MI) and venous thromboembolism (VTE) across constellations of C-reactive protein (CRP) and body mass index (BMI). (A) Men, MI. (B) Men, VTE. (C) Women, MI. (D) Women, VTE.

only male subjects [16]. Third, the incidence rates of VTE increased with higher predefined combined categories of CRP and obesity measures in women, but not in men.

Recently, Olson *et al.* investigated the effect of the association of CRP, albumin, white blood cell count and platelet count on VTE risk in a large mixed cohort of white and black Americans (n = 30 239), with a follow-up of 4.6 years (the REGARDS study) [15]. In agreement with our findings, their data suggested that the association between BMI and VTE was partially (13% and 21% in the overweight and obese categories, respectively) mediated by CRP [15]. Unfortunately, they did not report gender-specific analyses.

Inflammatory substances released by adipose tissue induce insulin resistance and endothelial dysfunction, which can promote atherosclerosis and arterial thrombosis [31]. Furthermore, inflammation stimulates the synthesis of plasminogen activator inhibitor 1, tissue factor, fibrinogen, and potentially other factors involved in the coagulation cascade [32,33]. Plasma levels of plasminogen activator inhibitor 1 and factor VIII are elevated in obesity [34], and high levels of these factors have been associated with increased VTE risk in several studies [14,35]. Unlike for MI, the increased risk of VTE in obesity is not dependent on central obesity. In fact, a high HC, especially in women, is a strong risk factor for VTE [20,36]. HC is, to a greater extent, correlated with subcutaneous fat [37], which shows a much lower correlation with inflammatory markers [6]. In the INTERHEART study, the investigators found an inverse relationship between HC and the risk of MI [38], which supports the concept of different causal pathways for VTE and MI. Atherosclerosis is a key component for the development of MI, but does not play a central role in the development of VTE [39–41]. Obesity-induced inflammation may enhance both atherosclerosis and coagulation, and thereby promote the risk of MI in obese individuals. In contrast, the procoagulant effects of obesity-induced inflammation alone may not be sufficient to cause VTE. Thus, other mechanisms, such as hampered venous flow in obese individuals resulting from increased intra-abdominal pressure, or the procoagulant effects of adipokines or extracellular vesicles [42], might be more important for VTE risk.

The main strengths of our study are the prospective design with repeated measurements, long-term follow-up,

large number of participants, and validated VTE and MI events. All hospital care in the region is exclusively provided by a single hospital, which facilitates the completeness of our outcome registries. However, it is possible that a few cases (e.g. in nursing homes) could have been treated without a confirmed diagnosis. Likewise, pulmonary embolisms presenting as out-of-hospital sudden deaths might have been misclassified. The use of frozen blood samples for analysis may introduce bias if the stability of the measured biomarker is affected by freezing, thawing, or by storage itself. In the present study, CRP was analyzed in thawed serum aliquots after 12 years (Tromsø 4) or in consecutive batches during the course of the study (Tromsø 5 and Tromsø 6), with no freezing–thawing cycling before measurement. Previous studies examining CRP stability in frozen samples found a high correlation between CRP values obtained before and after storage [43,44]. As CRP is merely a marker of inflammation, and probably not a causative agent [45], inflammatory activity not detected by measurement of CRP might still be present. However, levels of CRP are closely related to levels of both circulating and adipose tissue tumor necrosis factor and interleukin-6 [46,47]. Invasive samples are required for direct measurement of inflammatory activity in the adipose tissue, and these are more suited for experimental studies. Both anthropometric measures and CRP are modifiable risk factors, and the time from registration of exposure to end of follow-up is, despite repeated measurements, relatively long, which increases the chance of regression dilution. However, subjects tend to have stable CRP concentrations; the intraindividual correlation coefficient of serum levels of CRP measured years apart is ~ 0.5, which is comparable to that of cholesterol [48]. As body weight tends to increase with age [49], the inflammation associated with obesity probably also increases, potentially leading to underestimation of the true risks. Statin therapy lowers CRP levels [50], and, unfortunately, we did not have reliable information on statin use. However, the degree of exposure misclassification will be similar for the two outcomes.

In conclusion, our findings suggest that CRP is associated with the risks of both MI and VTE, and partly mediates the association between obesity and MI and VTE, particularly in women.

Addendum

L. D. Horvei contributed to data analysis and writing of the manuscript. G. Grimnes contributed to data interpretation and revision of the manuscript. K. Hindberg and T. Wilsgaard provided statistical support and contributed to revision of the manuscript. E. B. Mathiesen, I. Njølstad, and J. Brox contributed to data collection and revision of the manuscript. S. K. Brækkan contributed to data collection, and interpretation and revision of the manuscript. J.-B. Hansen contributed to the conception and design of the study, data collection, and interpretation and revision of the manuscript.

Acknowledgements

K. G. Jebsen TREC is supported by an independent grant from the K.G. Jebsen Foundation. L. D. Horvei is in receipt of a grant from the Northern Norway Regional Health Authority.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pearson correlation coefficients between anthropometric measures and C-reactive protein (CRP).
Table S2. Joint effects of high BMI and high CRP on the risks of myocardial infaction (MI) and venous thromboembolism (VTE).

References

1 Braekkan SK, Hald EM, Mathiesen EB, Njolstad I, Wilsgaard T, Rosendaal FR, Hansen JB. Competing risk of atherosclerotic risk factors for arterial and venous thrombosis in a general population: the Tromso study. *Arterioscler Thromb Vasc Biol* 2012; **32**: 487–91.
2 Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. *Circulation* 2008; **117**: 93–102.
3 Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006; **113**: 898–918.
4 Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular risk factors and venous thromboembolism incidence: the longitudinal investigation of thromboembolism etiology. *Arch Intern Med* 2002; **162**: 1182–9.
5 Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006; **444**: 881–7.
6 Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 2005; **96**: 939–49.
7 Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. *J Am Coll Cardiol* 2007; **49**: 2129–38.
8 Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol* 2011; **29**: 415–45.
9 Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000; **148**: 209–14.
10 Anty R, Bekri S, Luciani N, Saint-Paul MC, Dahman M, Iannelli A, Amor IB, Staccini-Myx A, Huet PM, Gugenheim J, Sadoul JL, Le Marchand-Brustel Y, Tran A, Gual P. The

inflammatory C-reactive protein is increased in both liver and adipose tissue in severely obese patients independently from metabolic syndrome, Type 2 diabetes, and NASH. *Am J Gastroenterol* 2006; **101**: 1824–33.
11 Hald EM, Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Brox J, Hansen JB. High-sensitivity C-reactive protein is not a risk factor for venous thromboembolism: the Tromso study. *Haematologica* 2011; **96**: 1189–94.
12 Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein and risk of venous thromboembolism in the general population. *Arterioscler Thromb Vasc Biol* 2010; **30**: 1672–8.
13 Kamphuisen PW, Eikenboom JC, Vos HL, Pablo R, Sturk A, Bertina RM, Rosendaal FR. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. *Thromb Haemost* 1999; **81**: 680–3.
14 Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Tracy RP, Aleksic N, Folsom AR. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). *Am J Med* 2002; **113**: 636–42.
15 Olson NC, Cushman M, Lutsey PL, McClure LA, Judd S, Tracy RP, Folsom AR, Zakai NA. Inflammation markers and incident venous thromboembolism: the REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. *J Thromb Haemost* 2014; **12**: 1993–2001.
16 Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997; **336**: 973–9.
17 Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. *BMJ* 2010; **340**: c2289.
18 Mahmoodi BK, Gansevoort RT, Veeger NJ, Matthews AG, Navis G, Hillege HL, van der Meer J. Microalbuminuria and risk of venous thromboembolism. *JAMA* 2009; **301**: 1790–7.
19 Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njolstad I. Cohort profile: the Tromso Study. *Int J Epidemiol* 2012; **41**: 961–7.
20 Horvei L, Brækkan S, Mathiesen E, Njølstad I, Wilsgaard T, Hansen J-B. Obesity measures and risk of venous thromboembolism and myocardial infarction. *Eur J Epidemiol* 2014; **29**: 821–30.
21 MORGAM project. MORGAM Manual. Morgam Project e-publications 2001. Available from URL: http://www.thl.fi/publications/morgam/manual/followup/form22.htm [cited 2016 April 11].
22 Thiebaut AC, Benichou J. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. *Stat Med* 2004; **23**: 3803–20.
23 Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107**: 499–511.
24 World Health Organization. *Obesity: Preventing and Managing the Global Epidemic; Report of a WHO Consultation*. Geneva: World Health Organization, 2000.
25 Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev* 2012; **13**: 275–86.
26 Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. *Stat Med* 2000; **19**: 1141–64.
27 Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999; **282**: 2131–5.
28 Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obes Rev* 2013; **14**: 232–44.
29 Quist-Paulsen P, Naess IA, Cannegieter SC, Romundstad PR, Christiansen SC, Rosendaal FR, Hammerstrom J. Arterial cardiovascular risk factors and venous thrombosis: results from a population-based, prospective study (the HUNT 2). *Haematologica* 2010; **95**: 119–25.
30 Folsom AR, Lutsey PL, Astor BC, Cushman M. C-reactive protein and venous thromboembolism. A prospective investigation in the ARIC cohort. *Thromb Haemost* 2009; **102**: 615–19.
31 Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. *Nat Rev Cardiol* 2009; **6**: 399–409.
32 Faber DR, De Groot PG, Visseren FLJ. Role of adipose tissue in haemostasis, coagulation and fibrinolysis. *Obes Rev* 2009; **10**: 554–63.
33 Joseph L, Fink LM, Hauer-Jensen M. Cytokines in coagulation and thrombosis: a preclinical and clinical review. *Blood Coagul Fibrinolysis* 2002; **13**: 105–16.
34 De Pergola G, Pannacciulli N. Coagulation and fibrinolysis abnormalities in obesity. *J Endocrinol Invest* 2002; **25**: 899–904.
35 Meltzer ME, Lisman T, de Groot PG, Meijers JC, le Cessie S, Doggen CJ, Rosendaal FR. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood* 2010; **116**: 113–21.
36 Severinsen MT, Kristensen SR, Johnsen SP, Dethlefsen C, Tjonneland A, Overvad K. Anthropometry, body fat, and venous thromboembolism: a Danish follow-up study. *Circulation* 2009; **120**: 1850–7.
37 Seidell JC, Pérusse L, Després J-P, Bouchard C. Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. *Am J Clin Nutr* 2001; **74**: 315–21.
38 Yusuf S, Hawken S, Ôunpuu S, Bautista L, Franzosi MG, Commerford P, Lang CC, Rumboldt Z, Onen CL, Lisheng L, Tanomsup S, Wangai P, Razak F, Sharma AM, Anand SS. Obesity and the risk of myocardial infarction in 27000 participants from 52 countries: a case-control study. *Lancet* 2005; **366**: 1640–9.
39 Hald EM, Lijfering WM, Mathiesen EB, Johnsen SH, Lochen ML, Njolstad I, Wilsgaard T, Rosendaal FR, Braekkan SK, Hansen JB. Carotid atherosclerosis predicts future myocardial infarction but not venous thromboembolism: the Tromso study. *Arterioscler Thromb Vasc Biol* 2014; **34**: 226–30.
40 Reich LM, Folsom AR, Key NS, Boland LL, Heckbert SR, Rosamond WD, Cushman M. Prospective study of subclinical atherosclerosis as a risk factor for venous thromboembolism. *J Thromb Haemost* 2006; **4**: 1909–13.
41 van der Hagen PB, Folsom AR, Jenny NS, Heckbert SR, O'Meara ES, Reich LM, Rosendaal FR, Cushman M. Subclinical atherosclerosis and the risk of future venous thrombosis in the Cardiovascular Health Study. *J Thromb Haemost* 2006; **4**: 1903–8.
42 Braekkan SK, Siegerink B, Lijfering WM, Hansen JB, Cannegieter SC, Rosendaal FR. Role of obesity in the etiology of deep vein thrombosis and pulmonary embolism: current epidemiological insights. *Semin Thromb Hemost* 2013; **39**: 533–40.
43 Ishikawa S, Kayaba K, Gotoh T, Nakamura Y, Kario K, Ito Y, Kajii E. Comparison of C-reactive protein levels between serum and plasma samples on long-term frozen storage after a 13.8 year interval: the JMS Cohort Study. *J Epidemiol* 2007; **17**: 120–4.

44 Lewis MR, Callas PW, Jenny NS, Tracy RP. Longitudinal stability of coagulation, fibrinolysis, and inflammation factors in stored plasma samples. *Thromb Haemost* 2001; **86**: 1495–500.
45 Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 2010; **375**: 132–40.
46 Maachi M, Pieroni L, Bruckert E, Jardel C, Fellahi S, Hainque B, Capeau J, Bastard JP. Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. *Int J Obes Relat Metab Disord* 2004; **28**: 993–7.
47 Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999; **19**: 972–8.
48 Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; **111**: 1805–12.
49 Baum CL II, Ruhm CJ. Age, socioeconomic status and obesity growth. *J Health Econ* 2009; **28**: 635–48.
50 Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 2005; **352**: 20–8.